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The application of gene-set analysis to identify the molecular genetic correlates of human cognitive abilities

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Declaration

I hereby declare that this thesis is of my own composition, and that it contains no material previously submitted for the award of any other degree. The work reported in this thesis has been executed by myself, except where due acknowledgement is made in the text.

William David Hill

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Abstract

Individual differences across seemingly disparate cognitive tests are not independent. This general factor of cognitive ability allows around half of the variation in a diverse battery of cognitive tests to be explained in terms of individual differences along a single dimension. An individual's position on this dimension, as ascertained using standardised tests of cognitive ability (intellectual quotient (IQ) tests), has been shown to be predictive of important life events ranging from educational and occupational success, to enjoying good health and longevity. Genetic differences have been shown to be associated with differences in cognitive ability and recent molecular genetic research has demonstrated that variants in linkage disequilibrium with common single nucleotide polymorphisms (SNPs) can explain around 50% of the variation in general cognitive ability.

The goal of this thesis was to build on these findings by applying gene-set analysis methods to examine genome-wide association data sets to test guided hypotheses regarding the mechanisms and genetic architecture of human cognitive differences. Gene set analysis is a method that can lead to an increase in statistical power and help derive functional meaning from the results of genome wide association studies (GWAS). Existing GWAS data sets provided by the Cognitive Ageing Genetics in England and Scotland (CAGES) consortium, the Brisbane Adolescent Twin Study (BATS) and the Norwegian Cognitive NeuroGenetics (NCNG) cohort were used. The individuals in each of these groups have also completed a battery of cognitive tests enabling the extraction of a general factor of fluid cognitive ability and a measure of crystallised ability.

In Chapter 3, the role of synaptic plasticity was examined using data derived from proteomic experiments on human and animal brain tissue which details the molecular constituents of the postsynaptic density and the associated components of the glutamatergic synapse. These components include: the α-amino-3-hydroxy-5-methyl-4-isoxazoiepropionic acid receptor complex (AMPA-RC), the *N*-methyl-D-aspartate receptor complex (NMDA-RC), and the metabotropic glutamate 5 receptor complex (mGlu5-RC). Using a competitive

test of enrichment it was shown that the genes responsible for the proteins of the NMDA-RC were associated with fluid cognitive ability. This study (published as Hill et al., 2014) indicates that individual differences in synaptic plasticity may underlie some of the differences in fluid cognitive ability.

In Chapter 4, rather than using traditionally defined linear pathways, the focus was on a gene set created by grouping genes according to their cellular function. Linear pathways, such as the glutamatergic system share proteins, a property which can be exploited by utilising horizontal pathway analysis, also termed functional gene group analysis. In a functional gene group analysis genes are grouped according to their cellular function such as ligand gated ion channels, neurotransmitter metabolism, and G protein relays. This chapter (published as Hill et al., 2014) examined the role that heterotrimeric G proteins play in cognitive abilities as previous work has indicated a role for them in individual differences in human cognitive ability. The analyses carried out in this chapter indicate that whilst heterotrimeric G proteins may be required to engage in cognitive tasks, genetic variation in the genes that code for these proteins is not associated with normal variation in cognitive ability.

Chapter 5 examined the role of functional SNPs, defined as those that have been shown to be able to alter protein expression. Previous research has shown an association between genotype and methylation status and between genotype and gene expression in human cortical tissue. Using the results of previous research, gene sets were assembled which detailed SNPs known to alter methylation status and gene expression in the frontal cortex, the temporal cortex, the pons, and the cerebellum. In addition, the bioinformatics database dbQsnp was mined to assemble a SNP set detailing SNPs in known promoter regions. Finally, a gene set was made using published literature to capture SNPs affecting microRNA. Two complementary statistical methods were used to examine these sets for an association with general cognitive ability. The results of these analyses indicate that these gene sets are not more associated with cognitive ability beyond what would be expected by chance.

Chapter 6 exploits the current knowledge of the molecular genetics of non-syndromic autosomal recessive intellectual disability (NS-ARID). The 40 genes associated with NS-ARID have a large deleterious effect on cognitive ability and appear to do so without the cognitive deficit being the product of obvious pathology. These 40 NS-ARID genes were examined as a gene set for an enriched association with cognitive abilities. Additionally, the biological systems that these genes are involved in were examined using an automated literature mining tool. These systems were then examined for an enriched association with general cognitive ability. When examining the 40 NS-ARID genes as a set there was no evidence that they were associated with cognitive abilities. The results of the literature search provided 180 additional gene sets based on the relationship between the 40 NS-ARID genes. These gene sets were examined for an enriched association with cognitive ability where the sodium ion transporter gene set (GO:0006814) was shown to be significantly enriched in the CAGES data set, but not BATS data set, for fluid ability. This could indicate that whilst the same genes are not involved in both intellectual disabilities and in cognitive abilities, the genes that can contain mutations resulting in intellectual disabilities are found in pathways that govern the normal range of cognitive ability.

The results of this thesis indicate that common SNPs which tag causal variants are not randomly distributed across the genome but are clustered in genes that work together as part of a larger mechanism. In addition this work provides working examples of how multiple data sources that can be utilised to construct gene sets designed to explore the known relationship between genotype and cognitive ability and to utilise GWAS data sets to prioritise groups of genes.

Contents

Chapter 1: Introduction to intelligence

1.1 Intelligence

The degree in which people are capable of cognitively challenging activities is one such way that individuals can differ. Differences in this capacity can be referred to as differences in intelligence (abbreviated to as intelligence from this point), however loose verbal descriptions of intelligence make subsequent quantification impossible and with the myriad of tests used, often with seemingly unrelated material, vocabulary or reaction time for example, it is not possible to describe intelligence in terms of the tests used in its assessment. Indeed, such verbal descriptions are not always agreed on by psychologists studying intelligence (Neisser et al., 1996). Despite this, individual differences in standardized tests of intelligence, IQ tests, have been shown to be predictive of educational attainment (Deary, Strand, Smith, & Fernandes, 2007a), everyday decision making (Gottfredson, 1997) and even of mortality (Calvin et al., 2011). This makes understanding the origins of these differences important to intelligence researchers and those outside the field alike.

Regarding the source of these intelligence differences it has been shown that the more similar individuals are in terms of their genetic makeup the more similar their performance on intelligence tests, demonstrating a heritable component to intelligence. This finding has been shown using methods which compare parents to their offspring, as well as comparing twins, (Bouchard & McGue, 1981) as described in section 2.1-2.2. More recently, this relationship between genotype and phenotype has been shown using common SNPs from unrelated individuals using the Genome-Wide Complex Trait Analysis (GCTA) method (Yang et al., 2010; Yang, Lee, Goddard, & Visscher, 2011). However, whilst GCTA does provide an estimate of the proportion of variance attributable to genetic variants correlated with common DNA variants, like the twin method, it does not indicate which genetic variants are of particular importance and so cannot be used to examine the biological systems that are responsible for intelligence using current sample sizes.

With these current limitations in mind, the goal of this thesis is to build on the finding that there is a genetic element to intelligence and begin to prioritize specific groups of genes selected in accord with a particular theory regarding intelligence differences. Chapter 3 examines the role of synaptic plasticity using data derived from proteomic experiments on human and animal brain tissue. These data detail the molecular constituents of the postsynaptic density and the associated components of the glutamatergic synapse including the α-amino-3-hydroxy-5-methyl-4-isoxazoiepropionic acid receptor complex (AMPA-RC), the *N*-methyl-D-aspartate receptor complex (NMDA-RC) and the metabotropic glutamate 5 receptor complex (mGlu5-RC).

Chapter 4 examines a different set of genes expressed in the synapse. This set corresponds to the heterotrimeric G proteins, created using functional gene group analysis where genes are grouped according to their cellular function, such as ligand gated ion channels and neurotransmitter metabolism rather than according to classically defined vertical pathways such as the glutametergic system. This method has the potential to increase power and the heterotrimeric G proteins have previously been associated with intelligence (Ruano et al., 2010).

Chapter 5 looks for an enriched association between functional single nucleotide polymorphisms (SNPs), defined as those that can alter protein expression through a known association with methylation, gene expression or being found in promoter regions or effect microRNA.

Chapter 6 builds on the knowledge of the molecular genetics of intellectual disabilities to determine if the same genes and biological systems are involved in both intellectual disability and the normal range of intelligence differences.

1.2 The structure of Intelligence

Before the empirical work in this thesis, addressing the search for the molecular underpinnings of intelligence is presented; it is important to have an operational definition of

what intelligence is at the phenotypic level. As mentioned, such verbal definitions as "problem-solving ability" or "processing capacity" do not help with establishing an operational definition of intelligence, nor do they help with the question of whether intelligence is composed of a single ability or if it is best described in terms of multiple independent or dependent abilities. The next sections detail the development of the idea of the phenotype of intelligence. Whilst the notions of intelligence being a unitary construct predate the foundation of differential psychology (Spencer, 1855) it also predates the statistical method of factor analysis to test this hypothesis, and so, this review begins by detailing the work of the discoverer of the general factor of cognitive ability as well as a pioneer of factor analysis, Charles Spearman. Following this, hierarchical models of intelligence will be discussed before looking at models that do not include a general factor. Finally, the predictive validity of the general factor will be addressed.

1.3 Spearman's Two-factor Theory of Intelligence

Charles Spearman (1863 - 1945) invented one of the first forms of factor analysis and was the first to use this method to address the question of whether intelligence can be described in terms of individual differences along a single dimension (Spearman, 1904). Factor analysis allows the researcher to test if cognitive performance across a wide variety of tests can be described by a smaller number of dimensions termed factors. A factor, in the parlance of factor analysis, refers to an underlying hypothetical variable that cannot be observed and can also be called a latent variable. Whereas latent variables cannot be directly observed, they can be sampled and, in the case of cognitive ability, this sampling takes the form of batteries of cognitive tests to derive a latent measure of intelligence or performance on intellectually engaging material such as that found in schools which can be used to derive a latent variable describing educational attainment. A factor or factors will emerge following a factor analysis if there are non-zero correlations between all or most of the cognitive abilities required to score well in the test battery.

Spearman (1904) applied an early form of factor analysis to teachers' ratings of the rank order of 22 pupils' performance across different school subjects. Included in the school subjects of Classics, French, English, Mathematics, and Music was a test of Pitch discrimination, enabling Spearman to test an earlier hypothesis of Galton's that intellectual ability was linked to seemingly more basic processes including information intake (Galton, 1883). What Spearman found was that the pupil's ranks between any two subjects was positively correlated, a phenomenon termed a positive manifold. In addition to this, following the inclusion of the pitch discrimination task, the positive manifold remained, indicating the link first suggested by Galton between an individual's level of intellectual ability and the keenness of the senses. Spearman found that a single factor could explain some of the variance on each test. Spearman termed this latent trait *g* in an effort to move it from a verbal definition of general intelligence to a scientific construct describing a universal source of variance for diverse cognitive abilities.

In order to show the importance of this general cognitive trait, Spearman used the observed correlations between the subjects and *g* to derive the degree to which each pair of subjects correlated due to the variance each pair shared with *g*. Once the variance attributable to *g* had been removed from each test, no residual correlations between the subjects remained, indicating that the observed correlations only occurred due to each subject providing a measure of *g*.

Spearman used this finding to construct his two-factor theory of intelligence, where *g* was the sole source of communality and accounted for 63% of the observed variance. Although the statistical methods deviate from what is used today, when factor analysis is applied to sample sizes larger than that used by Spearman a general factor accounting for 63% appears an overestimation when considering modern estimates of around 40% (Carroll, 1993).

The remaining variance in his battery of tests, termed *s*, was attributable to both the variance specific to each test and to measurement error. Whilst termed the two-factor theory,

it is important to note that test specific variance did not constitute a factor as there would be as many independent sources of variance, termed *s*, as there were tests used.

1.3.2 Criticisms of Spearman's two factor theory

Notably absent from Spearman's two factor theory of intelligence, when compared to the psychometric models of today, are group factors. Group factors reflect the shared variance of tests that is not attributable to *g* but rather to domain-specific factors such as memory or information processing speed (Carroll, 1993). Whilst Spearman's early form of factor analysis was limited in the sense that it could only be used on a correlation matrix where a single underlying factor provided the only common source of variance his data were reanalysed by Carroll (1993). Carroll (1993) found that Spearman's hypothesis was supported by this data. However, the method devised by Spearman is only appropriate when a single factor is present. If more than one common factor exists Spearman's method will not work. Additionally, Spearman also constructed his test battery in such a way that no two tests were measuring the same ability outside of *g*. Spearman contended that in addition to measurement error the variance in any test was due to a completely general source, *g*, or a completely specific source and so attributable to *s*. If groups of tests were found to correlate more highly with those of similar content, such as groups of tests each stressing speed of response for example, Spearman concluded that they were each measuring the same *s* indicating that they were equivalent forms of the same test. This strict dichotomy of total generality and total specificity was found to be insufficient to explain such clusters of correlations which were soon found to be common in large batteries of tests (Burt, 1917) leading to the notion of group factors. A group factor would be a source of variance that is more general than the *s* of Spearman's model but does not have the same level of generality across the range of cognitive abilities that *g* would have. By 1927 a wealth of data indeed indicated that these group factors were the norm in large data sets and led Spearman to concede that group factors did exist (Spearman, 1927).

1.4. Carroll's hierarchical three factor model

Carroll's model accounts for the positive manifold discovered by Spearman with a domain-general factor. However, whilst Spearman's method involved the extraction of first-order factors by directly examining the correlation matrices, Carroll's model includes second and third-order factors. Second-order factors arise when multiple first-order factors are derived from the correlation matrix, which in turn correlate with each other. This allows for a further factor analysis to determine if the variance in the first-order factors is better explained by a smaller number of factors. Carroll (1993) reanalysed over 430 data sets drawn from predominantly English speaking groups to show that cognitive abilities were organised into three hierarchical levels. At the first level are narrow abilities that are measured by single tests. The second level features abilities that are much more general than the first level but still constrained into particular aspects of cognitive ability and include such abilities as fluid ability (*gf*) involving problem solving with novel information and crystallised ability (*gc*) utalising previously learned knowledge, as well as memory, visual perception, auditory perception, retrieval ability, mental speed and processing speed (see Figure 1.1). However, these factors were correlated with each other indicating a third-stratum factor which accounted for around 50% of the observed variance in test scores. The group factors load highly on the *g* factor where in adulthood these loadings are .80 and above.

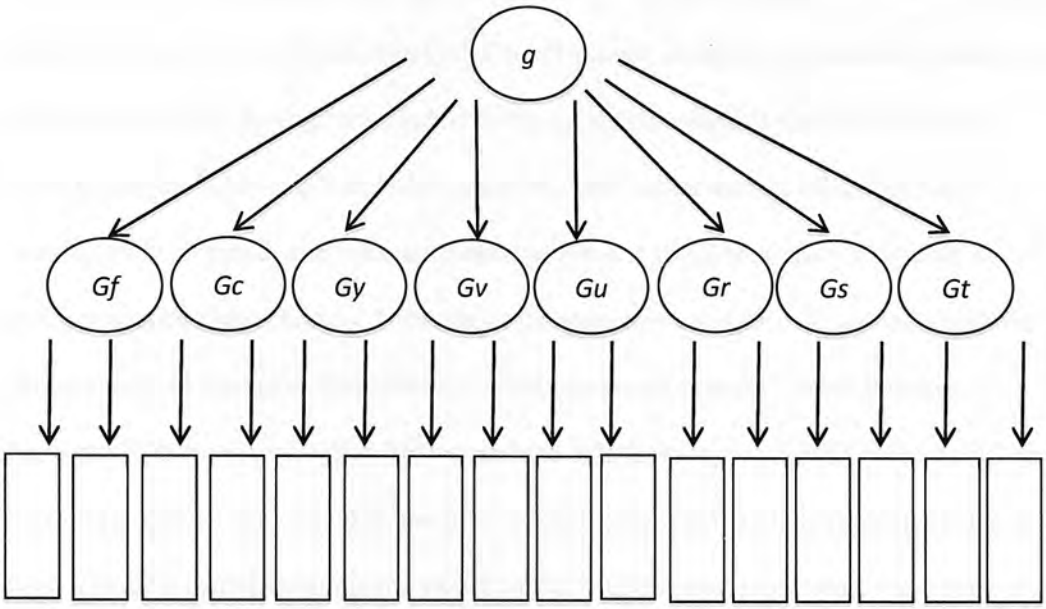

Figure 1.1 Graphical representation of the distribution of variance in Carroll's model. Moving up through the levels, variance becomes more general and less specific to any cognitive domain or test. The first level shows the specific tests. The second shows that tests which tax similar abilities correlate more highly with each other than tests that measure other abilities indicating the presence of group factors such as fluid intelligence (G*f*), crystallised intelligence (G*c*), general memory and learning (G*y*), broad visual perception (G*v*), broad auditory ability (G*u*), broad retrieval ability (G*r*), broad cognitive speediness (G*s*), and processing speed (G*s*). At the top of the hierarchy is general cognitive ability.

1.4.2 Criticisms of Carroll's hierarchical three factor model and the *g*-VPR model

Whilst the wealth of data collected by Carroll indicates that the three stratum view of human intelligence accounts for the positive manifold and the existence of group factors, the accuracy of this model was questioned by a study which compared Carroll's model to both the Cattell and Horn *gf – gc* model and an earlier hierarchical model (Vernon, 1964, 1965). Vernon's model incorporated a *g* factor and so accounted for the positive manifold between cognitive abilities in addition to the two group factors of verbal educational and perceptual motor ability. Using a sample of 436 adults who had completed 42 tests of mental ability and

confirmatory factor analysis Johnson and Bouchard Jr (2005b) showed that Vernon's model provided a better fit to the data than both Carroll's three stratum model and the Cattell Horn *gf – gc* model. In addition to this finding Johnson and Bouchard Jr (2005b) extended Vernon's model to include four levels of ability. Like earlier models these four were hierarchically arranged with the least general abilities at the first-stratum, followed by multiple second-order abilities. These are more general but still focused around a specific skill set, such as the distinction between verbal comprehension and verbal fluency. Importantly, these second-order factors can load onto more than one third-order factor. Three third-order factors were included, the first two being the verbal and the perceptual factors from Vernon's model. Johnson and Bouchard Jr (2005b) included an additional factor of rotational ability which is measured by tests involving the mental manipulation of stimuli. These third-order factors also show strong correlations between them leading to the need for a fourth level consisting of a single domain general factor *g*. This four-stratum model (*g*-VPR model) provides a better fit to the data than other models and has been replicated (Johnson & Bouchard Jr, 2005a) and found to provide a better fit when compared to the Cattell Horn Carroll model which included a general factor (Major, Johnson, & Deary, 2012).

1.5 Theories of multiple intelligences

Spearman's idea that a diverse battery of cognitive tests yielded a single factor indicating general intelligence was challenged by Thurstone (1938b) who postulated that the positive manifold was a result of impure tests which measured multiple independent abilities. In his own analysis of 240 university students he found that seven "Primary mental abilities" (PMA)-verbal comprehension, word fluency, arithmetic, spatial ability, memory, processing speed, and reasoning ability-were evident. Thurstone contended the positive manifold existed because each test used by Spearman drew on multiple PMAs and that if tests could be designed that would only measure a single PMA no such general factor would be found.

Thurstone's conclusions rested on rotating his factors to simple structure with, at

first, orthogonal vectors. The aim of simple structure is to aid in the psychological interpretation of what the factors represent by ensuring the tests have large loadings on a small number of factors, and near zero loadings on others. Ideally tests would load on a single factor. The ease in interpretation can be seen when, for example, tests that incorporate verbal abilities load highly on one factor following rotation to simple structure. This underlying factor can be called a factor of verbal ability.

Methods of rotation do not alter the degree to which each test will correlate with other tests but rather alter the position, and therefore the loading, of the latent variable that is used to account for the variance seen in the tests. Whilst Thurstone did expressly state that his method would allow for a single general factor to be extracted (Thurstone, 1938b) the use of orthogonal vectors is ill suited to this task. The general factor of cognitive ability arises as a result of each test positively correlating with every other test in a battery. Whilst rotating the factors to simple solution using orthogonal vectors does not eliminate this, it does lead to the variance that is attributable to a domain general factor being redistributed amongst the tests new loadings on the orthogonal factors. This form of rotation can provide a level of clarity when naming the factors found to be present in a test battery however, it does result in the loss of clear evidence for a domain general factor. A further point illustrating the unsuitability of rotation to simple structure using orthogonal factors lies in the fact that some tests may load highly on multiple factors following rotation making simple structure impossible to achieve. Building on his earlier work with factor rotation (Thurstone, 1938a) devised a method by which the factors can be rotated but with oblique vectors. The advantage of using oblique vectors allows a closer approximation to simple structure and accounts for the positive manifold as now the factors themselves are permitted to correlate if the data allows. This method allows for a domain general factor to be extracted as it is now accounted for by the correlation between the oblique factors. This form of analysis based on the extraction of factors from the correlation of factors is known as a hierarchical factor analysis.

The *g* factor derived only accounted for 31% of the variance it should be noted that

the data came from university students, a sample that by definition, are in the upper end of the distribution for intelligence. This would constrain the differences between them in terms of a general factor and inflate domain specific cognitive abilities. Indeed this is what was found when Thurstone applied his tests to a sample of school children.

Howard Gardner's theory of Multiple Intelligences (MI) (Gardner, 1983) begins with the assumption that there is no single general factor. The positive manifold observed is a consequence of psychological tests only measuring a small subset of what could be termed intelligence. Gardner also asserts that multiple forms of intelligence exist and are independent. Gardner identifies seven forms of intelligence including linguistic ability, logico-mathematic ability, spatial intelligence, kinesthetic intelligence, naturalistic intelligence being the ability to deal with elements in the natural environment, intrapersonal intelligence and interpersonal intelligence. Whilst each of these abilities can be named and described to be single abilities, over 100 years of psychometric studies into the structure of intelligence has shown that many of these abilities are not independent and the positive manifold can be found between tests that are cognitive in nature. The MI theory of intelligence was explicitly tested in the work of (Visser, Ashton, & Vernon, 2006), using the Wonderlic Personnel Test. These data showed there to be a strong *g* factor that had high loadings on Gardner's Linguistic, Logical/Mathematical, Spatial, Naturalistic, Interpersonal intelligences. There were also lower loadings for tests of other abilities, particularly Kinesthetic intelligence. An additional problem for the MI theory was that once the effect of *g* was removed there were only weak correlations between tests thought by Gardner to measure the same form of intelligence.

1.6 Cattell and Horn fluid and crystallised model

In contrast to single *g* models Cattell proposed that what appeared to be a general ability was in fact composed of two highly co-operative systems (Cattell, 1963). Cattell named these two factors Crystallised ability (*gc*) and Fluid ability (*gf*) to distinguish between

mental activities which required the individual to draw upon a body of acquired knowledge and, in the case of *gf*, tasks that required adaptive thinking to solve problems featuring novel information. The link between these two factors is that an individual's level of *gc* would be a product of their level of *gf*, specifically how it is invested. Cattell theorized that the more time an individual spent engaged in study or cultural pursuits the greater their level of *gc*. Additionally those with a high level of *gf* would require less exposure to information before they could assimilate information meaning that they would also have a high level of *gc*.

The tests that are the best measures of *gc* will draw heavily on scholastic knowledge and verbal ability and is typically measured using tests of vocabulary. The tests that are the best measures of *gf* will not draw on verbal ability but rather include tests which include novel information and will be less affected by the culture of the tests taker than tests of *gc*. Following their discovery of *gf* and *gc* additional group factors of visual inspection speed, fluency in recall of learned information, auditory thinking and quantitative reasoning were later added (Cattell, 1971, 1987; Horn, 1985). The *gf-gc* distinction made by Cattell is supported by evidence from ageing research where, *gf* shows the same age related decline as other physical attributes, whereas *gc* is more robust to the effects of ageing (Tucker-Drob & Salthouse, 2008).

1.6.2 Criticisms of the Cattell and Horn fluid and Cryatallised model

The *gf-gc* model asserts that there is no single factor corresponding to general intelligence (Cattell, 1971; Horn, 1985). This argument stems in part from the belief whilst a general factor can be extracted it would vary between batteries, making it an artifact of the tests used. This was directly tested using a hierarchical confirmatory factor analysis using 400 individuals from the Minnesota Study of Twins Reared Apart (Johnson, Bouchard Jr, Krueger, McGue, & & Gottesman, 2004; Johnson, te Nijenhuis, & Bouchard, 2008). Johnson et al. (2004) found that the *g* factor formed using three separate test batteries, the Hakstian and Cattell Comprehensive Abilities Battery, the Hawai Battery including Raven's Matrices

and the Wecshler Adult Intelligence Scale, correlated .99 .99 and 1. This result was replicated by Johnson et al. (2008) and provides strong evidence that *g* is not merely a statistical artifact created by factor analysis, but rather all these batteries are providing a measure of the same domain general ability. This indicates that that the extraction of a general factor is valid, because such a factor is not merely a creation of the test but rather the *g* factors from different tests are all measures of one trait.

The positive manifold found in tests of cognitive ability is one of the most replicated findings in psychology and indicates a trait common to seemingly disparate cognitive tasks. Models that do not acknowledge the positive manifold are not consistent with the data. However, as indicated, a model containing *g* alone is insufficient to describe the full structure of intelligence differences, instead meaningful sources of variance are arranged hierarchically with the most domain general source, *g,* at the top and test specific sources acting at the base of the four levels (Johnson & Bouchard Jr, 2005b). Contrary to positions such as those of Gould (1981) the choice of models is not a matter of opinion but one of empirical fact, where confirmatory factor analysis has been used to compare models the *g*-VPR model has consistently shown to be the best fit to the data (Johnson & Bouchard Jr, 2005a, 2005b; Major et al., 2012). Whilst the number of factors in addition to *g* would vary as a function of the tests used in the *g*-VPR theory (Major et al., 2012) the presence of *g* is ubiquitous due to the positive manifold. This degree to which this common source of variance is measured by a test will determine if the test can be used to predict future educational and occupational success as well as future health and mortality.

1.7 The predictive value of intelligence

Whilst the *g* factor remains a ubiquitous feature of any battery of cognitive tests which tap multiple domains in any sample representative of the distribution of mental abilities it does not indicate that the tests provide a measure of anything that exists outside of the tests themselves. In order to establish that these tests are measuring something useful the

results must be compared with an external criterion. The predictive validity of a test, in this case an intelligence test, pertains to the ability to use the score to predict an outcome on a different measure. Intelligence as established by Spearman being the general factor of cognitive ability predicts performance for every type of behavior which requires learning new information and reaching decisions based on the sound use of judgment. This includes school grades, occupational success and social mobility as might be expected, but intelligence scores also predict health outcomes and even mortality.

1.8 Education and social mobility

Some of the earliest forms of cognitive test were used in educational settings to find which children were failing due to lack of ability (Binet, 1905) and which were failing due to other reasons and with the discovery of *g* being made using data from school teachers (Spearman, 1904) it should not be surprising that tests of cognitive ability predict academic achievement. Using longitudinal data, the association between intelligence and educational success appears to be in the range of 0.40 – 0.63 (Jencks, 1979) with a more recent meta-analysis finding similar results (Strenze, 2007). This relationship is greatest at the level of primary school, r = 0.60 – 0.70, following which a decrease can be seen at each stage of education culminating at university level, where the relationship drops to r = 0.40 – 0.50 (Jensen, 1998). This decrease is not attributable to the importance of factors besides intelligence taking precedence, but rather is the consequence of selective dropout from schooling, where those of low cognitive ability fail to progress to the upper levels of education. From this selective dropout there is a restriction of the range of cognitive ability which constrains the correlation between intelligence and education. Using a longitudinal design of 70,000 school children (Deary et al., 2007a) found that the *g* factor from a number of cognitive tests taken at age 11 years correlated at 0.81 with a general factor composed of national exam grades at age 16. The exams in question were from the General Certificate of Secondary Education (GCSE). Although there is some scope to select subjects, the exams are

compulsory and taken prior to the minimum age of school leaving, meaning that these results, with the exception of those who suffer from learning disabilities, include data from almost the full range of the distribution of intellectual ability. The association between each subject with *g* was then examined where correlations ranged from 0.43 (Art and Design) to 0.77 (Mathematics) indicating that performance across the 25 subjects was substantially associated with differences in *g*.

Socioeconomic success, as typically measured through income, occupation and level of education, can also be predicted by intelligence tests (Jensen, 1998). Intelligence test scores have also been shown to be a better predictor of job success than any other variable including personality and even on the job experience (Schmidt & Hunter, 2004).

In a meta-analysis of 135 longitudinal studies (Strenze, 2007) examined the role that general intelligence, education and occupational status play in determining socioeconomic status. Adult social class has been shown to be an important predictor of both access to resources and mortality (Smith, Hart, Watt, Hole, & Hawthorne, 1998). (Deary et al., 2005) investigated the role that childhood cognitive ability played in social status in middle age. Importantly the cognitive tests were administered during the years of compulsory education meaning that each child had been in education for the same number of years. In a sample of 242 males, childhood IQ was associated with SES in midlife. This relationship held for both upward and downward social mobility where the men's fathers SES was used as the point of reference. This was extended by (von Stumm, Macintyre, Batty, Clark, & Deary, 2010) using a sample of 6281 men to show that education acts as a mediating variable between general intelligence and socioeconomic status and again found that intelligence plays a greater role in socioeconomic status than social class of origin.

1.9 Intelligence and mortality

Whilst the notion that health can impact upon cognitive abilities has been shown

(Comijs et al., 2009; Kivipelto et al., 2005; Rafnsson, Deary, Smith, Whiteman, & Fowkes, 2007) the now established field of cognitive epidemiology seeks to examine the reverse direction of association. Cognitive epidemiology grew from two studies (O'Toole, 1990; O'Toole & Stankov, 1992) linking cognitive ability and mortality whilst these initial studies did employ a longitudinal design the participants were only in middle age when mortality was examined meaning that causes of death had yet to be established. Additionally, these studies by O'Toole (1990) & O'Toole and Stankov (1992) only utilised data drawn from males and so any effect of sex on the link between intelligence and health could not be ascertained. Whalley and Deary (2001) used data from the Scottish Mental survey of 1932 where almost every 1921-born Scottish child was given the same cognitive test at age 11 years to reduce this concern. By following them up in old age it was shown that the test taken in childhood could be used to predict survival into old age. This effect was found across the distribution of scores and was not altered by adjusting for socioeconomic position. Indeed, this link between cognitive test scores taken in young adulthood and mortality in middle age has also been found in a sample of 1 million Swedish males (Batty et al., 2009). A systematic review and meta-analysis conducted by Calvin et al. (2011) gathered the results of 16 unrelated studies using a total of 1,107,022 participants. Calvin et al. (2011) found that a 1 standard deviation increase in cognitive ability was associated with a 24% reduction in the risk of death over the follow-up period ranging from 17 to 69 years with no effect of sex. This result remained once adjusted for childhood SES, but was attenuated by 34% by controlling for adult SES and by 54% by controlling for the level of education. However, it maybe that education and adult SES are both providing an imperfect measure of intelligence and that by including these variables the model is over adjusted.

Increasing levels of intelligence may be associated with an increased ability to assess the risk factors that contribute toward disease. Engaging in behaviours that are adverse to health will increase the prevalence of disease, and environmental risk factors such as smoking (Martin, Fitzmaurice, Kindlon, & Buka, 2004) and binge drinking (Batty, Deary, &

Macintyre, 2006) have been linked to intelligence.

Dealing with medical conditions requires the learning of new, and in some cases seemingly abstract, information and using it to reach a decision (Baker, Parker, Williams, & Clark, 1998). Individuals who have a low level of education have been found to be less likely to seek medical intervention with their symptoms (Williams, Baker, Parker, & Nurss, 1998) and are less equipped to deal with a condition, such as diabetes, which require consistent observation and self-medication (Williams et al., 1995).

One theory postulated as to the link between intelligence test scores and longevity is that IQ tests provide a measure of system integrity (Deary, 2012). The system integrity (SI) hypothesis states that tests of general cognitive ability tap into a latent trait of how well an individual's body is put together. The SI theory also states that the link between mortality and intelligence is due to both physical health and cognitive ability requiring a physique that is free from defect. SI theory contrasts with the common-cause hypothesis as in SI both mortality and intelligence are thought to measure the same latent trait of bodily integrity meaning that the two should correlate in youth or before the presence of environmental insult.

The common cause theory, originally postulated to account for the correlation between sensory discrimination and cognitive functioning in old age, states that health and intelligence are linked in old age as a third variable, age, acts on them both. Evidence that supports the SI theory includes the finding that reaction time is correlated with *g* and was found to be a better predictor of death than IQ in a population based sample of 898 participants (Deary & Der, 2005). Indeed, once reaction time was controlled for the link between *g* and mortality was substantially attenuated and no longer statistically significant indicating that reaction time and *g* both share variance and this shared variance is predictive of mortality as would be required for the SI theory. Whilst this evidence is suggestive of a latent trait of system integrity both reaction time and *g* are cognitive abilities and a more parsimonious explanation for their correlation with mortality is that *g* and reaction time measure cognitive ability which in turn is correlated to mortality. A stronger test of SI would

be to find a measure of the latent trait for integrity that cannot be explained by the positive manifold between cognitive abilities. One such measure is fluctuating asymmetry. This is based on the notion that achieving perfect symmetry involves no defects and if defects were present they would be random across the body leading to a deviation from perfect symmetry. A meta-analysis with a total of 1871 individuals concluded that there was a small but consistent correlation of $r = 0.12 – 0.20$, between measures of symmetry and intelligence test scores (Banks, Batchelor, & McDaniel, 2010).

These findings provide strong evidence that tests of cognitive ability are measuring something that exists outside of the context of the test. By being able to predict education, job success and mortality it is clear that tests of cognitive ability are measuring something of importance. The predictive validity of tests of intelligence underscores the importance of understanding the etiology of these differences. Intelligence has also been found to be highly familial indicating that in order to understand the etiology of intelligence differences genetic effects must be considered.

Chapter 2: Introduction to the Genetics of intelligence

2.1 Introduction

This section will provide an introduction to the basic principles of genetic theory beginning with the work of Mendel describing models of single gene phenotypes before moving on to discuss how these laws apply to quantitative traits. Following this, the classical twin method will be described, followed by molecular genetic methods including linkage and association. Finally multi-SNP methods including GCTA and gene set analysis will be discussed.

Much work has been done to elucidate the genetic contributions to general intelligence utilizing behavioural genetic methodology. This section will provide a concise overview of the methods used in linking genotypic variation to phenotypic variation. Prior to the work of Mendel in the 1800's (Mendel, 1865) the accepted wisdom for a phenotype being passed down was one of blended inheritance understood as the progeny of any pairing manifesting an intermediary phenotype somewhere between that which was demonstrated in each of the parents. Mendel used garden peas to show that when pairing plants with different phenotypes, one with a wrinkled pea and one with a smooth pea, the resulting offspring were all smooth rather than a blend. The next step in his experiment involved the self-fertilization of the offspring where Mendel observed 0.75 of this generation displayed the smooth pea phenotype whilst 0.25 demonstrated the wrinkled pea phenotype.

These results demonstrated that the phenotype is not lost between generations but rather is retained and may be seen in later generations. Mendel theorized that each of the plants contained two elements, today referred to as alleles, of which one is inherited from each parent. In this way the genetic potential for phenotypic variation is maintained across the generations, formalised as the particulate law of inheritance. Mendel also theorized that, based on the observed proportion of phenotypes in the third generation of pea plants, one of

the two alleles would be dominant in the sense that only one of the two alleles would need to be present to fully express the associated phenotype. The other allele was described as recessive, and in order to express the phenotype associated with the recessive allele two must be present, one inherited from each parent. The observations Mendel made explained the frequencies of the phenotype observed in the third generation plants and the absence of the wrinkled phenotype from the second generation plants. These findings were used to form the Law of Segregation.

Mendel also used his observations to examine multiple phenotypes and discovered what became to be known as The Law of Independent Assortment. This was shown by demonstrating that two dominant traits alleles corresponding to two different traits were inherited independently, although this is only true in the absence of both pleiotropy and linkage disequilibrium (Falconer & MacKay, 1996).

Whilst Mendel's work provides an elegant account of how phenotypic variation is transmitted and retained across the generations many traits of importance, including intelligence, are continuously distributed across the population and seemingly defy Mendel's laws. The imposed distinction between Mendelian traits, single gene traits inherited in the dominant recessive manner outlined above, and quantitative traits including height, weight and intelligence was not resolved until the work of Fisher (1919). Fisher united the mechanistic account of Mendel's works with his knowledge of statistics to show that if multiple genes are involved in the expression of a phenotype then across a population that phenotype will be normally distributed. In behavioural genetic research, the role that genes play in contributing to the variance of a trait is shown by comparing patterns of covariance between the phenotypic traits and the degree to which individuals share genes. This is used in methods such as comparing the difference between monozygotic twins and dizygotic twins in the classical twin design (Neale & Cardon, 1992). By using related groups such as the twin method it is possible to derive the proportion of variance in a trait that is attributable to genetic or environmental effects.

The variance components approach (Neale, Boker, Xie, & Maes, 1999; Neale & Cardon, 1992) using twin or pedigree data is used to estimate the relative genetic and environmental contributions to any trait. By way of the classic twin design the total observed variance for a trait can be divided into four sources: A, additive genetic effects; D, non-additive genetic effects; C, shared environmental effects; and E, non-shared or unique environmental effects. However, D and C are confounded in data sets where twins are raised together and cannot be estimated in the same model. Additive genetic effects refers to a genetic effect where the effects of each contributing allele sum (Falconer & MacKay, 1996).

Non-additive genetic effects describe instances of where the observed phenotype is not the simply the sum of each contributing allele but rather the product of an interaction. This interaction can take place between loci, referred to as epistasis, or can be an intra-loci interaction, referred to as dominance. Shared environmental effects refers to elements in the environment that both twins were exposed to and can include socio-economic status. Unique environmental effects, together with measurement error, reflects instances from the environment that the twins do not have in common and can include, peer group, periods of illness, or employment.

Estimates for the components are derived according to the principles that monozygotic twins (MZ) are both genetically identical and live in the same household. This means that a correlation between MZ twins' performance on a tests would be an estimate of A+C. Therefore any differences that exist between them are the product of E, their unique environment. Dizygotic twins (DZ) share, on average, only 50% of their genes. If they are raised in the same household a correlation between their performances on a test would be an estimate of 0.5A + C. The difference in their abilities would be a product of their unique environment and the genetic differences that exist between them. Using this logic, and assuming no genetic interactions or more similar environments for MZ twins, it is clear that the difference between the magnitude of the correlation between the MZ twins and the DZ twins is due to the lower genetic similarity of the DZ twins. Indeed, the contributions of

additive genetic effects can be defined as the difference between the MZ correlations and the DZ correlations multiplied by 2.

$$A = 2(rMZ - rDZ)$$

The MZ correlation is a product of the total additive genetic effect in addition to the full shared environmental effect and so magnitude of the unique environmental effects can be estimated subtracting the MZ correlations from 1.

$$E = 1 - rMZ$$

Variance attributable to the shared environment can be estimated by subtracting the additive genetic effects from the MZ correlations.

$$C = rMZ - A$$

This logic can also be extended to multivariate analysis using a cross-trait, cross-twin design. By correlating the score from one twin with the scores from a separate measure in the other twin, the degree to which the two traits are genetically independent can be derived. If the MZ twin cross correlations are greater than the DZ cross correlations then the two measures are not genetically independent and a genetic correlation can be seen. This measure is statistically independent of the additive genetic variance and so a genetic correlation can be high even when the contribution made by additive genetic effects is negligible.

2.2 Application of behavioural genetic methodology to intelligence

The earliest attempt to link genotype with intellectual ability was conducted by Francis Galton who examined eminence, defined as the ability to rise through social

hierarchy (Galton, 1892). By examining historical records he found that the sons of those deemed to be eminent men were more likely to display eminence themselves. Whilst this work did not control for the inheritance of wealth and social connections that would also contribute towards an individual's rise to power, Galton did suggest that twin studies would make a valuable contribution towards his hypothesis that intelligence was passed down. Indeed, the finding that there is a substantial genetic component responsible for variance in *g* has been consistently replicated. In a review containing 30 studies of general cognitive ability (Nichols, 1978) showed that the total genetic contribution to the phenotype, termed broad sense heritability, was around 0.44. Nichols (1978) also conducted his own analysis utilizing data drawn from the National Merit Twin Study containing data from around 3000 sets of same sex twins where he estimated the broad sense heritability to be around 0.70 once corrected for reliability error in zygostiy as well as assortative mating. Bouchard and McGue (1981) conducted an extensive review of the literature comparing the correlations between intelligence test scores and different degrees of relatedness. In a comparison of over 10,000 MZ and DZ twins the correlation between MZ twins was on average around 0.86 with the DZ twins being 0.60 showing that as genotypic similarity increased so too did phenotypic similarity indicating large genetic influences acting on intelligence (see Figure 2.1). Also apparent here is the finding that MZ twins adopted apart show substantial correlations on tests of intelligence.

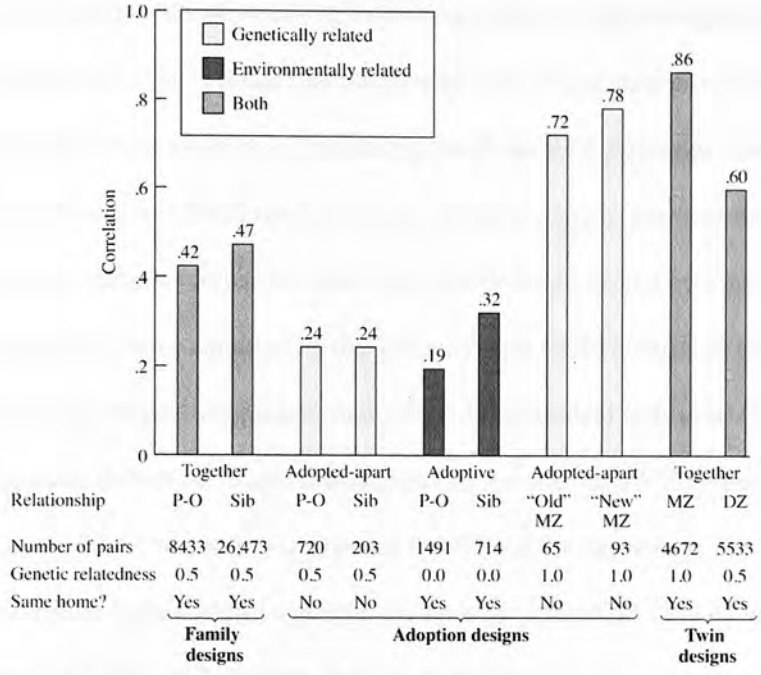

Figure 2.1 Average correlations for family designs, adoption designs and twin designs. P-O = parent-offspring, Sib = sibling. Figure assembled using the reviews of Bouchard and McGue (1981) as revised by Loehlin (1989). For the adopted MZ twins raised apart, Old MZ data excludes the data of Burt (1966) New includes the data of Bouchard, Lykken, McGue, Segal, and Tellegen (1990) and Pedersen, McClearn, Plomin, and Nesselroade (1992). Figure adapted from Behavioral genetics (5th ed., Figure 8.7, p. 157) by Plomin, DeFries, McClearn, and McGuffin (2007).

A heritability estimate was derived with these data by Plomin, DeFries, McClearn, and McGuffin (2001) where model-fitting analysis was used to show that around half of the phenotypic variation in *g* was attributable to genetic differences between the individuals in these studies. The studies included in the review by Bouchard and McGue (1981) were predominantly collected from studies using European and North American samples but comparable estimates for the genetic effects on intelligence have also been observed in Japan, East Germany and rural and urban Indian samples (Plomin et al., 2001).

Pleiotropy, the degree to which the same genes are involved in multiple traits can be examined by extending the twin design to encompass multivariate genetic analysis. For the study of cognitive abilities the degree to which genetic effects are shared between *g* and

second order cognitive domains can be examined to derive a genetic correlation. Using this methodology Petrill (1997) found that the genetic correlations between *g* and specific cognitive abilities were close to 1.0 indicating a high level of pleiotropy. Indeed, Rijsdijk, Vernon, and Boomsma (2002) used data from 194 twin pairs to examine the distribution of additive genetic variance across the three level hierarchy described by Carroll (1993). The additive genetic variance captured by the general factor could be used to explain an average of 31% of the phenotypic variance in each of the 12 individual tests used (Range 8 -53%). Additive genetic factors were also found to act on *g* using the *g*-VPR model of intelligence (Johnson et al., 2007) where they accounted for 77% of the variance in the fourth striatum *g*. For the third level factors additive genetic variance accounted for 78% of Verbal ability, 77% of Perceptual and 75% of Rotational abilities. In the second order factors, with the exception of content memory, additive genetic variance accounted for 33% of the variance. This finding is consistent with the notion that genes work on cognitive abilities at the general level rather than a separate group of genes for each ability, this evidence of pleiotropy between cognitive abilities led to the Generalist Genes Hypothesis (Plomin & Kovas, 2005).

The heritability of *g* is not static but rather it increases across the lifespan (McCartney, Harris, & Bernieri, 1990) where in young children it increases from 30% between the ages of 2 and 4 (Spinath, Ronald, Harlaar, Price, & Plomin, 2003) to 80% during adulthood (Edmonds et al., 2008; Jacobs, van Os, Derom, & Thiery, 2007). Using a combined sample of over 11,000 twin pairs drawn from 6 samples across the USA, Australia, The Netherlands and Great Britain, Haworth et al. (2010) investigated the increase of heritability across childhood through to early adulthood. Haworth et al. (2010) found statistically significant increases in heritability from 41% at age 9 to 55% at age 12 and again from age 12 to age 17 where heritability was found to be 66%. This effect was attributed to a gene/environment correlation, which refers to the ability of individuals to select, modify and create environments in accord with their genotypes.

Following from the finding that heritability is not stable across the life span, work

has been done to examine which genetic influences have an effect on both the stability and the changes in intelligence differences. By examining verbal ability and non-verbal ability in over 360 twin pairs measured at ages, 5, 7, 10, 12, and 18 years of age, Hoekstra, Bartels, and Boomsma (2007) found that the heritability of non-verbal ability increased from 64% to 74% whereas verbal ability increased from 48% to 84%; the genetic correlation between verbal and non-verbal abilities increased from 0.62 to 0.73. The increasing heritability (Edmonds et al., 2008; Hoekstra et al., 2007; Spinath et al., 2003) indicates the increasingly important role of genetic factors from childhood to early adulthood.

2.3 Molecular genetics

Two classes of methodology exist for mapping genes to traits and to disease: linkage and association. Linkage examines family groups and is based on the idea of Mendel's Law of Assortment. Using linkage methodology, variants are linked to disorders or to quantitative traits in instances where there is a violation of the Law of Independent Assortment. This can be seen when there is coinheritance of a specific DNA variant and a disorder or level of a quantitative trait within the families. Linkage has previously been used successfully to link genes to phenylketonuria and cystic fibrosis (Kerem et al., 1989), where a small number of genes have a very large effect. However, the low success rate of linkage when applied to quantitative traits indicates that the alleles that contribute are of small effect or that complex traits are highly heterogeneous and would require a greater level of statistical power to find them. Indeed, Risch and Merikangas (1996) examined the numbers of individuals required using linkage (affected sibling pairs) and association (the transmission disequilibrium test, TDT) methods to attain statistical significance at a power of 0.80. The TDT uses affected children and their heterozygous parents to test for the equality of transmission of the variant under investigation. At a diallelic locus, under the conditions of the null hypothesis, each allele should be present in 50% of the affected children. However, if this loci is associated with the disorder then a statistically significant deviation from equal transmission of each allele is

observed. They found that as the relative risk fell to 2 or below, the numbers of participants required became unfeasibly large for linkage methods requiring 296,710 affected siblings at an alpha level of 0.01 when compared to the 5823 required using association. Whilst these calculations were carried out using the TDT they do generalise to other forms of association. Additionally, it is a far more simple matter to assemble large cohorts if they do not have to be composed of sibling pairs.

Association analysis, referred to as allelic association in the context of genetic studies, involves correlating a marker allele with a trait. Association analysis can be used for candidate gene designs, the fine mapping of linkage regions and for genome wide analysis. The case-control design is one such form of association where allelic frequency between two groups is compared. One group consists of unrelated affected individuals and the other is a group of controls matched for age, sex and ethnicity. By controlling for these factors the allelic frequency between the two groups can be compared. This design extends to the use of quantitative traits such as intelligence, but rather than comparing allelic differences between two groups, an additive linear model is used to assess if allelic variation co-occurs with phenotypic variation. It should be noted that many of the same issues exist in the use of quantitative designs as case control designs examining disease status as they are both particularly vulnerable to false positives in instances where there is an underlying population substructure known as population stratification. When examining cognitive abilities, in instances where the frequency of a particular allele is correlated with cognitive test scores and is attributable to the diversity in background population the study is said have population stratification. Whilst this can be ameliorated by using a cohort design where participants are drawn from the same population stratification can still occur.

One of the most promising uses for association methods is their application to genome wide association studies (GWAS). GWAS combine the strengths of linkage analysis methods to search across the genome without an a priori hypothesis regarding which areas are more important with the increase in statistical power and resolution afforded to the

association methods. A GWAS makes use of between 500,000 – 1,000,000 single nucleotide polymorphisms (SNPs) which form the unit of genetic variation, where variation refers to a single base pair change in an individual's genotype at a specific genomic region. SNPs are spread across the genome and this single base pair substitution is the most common form of genetic variation between individuals (1000 Genomes Project Consortium, 2010).

The low rate of success for linkage studies to find regions linked with common diseases and traits contributed to the notion that the genetic architecture of common disorders is quite different from the architecture of rare disorders. This idea was further cultivated by the finding that some common variants were associated with common diseases such as *APOE* with Alzheimer's disease (AD) (Corder et al., 1993). This association between the *APOE* e4 allele and AD was found in 234 individuals where there was a highly significant additive trend for the e4 allele with each additional allele increasing the risk of contracting AD by a factor of 2.84 (95% CI 2.03 – 3.96). The presence of the e4 allele was also shown to be associated with an earlier onset of the disease and an earlier age of death again with a dose dependent effect of the e4 allele. Also a missense variant in *PPARG* has been associated with diabetes (Altshuler et al., 2000). The finding that common variants were associated with common diseases, such as the *APOE* e4 allele and Alzheimer's disease, in these studies led to the development of the common disease common variant hypothesis (CD/CV) (Reich & Lander, 2001).

The CV/CD hypothesis states that if a disorder in the population is common, so too must be the genetic variants responsible. This would indicate that the variants involved would have a small effect size or penetrance as, if a common variant is defined as one where the minor allele is present in, for example, 5% of the population, and if penetrance was complete, every common disorder would also be present in at least 5% of the population. That is to say, a perfect correlation between the presence of the disorder and the minor allele would mean the disorder would be present in 5% of the population. However, should such common variants, also have a low penetrance and only increase the propensity for disease then the

presence of the minor allele would not be perfectly correlated with a disorder. A second implication of the CV/CD hypothesis is that if common disorders are heritable and each common variant responsible has low penetrance, then there must be multiple contributing common variants.

In order to conduct a GWAS a large proportion of the genome must be interrogated, this is made possible through the use of DNA microarrays, referred to as chips. A chip allows up to 1 million SNPs to be processed simultaneously reducing both the time to process and the cost per genotype, making large sample sizes more feasible. Whilst this number of SNPs represents only a small fraction of the 3 billion nucleotide pairs found in the human genome the SNPs used are selected in order to best exploit the lack of independence between SNPs. This correlation between an allele at one position in the genome and identity of a SNP in another is referred to as linkage disequilibrium (LD). LD occurs due to recombination events that take place during meiosis. Two SNPs on a chromosome may begin as being perfectly correlated with each other but as multiple recombination events occur across many generations these markers may move into linkage equilibrium, that is to say, they are no longer correlated. Whilst this would depend on certain assumptions, primarily random mating in a population of fixed size, it does illustrate that LD is population specific. Indeed different human populations have very different patterns of LD. African populations, are a far older population and so a greater number of recombination events have occurred, meaning a lower level of linkage disequilibrium is observed in African populations when compared to European or Asian populations. As these groups initially began as samples drawn from the original African population, the population size would differ along with the number of generations since their founding. The SNPs that are selected to capture genetic variation across loci are referred to as tag SNPs and these are specific to a population. By exploiting LD over 80% of variation in commonly occurring SNPs can be captured in European populations using a subset of between 500,000 – 1 million tagging SNPs. The property of LD can also be exploited to gain additional coverage of the genome as if two SNPs are correlated the

identity of a SNP at a locus will be informative of the identity of a SNP at another locus. This means that researchers will only have to genotype a part of the genome and from this knowledge of the identity of the variants from a much greater portion can be known.

2.4 Molecular genetics and cognitive abilities

The search for specific variants associated with intelligence has been disappointing. There is evidence that the e4 allele of the *APOE* gene has a detrimental effect on cognitive abilities with an effect size of d = -0.05 derived from a meta-analysis of 77 studies using 41,000 individuals (Wisdom, Callahan, & Hawkins, 2011). Other than *APOE,* results from candidate gene studies in cognitive ability have failed to replicate in larger more powerful studies than those in which they were discovered (Chabris et al., 2011; Houlihan et al., 2009). A notable exception to this is rs12206087, rs11584700 and rs4851266 which have been found to be associated with the number of years of education in 126,559 individuals (Rietveld et al., 2013) which were subsequently replicated in a sample of 34,397 by 23andMe. Indeed, GWAS for cognitive ability have so far produced only a small number of SNPs that survive correction for multiple comparisons made. The apparent lack of SNPs associated with intelligence coupled with the finding from behavioural genetics research that intelligence is substantially heritable has been deemed the missing heritability problem (Maher, 2008). This problem is most noticeable in cases such as intelligence, where the phenotype is complex and highly polygenic. Indeed in sample sizes of > 17,000 no SNPs have been associated with intelligence using the GWAS method (Benyamin et al., 2013). However, in other polygenic traits such as schizophrenia sample sizes of greater than 20,000 were required before specific variants were found (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011) and as their sample size grew larger additional loci were discovered. This same pattern has been observed for multiple traits including height where, as sample size increases so too does the number of significant loci

(Visscher, Brown, McCarthy, & Yang, 2012) consistent with the notion of the CD/CV hypothesis.

2.5 Multi-SNP analysis in GWAS

One of the issues regarding the lack of findings using the GWAS approach is the stringent correction for multiple comparisons that needs to be made. This issue is of particular concern as the expected effect size of any SNP in a highly polygenic trait is expected to be small. This helps to ensure that SNPs of small effect do not cross the genome wide significance threshold of 5×10^{-8}. However a new method developed (Yang et al., 2010; Yang et al., 2011) referred to as Genome Wide Complex Trait Analysis (GCTA) can derive a heritability estimate using SNP data from populations bypassing the need for twins and does not rely on the equal environment assumption. This method was applied to the data drawn from 3,511 individuals in a study by Davies et al. (2011)to show that additive genetic effects in LD with common SNPs could be used to derive a heritability estimate of 0.51 for fluid cognitive ability and 0.40 for crystallised ability. This result using GCTA, has been replicated (Chabris et al., 2011) and extended to include children (Benyamin et al., 2013). The lack of any significant SNP combined with GCTA being able to derive a heritability estimate using common SNPs indicates that there are common SNPs with very small effects that do not survive correction for multiple comparisons. The heritability estimates provided by GCTA indicate the lower bound of the full heritability estimate as GCTA uses common SNPs where the twin method can capture any genetic variant that is passed down.

The GCTA method has also been extended to cover multivariate analysis where the pleiotropic effects of genes associated with intelligence was demonstrated using molecular genetic evidence (Trzaskowski et al., 2013a) to show that there are strong genetic correlations between mathematics reading ability and general cognitive ability. In addition to the finding that the same genes explain a substantial portion of the correlation between

cognitive phenotypes, multivariate GCTA has also been used to show a high genetic correlation between intelligence and educational attainment of 0.95 and between intelligence and SES at 0.26 (Marioni et al., 2014a).

Despite the advances made by using GCTA it does not inform us which genes are more important or what their likely function may be. Due to the highly polygenic nature of intelligence and the small effect sizes expected to be involved, a potential strategy to increase power using the sample sizes currently available is to sum the effects of multiple SNPs. This branch of methodology has been used successfully by Davies et al. (2011) to show that, by deriving a gene based statistic using every SNP located within a gene, that the *FNBPL1* gene was associated with intelligence in older adults. This finding was replicated in children (Benyamin et al., 2013).

SNPs can also be summed across genes using a pathway design, more accurately referred to as gene-set analysis. The goal of gene set analysis is to examine if the test statistics from a preselected group of genes shows a consistent deviation from chance. As it is well known that genes do not act in isolation; rather, complex networks of molecules act together each under some degree of genetic influence (Schadt, 2009). Indeed, the loci so far associated with height have been found to cluster in the Hedgehog, TGF-band growth hormone pathways (Allen et al., 2010). Gene sets drawn for analysis can be grouped by their biological function such as being involved in glial cells (Goudriaan et al., 2013) or specific synaptic components (Ruano et al., 2010).

One of the limitations of gene-set analysis is that it is dependent on the accurate definition of the sets involved, as better annotation is expected for more well studied biological processes. Gene-set analysis has provided a valuable addition in uncovering the mechanisms of disease using GWAS data. In a series of studies (Abraham & Cho, 2009a; Abraham & Cho, 2009b; Dong, 2008; Yoshida, Nakaya, & Miyazaki, 2009) the pathway (Il)-12 and Il-23 was studied in relation to its importance to Crohn's disease. Whilst only three genes at two loci showed genome wide significance three genes were subsequently

replicated in the same pathway. Further studies also found that the genes in the (Il)-12 and Il-23 pathway were associated with Crohn's disease. This illustrates that multiple genes acting to reduce the efficiency of a specific process can result in disease. It is also important to note that the most significant gene may not be the most accessible target for therapeutic intervention.

Whilst wide variation exists with regards to the statistical methodology used to determine if a gene set is significantly associated with a trait they fall into two classes, self-contained tests and competitive tests of enrichment. The difference between the two lies in the null hypothesis being tested. The self-contained test only examines the genes in the set under consideration. The whole set is treated as the unit of association and the test is used to show if variation across the all of the genes in the set is associated with phenotypic variation. This does offer a number of advantages, chiefly amongst them are only the genes in the gene set are needed. Additionally, self-contained tests are typically extremely robust to any effects of LD as they can use phenotype permutation where the observed phenotype for each participant is permuted before the gene set statistic is derived after each permutation. The observed phenotype is then compared to a null distribution made up from the permuted scores to assess if the gene set based statistic shows a greater level of association with the observed phenotype than the null distribution. Whilst this has the advantage of controlling for LD and gene set size after each permutation, it does suffer from genomic inflation resulting in an increase in type 1 error rates.

Competitive tests of enrichment determine if the gene set of interest has a greater weight of evidence for its association with a trait than genes drawn from outside the set. It does require, however, that a there is sufficient coverage across the genome to make such a comparison valid. Competitive tests, whilst robust to the effects of genomic inflation, can also suffer from an increased rate of both type 1 and type 2 errors if properties such as LD and gene set size are not controlled for. Indeed, several authors have not controlled for the number of SNPs in their gene sets of interest (Askland, Read, & Moore, 2009; Torkamani,

Topol, & Schork, 2008; Walsh et al., 2008) meaning that these results should be interpreted with caution. Failure to control for the number of SNPs can create type 1 errors when the set of interest is composed of large genes. The more SNPs there are in a gene the more likely it is that there will be extremely small p-values associated to the SNPs of that gene. The implication of this is that a gene set with a large number of SNPs will have a greater weight of evidence associating it with a trait by virtue of its size alone. LD between SNPs must also be considered, as a gene with high levels of LD between the SNPs within it would contain fewer points that are free to vary than a smaller gene with much lower levels of LD. These two problems are not mutually exclusive and both gene size and LD must be controlled for in order to avoid type 1 error inflation. The competitive test is to be preferred however as it allows for the assessment of the contribution of a gene set against other genes and so can be used to prioritize sets.

2.6 Summary

The results from twin estimates of heritability provide strong evidence that an individual's genotype is predictive of their level of cognitive ability, whilst GCTA indicates that common SNPs, those with a minor allele frequency of >5%, tag the variants responsible. Although, the common SNPs that tag this heritability are currently unknown, gene-set analysis provides a method that can help prioritise common SNPs based on what genes they are located in and, what biological mechanisms these groups of genes are involved in. General cognitive ability was examined because, as indicated previously in this thesis, general cognitive ability is predictive of educational attainment (Deary et al., 2007a), everyday decision making (Gottfredson, 1997) and of mortality (Calvin et al., 2011) and so finding molecular mechanisms associated with intelligence can have implications for health and education. Another reason for the focus on intelligence is that GWAS have shown that complex traits are highly polygenic with the variants involved consisting of a large number of loci, each making only a small contribution to the heritability (Munafò & Flint, 2014) this includes so called

endophenotypes. In this context, an endophenotype is phenotype that is thought to be closely related to the biological processes that result in the phenotype of interest so, for example, reaction time could be used to understand intelligence. However, evidence to suggest that the effect size is greater for variants responsible for end phenotypes is lacking (Flint & Munafò, 2007).

2.7 The current thesis

The aim of this thesis is to find associations between gene sets and cognitive abilities with the aim of elucidating the possible mechanisms by which genetic variation leads to variation in cognitive abilities. This will be done by examining gene sets selected according to theoretical reasons for their involvement in intelligence. Whilst the variants considered are selected in a similar fashion to candidate gene studies the methods contrast. In a number of important ways. Firstly, multiple variants are considered together in order to capture a greater proportion of variance and to increase statistical power. Candidate gene designs have failed to replicate in larger more, powerful samples (Chabris et al., 2011; Houlihan et al., 2009) and by capturing more variance using multiple genes this problem can be ameliorated. Secondly, in this thesis competitive tests are used to examine if the genes selected show a greater weight of evidence than genes that are not included in the gene set of interest. Candidate gene designs typically assess if the variant shows a non-zero effect, however in a genome wide data set there are more low p-values than would be expected under the null hypothesis. Chapter 3 examines the mechanisms responsible for synaptic plasticity namely postsynaptic density and its associated components for an enriched association with intelligence. Chapter 4 details the analysis of another synaptic component, heterotrimeric G proteins, which have been shown to be involved in intelligence differences (Ruano et al., 2010). We sought to examine this claim with a greater sample size and a competitive test of enrichment.

Chapter 5 broadens the scope of the investigation to consider functional SNPs. These

are defined as SNPs that have been shown to produce variation in gene expression or function either through their effect on methylation, micro RNA or their role in promoter regions. Chapter 6 takes genes that have been shown to be associated to extreme ranges of cognitive ability namely intellectual disability. Here we seek to determine if the genes involved in intellectual disabilities are also involved in the normal range of human cognitive ability.

Chapter 3: Description of samples and methods.

This chapter will provide an overview the samples and tests along with the statistical methods used to construct the phenotypes for genetic analysis. In addition, this chapter will describe the methods for genotype extraction and the methods used to conduct a GWAS on each of the samples. These GWAS form the discovery and replication data sets required to conduct the gene set analyses found in the empirical chapters of this thesis.

3.1 Participants of the discovery cohort

The analyses conducted throughout this thesis used data from the samples of the Cognitive Ageing and Genetics in England and Scotland (CAGES) consortium. The sample consists of 3511 healthy middle-aged and older individuals and is composed of five cohorts; the Lothian Birth Cohorts of 1921 and of 1936 (LBC1921 and LBC1936) (Deary, Gow, Pattie, & Starr, 2012), the Aberdeen Cohort of 1936 (ABC1936) (Whalley et al., 2011), and the Manchester and Newcastle Longitudinal Studies of Cognitive Aging Cohorts (Rabbitt et al., 2004).

The cohorts of LBC1921, LBC1936 and ABC1936 were drawn from the sample of individuals who took part in The Scottish Mental Survey of 1932 (SMS1932) (Scottish Council for Research in Education, 1933) and The Scottish Mental Survey of 1947 (SMS1947) (Scottish Council for Research in Education, 1949). The SMS1932 was conducted on the 1st of June 1947 and was established to provide a measure of the level of cognitive functioning of all individuals in Scotland who were born in 1921 and attending school. The SMS1932 was conducted in order to both discover the rates of mental deficiency and to obtain information regarding the distribution of intelligence across the population. A total of N = 87,498 (43,288 females) were tested. It has since been found that a number of private schools did not participate and some individuals took the test a few days later and a number of schools and individuals were missed to an insufficient number of papers being

delivered.

Each individual in SMS1932 completed the same test, the Moray House Test No. 12 (MHT) (Scottish Council for Research in Education, 1933), with the same instructions and time limit. The MHT was a version of one in a series of tests devised by Godfrey Thomson for school selection between primary and secondary school. Administered to 11-year-old children, the MHT would be used to select children better suited to more academic schooling. The MHT is composed of 71 items where a maximum score of 76 is attainable in the 45 minutes provided for its completion. The test includes eight practice items and two short picture tests used to assess the ability of those who could not use the MHT due to a low level of cognitive ability. The main body of the test is composed of 12 categories of items: following directions (14 items), same opposites (11), word classification (10) analogies (8), practical items (6), reasoning (5), proverbs (4), arithmetic (4), spatial items (4), mixed sentences (3), cipher decoding (2), and four other items.

Whilst the goal of SMS1932 was to ascertain the level of cognitive ability for the population of Scotland, the MHT was a test of verbal ability designed to indicate which children would be most suitable for a grammar school education, as such it was not designed to yield an estimate of a child's IQ. In order to establish the validity of the MHT as a measure of general cognitive ability a subsample of SMS1932 was retested the following summer using the Stanford Revision of the Binet scale. Of the 1000 individuals (500 female) selected, 847 were born in June, 101 in May and 52 across June and July. Whilst these individuals were selected from across the educational areas the males of the sample scored 4 points higher than the population and the females scored 2.5 points above the average. The MHT was shown to be a good indicator of general cognitive ability with a strong correlation with the Stanford Revision of the Binet Scale of 0.80 (0.81 male, 0.78 female)(Scottish Council for Research in Education, 1933).

The SMS1947 was conducted to test the hypothesis that the average IQ of the United Kingdom was decreasing due to low IQ individuals producing a greater number of offspring

than those of a higher IQ bringing the average IQ down (Scottish Council for Research in Education, 1949). The data gathered 15 years previously from the SMS1932 enabled a population wide comparison to be made. SMS1947 was conducted on the 4th June 1947 where almost all children born in 1936, N= 70,805 (33,996 females), were tested using the same instructions and test as used in SMS1932. The results of this comparison with SMS1932 produced evidence contrary to the original aims and are one of the earliest examples of the Flynn effect (Flynn, 1999) which describes the observation that tests of cognitive ability have raised between generations.

The Lothian Birth Cohort of 1921 (LBC1921) was begun in 1999 with the aim to find the genetic determinants of cognitive ageing. LBC1921 consists of individuals, most of whom originally took part in The Scottish Mental Survey of 1932 (SMS1932) (Deary, Whiteman, Starr, Whalley, & Fox, 2004; Deary, Whalley, & Starr, 2009; Scottish Council for Research in Education, 1933). Recruitment into LBC1921 from the individuals of the SMS1932 was carried out by examining the Community Health Index (CHI) for individuals registered with a General Practitioner (GP) who were born in 1921 and living in the Edinburgh area. Due to the National Health Service of the United Kingdom over 99% of the population of Scotland were registered with a GP. On request the individuals who were living independently in the community were contacted by their GP and invited to take part in the follow up to SMS1932. Individuals were also recruited into LBC1921 through advertisements in the local, regional and national newspapers. A total of 1120 potential participants were identified and contacted through the examination of CHI with 728 responding to the request to participate. Of these 501 were found to be eligible with 260 going on to take part. The advertisements placed in the media generated 423 potential participants with 368 of these being found to be eligible to join LBC1921. From these individuals 321 agreed to take part with 290 participating in LBC1921. Through these methods a total of 550 (316 females) independent, healthy individuals living in Edinburgh or the surrounding regions, were included in LBC1921 with mean age was 79.1 years (SD =

0.6) (Deary et al., 2004). Venous whole blood was collected for DNA extraction following informed consent. Ethical approval was granted by The Lothian Research Ethics Committee.

The Lothian Birth Cohort of 1936 (LBC1936) was started in 2004 and was conducted to find genetic and environmental factors which contribute to cognitive ageing. LBC1936 is composed of individuals most of whom originally took part in The Scottish Mental Survey of 1947 (SMS1947) (Scottish Council for Research in Education, 1949). In order to locate and recruit individuals from SMS1947 into LBC1936 the CHI was used in conjunction with media advertising. By using the CHI 3810 potential participants were identified and 3686 were invited to participate. A total of 2318 responded to the invitation and 1126 were found to be eligible to join 97 of which were gathered via advertisements placed in the media. This led to a total of 1091 (543 females) mostly healthy independent individuals from Edinburgh and the surrounding regions being recruited. Figure 3.1 illustrates the recruitment procedures used to gather LBC1936. The mean age of this cohort was 69.5 years (SD=0.8) (Deary et al., 2007b). Venous whole blood was collected for DNA extraction following informed consent. Ethical approval was granted by Scotland's Multicentre Research Ethics Committee and the Lothian Research Ethics Committee.

The Aberdeen Birth Cohort of 1936 (ABC1936) was recruited between 1999 and 2003 and was formed from the re-testing of individuals from the SMS1947 with the aim to determine childhood influences on future dementia risk (Whalley et al., 2011). Individuals who sat the SMS1947 in schools in Aberdeen and still living locally were traced using the CHI where 664 potential participants were identified. Of these 17 were excluded due to recent bereavement or life-threatening illness and 506 agreed to take part. The final sample size was 498 individuals (255 females) with a mean age of 64.6 (SD = 0.9) years. Like the Edinburgh cohort, those included in ABC1936 are healthy older individuals. Each had venous whole blood collected for DNA extraction following informed consent. The Grampian Research Ethics Committee granted ethical approval.

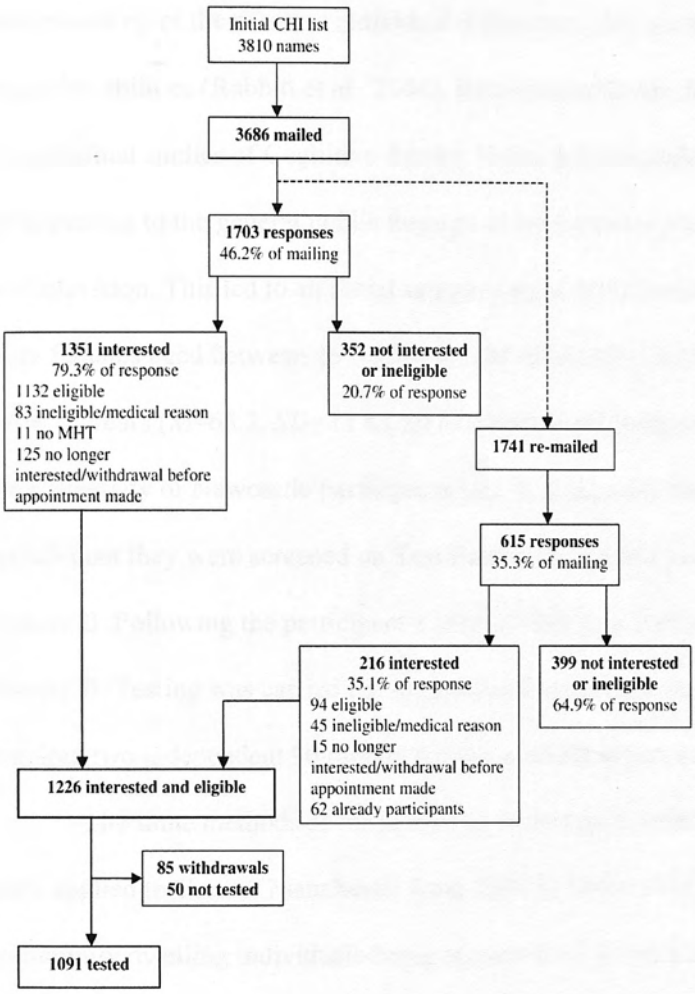

Figure 3.1. Illustrating a flow chart describing the recruitment process for LBC1936. Figure taken from Deary et al. (2007b)

The Manchester and Newcastle cohorts were created with the intent to further the understanding of the role that individual differences play on the effects of ageing on cognitive abilities (Rabbitt et al., 2004). Recruitment for the Manchester and Newcastle Longitudinal studies of Cognitive Ageing began in Newcastle-upon-Tyne in 1983 and 1984 by appealing to the general public through advertisements placed in local newspapers, radio and television. This led to an initial sample size of 2052 healthy individuals of whom 1539 were females aged between 46 – 92 years (M=67.4, SD=14.3) with the males age range from 49 to 86 years (M=65.2, SD=11.8), all of whom lived independently in the community. At the University of Newcastle participants took two separate batteries of cognitive tests. At recruitment they were screened on Test Battery A and two years later they performed Test Battery B. Following the participant's second visit Test Battery A was alternated with Test Battery B. Testing was carried out in groups of between 5 and 20 and each test battery required two independent 90 minute testing sessions supervised by two examiners.

The same methods as those used to recruit participants from Newcastle-upon-Tyne were applied to Greater Manchester from 1984 to 1986. This resulted in 2193 healthy community dwelling individuals being recruited, of whom 1503 were female whose ages ranged from 50 – 92 years of age (M=64.4, SD=7.8) with the age range of the males being from 45-93 years (M=65.6, SD=7.7). In both the Manchester and Newcastle samples additional participants were recruited until 1994 with assessments of survivors through to 2003 occurring every two years. Venous whole blood was taken for DNA extraction from 805 of the Manchester cohort (572 females) and 758 of the Newcastle cohort (536 female) following informed consent. Ethical approval was granted by the University of Manchester.

3.2 Participants of the replication cohorts

This study also makes use of two replication cohorts. Two were included due to their differences in terms of both demographics and sample size. The Norwegian Cognitive NeuroGenetics Sample (NCNG) (Espeseth et al., 2012) has an age range of 50-70 years

which is comparable to the CAGES discovery cohort. The age range of the Brisbane Adolescent Twin Study (BATS) is 15.4 to 29.6 years (M=16.6, SD=1.5 years). However, whilst NCNG is closer in terms of age, it differs in that it is of considerably smaller size (NCNG, N= 670; BATS, N= 2062) and thus has less power to replicate any significant associations found.

The NCNG was established to study the normal range of cognitive ageing with particular focus on how genetic factors, brain morphology and brain function may contribute to individual differences. Individuals were recruited to the NCNG sample via advertisements placed in newspapers asking for individuals from the Oslo and Bergen urban areas to participate. Individuals with a history of psychiatric or neurological disorder or a history of substance abuse were excluded from the sample as were those who had a score on the depression inventory indicating undiagnosed depressive mental illness. Following testing, individuals with a score of below 1 *SD* of their age norm on intelligence or memory scores were also removed. This resulted in a final sample size of 670 healthy individuals (457 females) with an age range of 18-79 years (Mean = 47.6, SD =18.3). Participants were drawn from and tested in Bergen (n = 171) and Oslo (n = 499). Permission to take and store blood samples for genotyping along with cognitive and MRI data in a bio-bank and to establish a registry for relevant information was granted by the Norwegian Department of Health. Ethical approval was granted by the REK Sørøst (Norwegian Ethical Committee), NCNG: project ID S-03116.

The BATS sample is formed from healthy sets of twins and their siblings who were drawn both for the Brisbane Adolescent Twin Study (Wright & Martin, 2004) along with those individuals who were recruited for participation in cognition and imaging studies (de Zubicaray et al., 2008; Wright et al., 2001). These participants were recruited from primary and secondary schools in South East Queensland. Recruitment from the schools followed the approval of the Department of Education who aided in the identification of twins and their

non-twin siblings. Screening questionnaires were used to identify those whose health may impact on their cognitive performance and aimed to identify episodes of psychiatric disorder, medical illness, substance abuse, closed head injury or impairments of vision and/or hearing. The total sample size for BATS was 2032 (1093 females) drawn from a total of 923 families (339 MZ twin pairs, 1 set of MZ triplets) with an age range from 15.4 - 29.6 years of age (M=16.6, SD=1.5). The studies were approved by the Human Research Ethics Committee at the Queensland Institute of Medical Research, as well as the institutional ethics boards at the University of Queensland and the Wesley Hospital.

3.3 Cognitive phenotypes

In the following studies up to four cognitive phenotypes will be utilised. These are fluid cognitive ability (*gf*), crystallised cognitive ability, memory and processing speed. The fluid-crystallised distinction was used as it captures the differential effects of ageing on human cognitive abilities. Where *gf* shows a pattern of decline similar to that of physical capabilities (Tucker-Drob & Salthouse, 2008), crystallised ability shows very little decline into old age. Due to the older age of the majority of participants used in the cohorts here maintaining the fluid-crystallised distinction should help to control for the differential effects of ageing across the range of human cognitive abilities.

In each of the Scottish cohorts a score for *gf* was derived by using the raw score from each test and implementing a principal components analysis. Using regression, the first un-rotated component was derived and indicated a single general component in each of the three cohorts (Tables 3.2-3.9). This was then extracted before the effects of age and sex were statistically controlled for by using a linear model with the component score being the dependent variable with age and sex used as predictor variables. The standardised residuals that were extracted from this model were used as each individual's *gf* score adjusted for both age and sex.

For the LBC1921 cohort, *gf* was derived from the Moray House Test (Deary et al.,

2004), Raven's Standard Progressive Matrices (Raven, Court, & Raven, 1977), phonemic verbal fluency (Lezak, Howieson, & Loring, 2004), and Wechsler Logical Memory scores (Wechsler, 1987). The Raven's standard progressive matrices test (Raven et al., 1977) involves the participant being presented with an abstract image with a section removed. The participant is instructed to select one from the six available "pieces" that would complete this image. The instructions are presented verbally to the participant and the first two problems are used as examples to ensure the participant understands the task. Later tasks involve the participant being presented with a 2x2 and later a 3x3 matrix containing abstract symbols where, as previously, one is missing. Elements within the matrix follow a logical progression from one to the next, meaning that the identity of the missing element can be derived by observing the elements which are present. The participant must select from between six to eight symbols of which, only one is the correct response. The test has no time limit and is scored by summing the number of correctly solved problems. The maximum score is 58.

The verbal fluency test (Lezak et al., 2004) is a timed test where the participant should name as many words as possible beginning with the letters "C", "F" and "L". Performance on each letter is measured separately with one minute for each letter, with the final score being the sum of the performance on each letter. Proper names are not allowed and the score is the total number of words for each of the three letters.

The Wechsler Logical Memory scores (Wechsler, 1987) consist of a short story being read out aloud to the participant. Following this the participant is to immediately recall 25 pieces of information from the story. Marks were given for each correct detail with a maximum score of 25. Whilst this test can also be implemented with a measure of delayed recall, only the immediate recall score was included here.

The fluid ability component for LBC1936 was formed from six non-verbal tests from the Wechsler Adult Intelligence Scale III^{UK} (WAIS-III^{UK}) : Digit Symbol Coding, Block Design, Matrix Reasoning, Digit Span Backwards, Symbol Search, and Letter-number Sequencing (Wechsler, 1998a). In the Digit symbol coding test participants are presented

with two rows, with the upper row containing the digits 1-9 in a randomised order for a length of 20 cells. The lower row is blank with 20 spaces provided in which the participant is to place their answers. The participant is provided with a key whereby each digit corresponds with a unique novel symbol. The participant's task is to place in the lower row the corresponding symbol from the key ensuring the digit symbol pairings from the key remain intact. The test also includes seven practice items to minimize the effects of learning to test score. The score is the number correctly filled in within 120 seconds with a maximum of 133.

The Block Design Task involves the participant completing an increasingly difficult series of patterns using a number of coloured cubes. The test has 14 problems to solve, the first four of which are practice trials to familiarize the participants with the test instructions. The next five trials feature problems using four cubes with a 60 second time limit for completion where the last five use the full complement of nine cubes. Scoring is dependent on time taken to complete the pattern with a higher score awarded for rapid completion of the task.

The matrix reasoning task involves the participant selecting from five possible answers which best fits a pattern presented to them and is in keeping with the progression from one element to the next. Participants are presented with between four and nine cells arranged either as a line or in a typical matrix configuration. There are three practice items and a total of 26 problems.

The Digit Span Backward test involves a series of numbers being read aloud to the participant whereby they must repeat them back to the examiner in reverse order. A practice trial is provided for the participant. After instruction seven items are presented to the participant each of which contains two strings of digits, each of the same length, and each string is presented separately. The first item contains two digits and as item number progresses the number of digits increases by one with the final item containing eight digits. One point is awarded for each correct string with a maximum of 14 points in total.

The Symbol Search test involves participants being presented with a row of seven symbols the first two are the target symbols the remaining five are the search symbols. The participant is required to examine the row and indicate if either of the two target symbols is presented in the search symbols by marking either the yes or the no box. The test features three rows of sample items at which point the participant is instructed in how to complete the test correctly and three practice rows where the examiner can gauge as to whether the participants understand the test. There are 60 rows in total with a time limit of 120 seconds for the completion of the test. Scoring is carried out by totaling the correct responses minus the number of incorrect responses meaning the participant must perform the test both quickly and accurately.

In Letter-Number-Sequencing test the examiner reads aloud a string of both digits and numbers. The participant is instructed to repeat first the numbers, in ascending order, followed by the letters in alphabetical order. There are two practice strings where the participant first receives instruction and then the correct answer. This was followed by five practice trials. The test contains seven items with 3 letter digit strings in each where the initial item contains one letter and one number with an additional letter or digit in each subsequent item with the final item containing an eight letter/digit combination. A correct answer results in one point with a maximum of 21 for this test.

The measure of general fluid ability for ABC1936 was formed from: the Rey Auditory and Verbal Learning Test (R-AVLT) (Lezak et al., 2004), the Uses of Common Objects (Guildford, Christensen, Merrifield, & Wilson, 1978), Raven's Standard Progressive Matrices (Raven et al., 1977), and Digit Symbol from the Wechsler Adult Intelligence Scale Revised (WAIS-R: Wechsler, 1981).

The RAVLT was modified for use in this study. Here, the RAVLT is a test of cumulative recall. The examiner reads a list of 15 words to the participant, who is to repeat as many of them as possible. After which the same list is read out again by the examiner where the participant is instructed to repeat as many as they can including words recalled

from previous trials. The word list is presented a total of five times to the participant and their score is the total number of recalled words summed across the five presentations.

The Uses of Common Objects test involves participant's giving as many uses for three common objects, a felt hat, a bottle, and a clip, as they could. The participants score was the total number of correct uses for all three objects.

The measure of fluid ability in the Manchester and Newcastle aging cohort was derived using the two parts of the Alice Heim test 4 (AHT-4) (Heim, 1970) and the four sub-tests of the Culture Fair Test (Cattell & Cattell, 1960). The AHT-4 test is divided into two sections the first of which contains 65 problems which require knowledge of verbal opposites, analogies, synonyms and basic arithmetic to solve. The participant is initially presented with 15 practice items. This is followed by 50 scored items where one mark is awarded for each correct answer within a 10 minute time limit. Part two is a non-verbal test whereby the participant is presented with a test figures and a rule. They must select from a series of five figures and indicate which one is the correct answer according to the given rule. This test must be completed in 10 minutes and the score is the total correct in this time period.

The Cattell culture fair test, Scale 2 form A (Cattell & Cattell, 1960) is composed of four non-verbal tests. The first contains three images where by a rule is in place dictating the change between images. The participant must derive the rule and select an additional image from a choice of five. There is only one answer that is correct in keeping with the progression of the three presented images. Three practice items are included and the time limit for the test is three minutes. The maximum score in the series sub-test is 12. The second part is a test of classification whereby the participant must state which of the five items does not belong to the class of the remaining four. The time limit for this test is four minutes. The first two are practice items and the maximum score is 14. The third is the matrices test in which the participant is presented with a 2x2/3x3 matrix with an element missing. The participant is instructed to select from five options which element is correct in keeping the

logical relations between elements in the matrix. There are three examples and the maximum score is 12. The time limit for this test is three minutes. The fourth part of the test deals with logical conditions where the participant is presented with a series of overlapping shapes drawn on a side of paper. In the diagram of overlapping shapes there is a dot. The participant must select from five images, one of which corresponds to the logical placement of the dot in the first image. For example the dot maybe outside the square, but inside of the circle and the participant would have to select from the five images one which also has the dot outside the square, but inside of the circle. There are three test items and the maximum score is eight with a 2.5 minute time limit.

Age at test and sex were controlled using residualisation and these standardised residuals for each of the tests were then subjected to a maximum likelihood factor analysis provided by Dr Mike Allerhand from the Centre for Cognitive Ageing and Cognitive Epidemiology. A general factor was extracted using regression and missing data points were accounted for by sampling the posterior distribution of factor scores for each subject using Mplus (Muthen, Asparouhov, & Rebollo, 2006). Table 3.1 shows the tests used and the number of participants by cohort for the construction of the *gf* variable.

Table 3.1. The tests used for the construction of the fluid ability component/factor (*gf*) are shown for each cohort of the discovery sample.

	LBC1921	LBC1936	ABC1936	Manchester	Newcastle
	MHT	Block Design	Raven's Progressive Matrices	Alice Heim 4 (part 1)	Alice Heim 4 (part 1)
	Raven's Progressive Matrices	Matrix Reasoning	Digit Symbol	Alice Heim 4 (part 2)	Alice Heim 4 (part 2)
	Logical Memory	Digit Span Backwards	Uses of Common Objects	Cattell Culture Fair (Part 1)	Cattell Culture Fair (Part 1)
	Verbal Fluency	Symbol Search	R-AVLT	Cattell Culture Fair (part 2)	Cattell Culture Fair (part 2)
		Digit Symbol Coding		Cattell Culture Fair (part 3)	Cattell Culture Fair (part 3)
		Letter number Sequences		Cattell Culture Fair (part 4)	Cattell Culture Fair (part 4)
N	517	1005	426	805	754

Abbreviations: MHT, Moray House Test No 12; R-AVLT, Rey Auditory Verbal Learning Test.

Table 3.2. The results of the principal components analysis for the 4 tests used to establish a general fluid measure in LBC1921. These results show a single component can account for 55.9% of the variance indicating the tests used are a good measure of general ability.

	Initial eigenvalues		
Component	Total	Percentage of variance	Cumulative percentage
1	2.237	55.919	55.919
2	0.845	21.126	77.045
3	0.650	16.244	93.289
4	0.268	6.711	100

Table 3.3. Illustrates the loadings for the tests used in LBC1921 on the first un-rotated component.

Tests used	Correlation with first un-rotated component
MHT	0.772
Logical Memory Score	0.711
Verbal Fluency	0.739
R.S.P.M.	0.741

Abbreviations: MHT, Moray House Test No 12, R.S.P.M., Raven's Standard Progressive Matrices.

Table 3.4. The results of the Principal components analysis for the 6 tests used to establish a general fluid measure in LBC1936. These results show a single component can account for 52.6% of the variance.

	Initial eigenvalues		
Component	Total	Percentage of variance	Cumulative percentage
1	3.160	52.662	52.662
2	0.840	14.007	66.669
3	0.757	12.612	79.280
4	0.446	7.425	86.705
5	0.421	7.011	93.716
6	0.377	6.284	100

Table 3.5. Illustrates the loadings for the tests used in LBC1936 on the first un-rotated component.

Tests used	Correlation with first un-rotated component
Symbol search	0.772
Digit Symbol	0.711
Matrix Reasoning	0.739
Letter-Number Sequencing	0.741
Digit Span Backward	0.658
Block Design	0.728

Table 3.6. The results of the Principal components analysis for the 4 tests used to establish a general fluid measure in ABC1936. These results show a single component can account for 48.9% of the variance indicating the tests used are a good measure of general ability.

	Initial eigenvalues		
Component	Total	Percentage of variance	Cumulative percentage
1	1.957	48.916	48.916
2	0.816	20.407	69.323
3	0.743	18.567	87.891
4	0.484	12.109	100

Table 3.7. Illustrates the loadings for the tests used in ABC1936 on the first un-rotated component.

Tests used	Correlation with first un-rotated component
R-AVLT	0.630
Uses of Common Objects	0.612
R.S.P.M.	0.769
Digit Symbol	0.770

Abbreviations: R-AVLT, Rey Auditory Verbal Learning; R.S.P.M., Raven's Standard Progressive Matrices.

Table 3.8. Illustrates the loadings for the tests used in the Newcastle cohort on the first un-rotated factor.

Tests used	Factor loadings
AH41	0.819
AHF2	0.830
CF1	0.713
CF2	0.552
CF3	0.658
CF4	0.573

Abbreviations: AH41, Alice Heim test 4 test section 1; AH42, Alice Heim test 4 test section 2; CF1 The Cattell culture fair test, Scale 2 form A section 1; CF2 The Cattell culture fair test, Scale 2 form A section 2 ;CF3 The Cattell culture fair test, Scale 2 form A section 3; CF4 The Cattell culture fair test, Scale 2 form A section 4.

Table 3.9. Illustrates the loadings for the tests used in the Manchester cohort on the first un-rotated factor.

Tests used	Factor loadings
AH41	1.00
AHF2	0.977
CF1	0.765
CF2	0.574
CF3	0.823
CF4	0.687

Crystallised ability describes the level of knowledge an individual has acquired over the life course (Horn, 1994). It is typically assessed by means of language-based tests including reading ability or measurements of vocabulary. For LBC1921, LBC1936 and ABC1936 this was represented by the score from the National Adult Reading Test (NART) (Nelson & Willison, 1991). The NART contains a list of 50 words of increasing irregularity which the participant must read out aloud. All of the words are irregular in the sense that they do not conform to the Standard English rules of pronunciation. The test is untimed and number of correctly pronounced words forms the participant's final score.

For the Manchester and Newcastle cohorts, sections A and B from the Mill Hill Vocabulary Test (Raven, 1965) were used. The Mill Hill vocabulary test consists of a set A and a set B. In set A the participant must select from six possible synonyms for the most exact match to the test word. Set A consists of 34 problems with the first being an example problem to aid the participant in how the test is to be carried out. Set B consists of the test word alone, which the participant has to define on paper. Set B contains 34 problems where the first is already filled in to instruct the participant in how to answer each problem. Each set was administered without a time limit and the score was the number of correct responses with a maximum score of 66. Parts A and B were summed.

Verbal declarative memory (memory) was measured by a single test in each cohort. In the LBC1921 cohort, the total score from both the immediate and delayed recall sections of the Logical Memory test from the Wechsler Memory Scale-Revised (Wechsler, 1987) was used. In LBC1936, it was the total from the immediate and delayed recall sections from the Logical Memory test from WMS-III (Wechsler, 1998b). In ABC1936, the modified version of the R-AVLT (Lezak et al., 2004) was used. In the Manchester and Newcastle cohorts, a cumulative verbal recall task (Johnson et al., 2008; Rabbitt et al., 2004) was used in which four presentations of a list of 15 six letter nouns was read aloud to the participant. A recall phase was administered between each presentation where the participants were instructed to

write down as many of the words as they could recall. The final score was the total recalled across all four presentations. The raw scores from each of the tests representing memory were subjected to a linear regression with age and sex as predictors and the test score as the dependent variable. The standardised residuals from these models were used for all subsequent analyses.

Information processing speed (speed) was measured in each cohort using a single test. The digit symbol subtest of the WAIS-III $^{U.K}$ (Wechsler, 1998a) was carried out by LBC1921 and LBC1936, whereas in ABC1936 the WAIS-R version (Wechsler, 1981) was used. The Savage Alphabet Coding Task (Savage, 1984) was used in the Manchester and Newcastle cohorts. In this test, participants were presented with two rows: the top one contains random letters, the bottom row is blank and is for the participant to answer. Additionally, the participant is presented with a key instructing how the 15 different letters are to be recoded into the bottom by means of substituting one letter for another ("A" becomes "T"). Participants encode as quickly as possible the 200 letters in two minutes. There are four sets of trials whereby the code remains constant. The score for the participant is the total correct summed across the four trials.

The raw scores from each of the single tests representing speed were subjected to a linear regression with age and sex as predictors and the test score as the dependent variable. The standardised residuals from these models were used for all subsequent analyses. Table 3.10 shows the tests used to represent crystallised ability, memory and processing speed in each of the five cohorts of the discovery sample.

Table 3.10. Showing the number of participants available for each test by cohort.

	LBC1921	LBC1936	ABC1936	Manchester	Newcastle
Crystallised ability	NART	NART	NART	Mill Hill	Mill Hill
N	515	1003	420	770	750
Memory	Logical Memory	Logical Memory	R-AVLT	Cumulative verbal recall	Cumulative verbal recall
N	514	1002	338	769	743
Processing Speed	Digit symbol	Digit symbol	Digit symbol	The Savage Alphabet Coding Task	The Savage Alphabet Coding Task
N	301	999	380	773	729

Abbreviations: NART, National Adult Reading Test: R-AVLT, Rey Auditory Verbal Learning Test

3.4 Cognitive phenotypes for the replication cohorts

In the replication cohorts only fluid ability was measured as this was the only phenotype in need of a replication sample. For the NCNG sample a *gf* factor was constructed using a single test, the Matrix Reasoning subtest from the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1999). The construction of the *gf* phenotype was performed by collaborators in at the Department of Clinical Medicine in the University of Bergen. The phenotype was produced by using the Matrix reasoning score as the dependent variable in a linear regression model where age and sex were included as predictors. The standardised residuals extracted from this model formed the *gf* score for NCNG.

For the BATS sample, performance IQ was used as a measure of *gf* ability. This variable was derived by collaborators at the Genetic Epidemiology, Molecular Epidemiology and Neurogenetics laboratories of QIMR Berghofer. Performance IQ was measured using the scores from two tests the Spatial, and the Object Assembly from the Multidimensional Aptitude Battery (Jackson, 1984). The Spatial subtest involves participants to mentally rotate

figures in order to select the correct answer from 5 alternatives. There are 50 items of increasing difficulty in this test. The object assembly test consists of 20 items which represent pieces in a typical jigsaw puzzle presented in an incorrect order. The participant is instructed to arrange these items into the correct order and choose the alternative illustrating the correct series of items. Each section was administered with a seven-minute time limit with the tests and performance IQ scores being derived according to the manual which produces scores standardised by age and sex.

3.5 Genotyping

DNA was extracted from the 3782 participants of the discovery cohorts before it was genotyped for 599,011 common single nucleotide polymorphisms (SNPs) using the Illumina610 QuadV1 chip (Illumina, Inc., San Diego, CA, USA). Following quality control, 549,692 SNPs were retained in 3,511 participants (2,115 females). The reasons for the removal of individuals from the discovery cohort included unresolved gender discrepancies, relatedness or call rate < 0.95, as well as evidence of non-Caucasian descent. SNPs were removed from the analysis in the event that they had a call rate of < 0.98, minor allele frequency of < 0.01 and a Hardy-Weinberg equilibrium test of $P < 0.001$.

In order to identify individuals who were not of Caucasian origin multidimensional scaling (MDS) was carried out using an Identity By State (IBS) distance matrix on the genotyped data and incorporated unrelated HapMap samples. These analyses were performed by Davies et al. (2011). Individuals were removed if their genotype was visibly outside the cluster corresponding to the Utah residents with Northern and Western European ancestry (CEU). The results of the MDS analysis indicated that a small subgroup of individuals, particularly from the Manchester and Newcastle cohorts, were located at the edge of the CEU cluster. Whilst this is suggestive of population stratification, the notion was explored further by re-running the MDS using only the SNPs and individuals who had passed the quality control. Following this, regression analysis was performed in each of the

cohorts to measure the effect of the first four MDS components on the cognitive phenotypes. There was a small but significant effect of stratification on the phenotype in the Manchester cohort. In order to correct for this and any stratification in the other cohorts, the first four MDS components were fitted as covariates in the single marker analysis.

Imputation was performed in each cohort using the MACH (Li, Willer, Ding, Scheet, & Abecasis, 2010) software (v1.0.16) to the HapMap phase II CEU (NCBI build 36 release 22) reference panel. Imputed SNPs were omitted from analysis if they had an imputation quality score of < 0.3 and a minor allele frequency of < 0.005.

For the NCNG sample, DNA was extracted from blood using the Qiagen Gentra Autopure LS system (Qiagen, Valencia, CA, USA). Genotyping took place on the Illumina Human 610-Quad Beadchip (Illumina, Inc., San Diego, CA, USA). Quality control was implemented using the "check.marker" function from the R package GenABEL (Aulchenko, Ripke, Isaacs, & van Duijn, 2007). Identity-by-state (IBS) was used to assess cryptic relatedness, with cases where IBS threshold exceeded 0.85 being removed. Population structure was assessed using multidimensional scaling analysis where individuals who were suspected of possible recent non-Norwegian ancestry were removed. Individuals were also removed if the heterozygosity value was greater than two standard deviations from the sample mean or where sex could not be determined. SNPs were excluded if the call rate was < 0.95, a minor allele frequency of < 0.01 and a Hardy-Weinberg Equilibrium (exact test) *P*-value of < 0.001. The final sample consisted of 554,225 SNPs in 670 individuals.

In the BATS sample, DNA was extracted from the blood of 2104 participants and was genotyped using an Illumina Human 610-Quad chip (Illumina, Inc., San Diego, CA, USA). Quality control was then implemented leading to 529,379 SNPs being retained in 2062 (1,093 female and 969 male) participants. Individuals were removed due to unresolved gender discrepancies or evidence of non-Caucasian descent. SNPs were removed if they met the criteria of call rate < 0.95, minor allele frequency < 0.01 and a Hardy-Weinberg equilibrium test of P < 0.00001 (Medland et al., 2009). Multidimensional scaling analysis of

SNP data showed three components. To control for population stratification, all three components were entered as covariates along with age and sex in the analyses.

3.6 Genome Wide analysis and Meta-analysis of the four cognitive phenotypes.

Additional analysis carried out in in the following chapters will follow on from the single marker analysis carried out by the author in the discovery and replication cohorts. In the discovery cohorts single marker analysis was conducted in Mach2QTL (Li et al., 2010), in order to use imputed genotypes, using an additive linear model to gauge the effect of genotype on phenotype. The number of effect alleles at each locus was counted where the range of these estimates was from 0 – 2. A separate additive model was conducted in each cohort for each of the cognitive phenotypes. The first four multidimensional scaling components were included as covariates in each additive model before the results were meta-analysed using an inverse variance weighted model carried out in METAL (Willer, Li, & Abecasis, 2010) providing a measure of the weight of evidence for the association between each SNP across the five cohorts. The results show that no single SNP in any of the four cohorts attained the level of $5x10^{-8}$ to reach genome wide significance. Figures 3.2 – 3.5 plot these associations.

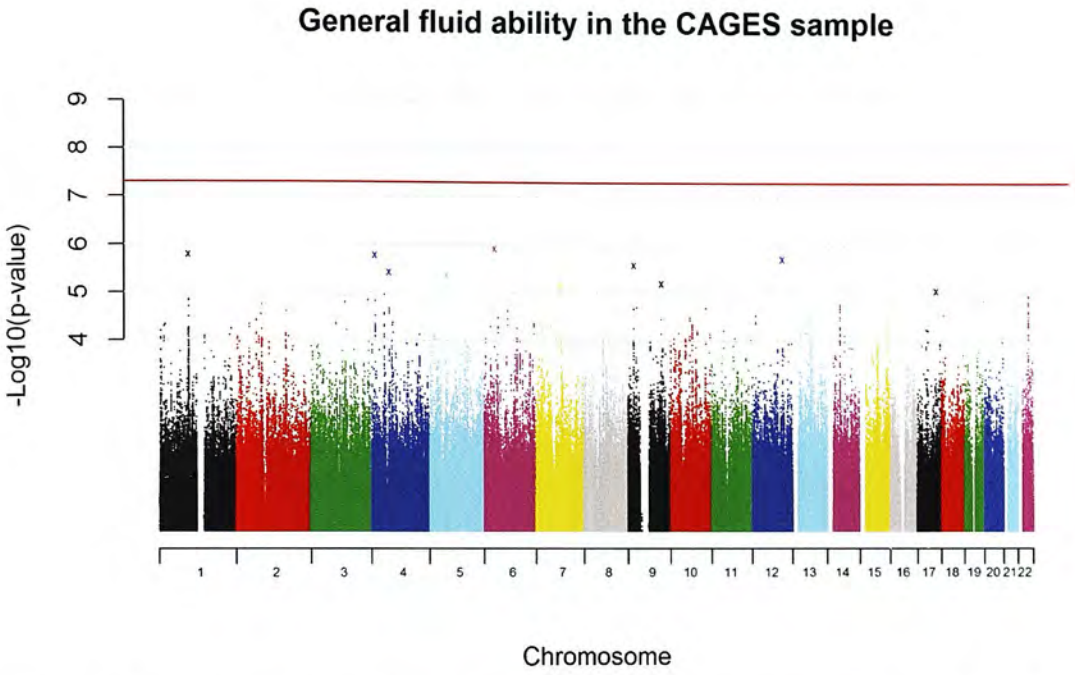

Figure 3.2. Manhattan plot for the meta-analysis conducted in the CAGES sample for fluid ability. Each point represents a SNP in its position along the chromosome of the x axis and the –log10 of the p value for each SNP is located on the y-axis. The red line indicates level of significance required for genome wide significance. Imputed data were used.

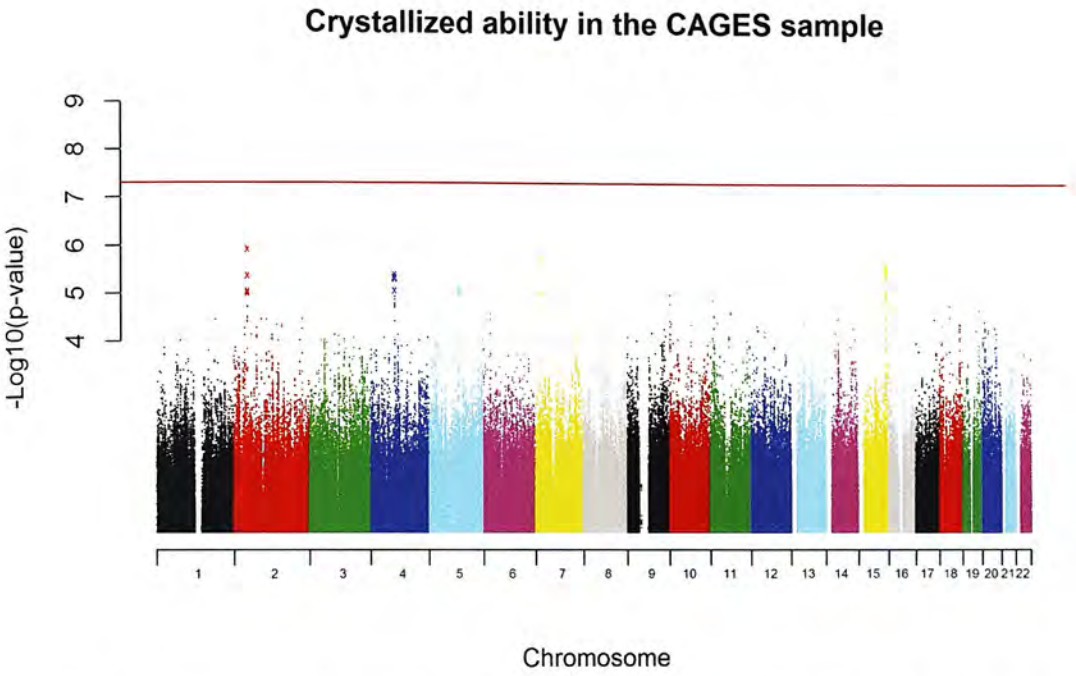

Figure 3.3. Manhattan plot for the meta-analysis conducted in the CAGES sample for crystallised ability. Each point represents a SNP in its position along the chromosome of the x axis and the –log10 of the p value for each SNP is located on the y-axis. The red line indicates level of significance required for genome wide significance. Imputed data were used. The red line indicates level of significance required for genome wide significance.

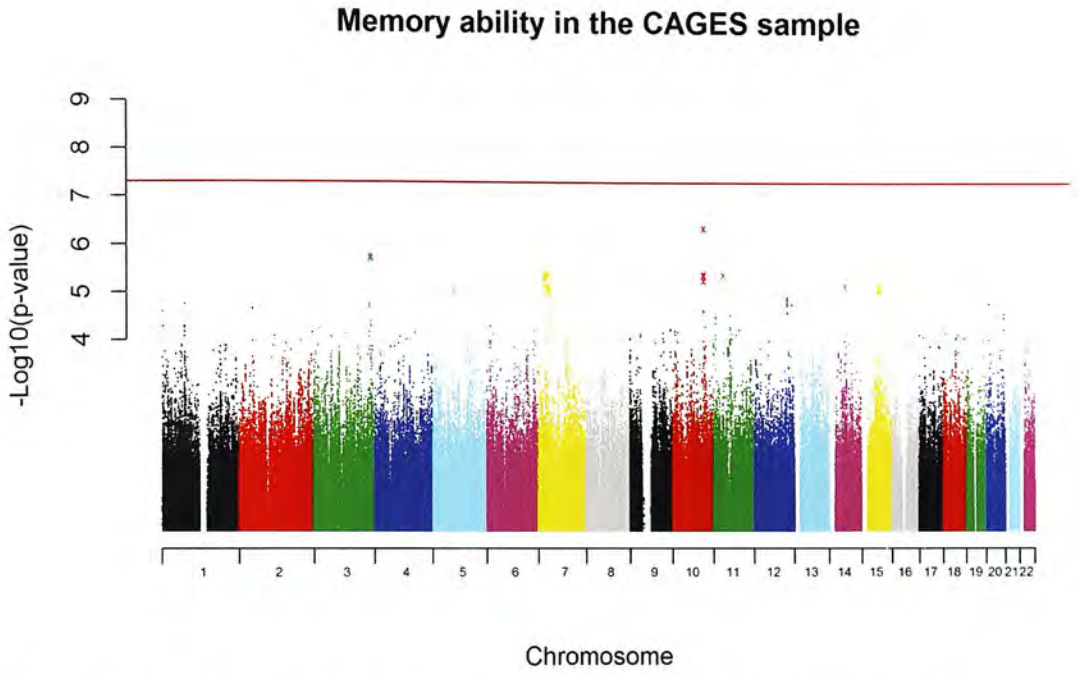

Figure 3.4. Manhattan plot for the meta-analysis conducted in the CAGES sample for memory ability. Each point represents a SNP in its position along the chromosome of the x axis and the –log10 of the p value for each SNP is located on the y-axis. The red line indicates level of significance required for genome wide significance. Imputed data were used. The red line indicates level of significance required for genome wide significance.

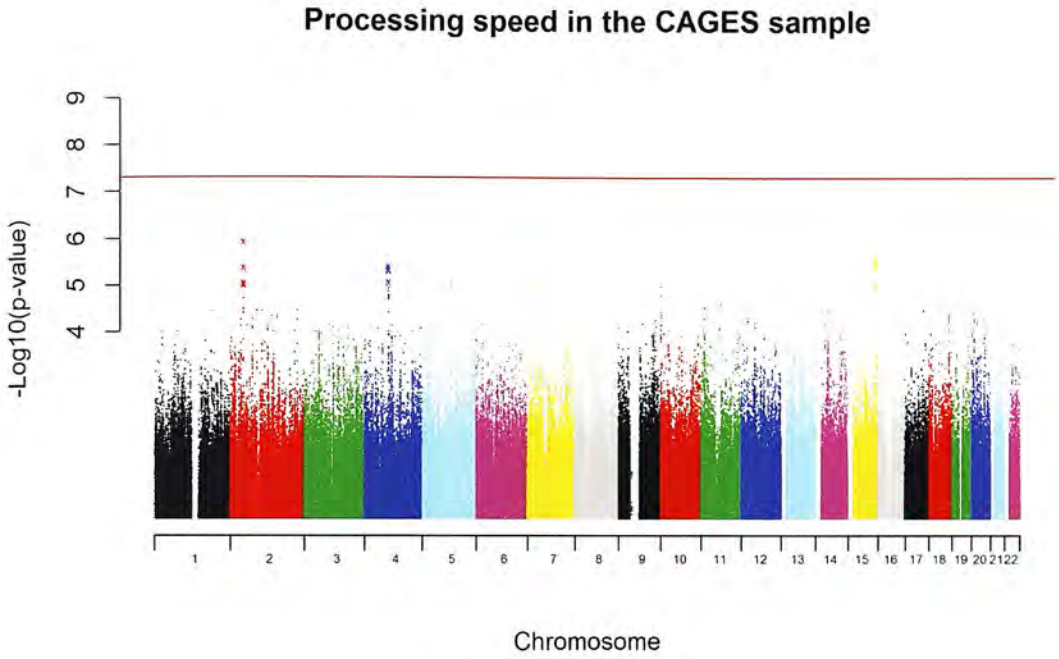

Figure 3.5. Manhattan plot for the meta-analysis conducted in the CAGES sample for processing speed. Each point represents a SNP in its position along the chromosome of the x axis and the –log10 of the p value for each SNP is located on the y-axis. The red line indicates level of significance required for genome wide significance. Imputed data were used. The red line indicates level of significance required for genome wide significance.

3.7 GWAS of the replication cohorts.

Single marker analysis was conducted separately in each of the replication cohorts by collaborators in the University of Bergen (NCNG) and at QIMR Berghofer (BATS). For the NCNG, PLINK (Purcell et al., 2007) was used. For the BATS sample, MERLIN (Abecasis, Cherny, Cookson, & Cardon, 2002) was used as this allows for relatedness between the twins and their siblings to be controlled for. Figure 3.6 and 3.7 shows that in both NCNG and BATS cohorts no SNP reached genome wide significance.

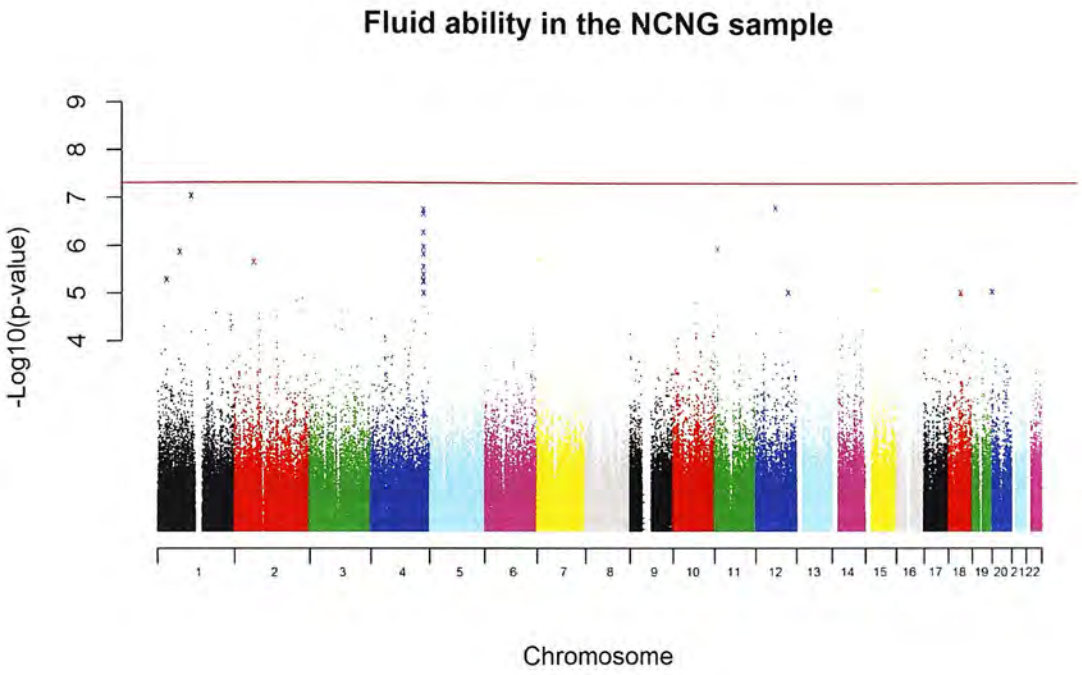

Figure 3.6. Manhattan plot for the GWAS conducted in the NCNG sample for fluid ability. Each point represents a SNP in its position along the chromosome of the x axis and the –log10 of the p value for each SNP is located on the y-axis. The red line indicates level of significance required for genome wide significance. The red line indicates level of significance required for genome wide significance.

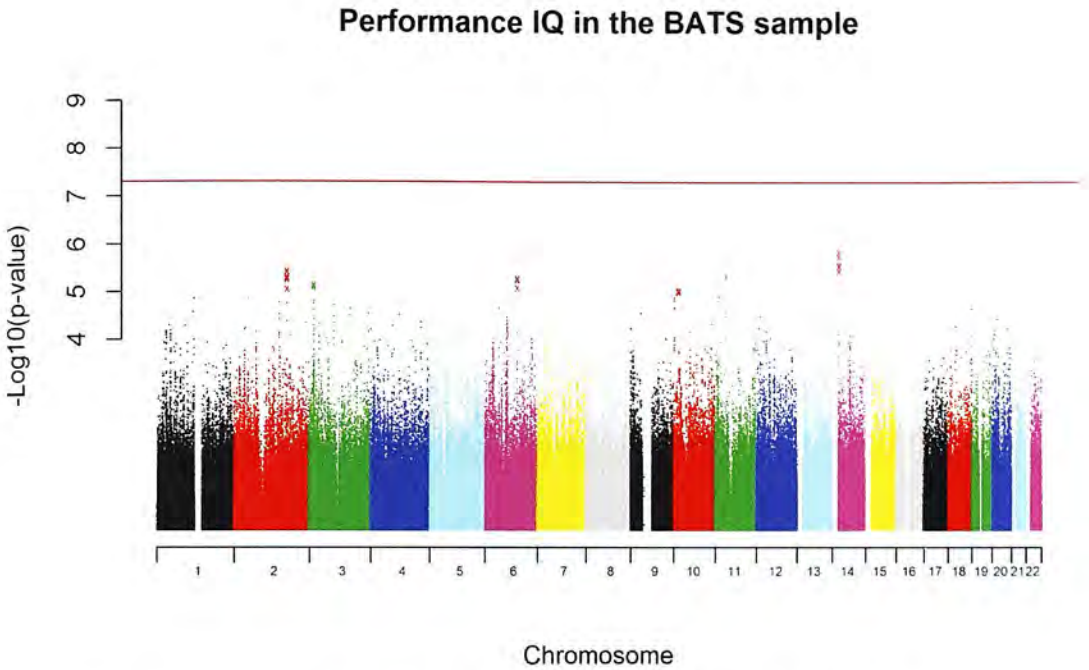

Figure 3.7. Manhattan plot for the GWAS conducted in the BATS sample for performance IQ. Each point represents a SNP in its position along the chromosome of the x axis and the –log10 of the p value for each SNP is located on the y-axis. The red line indicates level of significance required for genome wide significance. The red line indicates level of significance required for genome wide significance.

3.8 Summary

The GWAS described above form both the discovery and replication cohorts for the gene-set analyses described in this thesis. Whilst GWAS methodology has been conducted on these cohorts before (Davies et al., 2011) the current work makes use of imputed data. The results of where, as in the original report by Davies et al. (2011), no SNP was significant at the genome-wide level. Additionally *FNBP1L* was no longer statistically significant. Where changes in this data processing pipeline are present, such as the lack of meta-analysis in chapter 5 or the absence of gene based statistics in chapter 6, it will be noted in the relevant statistical methodology sections. In the next chapter, the genes of the postsynaptic density (Bayes et al., 2010; Collins et al., 2006) will be examined for an enriched association with cognitive abilities. Chapter 5 examines the

genes found in the heterotrimeric g proteins as these have previously been linked to intelligence differences (Ruano et al., 2010). Chapter 6 utilises these GWAS data sets to examine SNPs previously linked to gene expression (Bacanu et al., 2013; Gibbs et al., 2010; Richardson, Lai, Parnell, Lee, & Ordovas, 2011; Tahira et al., 2005) to assess if these SNPs also show an enriched association with intelligence. Chapter 7 examines the link between genes involved in non-syndromic autosomal recessive intellectual disabilities (Musante & Ropers, 2014) to query whether genes involved in large deviations of IQ are also enriched for quantitative trait loci involved in the normal range of intelligence differences.

Chapter 4: Is Intelligence influenced by genetic variation in components of postsynaptic signalling complexes assembled by NMDA receptors and MAGUK proteins?

4.1 Introduction

Individual differences in general cognitive ability have repeatedly been shown to be under genetic influence where common single nucleotide polymorphisms (SNPs) tag variants that account for between 25% to 50% of the variation (Benyamin et al., 2008; Davies et al., 2011). Efforts to elucidate the identity of genes involved in intelligence using the candidate gene design have been unsuccessful, as indicated by their subsequent failure to replicate in larger, more powerful samples (Chabris et al., 2011; Houlihan et al., 2009). However, as techniques such as GCTA have shown (Yang et al., 2010; Yang et al., 2011) using the net effect of multiple SNPs may increase statistical power as a greater proportion of variance can be captured. Gene-set analysis offers a way to examine the joint contribution of multiple SNPs grouped according to their involvement in a particular process or other criteria. In order to carry out a gene-set analysis a system must be selected that could be involved in intelligence. In this chapter, gene-set analysis is used to examine if the genes responsible for synaptic plasticity are also involved in intelligence. Firstly, the rationale behind the selection of a system involved in synaptic plasticity is discussed. Secondly, the components selected are described before a study is presented utalising gene-set analysis to determine if variants in them are involved in intelligence differences.

4.2 Selection of a candidate system

Candidate biological phenotypes implicated in cognitive differences center on the central nervous system, including variation in white matter integrity (Lopez et al., 2012;

Penke et al., 2012) and brain volume (McDaniel, 2005; Stein et al., 2012). However, to explore the genetic foundations of intelligence further, a more specific target is preferable. The goal of this chapter is to examine the weight of evidence for a synaptic system, the postsynaptic density (PSD) and its components, being associated with intelligence. The role that genetic variation at the synapse plays in intelligence has been explored before using the candidate gene design. For examine, the role that dopamine plays in intelligence can be examined by looking for an association between allelic variation in genes such as *COMT* (Barnett, Scoriels, & Munafò, 2008) and intelligence. Such designs have been criticised due to their lack of power to detect the likely small effects attributable to a single allele in a complex trait (Chabris et al., 2011). This chapter builds on the methodological findings provided by GCTA and polygenic scoring analysis that, by increasing the number of variants examined, there is the potential to increase statistical power. However, rather than utilising all SNPs (Yang et al., 2010) or a subset which show an agreed level of association (Purcell et al., 2009), here, groups of genes that work together to perform a specific biological function, synaptic plasticity, formed the unit of association. The synapse, particularly the postsynaptic density, is a rich target system both because of the large number of genes expressed and knocking out *Dlg2*, *Dlg3* or *Dlg4* which code for the proteins of the PSD in mice have a known effect on cognitive ability (Nithianantharajah et al., 2013). In humans the importance of the PSD has been shown using human cortical tissue in conjunction with proteomic profiling to show that mutations in the genes of the PSD are associated with 133 neurological and psychiatric disorders (Bayes et al., 2010; Grant, 2012). The postsynaptic density is a region on the dendrites that is enriched for structural proteins and signalling molecules and glutamate receptors. The signalling proteins of the PSD include calcium-dependent kinases (CaMKIIα, CaMKIIβ) as well as scaffolding proteins like postsynaptic density-95 (PSD-95) which tether glutamate receptors to signalling complexes. The PSD also contains scaffolding proteins such as GKAP, SHANK and HOMER, which are involved in dendritic spine growth, the regulation of synaptic plasticity as well as those proteins which fix signalling

complexes in place (Naisbitt et al., 1999; Sala et al., 2001; Sheng & Kim, 2011).

4.3 From single proteins to protein complexes

The notion that sensory information is processed in the brain by means of the patterns of action potentials (Adrian, 1928) led Hebb (1949) to postulate the existence of a mechanism capable of recognising such patterns and eliciting structural and functional change in the neuron allowing the brain to learn. The existence of such a mechanism was confirmed by Bliss and Lømo (1973) who used 18 anaesthetised rabbits to show that, when the system of axons leading from the entorhinal cortex to the dentate granule cells of the hippocampal formation (a system known as the perforant path) was stimulated with an electrode emitting 20Hz for 15 seconds, there was an increase in the amplitude and a decrease in the latency of subsequent population spikes. This effect, termed long-term potentiation (LTP), lasted for between 30 minutes to 10 hours after stimulation. Further research has shown that this ability of the neuron to alter itself also works to reduce the efficiency of the synapse, referred to as long-term depression (LTD). Where short duration but high frequency stimulation (100 Hz for 1 second) drives LTP, protracted stimulation at a lower frequency (5 Hz for 10 minutes) initiates LTD (Zorumski & Izumi, 2012). By means of LTP and LTD the brain has the ability to affect physical change in accord with environmental input.

Crucial to LTP and LTD taking place are the *N*-methyl-D-aspartate receptors (NMDAR). NMDARs are inotropic receptors where, upon binding with glutamate, the ion channel opens. However, the NMDAR also functions as a coincidence detector which serves to monitor activity at the pre- and postsynaptic regions. Due to the voltage dependent Mg^{2+} block of the calcium channel at the NMDAR, the glutamate release from the presynapse must occur simultaneously with the depolarisation of the postsynaptic region. Once these two events take place Ca^{2+} can enter the postsynaptic region where they encounter signalling complexes within the PSD. The first piece of evidence indicating the presence of such a

signalling system within the postsynaptic region was produced by Migaud et al. (1998) who examined the postsynaptic density 95 (PSD-95) protein, which normally binds with the NMDAR, and its role in both synaptic plasticity and learning in a mouse model. Using mice generated to lack PSD-95, Migaud et al. (1998) found that these mice produced significantly greater levels of NMDA induced LTP than wild type mice. In addition, the modified mice performed significantly poorer at a water maze test. This test of spatial memory is known to require hippocampal NMDA function (Morris, Anderson, Lynch, & Baudry, 1986; Tsien, Huerta, & Tonegawa, 1996). Importantly, the modified mice showed no signs of neurological abnormality, and NMDARs were present to the same extent in both wild and mutant mice. What these results show is that the NMDAR itself was left intact and unmodified by disruption to the PSD-95 protein. However, both LTP and learning were perturbed, indicating the presence of a signalling complex within the PSD tethered to the NMDARs requiring an influx of Ca^{2+} to function being located beneath the NMDAR. Indeed, biological functions are often performed by groups of proteins acting together in macromolecular "machines" (Alberts, 1998).

Proteomic studies investigating synaptic plasticity have used this link between PSD-95 and plasticity to try to elucidate the other proteins involved by isolating complexes containing NMDAR PSD-95. Early studies (Husi & Grant, 2001) using proteomic methods showed that these complexes were between 2,000 and 3,000 kDa suggesting a higher degree of molecular variation than if NMDAR complexes were composed of PSD-95 and a receptor channel alone. Indeed, it was shown that 77 proteins were found in these NMDAR complexes and that these molecules fell into 5 categories of neurotransmitter receptor, cell adhesion, adaptors, signalling enzymes, and cytoskeletal proteins (Husi, Ward, Choudhary, Blackstock, & Grant, 2000). Furthermore, interference with any of these classes of proteins resulted in a disruption to the induction of synaptic plasticity, indicating that the total protein complex is the functional unit rather than any one protein captured by such groupings.

These results show that, by starting with a single molecule linked to learning and

synaptic plasticity, PSD-95, it is subsequently possible to find the complexes that contain PSD-95 using proteomic tools. The importance of this is that it removes the focus away from a single molecule and exposes a greater set of components, all of which are potentially involved in the same process. The relevance for this in intelligence research is underscored by the finding that intelligence is a highly polygenic trait and by examining groups of molecules with a known effect on learning and brain physiology (synaptic plasticity), it may be possible to simultaneously increase statistical power and provide a more mechanistic account of intelligence differences.

The NMDAR is a part of the postsynaptic density (PSD) also examined here due to the role that mutation in genes expressed in the PSD plays in many dozens of neurological and cognitive disorders (Bayes et al., 2010; Collins et al., 2006; Kirov et al., 2012). The PSD can update its own responsiveness to subsequent input on very short and long time scales (Emes & Grant, 2012). At the genetic level, evidence suggests that the elaboration of complex learning involved duplication and subsequent divergence of genes in the PSD (Ryan et al., 2013). This was followed by strong conservation of function in the vertebrate line, (Emes et al., 2008) indicative of a finely-tuned system. The PSD, therefore, is a promising candidate for seeking genes in which variation is associated with intelligence.

4.4 The PSD and associated complexes

Among the proteins comprising the mammalian PSD, three complexes are of particular importance in mediating neural transmission: The NMDA-RC (*N*-methyl-D-aspartate receptor complex), mGlu5-RC (the metabotropic glutamate 5 receptor complex), and the AMPA-RC (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor complex) (Collins et al., 2006) (See Figure 4.1).

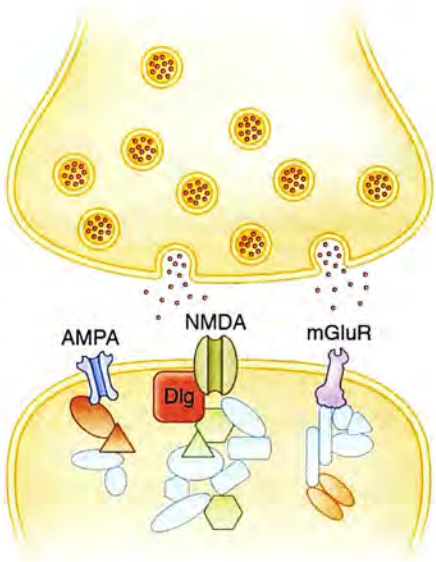

Figure 4.1: Schematic of a central nervous system excitatory synapse showing the proteins in the postsynaptic terminal organised into multi protein complexes assembled with glutamate receptors (AMPA, NMDA and mGluR receptors shown).

The AMPA-RC is the primary basis of rapid excitatory neurotransmission in the mammalian brain (Anggono & Huganir, 2012; Shepherd & Huganir, 2007); additionally, the induction of LTP is induced, in part, by the summation of AMPA mediated exciatatory postsynaptic potentials (EPSPs) (Collingridge, 1985). Using in vivo rat models it has been possible to show that an increase in the amplitude and duration of the excitatory postsynaptic potentials (EPSPs), produced by AMPA-RC activation, is associated with an increase in long term potentiation and performance in memory tasks (Stäubli et al., 1994).

Synaptic plasticity is dependent on both the NMDA-RC (Zorumski & Izumi, 2012) and mGlu5-RC (Mukherjee & Manahan-Vaughan, 2013). The mGlu5-RC, consisting of some 52 proteins forming the metabotropic $G\alpha_q$-linked G-protein coupled glutamate receptor (Kim, Lee, Lee, & Roche, 2008), is closely associated with longer-term modulation and

maintenance of long-term potentiation (Attucci, Carla, Mannaioni, & Moroni, 2001; Hermans & Challiss, 2001; Jia et al., 1998; Manahan-Vaughan et al., 2003). NMDA/MAGUK-RC is involved in rapid processing of information and updating of AMPA-RC responsiveness (Zorumski & Izumi, 2012). The NMDA-RC consists of neurotransmitter receptors, ion channels and signalling proteins scaffolded at the postsynaptic membrane where they function to convert information in patterns of action potentials into biochemical signals underlying memory and other aspects of cognition (Pocklington, Cumiskey, Armstrong, & Grant, 2006). Mutations in NMDA-RC have been implicated in the aetiology of over one hundred brain disorders, including those with cognitive deficits, such as schizophrenia, autism and intellectual disability, (Bayes et al., 2010; Frank et al., 2011; Kirov et al., 2012; Pocklington et al., 2006; Walsh et al., 2008) this supports the linkage of the NMDA-RC to both cognitive and psychiatric disorders.

4.5 Summary

By combining GWAS data sets on fluid cognitive ability, crystallised cognitive ability, memory, and processing speed with gene sets assembled using experimentally-determined sets of proteins detected in the PSD of human and mouse brains (Bayes et al., 2010; Collins et al., 2006) it is hypothesised that the PSD gene sets will show a greater weight of evidence for cognitive associations than genes drawn from outside these sets. To test this, all SNPs in genes and a 50kb boundary will be mapped to genes before a single statistic describing the weight of evidence for each gene is calculated (Liu et al., 2010). A competitive test of enrichment, Gene Set Enrichment Analysis (GSEA) (Subramanian et al., 2005; Wang, Li, & Bucan, 2007b), was used to test if gene sets corresponding to these components showed significant enrichment for the five cognitive phenotypes. The discovery samples were those of the CAGES consortium (Davies et al., 2011). Replication of significant findings was sought in two independent samples from Norway and Australia.

4.6 Materials and methods

4.6.1 Postsynaptic density gene sets

The genes responsible for the expression of the postsynaptic density and its subcomponents are available at the G2C database (http://www.genes2cognition.org/db/GeneList) see Appendix A. The size of the gene sets used along with the degree of overlap between the gene sets is shown in Figure 4.2.

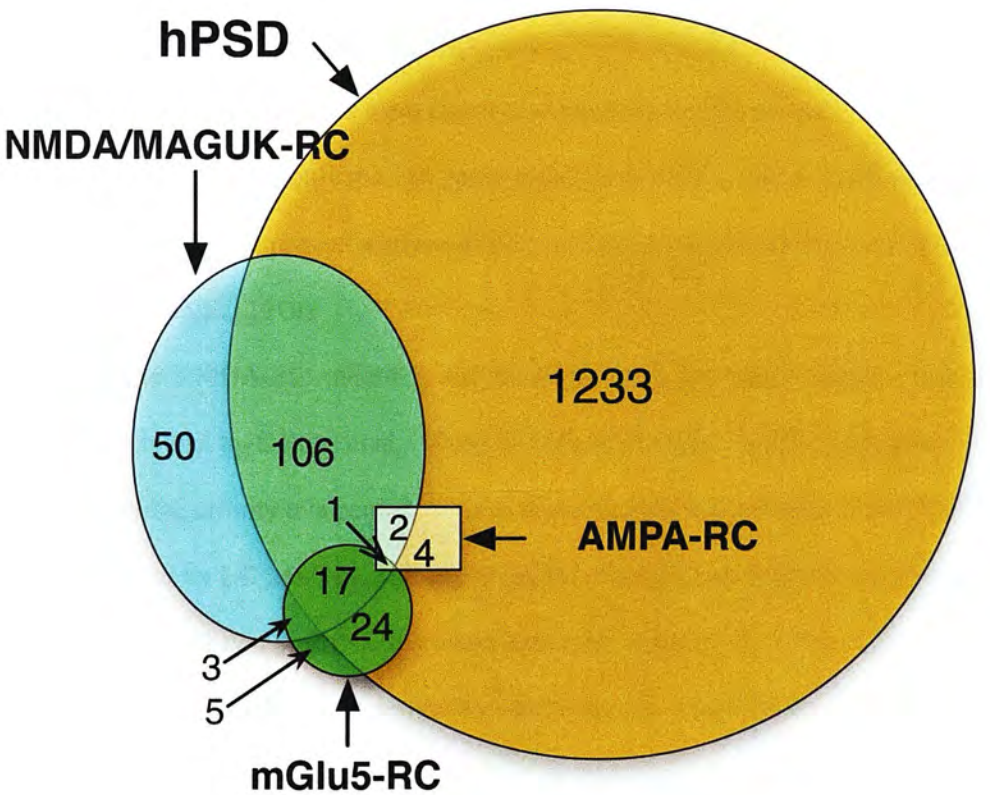

Figure 4.2. Venn diagram showing the overlap of three gene complexes and their relative genetic overlap within the proteins of the full human PSD (hPSD). Numbers of genes in each gene set and overlap of these are also shown. Note: The full hPSD consists of all genes associated with proteins in the human-derived postsynaptic density (Bayes et al., 2010). The genetic constituents of the AMPA-RC, mGlu5-RC, and NMDA-RC are taken from (Collins et al., 2006) mouse-based proteomic experiments.

The human postsynaptic density (hPSD) was ascertained based on experimentally identified proteins and taken from work and analyses performed by Bayes et al. (2010). Human postsynaptic densities were isolated from neocortical samples of 9 adults (mean age = 47.0 years, SD = 15.74, 3 females) who had undergone a variety of medically necessary neurosurgical procedures (Bayes et al., 2010). The protein preparations were pooled into three samples from three individuals, each sample containing normal non-diseased tissue from at least two of three cortical regions (Frontal, parietal, and temporal lobes). These three samples were then subjected to proteomic profiling using liquid chromatography tandem mass spectrometry (LC-MS/MS) (Bayes et al., 2010). The full set, consisting of 1387 genes, details genes whose proteins were found in at least two pooled samples, whereas the consensus set features only the 714 genes found in all three samples. Only autosomal genes were included in the present analyses (96.1% of the full hPSD and 96.4% of the consensus list) (Bayes et al., 2010).

The NMDA-RC, mGluR5, and the AMPA-RC gene sets were taken from previous work conducted by Collins et al. (2006). For the NMDA-RC, MASC complexes were isolated using affinity to a peptide derived from the carboxy terminus of the NR2B protein and analysed by LC-MS/MS. The identified list of proteins overlapped substantially with an NMDA receptor complex (NRSC) identified earlier (Husi et al., 2000). The earlier complex was an amalgamation of lists derived by immunoprecipitation from mouse forebrain with an NMDA receptor NR1 subunit antibody and the same NR2B carboxy terminal peptide. The combined NMDA-RC list consists of 186 genes of which 181 are autosomal and were included in this study. Genes coding for the mGlu5-RC were those identified using an antibody against mGluR5 protein in rat brain lysates (Farr et al., 2004). Of 52 mouse orthologues of these genes that have been identified (Collins et al., 2006), all 50 autosomal genes were included in the present analyses. The AMPA-RC comprised a set of nine proteins and corresponding genes isolated by immunoprecipitation using an antibody against the GluR2 protein (*Gria2*) (Collins et al., 2006). The seven autosomal genes from this set were

included in the present analyses.

4.6.2 Statistical analysis

First, gene-based statistics were derived using all markers that fell within gene boundaries. Gene-based statistics have the potential to increase statistical power in comparison with single marker designs. However, this depends on the underlying genetic architecture of the trait and in instances where there are multiple causal variants within a gene, as is likely with a highly polygenic trait such as intelligence, summing the small effects of each marker once may enable statistical significance to be attained which would otherwise be indistinguishable from background noise. In addition, gene-based statistics make it possible to assess the relative importance for intelligence of each gene in the PSD gene sets. A versatile gene-based test for genome-wide association studies (VEGAS) (Liu et al., 2010) was used to conduct gene-based analyses of association for each of the five cognitive phenotypes on the results of the meta-analysis described in chapter 3.

VEGAS derives a gene-based statistic by first converting the p-value associated with each SNP into an upper-tail χ^2 statistic with 1 degree of freedom. Next, the χ^2 statistics corresponding to each SNP within a gene is summed forming the gene-based statistic. The statistical significance for each gene is derived by simulating a multivariate standard normal random vector with correlations equal to those found between SNPs in the gene thus controlling for linkage disequilibrium (LD). Each vector contains as many elements as SNPs in the gene controlling for the number of SNPs per gene. This is an important consideration as, if the most significant SNP within a gene is used as the gene-based statistic (Wang et al., 2007b), a bias will be introduced where genes with a greater number of SNPs will be assigned a small p-value even in the absence of any true association between genotype and phenotype. The component variables are then squared to give the correlated χ^2 random variables. This vector is then summed to give a gene-based statistic consistent with the null hypothesis of no association. This procedure is then repeated multiple times to provide a null

distribution against which the empirical gene-based statistic can be compared. The empirical p-value for the gene is the proportion of simulated gene-based statistics which are greater than the observed gene-based statistic. The number of simulations used to ascertain statistical significance is determined using an adaptive procedure. Initially, 10^3 simulations is used which is increased to 10^4 should an empirical p-value of <0.1 be produced. In the event that when 10^4 simulations produces a p-value of <0.001 the number of simulations is increased to 10^6. Should a P-value of 0 be produced using 10^6 simulations, no more will be performed and the 0 should be interpreted as $p < 10^{-6}$. This constrains the minimum P-value that VEGAS can produce, however it should be noted that none of the genes used in this work attained a p-value of 0. The alpha level used in VEGAS is modified using a standard Bonferroni-correction where the number of genes is used as the denominator ($0.05/17787=2.811 \times 10^{-6}$). However, the overlap that occurs between genes means that some of these tests will be positively correlated, and so the Bonferroni-correction is likely to be overly conservative.

VEGAS was selected in part as it can be used on summary GWAS data and does not require access to the raw genotype data. This is an important consideration in genetic analysis as it allows this method to be used in the ever increasing sample sizes that are required in GWA studies and enables replication in larger sample sizes. Despite the use of summary rather than raw genotype data there appears to be little loss of information when using VEGAS to derive a gene-based statistic in comparison with phenotype permutation. Using a GWAS data set examining height in 3,611 unrelated individuals (Benyamin et al., 2008; Cornes et al., 2005; Medland et al., 2009) Liu et al. (2010) compared VEGAS with phenotype permutation method carried out in the PLINK (Purcell et al., 2007). This comparison took place using a subset of the full 17,787 genes that VEGAS uses due to the genotype based permutation method used by the PLINK set-based method being computationally intensive. The subset of 413 was selected from chromosome 22 with an additional 7 genes being selected on the basis that VEGAS indicated that their P-value was

$<10^{-3}$. The p-values produced by VEGAS and PLINK were then $-\log^{10}$ transformed and a Pearson's correlation showed that the two methods produced near identical results for the majority of genes tested ($r = 0.999$). A Spearman's rank correlation showed that the rank orders were highly matched ($r_s = 0.998$).

Using VEGAS, SNPs were assigned to genes according to their position on the UCSC Genome browser hg18 assembly with a gene boundary of ± 50kb of 5' and 3' UTRs. The gene-based statistic was then derived using each SNP within the specified boundary, with VEGAS controlling for the number of SNPs in each gene and the LD between those SNPs. Gene-based p-values were then –log(10) transformed and rank ordered for each phenotype.

Next, the specific gene-set enrichment hypotheses were tested using a competitive test of enrichment, GSEA (Subramanian et al., 2005; Wang et al., 2007b). As a method of gene-set analysis GSEA examines if the test statistic for a set of genes shows a small but consistent deviation from what would be expected under the null hypothesis. It has been shown that genes do not operate in isolation but rather form groups of proteins, which are part of pathways, or networks that underlie disease status or biological processes such as synaptic plasticity (Husi & Grant, 2001; Husi et al., 2000; Schadt, 2009)

These pathways and networks are then used as the unit of association enabling the weight of evidence for the association of the set, and the biological process the set is involved in, to be quantified as well as assist in a mechanistic interpretation of statistically significant results. GSEA is a competitive test, which contrasts with self-contained tests. The difference between the two lies in the nature of the null hypothesis being tested (Goeman & Bühlmann, 2007). Competitive tests compare the level of association between the gene-set with that of gene-sets drawn from throughout the genome. In this sense the null hypothesis tested is that the gene-set of interest displays the average level of association given the distribution of association from throughout the genome. Self-contained tests examine if there is a statistically significant association between the gene-set and the trait of interest, where

the null hypothesis tested is that there is no association between the gene-set and the trait. Whilst self-contained tests are more consistent with typical null hypothesis testing, competitive tests are robust to sources of genomic inflation (Devlin & Roeder, 1999; Wang, Li, & Hakonarson, 2010). In addition, self-contained tests are susceptible to an increase of type 1 errors due to the manner in which the null hypothesis is constructed. By permuting phenotype labels before the gene-set statistic is derived, creates a null distribution describing no association between genotype and phenotype. However, the observed gene-set statistic is calculated from the GWAS data where there are more low p-values than we should expect by chance meaning that randomly selected gene sets would have more low p-values than would be expected under the null hypothesis of no association. This problem is avoided by using competitive tests as the gene-set is compared against the average level of association in the same data set.

Competitive tests can be further divided into two categories: overrepresentation analysis and enrichment analysis. An overrepresentation analysis involves generating a list of significant genes from the observed data. This is done by selecting those genes that reach a predetermined level of statistical significance. The proportion of genes in the a priori gene-set that appear in the list is compared to proportion that is not. The gene-set is overrepresented if there is a greater proportion of genes from the a priori gene set amongst those data driven list than would be expected by chance. Enrichment methods rank all genes by the significance of their association with the trait of interest. A gene-set is enriched if it is ranked higher in the genome wide set than would be expected by chance. Enrichment methods have two main advantages when compared to overrepresentation analysis. The first is that they avoid the problem of establishing a threshold to determine which genes are examined as the null distribution is formed using all genes in a genome wide ranked set. The second is that they make use of the metric used to rank the genes rather than simply including them should they pass the threshold. This means that more weight can be given to genes in the gene-set that show a high level of association with the trait as opposed to

overrepresentation tests where a gene in a set with a p-value of 0.049, for example, is treated as having the same evidence for association as a gene with a P-value of 5×10^{-8}. By retaining these data there is the potential to increase the power to detect a gene set which influences intelligence.

As a competitive test of enrichment, GSEA uses a candidate list of gene identifiers and a genome-wide set of genes which are ranked by the strength of their association with a phenotype. GSEA tests whether gene identifiers in the candidate set fall higher in the genome-wide ranking than would be expected by chance. A running–sum Kolmogorov-Smirnov statistic weighted by the p-value from the genome-wide gene ranking set is derived. By weighting the p-value in this way the rank order and the distance between the ranks is used to determine if the gene-set shows enrichment for intelligence. This process is repeated, and the final enrichment p-value corresponds to the proportion of runs in which the test gene set ranked higher than the permuted set. Here, 15000 permutations were used. Statistical significance for the gene sets was to attain an uncorrected enrichment P-value ≤ 0.05 and/or *FDR*-corrected q-value of < 0.25 as per the developers' instructions (Subramanian et al., 2005; Wang, Li, & Bucan, 2007a). Whilst the use of both the p and FDR values is advised the mGluR5-RC was retained for further testing as it had a *FDR* <0.25.

In order to determine the validity of setting a *FDR* of 0.25 gene sets that reached this level, (the NMDA-RC and the mGlu5-RC) were compared against *P*- and *FDR* values derived from 1000 randomly-sampled gene sets of the same length (Ersland et al., 2012). The observed p- and *FDR* values were compared to the randomly sampled gene-sets of equal length and an empirical significance value was set for *P* and *FDR* values of the observed gene set as being smaller than 95% of those obtained in the random gene sets. Gene sets passing this criterion were taken forward to step six: replication in the BATS and NCNG cohorts. Figure 4.3 illustrates the total data processing pipeline including GWAS and meta-analysis.

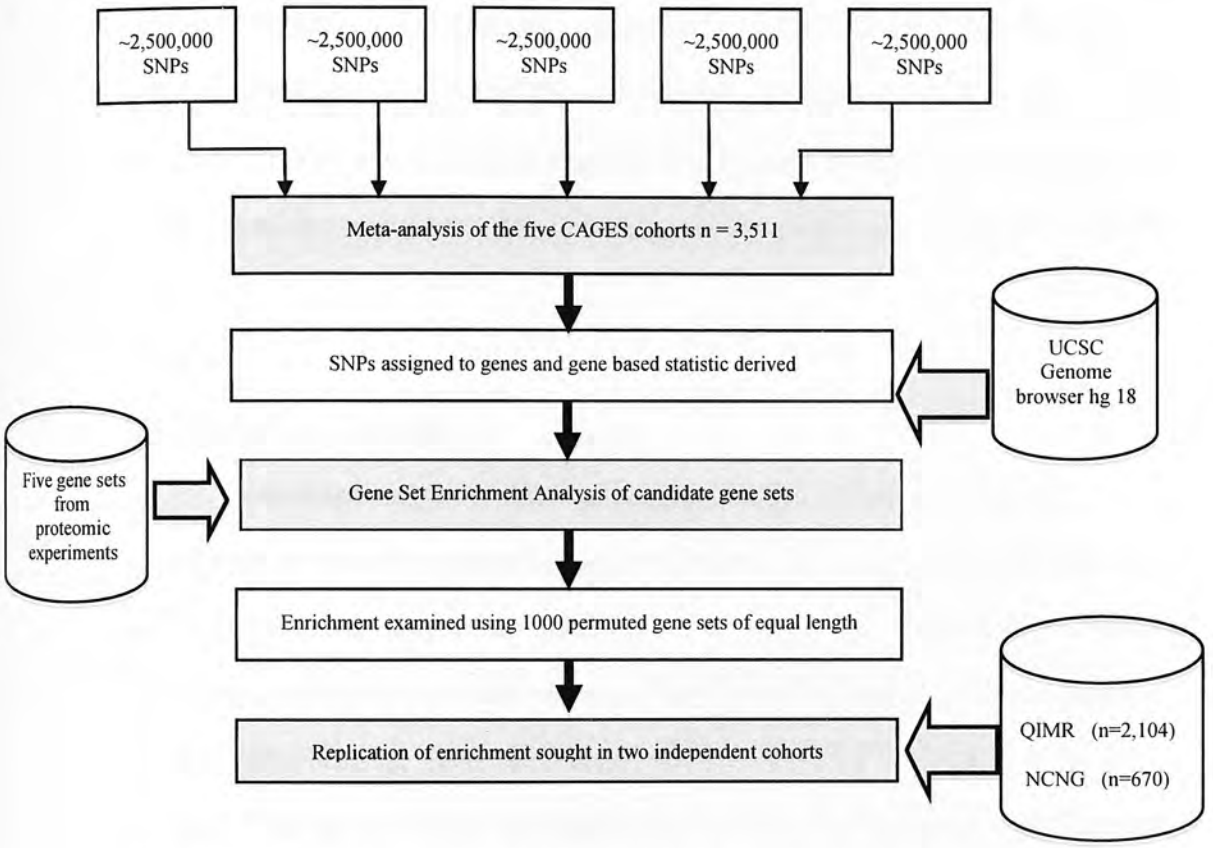

Figure 4.3. Total data processing stages from top to bottom. As described in chapter 3, the five cohorts from the CAGES consortium underwent single marker analysis (Li et al., 2010) separately before the results were meta-analysed (Willer et al., 2010). Next, SNPs were assigned to genes based on their position as indicated in the UCSC Genome browser hg 18 assembly and a gene based statistic was derived using VEGAS (Liu et al., 2010). A priori selected gene sets detailing the molecular composition of the PSD were brought in (Bayes et al., 2010; Collins et al., 2006; Husi & Grant, 2001) and enrichment of these sets in cognition was sought using GSEA (Subramanian et al., 2005; Wang et al., 2007b). Gene sets which were enriched were then compared to 1000 randomly selected gene sets of the same length to examine the strength of the enrichment found. Gene sets which survived this procedure were then taken forward for replication in two independent cohorts.

4.7 Replication

As only the NMDA-RC gene set met the criteria to be deemed significant against

any cognitive variable (as will be described below), it was the only set in which a replication was sought. Following replication the enrichment P-values from each of the three cohorts (CAGES, NCNG and BATS) were combined using Stouffer's Z-transform method (Whitlock, 2005a; Zaykin, 2011). The discovery cohort P-value was corrected for multiple comparisons using a Bonferroni correction for the 5 gene sets tested × 4 phenotypes; i.e., a correction for 20 tests (0.002 × 20 = 0.04) before being combined with NCNG and BATS.

4.8 Results

4.8.2 Gene-based association

Gene-based analysis of the meta-analytic SNP association data combining information from the five cohorts found no single gene significantly associated with any of the four cognitive phenotypes of fluid ability, crystallised ability, memory, and mental speed. The most significant gene-based p-values for fluid cognitive ability, crystallised ability, memory and processing speed, respectively, were for *BCAR3* ($P = 4.0\times10^{-6}$), *RFFL* ($P = 7.0\times10^{-5}$), *OR4P4* ($P = 4.0\times10^{-5}$), and *EIF5A2*, ($P = 4.9\times10^{-5}$). The gene with most evidence for association in the earlier GWA in this cohort (*FNBP1L* for *gf* (Davies et al., 2011)) ranked second in these analyses ($P = 1.9\times10^{-5}$). This slight difference is likely to be due to the use of imputed SNPs in the present analyses, by contrast with actual SNPs in the original analysis.

4.8.3 Enrichment analysis of postsynaptic density gene sets

Next, we test our principal hypothesis that variation in genes that code for the proteins in the PSD is involved in the normal range of variation of cognitive abilities. GSEA analyses were performed on each the six gene sets for each of the cognitive phenotypes. Of the five gene sets, the NMDA-RC was significant ($P = 0.002$, $FDR = 0.221$) for *gf* (Table 4.1). mGlu5-RC had an *FDR* also under 0.25, but had a P-value of 0.133. No significant support for enrichment was found for any of the other three phenotypes for any other gene

set (Table 4.1) nor was enrichment found for crystallised ability, memory, or processing speed (Tables 4.2 – 4.4). By comparison with 1000 randomly-ascertained sets of 181 genes, both the P-value and *FDR* obtained for the NMDA-RC was lower than that of 99.7% of the random gene sets in the *gf* phenotype. In the case of the association of mGlu5-RC with *gf*, comparison with 1000 randomly-sampled lists did not provide significant support for enriched association as it fell below the 95% threshold (observed P-value < 83.0%; *FDR* < 84.1% of random gene sets).

Table 4.1. Shows the results of enrichment analysis on five candidate gene lists from the PSD conducted on *gf* in the CAGES cohorts. The replication of the NRSC gene set in both BATS and NCNG cohorts is included.

Complex name	Number of genes	Empirical P-value	*FDR*
hPSD full	1386	0.628	0.705
hPSD consensus	714	0.242	0.542
NMDA-RC	181	0.002	0.221
mGlu5-RC	50	0.133	0.203
AMPA-RC	7	0.595	0.804
Replication Samples			
NMDA-RC (BATS)	180	0.012	0.012
NMDA-RC (NCNG)	180	0.371	0.371

Note: *FDR* is False Discovery Rate hPSD, postsynaptic density; NMDA-RC, *N*-methyl-D-aspartate receptor signalling complex/membrane-associated guanylate kinase associated signalling complex; mGlu5-RC, the metabotropic glutamate receptor complex 5; AMPA-RC, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor complex; BATS, Brisbane Adolescent Twin Study; NCNG, Norwegian Cognitive NeuroGenetics.

Table 4.2. Shows the results of enrichment analysis on five candidate gene lists from the PSD conducted on crystallised ability in the CAGES cohorts.

Complex name	Number of genes	Empirical P-value	*FDR*
hPSD full	1386	0.706	0.757
hPSD consensus	714	0.821	0.851
NMDA-RC	181	0.307	0.679
mGlu5-RC	50	0.327	1.000
AMPA-RC	7	0.501	0.887

Note: *FDR* is False Discovery Rate hPSD, postsynaptic density; NMDA-RC, *N*-methyl-D-aspartate receptor signalling complex/membrane-associated guanylate kinase associated signalling complex; mGlu5-RC, the metabotropic glutamate receptor complex 5; AMPA-RC, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor complex.

Table 4.3. Shows the results of enrichment analysis on five candidate gene lists from the PSD conducted on memory in the CAGES cohorts.

Complex name	Number of genes	Empirical P-value	*FDR*
hPSD full	1386	0.450	1.000
hPSD consensus	714	0.768	1.000
NMDA-RC	181	0.441	1.000
mGlu5-RC	50	0.819	0.892
AMPA-RC	7	0.637	1.000

Note: *FDR* is False Discovery Rate hPSD, postsynaptic density; NMDA-RC, *N*-methyl-D-aspartate receptor signalling complex/membrane-associated guanylate kinase associated signalling complex; mGlu5-RC, the metabotropic glutamate receptor complex 5; AMPA-RC, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor complex.

Table 4.4. Shows the results of enrichment analysis on five candidate gene lists from the PSD conducted on processing speed in the CAGES cohorts.

Complex name	Number of genes	Empirical P-value	*FDR*
hPSD full	1386	0.837	1.000
hPSD consensus	714	0.861	0.990
NMDA-RC	181	0.817	0.819
mGlu5-RC	50	0.619	1.000
AMPA-RC	7	1.000	1.000

Note: *FDR* is False Discovery Rate hPSD, postsynaptic density; NMDA-RC, *N*-methyl-D-aspartate receptor signalling complex/membrane-associated guanylate kinase associated signalling complex; mGlu5-RC, the metabotropic glutamate receptor complex 5; AMPA-RC, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor complex.

To ensure that the enriched association was not driven by a single gene, the most significant gene from the NMDA-RC set and the mGlu5-RC set were removed. Once *DNM2* was removed from the mGlu5-RC list, no significant evidence of enrichment with *gf* remained. However removing the top gene from the NMDA-RC gene set – *PRDX2* – attenuated the enrichment with *gf* but it remained significant ($P = 0.006$).

4.8.4 Replication

The enrichment of the NMDA-RC gene set in fluid cognitive ability was tested for replication in the Norwegian and Australian cohorts using identical methods to those used above in the discovery sample; i.e., gene-based analysis using VEGAS, followed by a GSEA unit-weighted analysis with 15000 permutations. Enrichment testing in the BATS and the NCNG cohorts yielded P-values of 0.012 and 0.371 respectively. The association remained

significant in BATS after removing the top gene (*RAB3A*) from the set ($P = 0.024$), indicating that multiple genes were contributing to the enrichment signal in both CAGES and BATS. A meta-analysis of these results for the NMDA-RC across the discovery cohort and two replication samples was determined using Stouffer's weighted Z-transform method (Whitlock, 2005a; Zaykin, 2011). The probability of obtaining these results across the three independent cohorts, corrected for multiple testing in the discovery cohort, and tested against the null hypothesis of no association was $P = 0.003$. By omitting the discovery cohort the enrichment of the NMDA-RC across BATS and NCNG remained significant ($P = 0.018$) supporting the enriched association of the NMDA-RC with fluid ability.

4.9 Discussion

The present study used a hypothesis-driven approach to test the joint effect of multiple genetic variants clustered in the same biological network on human intelligence differences. In drawing upon the synapse proteomic datasets the results suggested that SNP variation in the genes encoding the NMDA/MAGUK receptor complex is enriched for association with fluid cognitive ability as measured by the tests used in CAGES and BATS outlined in section 2.3. This finding linking NMDA-RC to fluid ability provides evidence that genetic variation in the macromolecular machines formed by MAGUK scaffold proteins and their interaction partners contributes to variation in intelligence.

By contrast with the NMDA-RC, other components of the PSD were not found to be significantly enriched for variation in cognitive abilities in this study. These results raise the question of why the NMDA-RC should be preferentially involved in fluid-type intelligence. The present results suggest that association of the NMDA-RC with *gf* does not simply follow from it being a part of the synapse or playing a role in the excitatory transmission system, as three other systems found in the synapse did not show enrichment and all are activated once the receptors bind with glutamate or are found only at glutamatergic synapses. However, the lack of an enriched association for the AMPA-RC or the mGlu5-RC could be due to the

small numbers of genes involved in their expression, meaning that even greater sample sizes would be required to detect an enrichment of these complexes.

The NMDA-RC is enriched for both learning and synaptic plasticity phenotypes in mice (Pocklington et al., 2006), and the same proteins have been shown to be involved in human learning disabilities (Pocklington et al., 2006). These findings validate the utility of rodent models for human cognitive function. Additionally, they suggest that combinations of SNPs in LD with common SNPs found within the genes of the NMDA-RC may result in variation in synaptic plasticity, which in turn is responsible for some of the observed differences in human intelligence.

Variation in the NMDA-RC has been implicated in schizophrenia (Fernandez et al., 2009; Kirov et al., 2012) and intellectual disability (Nithianantharajah et al., 2013; Tarpey et al., 2004; Walsh et al., 2008; Zanni et al., 2010) with mutations in individual scaffolding molecules SAP102/Dlg2 and PSD93/Dlg3 linked to these disorders respectively. The present finding of a link between intellectual function and variation in the NMDA-RC therefore supports a genetic link between schizophrenia and intelligence, in keeping with behaviour genetic (Toulopoulou et al., 2010) research, and also with recent polygenic risk studies of a sub-set of the present cohorts that indicated an overlap of polygenic risk factors for schizophrenia and for cognitive aging (McIntosh et al., 2013). The genetic link between schizophrenia and cognitive abilities appears to be region rather than variant specific. Where de novo copy number variation (CNV) at the NMDA-RC is associated with schizophrenia (Kirov et al., 2012) it is common SNP variation, in the same region, which shows an enriched association with the normal range of cognitive abilities. However, neither the common SNPs, nor CNVs associated with schizophrenia have been shown to be associated with intelligence differences in a non-elderly cohort (van Scheltinga et al., 2013).

Enrichment was found for fluid ability and not for crystallised ability, memory or processing speed. If gene effects directly impact on specific functions (rather than on general ability per se), then analyses targeting these specific functions (such as speed or memory) are

known to be significantly more powerful than are analyses of a composite or latent factor such as fluid ability (van der Sluis, Verhage, Posthuma, & Dolan, 2010). Here, the enriched association of the NMDA-RC was found for the fluid ability composite, rather than specific functions. This contrasts with work (Nithianantharajah et al., 2013) finding that specific cognitive deficits, such as visual discrimination tests of operating conditioning or attention, co-occur with variation in the genes of the NMDA-RC. Such findings are consistent with the current study as where Nithianantharajah et al. (2013) examined the effect of knocking out a single gene, here normal variation in the form of common SNPs was measured across the genes of the NMDA-RC. The finding that genetic association for the fluid ability phenotype proved the stronger indicator, then, is compatible with generalist genetic action as opposed to functional specificity (Plomin & Kovas, 2005).

Whereas the mGlu5-RC gene set showed weak evidence of enrichment in the initial GSEA analysis, this did not survive permutation testing. It was shown to be due to a single gene, *DNM2*, rather than an over representation of mGlu5-RC genes in the upper portion of the total gene list. This is in contrast with the NMDA-RC gene set where multiple genes were involved in the enrichment signal in both CAGES and in BATS, consistent with the notion that it is variation in the network and not in a single gene, which contributes to normal variation in fluid ability.

There were a number of limitations with this study. Firstly, the link between genetic variation in the NMDAR-RC and intelligence may exist for reasons other than synaptic plasticity. Activation of the NMDA-RC can also lead to the destruction of neurons and initiate neuronal death (Zorumski & Izumi, 2012). The use of gene-set analysis methods would not be able to determine the reason for the association, only its existence. A related problem pertains to the genes driving the association in the NMDA-RC set. Whilst this set does indeed show significant enrichment in the data sets tested here it is not clear if it is because the NMDA-RC genes set shows enrichment or if this set overlaps with other gene-sets which contain the true association. A related issue is that the SNPs of the NMDA-RC

may be in LD with SNPs that do not fall within the gene boundaries of the NMDA-RC gene set. This would mean that the NMDA-RC gene set is tagging the variants responsible for variation in intelligence. These questions can be addressed through the use of testing additional gene sets detailing other gene-sets as well as examining both the portion overlapping with the NMDA-RC as well as that which is unique to it. A potential solution to both of these issues would be to assess a synaptic plasticity phenotype using human cortical tissue. In this instance the same data processing pipeline could be used here (single marker GWAS, gene based-statistics, gene-set analysis) but with synaptic plasticity phenotypes rather than intelligence. Such GWAS have already been used to examine the link between genotype and methylation levels in human cortical tissue (Gibbs et al., 2010)

A second limitation is the manner in which the gene-based statistic was derived. The gene-based statistics derived by VEGAS and used by GSEA are assumed to be independent. However, genes do overlap and the inclusion of the 50kb boundary is likely to magnify this problem particularly in the presence of LD. This overlap could result in a number of genes lying close together being given a low P-value due to a single variant mapped to them all. This in turn could lead to the gene-set showing significant enrichment if these genes are all part of the same gene-set. It would of course be equally true that that multiple genes could have a high P-value for the same reasons. One solution for this would be to only map each SNP to a single gene but the criteria to determine how to best map them is unclear.

Thirdly, whilst these results indicate that the NMDA-RC shows enrichment for fluid abilities no effect size is included. In order to derive the proportion of variance accounted for by the genes of the NMDA-RC, GCTA (Yang et al., 2010; Yang et al., 2011) could be performed excluding SNPs found outside the NMDA-RC, providing a measure of the heritability that is attributable to the genetic effects specific to the NMDA-RC. However owing to the large standard errors reported by GCTA larger sample sizes will be needed before this analysis becomes viable. An additional problem of using GCTA in this way requires access to the genotyped data as it cannot currently be performed using summary

data.

Finally, different tests were used in each cohort to derive measures of *gf* which may have reduced any effect found. However the fact that different cognitive tests were used in each cohort should not be overemphasised as correlations between measures of intelligence are high and often in excess of 0.9 (Johnson et al., 2004; Johnson et al., 2008)

In summary, large scale molecular studies indicate that intelligence is polygenic (Benyamin et al., 2013; Davies et al., 2011) which is compatible with a range of genetic models, the most extreme of which would be that all genes matter with roughly equal effect. Here, using Gene Set Enrichment Analysis we tested the hypothesis that that some genes matter more than others. Specifically, we found that genes in pathways related to postsynaptic functioning are enriched. The results suggested that a major component of the postsynaptic region, the NMDA-RC, is preferentially associated with normal variation in intelligence. The NMDA-RC pathway appears to be specifically enriched for association with fluid ability, providing a lead towards understanding a source of some of the variation in human intelligence differences. Future work should include other synaptic components such as activity-regulated cytoskeleton-associated protein (ARC). ARC has been reliably associated with both LTP (Guzowski et al., 2000) and LTD (Waung, Pfeiffer, Nosyreva, Ronesi, & Huber, 2008) with ARC mRNA being transported to active synaptic regions via the dendritic spine where it is then translated and serves to modulate AMPA trafficking (Chowdhury et al., 2006). Whilst studies using unpublished ARC gene sets exist (Kirov et al., 2012), the validity of the list has yet to be established meaning a significant result could indicate the ARC set containing genes also used in other synaptic components. Additionally, a non-significant result of this ARC set being enriched for intelligence may be due to the omission of critical genes making up the molecular constituents of ARC.

Chapter 5: Are the genes of the heterotrimeric G-proteins associated with cognitive ability?

5.1 Introduction

This chapter extends from the previous chapter and again takes as its rationale the idea that genes expressed in the synapse are candidates for explaining individual differences in intelligence. A previous functional gene-set analysis has implicated common SNPs in heterotrimeric G-protein coding genes as being associated with differences in human intelligence (Ruano et al., 2010). This chapter consists of an examination of this "horizontal" approach, and a test of replication for the finding.

Aggregation of genes based on shared function has the potential to increase power, as the focus is on the combined effect of multiple genes rather than examining the effect of each SNP separately (Mooney, Nigg, McWeeney, & Wilmot, 2014), as well as to begin a molecular-mechanistic account of human intelligence differences. This aggregation can be performed by grouping genes according to the biological systems in which they are found, however such pathways may not be independent, and this approach can lead to the same genes appearing in multiple pathways. This lack of independence can be exploited for phenotypes such as intelligence, where multiple pathways may contribute toward the phenotype, by using an approach to examine sets of genes with the potential to influence multiple systems, so called generalist genes (Kovas & Plomin, 2006). Gene-sets influencing multiple pathways can be created by grouping genes according to their cellular function such as tyrosine kinase signalling or ligand gated ion signalling, an approach termed horizontal pathway analysis by Ruano et al. (2010). This horizontal method of grouping genes contrasts with traditionally defined pathways, also termed vertical pathways, as illustrated in Figure 5.1A.

The biology of the synapse is well suited to the use of horizontal groupings as there

is a high degree of overlap between the proteins in both the dopaminergic and the glutamatergic system for example. These two systems, along with other synaptic systems have been shown to regulate functions such as synaptic plasticity (Migaud et al., 1998; Yang et al., 2012). This indicates that genetic variation in the synapse may lead to disruption across multiple systems which are all involved in the same process making the genetic architecture of the synapse a suitable candidate system for horizontal analysis.

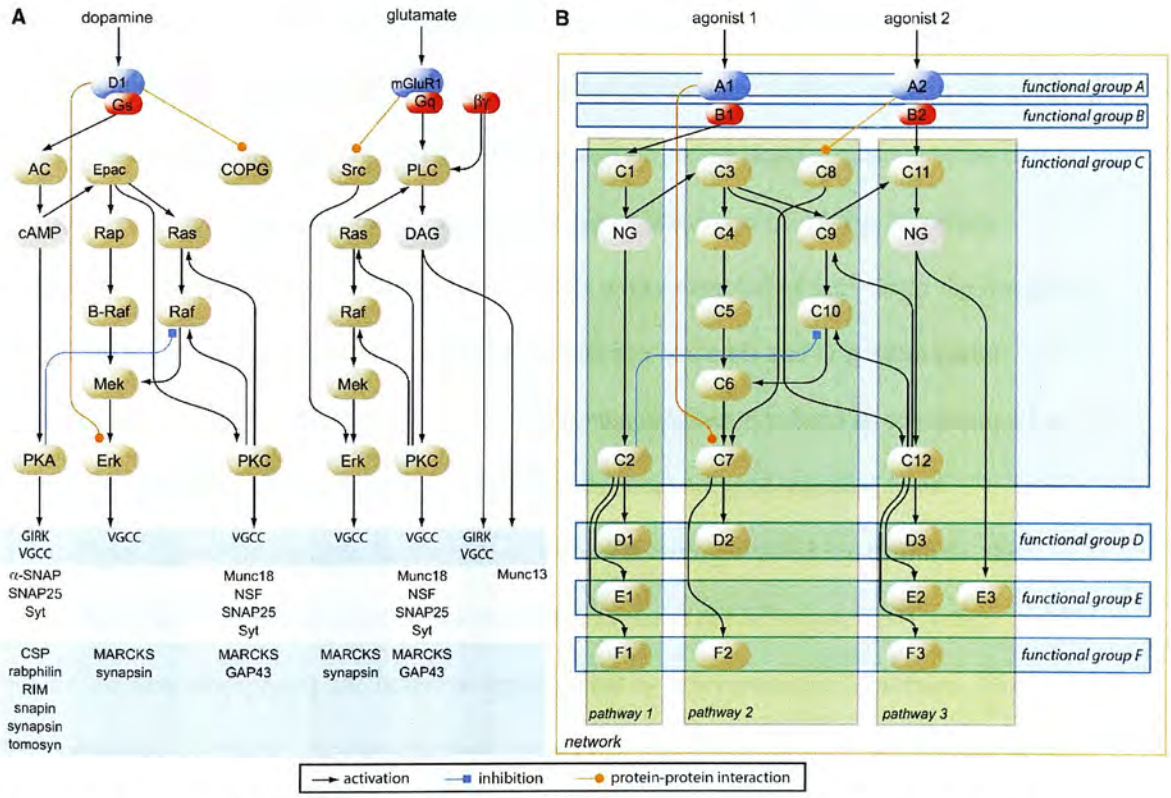

Figure 5.1. Section A illustrates the genes in a dopamine pathway and a glutamate pathway. As can be seen, these vertical or linear pathways share genes. Section B illustrates 3 pathways which have been broken up into 6 functional groups (A-F). These 6 groups are assembled according to the function of the proteins rather than the pathways they fall into. As proteins are known to act across pathways, grouping genes according to their functional category allows each gene-set to have an effect on multiple linear systems. (Figure taken from (Ruano et al., 2010))

The synaptic sets used by Ruano et al. (2010) were drawn from a total set comprised

of all genes expressed in the synapse. The 22 gene-sets taken from this total included 17 horizontal pathways and 4 vertical synaptic signalling pathways, along with a group of genes expressed in the synapse, but whose function was unknown at the time. Among the 23 groups was a set of 27 genes coding for heterotrimeric G-proteins. Heterotrimeric G-proteins form the internal mechanism of one of the four types of ion gate including, ligand gated ion channels, voltage gated ion channels, second messenger ion channels and G-protein gated ion channels. Ligand gated ion channels, such as the AMPA glutametergic ion channels, are opened in response to the neurotransmitter (the ligand) binding to the receptor. Voltage gated ion channels such as calcium ion channels are opened in response to the change in electrical potential near the channel being altered. This takes place during depolarization. Activation of the NMDA receptor, for example, along with sustained activation of AMPA receptors, results in an opening of the calcium channels along with a removal of mg^{2+} from the ion gate allowing ca^{2+} to freely enter. Both second messenger ion channels and G-protein gated channels are metabotropic ion channels where the receptor does not form an ion channel but rather upon binding with the appropriate ligand transduces a signal resulting in an intracellular signalling cascade. In the case of second-messenger-gated ion channels, the intracellular signalling cascade takes the form of the release of Ca^{2+} or cAMP (Cyclic adenosine monophosphate) the latter can be activated by heterotrimeric G-proteins. The heterotrimeric G-proteins can also regulate the opening of the ion channel directly following the activation of metabotropic neurotransmitter receptors including the mGlu5-R. Heterotrimeric G-proteins consist of three subunits α, β, and γ. Heterotrimeric G-proteins are activated in response to G-protein-coupled receptor binding (Oldham & Hamm, 2008). Of interest in accounting for general cognitive ability, as these same G-proteins are used in numerous synaptic signalling pathways, they potentially create a processing bottleneck which could affect a diverse range of cognition-related functions, in keeping with a role in general cognition.

Pathways were formed by Ruano et al. (2010) for each available gene coding for the

proteins of the heterotrimeric G-protein set (Of these 27 genes (see Table 5.2) 25 had SNP coverage in the Perlegen chip used) based on all SNPs located within the region spanning from 2kb upstream to 500bp downstream of the boundary of each member gene. The resulting SNP lists were tested for association using software to test for Joint Association of Genetic variants: (JAG: http://ctglab.nl/software/jag). This software uses phenotype permutation to create an empirical test for significant associations between the phenotype and the aggregated SNPs in a gene-set (Lips, Kooyman, de Leeuw, van Bochoven, & Posthuma, submitted). Tests used to assess the combined weight of evidence from multiple genes fall into one of two categories self-contained or competitive. Self-contained tests evaluate the association of SNPs in a pathway against a null hypothesis of no association. By contrast, a competitive test evaluates evidence for association in a candidate pathway against competing random selections of genes forming a baseline level of association (Wang et al., 2010). At the time of the Ruano et al. (2010) study only self-contained tests were implemented in the JAG algorithms but the software now implements competitive tests (see Lips et al. (2011)). The 23 gene-sets analysed by Ruano et al. (2010) were subjected to self-contained testing for association with four subtests of the Wechsler Intelligence Scale for Children in a sample of 627 children with ADHD. One gene-set – the group of 25 genes (359 SNPs) coding for heterotrimeric G-proteins – showed evidence for significant association: with an empirical p-value of 0.0015 against an experiment-wide α of 0.0022. This association was replicated in the UK ALSPAC cohort ($n = 1,507$, $p = 0.047$). The testing in ALSPAC differed slightly, in that two genes available in the discovery cohort – *GNB2* and *GNG11* – were omitted due to lack of coverage, with a total of 265 SNPs tested, mapped to 23 of the 25 genes used in the discovery cohort. G-protein coding genes, then, may be causally associated with intelligence, accounting for around 3.3% of variance in general ability (Ruano et al., 2010).

In this chapter replication of the association of G-protein coding genes with intelligence was sought. Additionally, crystallised ability was added along with fluid ability

in older age. As in the original Ruano et al. (2010) method, we used the self-contained option in JAG. In addition we used an alternative method – Gene-set Enrichment Analysis (GSEA (Subramanian et al., 2005; Wang et al., 2010; Wang et al., 2007a)– as a complementary analysis strategy. GSEA works with gene-level association statistics created by programs such as Versatile Gene Analysis System (VEGAS (Liu et al., 2010))and performs a competitive test of enrichment to determine if genes within the candidate pathway show a greater association to a phenotype than do equivalent sets of genes selected at random from outside the pathway. The self-contained test included here ensures that the same statistical methods were used as per the original report (Ruano et al., 2010), however significant results from a self-contained test should be followed up with competitive testing where deviation from the average level of association in the data set is sought. This is because in a GWAS data set there are more low p-values than would be expected under the null hypothesis of no association, meaning that randomly selected genes are more likely to have low p-values. These genes are then compared to a true null distribution where the link between genotype and phenotype has been broken by permuting the phenotype between participants. This means that the self-contained test used in JAG is sampling from a non-uniform distribution and assessing it against a uniform distribution for significance, resulting in an inflation of the type 1 error rate. As such their use without a competitive test should be interpreted with caution. The use of both self-contained and competitive methods of analysis provides a robust test of the original hypothesis that variation in heterotrimeric G-proteins is associated with intelligence. Gene-level statistics were also included to quantify the contribution made by each gene to both the fluid and crystallised phenotypes. In addition, due to the longitudinal nature of the Lothian and Aberdeen cohorts (Whalley et al., 2011) we were able to test for association both with current cognitive ability in older adults in five independent samples, and also for childhood (age 11) IQ scores in two of these samples.

5.2 Summary

By using horizontal gene grouping to assemble gene-sets coding for heterotrimeric G-proteins self-contained and competitive gene-set tests were performed on the CAGES data sets. Self-contained tests were used to determine if this gene-set showed association with fluid ability, crystallised ability, or age 11 IQ whereas competitive testing was used to determine if the weight of evidence for the heterotrimeric G-protein gene-set was greater than the average level of association for all genes. With α set at 0.05, a simulation-study of the power of JAG to detect the original reported-effect of 3.3% of total variation in our five-cohort meta-analysis lay between 0.78 and 0.87 depending on assumptions about the distribution of effects across the total set of SNPs in the pathway (see figures 5.3-5.7). Of course, given the winner's curse, the likely true effect of the gene-set, if replicable, is likely less than this.

5.3 Materials and methods:

5.3.1 Phenotypes

The original report detailed a statistically significant result between genetic variation in the heterotrimeric g proteins and general ability in a sample of 627 individuals whose ages ranged from 5 -19. As the samples used for discovery in the thesis are in middle to old age, the fluid and crystallised phenotypes were examined to take into account the differential effects of the ageing process on cognitive abilities (Tucker-Drob & Salthouse, 2008). An additional cognitive phenotype was added (age 11 IQ) to also explore the effects of ageing. See section 5.3.3 for full details.

5.3.2 Including covariates in self-contained tests.

The JAG method of analysis conducted to determine if there was a significant association between the heterotrimeric g-protein gene-set and cognitive ability uses phenotype permutation. As such the level of association between each SNP in the gene-set and cognitive ability is recalculated after each permutation. However, JAG does not offer the

user the ability to include covariates in these analyses. To solve this problem the 4 multidimensional scaling factors, used as covariates in the previous chapter for use with Mach2QTL, were used here along with age and sex in producing the standardised residuals for use from the factor and component scores.

5.3.3 Additional cognitive phenotypes

Due to the large age difference between the samples of the CAGES consortium and the discovery and replication samples used in the original discovery by Ruano et al. (2010) age 11 IQ was included in the analyses here. Age–11 IQ was assessed using the Moray House Test number 12(MHT) for both LBC1921 and LBC1936. The score on the MHT was corrected for age at the time of testing before being converted into an IQ-type score (mean = 100, SD = 15). Following this, sex and population stratification was controlled for by extracting standardised residuals as described in the other phenotypes presented here.

5.3.4 Candidate gene-set

A total of 33 genes in the human genome are responsible for the Heterotrimeric G-proteins of which 27 are ubiquitously found to be expressed at the synapse. The candidate gene-set tested by Ruano et al. (2010) consisted of 25 of the 27 synapse expressed heterotrimeric G-proteins. The cohorts in the present chapter were genotyped on a different platform that, whilst not including coverage of *GNB2*, nevertheless had significantly higher coverage of the remaining genes, with a total of 473 SNPs available for testing. For GSEA, imputed SNP data was used and coverage of all 27 genes was achieved. The analyses thus achieved better overall coverage of the theoretically relevant trait variants in G-protein genes.

5.3.5 Statistical analysis

In order to test for an association between the gene-set and the cognitive phenotypes

JAG was used. JAG is an example of a self-contained test where only the gene-set of interest is examined in order to test the null hypothesis that the gene-set is not associated with intelligence. As mentioned, this class of tests is more prone to false positives particularly in the presence of population stratification. JAG determines if there is a significant association between the gene-set and the trait in the following manner. Firstly, the p-values for each SNP in the gene-set are –log10 transformed before being summed. This forms the gene-set based statistic. This statistic is affected by the number of SNPs in the gene-set in that gene-sets which contain a greater number of genes will also have a higher $\sum$-log10(p-value). In addition to this there is the problem that the $\sum$-log10(p-value) will be high if there are multiple SNPs in the gene-set that are in strong linkage disequilibrium (LD) and have low p-values. These issues are dealt with in the second phase where the phenotype score is permuted across the participants before the level of association for each SNP with the phenotype is recalculated. The p-values for the same genes are then –log10 transformed and summed to create a null distribution for the gene-set, where the link between phenotype and genotype has been broken. The number of times the permuted gene-sets $\sum$-log10(p-value) was greater than the observed gene-set based statistic was divided by the number of permutations to derive a p-value describing the statistical significance for the gene-sets association with intelligence. By using phenotype permutations in this manner linkage disequilibrium and number of SNPs in the set is controlled for as the same SNPs, with the same haplotype structure, is included in each permutation. See Figure 5.2 for graphical representation of the JAG method for a self-contained test.

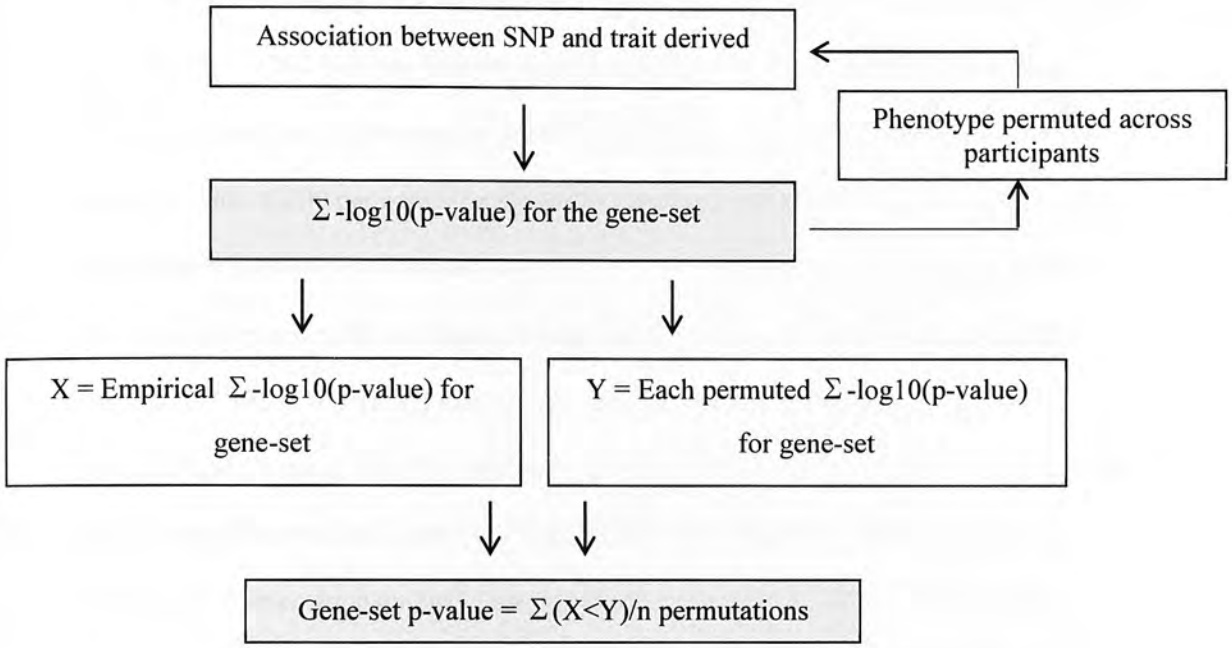

Figure 5.2. Illustration of the process used by JAG to derive a gene-set statistic and assess this for statistical significance. Firstly PLINK was used to derive the level of association between each SNP in the gene-set with the phenotype. The p-values for these SNPs are then –log10 transformed then summed to create the gene-set based statistic, labelled here as X. The phenotype is then permuted across participants breaking the link between genotype and phenotype. The level of association was then re-calculated from the permuted data set before being –log10 transformed then summed to form Y, the permuted gene-set statistic. Here, 10,000 permutations were used. The number of times Y was greater than X was summed before being divided by the number of permutations to derive the empirical p-value for the gene-set. As a self-contained test, JAG uses the same genes from the empirically derived gene-set statistic (Σ -log10(p-value)) in the null distribution, thus controlling for LD and the number of SNPs in the gene-set statistic.

For processing by JAG, a total of 473 SNPs were assigned to 24 genes based on their position in the UCSC Genome browser hg18 assembly with a 2kb upstream and 500bp downstream boundary, following the procedure in Ruano et al. (2010). The analysis was conducted with 10,000 permutations of the phenotype and an empirical p-value was derived. The cognitive phenotypes examined using GSEA were derived using the same procedures outlined in Chapter 3 with enrichment being examined using the data processing pipeline described in Chapter 4. Briefly, gene-based association statistics were computed on the combined GWAS using VEGAS which controls for both LD and the number of SNPs within a gene through simulation (Liu et al., 2010). SNPs were assigned to autosomal genes according to their position on hg18 Genome Browser assembly with a ± 50kb boundary around each gene used to capture regulatory elements. The full complement of 27 genes considered to form the heterotrimeric G-protein horizontal pathway were available in this analysis, which is two more than were available in the original paper due to insufficient coverage on the Perlegen chip (Ruano et al., 2010). GSEA was then used to determine if the 27 heterotrimeric G-protein genes were preferentially distributed in the upper portion of each genome wide ranked gene-set using a running-sum Kolomogorov-Smirnov (K-S) statistic weighted by the p-values of the gene-association statistic. These genome wide ranked sets were permuted 15,000 times to derive an empirical likelihood of association, describing the proportion of observed permuted K-S tests smaller than the original weighted K-S test statistic.

5.3.6 Power

Power was calculated for the JAG self-contained test through simulations. As the parameters required to accurately compute power would require prior knowledge of the genetic architecture of intelligence, a series of simulations were run to explore how power

fluctuates as a function of the number of SNPs the effect is distributed across and the total amount of variation this effect contributes toward. Simulations were carried out by assuming a non-zero effect for 10, 30, 100 and 300 SNPs. The percentage of variance explained by these effect SNPs was also varied where an effect size of 1, 2, 3, 4, 5 or 10% of the total variance was simulated. For the each of the SNPs with a non-zero effect an effect size was allocated by randomly sampling a normal distribution of effect sizes (mean = 0, SD = 1). A predicted phenotype was then calculated for each individual in each cohort using the effect sizes allocated to the effect SNPs. Next, 1000 phenotype vectors were generated within each sample by adding normally distributed noise with variance such that the predicted phenotype accounted for required percentage of total phenotypic variance (1,2,3,4,5 or 10%). For each of the 1000 phenotypes JAG was then used to calculate the gene-set based p-value in each cohort. These were then meta-analysed using Stouffer's weighted Z score to derive one p-value describing the strength of the association across the five cohorts. Power was calculated as the number of the 1000 phenotypes in which the meta-analytic p-value was less than 0.05. New effect sizes were allocated to the effect SNPs 100 times as illustrated in Figures 5.3-5.6 with Figure 5.7 showing the mean power for each condition simulated. These simulations indicate that there are only negligible fluctuations in power attributable to the number of SNPs the effect is spread across. Rather, power is largely a function of the total amount of variance explained by the SNP set. The article by Ruano et al. (2010) indicated that 3.3% of the variance explained could be attributed to the G-protein SNP set meaning that the present study would have between 0.775 and 0.867 power to detect the effect if it was present. The scripts used to derive the power calculations were provided by Christiaan de Leeuw of Center for Neurogenomics and Cognitive Research, Neuroscience Campus Amsterdam, Complex Trait Genetics, VU University Amsterdam, Amsterdam, The Netherlands, Institute for Computing and Information Sciences, Radboud University Nijmegen, Nijmegen, The Netherlands.

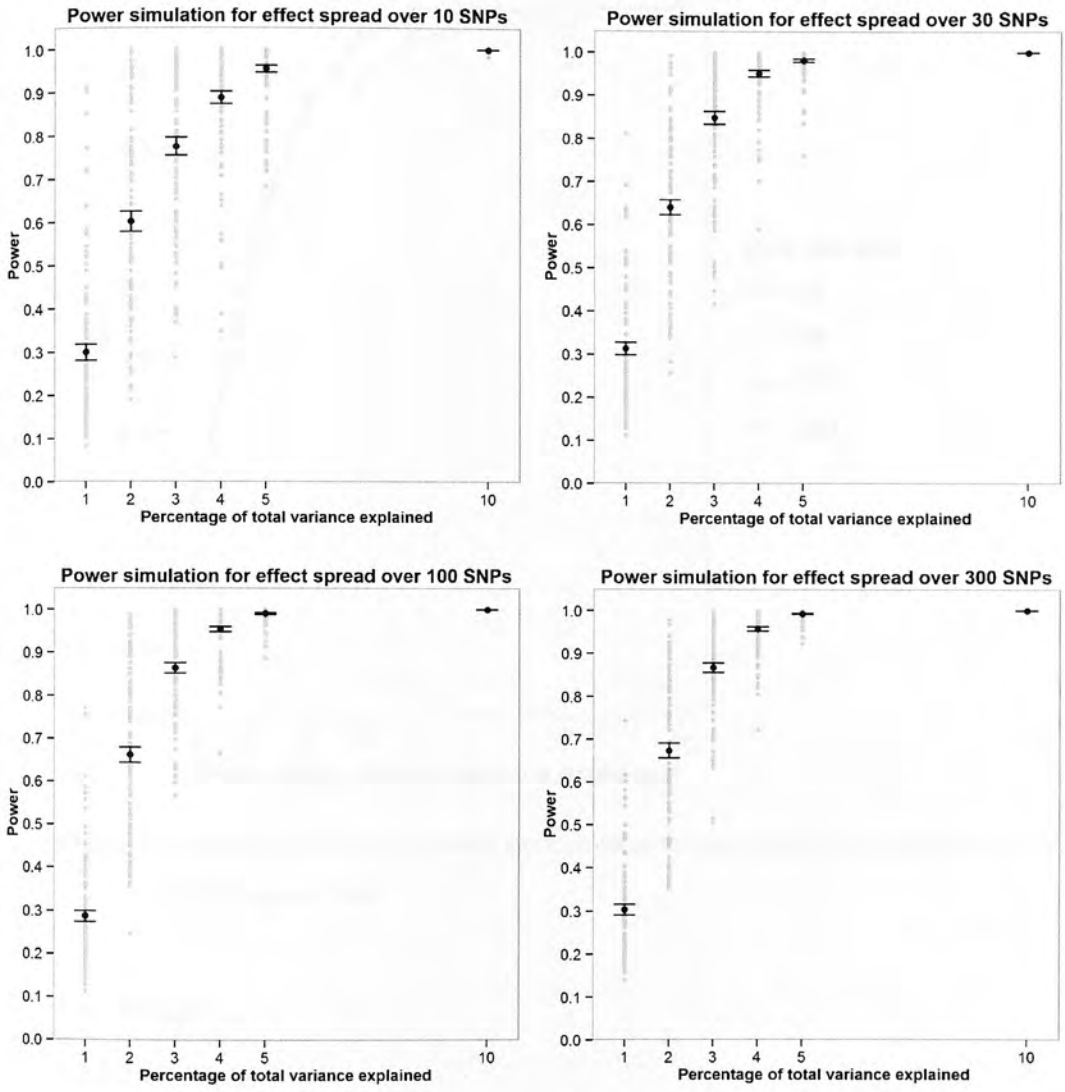

Figure 5.3-5.6 Simulations exploring how power fluctuates as a function of total variance explained and the number of SNPs the effect is spread across. For each effect size (1,2,3,4,5 or 10% of the total variance) 100 simulations were carried out. The mean power from these 100 simulations is shown with error bars depicting ±1standard error. Figure 5.3 shows the power calculation if the effect was spread over 10 SNPs where figures 5.4-5.6 show power if the effect is spread over 30, 100 and 300 SNPs respectively.

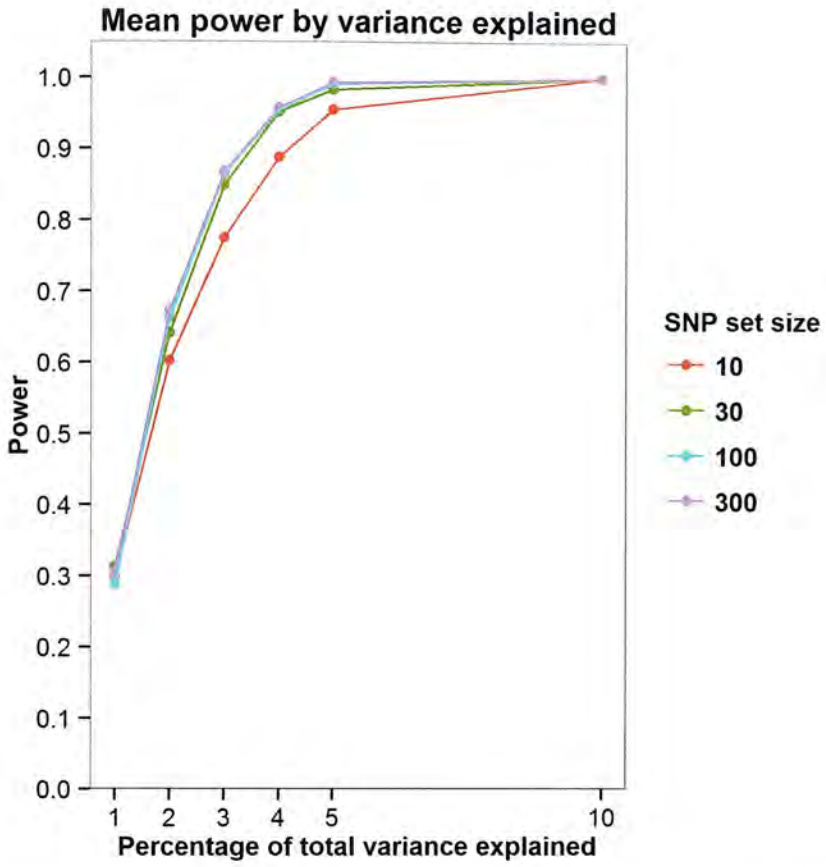

Figure 5.7. The mean power is plotted against variance explained for the number of SNPs the effect is spread over.

5.4.1 Results

5.4.2 JAG results

None of the SNPs from the G-protein groups reached genome wide significance in any of the cohorts examined. Using JAG, tests for association between SNPs in G-protein genes and either fluid or crystallised cognitive ability were non-significant in all five cohorts considered (See Table 5.1). Using Stouffer's Z, weighted by the square root of the sample size (Whitlock, 2005b; Zaykin, 2011) a single meta-analytic p-value was derived for evidence of association between the gene-set and the phenotype across the five cohorts. This revealed no significant evidence for association for either fluid ability ($p = 0.43$) or crystallised ability ($p = 0.98$).

Table 5.1. Association between fluid and crystallised ability and the G-protein gene-set for each of the five CAGES cohorts.

	Fluid ability			Crystallised ability		
Cohort	N	N SNPs	Empirical p-Value	N	N SNPs	Empirical p-Value
Lothian Birth Cohort 1921	505	468	0.68	515	468	0.66
Lothian Birth Cohort 1936	989	468	0.76	1003	468	0.97
Aberdeen Birth Cohort 1936	350	470	0.62	420	470	0.63
Newcastle	754	469	0.18	750	469	0.90
Manchester	805	469	0.20	770	469	0.57

5.4.3 GSEA results

We next conducted gene-set enrichment analysis of the 5-cohorts using GSEA. These analyses also showed no significant enrichment for either fluid ability ($p = 0.30$) or for crystallised ability ($p = 0.42$). The gene based statistics conducted using VEGAS indicate that one gene was nominally significant for crystallised ability and three for fluid ability. However, these did not survive correction for the 27 tests performed (see Table 5.2). These results indicate, then, that variation in the genes which code for heterotrimeric G-proteins are no more associated with variation in cognitive abilities than expected by chance.

We next tested for association with age-11 IQ in the two Lothian cohorts where this phenotype was available. These tests were conducted using the self-contained test in JAG. No significant evidence for association was present for either LBC1921 ($n = 464$, n SNP = 468, $p = 0.90$) or for LBC 1936 ($n = 947$, n SNP = 468, $p = 0.70$). Combined using Stouffer's method, these p values for age 11 IQ yield a meta-analytic p-value of $p = 0.88$.

Table 5.2. Gene based analysis results for Crystallised and fluid cognitive ability.

				Gene based P-values	
Gene name	N SNPs	Start position (bp)	Stop position (bp)	crystallised Ability	Fluid Ability
GNA11	69	3045407	3072454	0.280	0.340
GNA12	228	2734266	2850485	0.214	0.521
GNA13	54	60437294	60483216	0.844	0.705
GNA14	311	79228367	79453043	0.950	0.568
GNA15	88	3087190	3114766	0.640	0.561
GNAI1	186	79602075	79686661	0.251	**0.049**
GNAI2	32	50248650	50271790	0.541	0.684
GNAI3	102	109892708	109939975	0.804	0.937
GNAL	258	11679264	11871919	0.178	**0.002**
GNAO1	241	54782751	54948857	0.151	**0.070**
GNAQ	271	79525010	79836012	0.638	0.412
GNAS	127	56848189	56919645	0.717	0.752
*GNAT1**	34	50204046	50208953	0.235	0.588
GNAZ	138	21742668	21797221	**0.038**	0.807
GNB1	52	1706588	1812355	0.815	0.795
*GNB2***	35	100109310	100114728	0.677	0.956
GNB3	72	6819635	6826818	0.286	0.768
GNB4	94	180596569	180652065	0.946	**0.027**
GNB5	137	50200414	50270857	0.973	0.794
GNG10	130	113463681	113472347	0.236	0.907
GNG11	121	93388951	93393762	0.505	0.533
GNG12	239	67939736	68071730	0.611	0.725
GNG2	392	51396799	51506268	0.884	0.308
*GNG3**	38	62231708	62233246	0.922	0.186
GNG4	148	233777607	233880677	0.751	0.755
GNG5	136	84736593	84744850	0.836	0.953
GNG7	143	2462217	2653746	0.440	0.210

Note: * indicates genes without coverage in Ruano et al. (2010) ** Due to the use of imputed data the *GNB2* gene was incorporated into the GSEA analysis, but was not tested the JAG replication (Table 5.1). Nominally significant genes are in bold.

5.5 Discussion

We attempted to replicate an association between variation in genes coding for G-proteins and human intelligence differences (Ruano et al., 2010). Strengths of the present report include increased coverage of the G-protein genes than was available for the original report, and wide range of samples, two assessed both in youth and in old age, and also use of a competitive test of association using a distinct methodology, that of gene-based gene-set enrichment analysis (GSEA). The phenotype is highly similar to that used in the original report, with an identical analysis strategy and the same software. In no cohort was any significant association found, and this remained the case under meta-analysis.

Age effects are unlikely to have led to a discrepancy between the original report and those of the current study, for three reasons: first, there is a high genetic correlation, between childhood and old age measures of *g* (0.62) (Deary et al., 2012); second, we had available crystallised ability measures which are robust to ageing effects; and, third, we were able to directly test for association in participants using their IQ at age 11 in two samples. The null finding at both ages in the current study would indicate, then, that, across the life course, variation in heterotrimeric G-proteins does not contribute more than a slight degree to individual differences in intelligence.

The original replication sample had a genetic background similar to that reported here (UK Caucasian (Ruano et al., 2010). Differences in genetic background between the present samples and those in the original report could alter the direction of association of individual SNPs. This genetic heterogeneity, however, would not affect our power to detect a significant pathway, as pathway analysis derives the sign of association for each SNP freshly in the new samples.

One significant difference between the discovery cohort and the present samples is that the discovery sample consisted of children and adolescents diagnosed with ADHD. However, the ALSPAC validation sample was not drawn from a clinical population (Ruano et al., 2010). Sample differences, then, appear not to be able to account for the null finding in

the present report. The initial discovery sample was very small (N = 627) and although there was replication in the much larger ALSPAC sample, this replication was only just significant (p = 0.047). Given the null result in the current study, the original finding was likely due to failure to control for population stratification in the replication sample. In addition the original report makes use of only self-contained tests, rather than competitive tests. Competitive tests are to be preferred over self-contained tests due to them being less susceptible to the effects of genomic inflation and due to the conservative nature of testing against genes drawn from outside the pathway. Indeed, as shown in Table 1 of Ruano et al. (2010), prior to controlling for stratification a gene-set consisting of 900 genes and 22,325 SNPs detailing all synaptic genes was also statistically significant. Once the corrections had been implemented this group was no longer significant, demonstrating that controlling for stratification can affect the results of a self-contained test. However, no such steps were taken to control for the effects of genomic inflation when replication was sought in an independent sample of 1,507 individuals, and where the final p-value for the heterotrimeric G-proteins is stated as 0.047 it is unlikely to remain significant should any corrections need making.

Whereas SNP variation in heterotrimeric G-proteins appears unrelated to cognitive abilities, the grouping of genes according to their cellular function, rather than in vertical pathways nevertheless has the potential to elucidate genetic mechanisms which act in, and potentially disrupt, multiple systems (Ruano et al., 2010). In addition, while G-protein variation appears unrelated to normal variation in cognitive ability, the postsynaptic density per-se is rich in proteins – excitatory synapses of the human brain express over 1,500 genes and over 130 neurological and psychiatric disorders have been shown to arise from mutations in post-synaptic density genes (Bayes et al., 2010; Collins et al., 2006). Indeed, subsets within this large number of genes, form supramolecular complexes such as the *N*-methyl-D-aspartate receptor complexes (NMDA-RC) (Collins et al., 2006), which is preferentially involved in rapid processing of information and updating of AMPA

responsiveness (Zorumski & Izumi, 2012) and which has been associated with normal variation in human intelligence (Hill et al., 2014a). As knowledge of biological pathways increases, so too does the ability to utilise this information to aggregate the many thousands of small SNP based effects underlying intelligence (Benyamin et al., 2013; Davies et al., 2011; Trzaskowski, Yang, Visscher, & Plomin, 2013b) will increase, allowing testing for associations between psychological traits and candidate mechanisms.

The limitations of this study include heterogeneity of the phenotype both between the cohorts of the CAGES consortium used here as well as between the samples used by Ruano et al. (2010). The high correlations between g factors extracted by different tests (Johnson et al., 2004; Johnson et al., 2008) indicates that this is unlikely to have led to the failure to replicate the association here. A more problematic issue is the use of a single test in LBC1921 and LBC1936 to represent IQ at age 11. Whilst the MHT test does span multiple aspects of ability it has been described as a test of verbal ability in contrast with the WISC-III (Wechsler, 1991) used in the discovery sample of Ruano et al. (2010). The use of multiple disparate tests is to be preferred as this serves to reduce test specific variance and captures a greater proportion of common variance, provided the tests used assess multiple aspects of cognitive ability. However, despite the MHT being a test of verbal ability, it has been shown to correlate with the Stanford Revision of the Binet scale, a test of IQ, at 0.80 (0.81 male, 0.78 female) (Scottish Council for Research in Education, 1933).

Some methods of gene-set analysis, including JAG, can produce positive results if only a few genes or SNPs are strongly associated with the trait of interest which may be the reason for the initial association between the G-proteins and intelligence reported by Ruano et al. (2010). In the present study this was explicitly tested for by including gene-based statistics allowing for the quantification of each genes level of association to each phenotype to be quantified. In Table 2, *GNAL* shows no effect of association once multiple testing has been controlled for. It does however have a p-value of 0.002 associating it with fluid ability in these samples. This may indicate that *GNAL* is a part of a system that shows a true

association with fluid ability but that including it as part of the heterotrimeric G-protein set only serves to dilute this signal and that due to sample fluctuation this drove the initial finding of association between G-proteins and intelligence. However, it is equally probable that the seemingly low p-value for *GNAL* is simply a product of testing multiple genes.

Another limitation lies in the manner in which power was estimated. Whilst the original report indicates an effect size of 3.3% this is likely to be an over-estimation due to the winner's curse. The winner's curse describes the tendency for underpowered samples to yield inflated estimates of effect size when a statistical threshold must be crossed. This indicates that the present study may lack the required power to detect the effect. This is however still a relevant finding as it strongly suggests that if heterotrimeric G-proteins are involved in intelligence then the effect is less than 2.5% according to the power simulations run indicating 0.80 power to detect an effect between 2.5-3%.

Chapter 6: Do SNPs implicated in regulating protein expression show an enriched association with intelligence?

6.1 Introduction

This chapter continues the previous work in examining the aggregate effect of multiple SNPs selected according to their biological role. However, in contrast to the idea of selecting SNPs that fall within genes that are part of a known system, such as the NMDA-RC or genes that express heterotrimeric G proteins, the SNPs examined here were selected as they have been shown to be associated with regulating gene expression (Gibbs et al., 2010; Richardson et al., 2011; Tahira et al., 2005). Several of these gene-sets (the promoter SNP-set and the messenger RNA (mRNA) and methylation SNP-sets) have also been shown to be enriched for association with bipolar disorder and schizophrenia (Bacanu et al., 2013), indicating that they are relevant to GWA studies examining quantitative traits. In addition there is a known genetic overlap between schizophrenia and intelligence (Lencz et al., 2013) indicating that gene-sets relevant to one of these traits may be relevant to the other.

The ten SNP-sets included in this study are known to affect gene expression through a variety of mechanisms including SNPs found in promoter regions, SNPs found in microRNA seed sites, SNPs associated with methylation status, and SNPs associated with mRNA expression. Eight of the SNP-sets were taken from genome wide studies conducted using human cortical tissue where the phenotype being investigated was either methylation status or mRNA expression. Methylation is a mechanism that can alter gene expression and is an example of an epigenetic modification. Epigenetic modifications are defined as changes in the function of a gene which occurs without an alteration of the DNA sequence and can provide a means by which the transcription of a gene can be regulated beyond what would be expected from the DNA sequence alone (Egger, Liang, Aparicio, & Jones, 2004). DNA methylation, cytosine-5 methylation, within CpG dinucleotides, is an important mediator of

gene expression and the link between expression and methylation has been shown to be strongest in regions of the genome that have a high density of CpG sites, referred to as CpG islands. These CpG islands when found proximal to promoter regions show a negative relationship with gene expression, meaning the greater the level of methylation, the lower the level of gene expression. In order to assemble SNP-sets that could alter methylation and gene expression in the human brain, the results of a previously conducted GWAS examining the link between genetic variability, methylation and gene expression was used (Gibbs et al., 2010). The study utilised human cortical tissue taken from 150 individuals and across 4 regions, the frontal lobe, the temporal lobe, the pons, and the cerebellum. These SNP-sets were used in this chapter as the unit of association in an enrichment study carried out using the GWAS on fluid ability in the CAGES consortium. Eight SNP-sets were assembled from the work of Gibbs et al. (2010). Four of these sets were taken from the SNPs showing a significant association between genotype and level of methylation in the four cortical regions, the frontal lobe, the temporal lobe, the cerebellum, and the pons.

The other four SNP-sets taken from the study by Gibbs et al. (2010) consisted of SNPs showing a significant association between genotype and the level of mRNA. mRNA is part of the family of RNA molecules which moves genetic information to the ribosomes from the DNA. It is a crucial part of the central dogma of molecular genetics. The central dogma of molecular genetics describes the flow of genetic information from the protein coding genes in the DNA to the formation of proteins. Genetic information is stored in the DNA by the configuration of the four bases present (adenine, thymine, guanine, and cytosine). DNA molecules are double stranded and adenine will always pair with thymine whereas guanine will always pair with cytosine. In protein coding genes the information pertaining to each protein is stored in a series of three bases referred to as a codon. The first stage in protein formation involves the transcription of DNA to mRNA where the double stranded DNA molecule parts, to become single stranded. This single stranded section of the DNA molecule then pairs with amino acids to form mRNA (but with uracil replacing

thymine which binds with adenine), named messenger RNA due to its function of transporting the genetic information from the DNA molecule. The final stage is that the mRNA is then translated into a sequence of amino acids which form proteins. mRNA levels indicate the rate at which the gene is expressed and so the use of the SNP-sets used here detailing SNPs that are associated with mRNA levels provide a way of examining if this expression change is linked to fluid ability. Methylation has been shown to affect gene expression, which may mean that a variant associated with methylation would also be associated with mRNA levels. This would result in gene sets which overlap considerably and could reduce the power to find an association by increasing the burden of testing multiple hypotheses. However, only 4.8% of all SNPs with either a significant effect on methylation or mRNA were significant for both (Gibbs et al., 2010).

The ninth SNP-set consists of SNPs found in microRNA seed sites. MicroRNAs (miRs) are non-coding RNAs between 20 and 24 nucleotides in length. MiRs bind to their targets, miR recognition elements (MREs), which are found in the three prime untranslated regions (3'UTR) that follow the termination codon. By binding to the MRE's, miR can decrease the transcription of mRNA or degrade the transcript itself (Bartel, 2009). Within the MREs however, are areas that are more important for the binding of miR and as consequence of this, more important for down-regulating mRNA. These are the microRNA recognition element seed sites (MRESS) located in the 2-7 position of the MRE. The importance of the MRESS can be seen as the binding of miR to a single 7mer seed site has been shown to be able to repress translation, and miR repression of translation can be abolished with a single point mutation to areas within the MRESS (Brennecke, Stark, Russell, & Cohen, 2005). As these MRESS have been shown to be important for gene expression, genotypic variation in these regions may have the ability to produce variation in a phenotype by altering how the MRESS performs. In the current chapter SNPs that fell into regions that have been shown to be MRESS were taken as a functional SNP-set. The SNP-set was assembled based on the work of Richardson et al. (2011) who conducted a genome wide survey by combining the

SNP data taken from dbSNP build 132 from the 1000 genomes project with the bioinformatics resource http://microRNA.org detailing predicted MRESS. SNPs were assigned to the microRNA SNP set if they were located in a predicted MRESS. Using these data, 1 SNP-set detailing 26,708 SNPs was assembled for enrichment analysis with fluid ability in the CAGES sample.

The final functional category examined here was SNPs that were located within promoter regions. Promoter regions are found upstream of genes and RNA polymerase binds to these regions which then begins the process of transcription. SNPs in promoter regions may therefore be able to alter the transcription rate of a gene and so may explain some of the inherited variance of human cognitive abilities. Promoters are located near the start of the transcriptional start sites toward the 5' region and are between 100 and 1000 base pairs in length. SNPs in these transcriptional promoter regions have been proposed as candidates for cis-acting gene expression (Pastinen & Hudson, 2004). In order to assemble a SNP-set detailing the SNPs that fell in to promoter regions the database dbQSNP was used (http://qsnp.gen.kyushu-u.ac.jp/). This data base was assembled by utilising sequencing or single-strand confirmation polymorphism. By taking the SNPs that featured in this database a set of 6,411 SNPs was included.

6.2 Summary

The hypothesis examined here was that SNPs which can affect gene function and expression are more likely to be responsible in the creation of phenotypic variation than SNPs which do not appear to be functional. As the phenotype of interest here is intelligence, 8 of these sets were included as they have been shown to be associated in measureable changes, be it methylation or mRNA expression differences, in human cortical tissue. Using two complimentary methods of SNP-set analysis we aim to determine if these functional sets show an enriched association for intelligence using the GWAS data set of the CAGES sample. These SNP-sets and this data processing pipeline have already been used to show

that SNPs in promoter regions are enriched in schizophrenia and bipolar disorder in addition to showing that the SNPs associated with methylation and mRNA expression in multiple cortical regions are also enriched for schizophrenia (Bacanu et al., 2013). Here these methods are applied to the study of intelligence differences by utilising the GWAS on fluid ability phenotype described in chapter 3 to determine if these SNP-sets show evidence for enrichment for fluid ability.

6.3.1 Materials and methods

6.3.2 Phenotype.

Due to the large number of SNP sets examined in this chapter (10) only the fluid ability phenotype was examined in order to maintain statistical power.

6.3.3 Samples

These 10 SNP sets were examined for enrichment of fluid ability. The CAGES sample was used in conjunction with the GWAS carried out by the author.

6.3.4 Functional SNP sets

In total 10 SNP-sets were examined. The functional SNP-sets were taken from previous literature (Gibbs et al., 2010; Richardson et al., 2011) and existing bioinformatics resources http://qsnp.gen.kyushu-u.ac.jp/ (Tahira et al., 2005) and have been examined for enrichment for schizophrenia, bipolar disorder and major depression (Bacanu et al., 2013).

6.3.5 Methylation and mRNA expression QTL SNPs

The methylation SNP sets and the eQTL SNP sets were taken from the work of Gibbs et al. (2010). These sets were derived using human cortical tissue samples taken from the frontal lobe, the temporal lobe, the cerebellum, and the pons of 150 (47 female)

neurologically normal Caucasians who had donated their brains for medical research. This tissue was sourced from the University of Maryland Brain Bank, Baltimore (n = 114) or from the Department of Neuropathology, Johns Hopkins University, Baltimore (n= 36). Of the samples taken from the Department of Neuropathology, Johns Hopkins University, Baltimore, 10 were taken during routine autopsies and the remaining 26 as part of the National Institute on Aging-sponsored Baltimore Longitudinal Study of Aging.

Each of the subjects had four samples consisting of 5 grams of cortical tissue taken from the cerebellum, the frontal cortex, the temporal cortex and the caudal pons. Separate samples were taken for SNP genotyping, RNA extraction and to assess methylation. Each of the four cortical regions (Frontal lobe, temporal lobe, pons, and cerebellum) were analysed separately and by either mRNA expression profile or CpG methylation. This led to 8 genome-wide association analyses being performed. In each case, an additive linear model was carried out at each loci correlating allele dosage with phenotype variation. Corrections were made to account for the large number of tests per trait by using a phenotype permutation method carried out in PLINK. Here, a genome-wide empirical p-value was derived for each SNP using 1,000 permutations of the sample label for each trait using the max(T) function in PLINK. The max(T) function provides a method by which the large number of SNPs tested can be controlled for by comparing the observed test statistic for a SNP with the permuted distribution thus controlling for family-wise error rate. In contrast to the Bonferroni method the max(T) does not assume independent p-values between SNPs as phenotype permutation retains the pattern of LD between SNPs. In addition permutation based methods of this sort are not dependent on the phenotype being normally distributed.

In order to control for the number of traits examined in each cortical region, the fwer2fdr function in R was used to derive the false discovery rate threshold for the empirical p-values derived from the max(T) method. SNPs which exceeded this false discovery rate were however retained if there r2 LD value was >0.7 with a SNP that did not exceed the false discovery rate. The SNPs found to be significant were examined for

association in the CAGES cohort of fluid ability as described in chapter 3.

See Table 6.1 for details on the total number of SNPs tested by (Gibbs et al., 2010) and the total sample size used in their GWAS on methylation status and mRNA expression. See table 6.2 for the number of significant SNPs in each SNP-set.

Table 6.1. Illustrates the full sample sizes, number of probes and SNPs per assay type by tissue source. These are the final number after quality control.

	Methylation CpG				mRNA expression			
	Frontal lobe	Temporal lobe	Cerebellum	Pons	Frontal lobe	Temporal lobe	Cerebellum	Pons
Sample size	133	127	108	125	143	144	143	142
Probe	27532	27538	27310	27476	8984	9372	8984	8722
SNPs	1624830	1607740	1540472	1607740	1653451	1655958	1653451	1650475

Table adapted from Gibbs et al. (2010).

Table 6.2. Illustrating the number of significant quantitative trait loci (QTL) by tissue type for both CpG methylation and mRNA expression. The SNPs that were found to be significant were examined for enrichment in CAGES providing the SNP passed quality control in CAGES. The final number included in the association analysis for fluid cognitive ability is included below.

Tissue type	Methylation QTL	Methylation SNPs found in CAGES GWAS	mRNA expression QTL	mRNA expression SNPs found in CAGES GWAS
Frontal lobe	12135	10596	5515	5177
Temporal Lobe	16734	13658	5335	4039
Pons	11374	9473	3415	3274
Cerebellum	12102	10542	5244	4379

6.3.6 MicroRNA SNP-set

The SNP set representing SNPs that could disrupt or create microRNA seed sites were taken from Richardson et al. (2011) whose work is described below. This study was carried out to determine which SNPs fell in known or predicted microRNA seed sites. In order to address this question all SNPs from dbSNP build 132 were retrieved (retrieval date 31/11/2010) through the1000 genomes home page (1000 Genomes Project Consortium, 2010). Only SNPs that were reported as bi-allelic were retained in order to exclude those that were indels or copy number variants. A subset consisting of all 210,042 3'UTR SNPs was extracted and used in subsequent analyses to search against both known and predicted microRNA seed sites.

The full record of validated microRNA seed sites was downloaded from the miRecords database (Xiao et al., 2009). These were then pruned to include only those that had evidence of a loss of function by manually checking the literature, leaving 606 validated MicroRNA recognition elements. The genomic DNA co-ordinates for the microRNA recognition elements were then retrieved from Ensembl and, using the genomic DNA co-ordinates from dbSNP132, SNPs that fell in those regions were extracted. This led to a total of 31 SNPs being extracted.

In order to identify SNPs in conserved microRNA seed sites a genome-wide survey for SNPs that fell in computationally predicted conserved microRNA recognition elements was performed. Firstly, the data file "good mirsvr_score conserved microRNA" from http://microRNA.org was used to recruit a collection of Mir-mRNA interactions. This database was assembled using an algorithm which incorporates the most recent MiR prediction guidelines including seed-site pairing, site context, free energy as well as target conservation across multiple vertebrate species (Betel, Wilson, Gabow, Marks, & Sander, 2008).The file itself contains all predicted mRNA target motifs belonging to conserved

microRNA families. This was selected as whilst the conservation signal is used to predict which MREs are functional, the conservation signal being greater than the background signal for the most recent non-conserved mammalian specific miRNA families was unlikely to have been caused by the relatively short time between the emergence of the miRs and the occurrence of the new MREs within 3 'UTRs (Friedman, Farh, Burge, & Bartel, 2009). Therefore the use of the "good mirsvr_score conserved microRNA" data file enabled the number of false positives to be reduced as only conserved miR families are contained within it. An additional measure of conservation was also included by implementing a conservation score cut-off of Phastcon score >0.57 for the predicted miR targets. This cut-off is estimated to match the level of conservation across the mammalian genome (Betel et al., 2008; Siepel et al., 2005; Stark, Brennecke, Russell, & Cohen, 2003). The Phastcon scores were taken from the http://microRNA.org database. A mirSVR score of < -0.6 was used to select the top 12% of all predictions. The genomic DNA coordinates of these 197287 predicted MRESS were then compared to the genomic DNA coordinates taken from dbSNP132 and SNPs found in these regions were extracted. Using this method a total of 2723 SNPs interrupting mircoRNA recognition element sites were found.

In order to identify SNPs that fell in regions capable of creating new microRNA seed sites Ensembl variation Perl API tools (Build 61) was used to retrieve the 22 bases flanking the 5' and the 3' regions of each 3'UTR SNP from dnSNP132. Using this information the reverse complement for the mRNA transcribed from the negative strand was generated. The miRanda target prediction algorithm was with these data and a pairing score cut off of >150 and an energy cut off of <-20 was implemented to limit the discovery of false positives. Predicted MRESS that were created by SNPs were identified by filtering hits on the position of the target prediction on the mRNA and every SNP was located at position 23 of 45. This led to set of 22295 SNPs being derived that were predicted to create new microRNA seed sites.

6.3.7 Promoter SNP-set

SNPs in promoter regions were taken from http://qsnp.gen.kyushu-u.ac.jp/ (Tahira et al., 2005). The promoter regions in this database are defined as the regions that are 1.0kb upstream and 0.2 kb downstream of the transcriptional start regions. The transcriptional start regions have been previously defined experimentally as the 5' ends of full length complementary DNA (cDNA) clones. The SNPs within these regions were then found using sequencing or single-strand conformation polymorphism analysis (SSCP) and were taken from the database of transcriptional start sites (http://dbtss.hgc.jp/ (Tsuchihara et al., 2009; Yamashita et al., 2011; Yamashita, Wakaguri, Sugano, Suzuki, & Nakai, 2010). This led to a total of 6411 SNPs being downloaded.

6.3.8 Statistical analysis

The ten gene sets were used to perform two complementary analyses to determine if they contained a greater number of low p-values than would be expected under the null hypothesis. Professor Silviu Bacanu performed the analyses described below at the Virginia Institute for Behavioral and Psychiatric Genetics. The two tests used are modifications of programs designed to yield a gene-based statistic by combining the weight of evidence from across multiple SNPs located within a gene boundary. However, rather than using gene boundaries to determine which SNPs were included, here only SNPs from within the functional SNP-sets were included making each SNP-set the unit of association. Each of the tests used here tests for significance against the background level of association of the GWAS rather than 0. This is an important consideration, as a large GWA study has more low p-values than would be expected under the null hypothesis, meaning randomly selected SNPs will have a significant level of association more than 5% of the time if they are tested against 0. In order to prioritise candidates it is therefore important that the sets tested show a greater level of association than the average level of association found in the same GWAS.

The first test used was a modified Simes test (Li, Gui, Kwan, & Sham, 2011) which

operates as an extension to the Bonferroni method to control for the number of tests performed. Whilst this test does suffer from being overly conservative in situations were highly correlated tests are combined (such as when testing SNPs that are in high LD with each other), this issue is reduced here as SNPs were selected from cross the genome reducing the level of LD between them. The modified Simes test was included as a method to detect a small number of large signals. Whilst it should be noted that genetic variants for intelligence are well thought to be of small effect, each functional category here may only have a small number of SNPs that contribute toward intelligence meaning that the signal would be diluted by the inclusion of SNPs which are not involved in intelligence. The Simes test here would detect these signals from amongst the SNPs not involved. The Simes test performed was a Simes combination test. Here, if p(1),...p(n) are the n ranked p-values in the set, from smallest to largest, then the Simes test of the set is the min(n*p(1),n/2*p(2),n/3*p(3),...,n/(n-1)*p(n-1),p(n)).

The second test used is a VEGAS like (Liu et al., 2010) sum of squares test. This test is most suited to the detection of multiple small effects within each SNP set. However the inclusion of SNPs not involved in intelligence would serve to make these signals undetectable using the sum of squares test. A SNP-set statistic is derived for the sum of squares test by converting the beta weights from the GWAS describing the effect of the SNP on intelligence. Each beta weight is divided by the standard deviation of the beta weights estimated using the linkage disequilibrium information provided by the 1000 genomes project. Following this all the squared Z scores are summed to give the SNP-set statistic. Statistical significance for this statistic was derived through 50,000 permutations. SNPs from across the genome were selected for the permuted sets with LD being controlled using the 1000 genomes project data

6.4 Results

The ten functional SNP-sets were examined using both the Simes test to determine if

there were a small number of relatively large effects in each set, as well as a sum of squares test to find multiple, small effects. The Simes test did not detect any evidence of a small number of large signals coming from any of the ten functional gene sets. The use of the sum of squares test provided weak support for multiple small signals being found in the SNP-set corresponding to the promoter regions $p = 0.058$ but none of the other nine categories showed evidence of enrichment. See Table 6.3.

Table 6.3. Shows the details of each of the 10 functional SNP sets examined for enrichment using both the Simes test and the sum of squares test.

Functional SNP set	nSNPs in set	nSNPs in data	p-value Simes test	p-value sum of squares test
Promoters	6411	2643	0.164	0.058
MicroRNA	26708	2857	0.195	0.773
(eQTL) Temporal Cortex	5335	4039	0.994	0.895
(eQTL) Pons	3415	3274	0.991	0.971
(eQTL) Frontal Cortex	5515	5177	0.890	0.868
(eQTL) Cerebellum	5244	4379	0.885	0.734
(Methylation) Temporal Cortex	16734	13658	0.424	0.531
(Methylation) Pons	11374	9473	1.000	0.924
(Methylation) Frontal Cortex	12135	10596	1.000	0.978
(Methylation) Cerebellum	12102	10542	1.000	0.924

Abbreviations: eQTL, expression quantitative trait loci; SNP, single nucleotide polymorphism.

6.5 Discussion

None of the functional SNP–sets examined here attained a nominal level of significance. The lack of significance for the Simes test indicates that there are no SNPs

showing a large effect, whilst the sum of squares test indicates that none of the sets considered here contain multiple small signals of association above the background level of association. These findings give rise to the question of why SNP-sets with a known effect on gene expression, eight sets with a known effect on genes expressed in human cortical tissue, do not appear to contribute toward intelligence. The reasons for this finding are discussed below.

This lack of statistical significance contrasts with the work performed by Bacanu et al. (2013) using the data sets assembled by the Psychiatric GWAS consortium (PGC) where by using the same SNP-sets and the same data processing pipeline enrichment was found for multiple SNP-sets. One of the reasons for this could be due to the difference in statistical power indicated by the larger sample sizes used in studies of psychiatric conditions. Whilst the PGC data sets contained 9240 cases and 9519 controls for depression (Ripke et al., 2012), 7,481 cases and 9,250 controls for bipolar disorder (Sklar et al., 2011) and 9,394 cases and 12,462 controls for schizophrenia (Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011) in the present study data from 3,507 individuals was used. This indicates that the present study could be underpowered to detect enrichment using this method. Indeed, in the study by Bacanu et al. (2013) the phenotypes with the greatest number of subjects also had the most significant SNP-sets whilst depression, the smallest study had no hits at all supporting the notion that the current study may be underpowered. It should be noted that there has been less success in finding genes associated with depression than for schizophrenia or bipolar disorder and so the lack of enrichment in the study by Bacanu et al. (2013) may simply reflect the idea that these SNP-sets are not associated with depression.

In the current study the promoter region SNP-set was the closest to attaining statistical significance which may indicate that an increase in sample size and the corresponding increase power, enrichment for fluid ability may be detected in this SNP-set. This promoter SNP-set also showed the greatest evidence for enrichment in previous studies

examining schizophrenia and bipolar disorder (Bacanu et al., 2013) in addition, there is a known genetic overlap between schizophrenia and bipolar disorder (Purcell et al., 2009) and between schizophrenia and cognitive ability (Lencz et al., 2013) this indicates that there may be SNPs in common with these three phenotypes and adds to the weight of evidence that the lack of significance found in the promoter SNP-set may be due to a lack of power. It may also be the case however the failure to attain statistical significance is due to the SNPs in the promoter region playing no role in cognitive abilities.

Another possibility is that the SNP-sets tested here do not accurately capture what could be considered functional. Both the mRNA expression and the methylation SNP-sets were assembled using the results of a GWAS using mRNA expression and methylation as the phenotype being investigated. Whilst these analyses were carried out using stringent statistical methodology of phenotype permutation to establish statistical significance the sample size (n= 150) was modest by current standards. This raises the possibility that these SNP-sets contain false positives and omit true positives, meaning that should a signal be contained within these sets it will be too small to detect. The results attained by the Bacanu et al. (2013) indicate these SNP-sets do contain signal for psychiatric conditions and across schizophrenia, bipolar disorder, and depression and there is a trend for an increase in enrichment signal as sample size increases indicating the validity of the mRNA expression and methylation SNP-sets. However, whilst these sets may contain true positives for psychiatric conditions this may not be the case for intelligence as the variants missed by the initial GWAS conducted to assemble these sets (Gibbs et al., 2010) may have included some of the more crucial variants. It is also possible that the results of Bacanu et al. (2013) represent type 1 errors as the results have not been replicated in either schizophrenia or bipolar disorder.

A different method to examine the relevance of the methylation and mRNA expression SNP-sets would be to utilise a polygenic score test using methylation and mRNA expression phenotypes. Originally performed to show the level of genetic overlap between

schizophrenia and bipolar disorder (Purcell et al., 2009) the beta-weights of significant SNPs from one GWAS are used to predict a separate trait in another GWAS. The polygenic score test can be used here to derive a methylation and mRNA polygene score and by implementing different p-value cut offs it may be possible to include false negatives from these GWAS on methylation and mRNA expression. This would provide an additional method to test the importance of these sets with regards to intelligence differences.

The microRNA SNP-set was not found to be enriched for fluid ability here or for schizophrenia, depression or bipolar disorder (Bacanu et al., 2013). This set was also the largest SNP-set containing 26708 SNPs of which only 2857 were present in the CAGES GWAS. This discrepancy between the full set and the set that could be tested may mean important variants had to be omitted from the analysis as they failed quality control or were rare variants appearing less than 5% of the time. This lack of coverage was also present in the study by Bacanu et al. (2013) where no enrichment was evident after correcting for multiple comparisons for the microRNA SNP set for schizophrenia $p = 0.0893$, bipolar disorder $p = 0.0329$, or depression $p = 0.1968$. Additionally, the full set of microRNA SNPs was examined by (Richardson et al., 2011) to determine if the SNPs in this set were in LD with the SNPs found in GWA studies of disease. A data set consisting of 4817 GWAS associations were collected which included 3943 unique SNPs ($p < 1 \times 10^{-5}$). It was found that 87 of these SNPs were in LD ($r^2 > 0.8$, CEU population) whilst this overlap was found to be statistically significant it does however indicate that only a small number of the microRNA SNPs appear to be relevant to GWA studies.

Whilst the results of this study do not support the conclusion that loci where SNP genotype co-varies with gene expression are enriched for intelligence other bioinformatics resources are available for use to test this hypothesis. The Allen Brain Atlas (Hawrylycz et al., 2012) (http://www.brain-map.org/) is one such resource and provides an atlas of gene expression both across the cortex and of subcortical structures. The Allen Brain Atlas has been combined with GWAS data before where it was used to follow up the results of a

GWAS examining the surface area of the visual cortex (Bakken et al., 2012). The most significant SNP associated with the surface area of the visual cortex was located in *GPCPD1* using the Allen Brain Atlas it was possible to show that this gene was expressed to a greater extent in the visual cortical regions than elsewhere in the human brain.

However it may be possible to adopt the opposite strategy by beginning with genes which are preferentially expressed in cortical regions associated with intelligence, such as the areas indicated in the Parieto-Frontal Integration Theory (PFIT) of intelligence which includes the dorsolateral prefrontal cortex, inferior and superior parietal lobe (Jung & Haier, 2007). These sets could then be examined for enrichment in a GWAS conducted on human intelligence. By combining human brain maps of gene expression with imaging data on human intelligence it would be possible to prioritise genes based on their level of expression in the most trait-relevant areas of the brain.

6.9 Summary

None of the 10 SNP-sets examined here attained nominal statistical significance for enrichment in the CAGES GWAS for fluid ability. This result may be due to a lack of power or a failure to test SNP-sets relevant to the biology of intelligence as discussed above. Future studies should utilise larger sample sizes and draw upon additional bioinformatics resources describing the function of genes and their distribution across the cortex.

Chapter 7: Are genes associated with intellectual disability enriched for quantitative trait loci involved in intelligence differences?

7.1 Introduction

This chapter builds on the idea that it is possible to capture a greater amount of variance in a GWAS of intelligence by summing the effect of multiple SNPs with the aim of assisting in the elucidation of the genetic underpinnings of intelligence differences. The gene-set analysed in this chapter is composed of genes previously associated with intellectual disabilities and examines the notion that genes which are involved in the extreme range of intelligence differences are the same as those that underlie variation across the distribution of intelligence.

The notion that the underlying genetic architecture of intelligence can give rise to both the normal variation of intelligence differences and to the extremes of this distribution reflects a high degree of pleiotropy between the genetic aetiology of average ability and of low ability. This pleiotropy, referred to as, generalist genes, has been shown to be a ubiquitous finding in quantitative genetic studies (Plomin & Kovas, 2005), and contrasts with the notion of single gene disorders which produce phenotypes that are not the tail end extremes of the normal distribution but rather a deviation from normality.

Intelligence is both a highly heritable and polygenic trait, which along with the known pleiotropy between disability and ability in the normal range indicates that common variants underlying one may also be involved in the other. The present study uses a competitive test to examine SNP variation in sets of genes, along with an established method of text mining to examine the biological relationship between genes, in order to assess whether the genes responsible for non-syndromic autosomal recessive intellectual disabilities (NS-ARID) are enriched for QTLs associated with variation in the normal range of intelligence.

Intellectual disabilities (ID) are defined as a significant impairment in cognitive abilities. For a diagnosis of ID to be made the symptoms should be present before the age of 18 years and the individual, will have an IQ of less than 70, constituting a score of two standard deviations below the population mean (mean = 100, SD = 15)(American Psychiatric Association, 2000). Whilst a clear distinction is not always possible, ID can be further divided into syndromic ID and non-syndromic ID. Whereas in syndromic ID, the cognitive deficits are accompanied by a medical problem such as Phenylketonuria or fetal alcohol exposure, which could potentially cause ID, non-syndromic ID is characterised by the lack of any such pathology.

Parental consanguinity has been shown to place an individual at an elevated risk for ID (Bittles, 2001). As the offspring produced from consanguineous pairings will have a greater proportion of homozygous DNA, relative to the offspring produced from outbred individuals, there is an elevated risk of inheriting rare recessive disease causing variants. The effects of inbreeding are not, however, limited to the large shifts in IQ as seen in ID, but appear to also encompass variation in the normal range (Bashi, 1977). The finding that consanguineous pairings increase the risk of ID and appear to lower the normal range of IQ indicates that recessive variants in the population can alter intelligence. This makes genes containing known non-syndromic autosomal recessive intellectual disability (NS-ARID) associated variants, possible candidates for understanding normal variation in intelligence as they represent genes which, when mutations arise, can produce variation in cognitive ability without the presence of neurological disease. Forty such genes have been implicated in NS-ARID and these genes taken from Musante and Ropers (2014) formed the unit of association. Eight of these genes, *PRSS12, CRBN, CC2D1A, GRIK2, TUSC3, TRAPPC9, ZC3H14, MED23* were all found by examining consanguineous families suffering with NS-ARID (Basel-Vanagaite et al., 2006; Garshasbi et al., 2008; Hashimoto et al., 2011; Higgins, Pucilowska, Lombardi, & Rooney, 2004; Mir et al., 2009; Molinari et al., 2002; Motazacker et al., 2007; Pak et al., 2011). With the remaining 32, *ADK, ADRA2B, ASCC3, ASCL1,*

C11orf46, TTI2, RABL6, CASP2, CCNA2, COQ5, EEF1B2, ELP2, ENTPD1, FASN, HIST3H3, INPP4A, MAN1B1, NDST1, PECR, PRMT10, PRRT2, RALGDS, RGS7, SCAPER, TRMT1, UBR7, ZCCHC8, ZNF526, CRADD,KIAA1033, ST3GAL3, and *ZNF526* being identified using Next-generation whole exome sequencing and this group of 40 genes were used as the unit of association (Çalışkan et al., 2011; Hu et al., 2011; Najmabadi et al., 2011; Ropers et al., 2011).

We examined whether common genetic variation in genes associated with NS-ARID were enriched for quantitative trait loci associated with intelligence. These 40 NS-ARID genes were also considered as a gene set to determine if, in a GWA study of intelligence, the most significant SNPs were preferentially located in the NS-ARID gene set. It should, however, be noted that genes do not act in isolation, but rather operate in concert as a part of a network or a pathway (Schadt, 2009). By incorporating knowledge of which genes work together into our study we may better placed to elucidate the mechanisms involved in complex traits (Lee et al., 2012b) such as intelligence. Indeed, this is part of the rationale behind gene-set analysis that to examine genetic variation across groups of genes with a shared function can be more informative than testing single variants.

The large effect sizes of 2 standard deviations or 30 IQ points associated with the NS-ARID gene set (Musante & Ropers, 2014) may indicate that it is composed of genes in which functional variation is constrained due to the crucial role they play. This would mean that, although mutations can produce a large deleterious effect on intelligence, they might not be involved in variation in the normal range. However, the mutations in the genes responsible for NS-ARID can also be viewed as causing variation in the function of the biological systems they are a part of. Whilst mutations in the genes of the NS-ARID set lead to a large effect in the biological mechanisms they are in, common genetic variation throughout the rest of the system could result in more minor perturbations, which may underlie smaller shifts in cognitive ability. To quantify the biological relationships between the 40 genes in the NS-ARID set, a statistical text-mining analysis was used, Gene

Relationships Across Implicated Loci (GRAIL) (Raychaudhuri et al., 2009). This information was used to mine Gene Ontology (GO) (Ashburner et al., 2000) to extract gene sets indicated by relationships between the genes of the NS-ARID set. By prioritizing gene-sets linked to the shared function of the 40 NS-ARID gene-sets statistical power can be kept high as only sets presumed relevant to intelligence would be tested.

There were four goals to this series of analyses. Firstly, the common SNPs in the genes of the NS-ARID set were analysed to determine if there was an association with the normal range of intelligence in a GWAS data set. Secondly, the effects of multiple SNPs were combined into a gene-based statistic to explore the possibility of individual members of the NS-ARID gene set showing an association with fluid and crystallised ability. The third aim was to determine if in our GWAS of intelligence the most significant SNPs are preferentially found in the NS-ARID gene set. The fourth aim was to examine additional gene sets created using the relationships between the genes of the NS-ARID gene set and to test these additional sets for an enriched association with intelligence.

7.2.1 Materials and Methods

7.2.2 Phenotypes

The gene set under investigation were selected as mutations in these genes have been shown to produce large IQ deficits in childhood. Here the role of these genes in middle and old age was explored and fluid and crystallised ability were examined to account for the differential effects of ageing on cognitive abilities (Tucker-Drob & Salthouse, 2008)

7.2.2 Samples

The 40 genes of the NS-ARID gene-set were examined for enrichment of fluid and crystallised ability using the phenotypes from the CAGES sample described in sections 3.3. Replication for fluid ability was sought using the performance IQ phenotype measured in the BATS sample also described in sections 3.3

7.2.3 NS-ARID Gene set

The 40 genes (found in Table 7.1) that were examined for an enriched association with cognitive abilities have each been previously linked with NS-ARID (Musante & Ropers, 2014) indicating that mutations in these genes have a large and deleterious effect on cognitive abilities where a loss of 30 IQ points is required for a diagnosis of ID to be made. Whilst a sharp distinction between syndromic and non-syndromic ID is not always possible, the reduction in cognitive ability associated with the mutations in these genes is not merely the result of these mutations playing a causal role in other neurological disorders. Eight of these genes were identified from microsatellite based homozygosity mapping of large consanguineous families. These were followed up with mutation screening to identify the most likely gene responsible (Musante & Ropers, 2014). The remaining 32 were identified using Next-Generation Sequencing (NGS) methods including Whole Exome Sequencing (WES) and the enrichment and sequencing of exons from homozygous linkage intervals in consanguineous families (Musante & Ropers, 2014).

Table 7.1. Shows the 40 members of the NS-ARID gene-set and any disorder they have been associated with as well as the method used to discover the association.

Chr	Gene ID	Disorder
1	*HIST3H3*	NS-ARID
1	*RGS7*	NS-ARID, ASD
1	*ST3GAL3*	NS-ARID, MRT
2	*ADRA2B*	NS-ARID
2	*EEF1B2*	NS-ARID
2	*INPP4A*	NS-ARID
2	*PECR*	NS-ARID, ASD
3	*CRBN*	NS-ARID, MRT
4	*CCNA2*	NS-ARID
4	*PRMT10*	NS-ARID
4	*PRSS12*	NS-ARID, MRT
5	*NDST1*	NS-ARID
6	*ASCC3*	NS-ARID
6	*GRIK2*	NS-ARID, MRT
6	*MED23*	NS-ARID
7	*CASP2*	NS-ARID
8	*TTI2*	NS-ARID
8	*TRAPPC9*	NS-ARID, MRT
8	*TUSC3*	NS-ARID, MRT
9	*RABL6*	NS-ARID
9	*MAN1B1*	NS-ARID, MRT
9	*RALGDS*	NS-ARID
10	*ADK*	NS-ARID , ASD
10	*ENTPD1*	NS-ARID
11	*C11orf46*	NS-ARID
12	*ASCL1*	NS-ARID
12	*COQ5*	NS-ARID
12	*CRADD*	NS-ARID, MRT
12	*KIAA1033*	NS-ARID
12	*ZCCHC8*	NS-ARID
14	*UBR7*	NS-ARID, ASD
14	*ZC3H14*	NS-ARID
15	*SCAPER*	NS-ARID
16	*PRRT2*	NS-ARID
17	*FASN*	NS-ARID
18	*ELP2*	NS-ARID
19	*CC2D1A*	NS-ARID
19	*TECR*	NS-ARID
19	*TRMT1*	NS-ARID
19	*ZNF526*	NS-ARID

Abbreviation: NS-ARID, Non-syndromic autosomal recessive intellectual disability. ASD, Autism Spectrum disorder. MRT, Mental retardation. Note mental retardation is defined in the same way as NS-ARID (IQ <70) but is referred to here as mental retardation in line with the terminology used when first discovered.

7.2.4 Statistical analysis

Using data from the GWAS on fluid and crystallised ability described in chapter 3, single marker analysis was conducted examining the 5438 SNPs that were found within NS-ARID genes and within ±50kb of the known gene boundaries. To assess significance an alpha level of 9.194557e-06 (i.e., 0.05/5438) was used.

Secondly, a gene-based statistic was derived by combining the effect of each SNP within a gene and the 50kb boundary. Combining the effect of multiple SNPs has the potential to capture a greater proportion of variance which will lead to an increase in power (Hill et al., 2014b; Liu et al., 2010). Gene-based statistics were calculated using VEGAS (Liu et al., 2010) where a test statistic is calculated from the sum of test statistics within a gene region with Linkage disequilibrium (LD) being taken into account using the HapMap phase II CEU (NCBI build 36 release 22) reference panel for each gene and the 50kb boundary. The statistical significance of this statistic is calculated using simulations (see chapter 4 for full details of this method). With 40 genes in the NS-ARID set the alpha level was 0.00125 (0.05/40).

Thirdly, in order to examine the NS-ARID gene-set as the unit of association the gene-set analysis method INRICH (Lee, O'Dushlaine, Thomas, & Purcell, 2012a) was used. INRICH is an example of an over-representation test which are characterised by the use of a predetermined p-value threshold to extract the significant SNPs or genes from a GWAS. These significant hits are then compared to an a priori gene-set to determine if the most significant hits from a GWAS consistently fall within the regions described by the gene-set. The hypothesis tested by overrepresentation tests is if the SNPs that survive the p-value cut off are predominantly found in the genes of the a priori gene-set.

INRICH is used to examine independent genomic intervals generated using the genome wide data set selected according to SNPs where the p-value is below a

predetermined threshold. The use of independent intervals controls for the effects of overlapping genes as well as for linkage disequilibrium. The small number of genes in the NS-ARID set enabled the use of INRICH, as large gene-sets, defined by INRICH as being those consisting of over 200 genes, such as the postsynaptic density gene-sets examined in chapter 4 are unsuitable to test using INRICH as they expected to yield inaccurate p-values (Lee et al., 2012a) . The regions around these index SNPs are extended to include additional SNPs which pass a second p-value threshold and are in LD with the index SNP. The result of this is a number of genomic intervals which show evidence of association which is independent of the association found in the other intervals. This contrasts with gene-based statistics as LD can extend across multiple genes and the same SNPs can be mapped to multiple genes leading to correlations between genes if gene-based statistics are used. Additionally, overlapping genes in each of the candidate gene-sets are merged in order to avoid multi-counting physically clustered genes belonging to the same gene-set. The number of times these LD independent intervals overlap with the merged genes in the candidate gene-set is counted. This count or number of times the gene-set overlaps with the LD independent intervals is used as the gene-set statistic. In order to assess the statistical significance of the overlap between the intervals and the gene-set, interval based permutation is used. The intervals derived using the p-value and LD cut offs are matched for the number of SNPs, SNP density, and the number of genes that they overlap with in each permutation. These matched intervals are created across the genome and the number of times these intervals overlap with the gene-set is counted (Figure 7.1 illustrates the matching criteria). An empirical p-value for the gene-set is defined as the proportion of permuted statistics that are equal to or exceed the observed gene-set statistic.

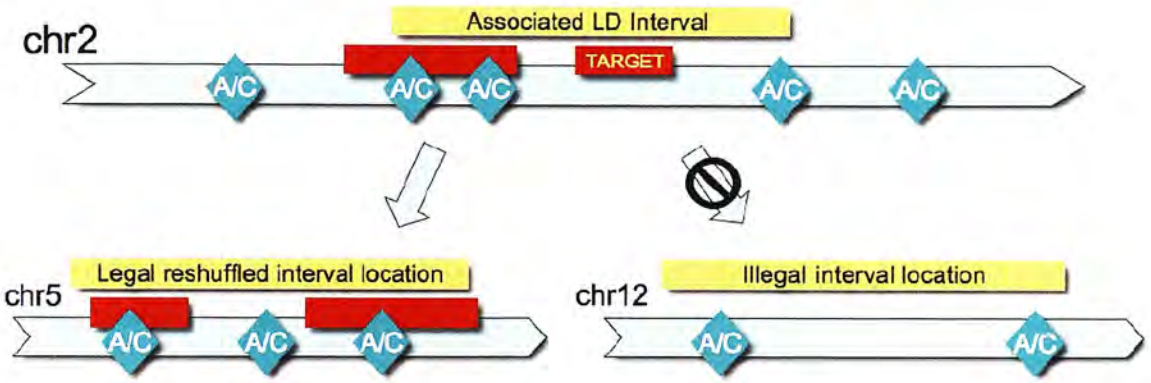

Figure 7.1. Illustrating how the intervals are matched using INRICH. Red indicates genes and yellow indicates the LD independent intervals whilst each blue diamond indicates a SNP. On the top illustrating the observed intervals on chromosome two, the LD interval overlaps three SNPs and with two genes, one of which is part of the gene-set. On the bottom on chromosome five, is an example of an interval that fulfils the genomic criteria for being included as part of the permuted intervals. The interval on chromosome five overlaps with three SNPs and two genes as per the observed interval on chromosome 2. The interval assembled on chromosome 12 does not fulfil the matching criteria as only two SNPs and zero genes overlap with the interval. This interval would therefore not be used as part of the null hypothesis. Figure adapted from (Lee et al., 2012a).

The hypothesis tested by INRICH would be that the most significant SNPs found in the CAGES GWAS aggregate in genes known to be involved in non-syndromic autosomal recessive intellectual abilities. Here, significant genomic intervals were identified using the clump function in PLINK (Purcell et al., 2007). Clump was used to form intervals by selecting index SNPs with a P-value of less than 0.0005, the region around each index SNP

was then extended across a 250kb range and included other SNPs if they were both nominally associated ($p<0.05$) with the cognitive phenotype and in moderate LD ($r^2 > 0.5$) with the index SNP according to the HapMap2 CEU reference panel. A total of 407 index SNPs were used for the fluid phenotype and 403 were found for crystallised ability. Genomic intervals were excluded from subsequent analysis if they did not overlap within 20kb (5' or 3') of any known gene according to the UCSC human genome browser hg 18 assembly. The statistical significance of any overlap between the genes of the NS-ARID gene set and the LD independent regions identified here was determined by creating permuted LD independent intervals matched with the intervals produced using the association p-value for the number of genes, SNP density and LD. 10,000 permutations were used to establish statistical significance.

The fourth analysis carried out here aimed to quantify the biological relationship between the genes of the NS-ARID set and to use this knowledge to test the systems and pathways that reflect these processes for an association with intelligence. Here, Gene Relationships Across implicated Loci (GRAIL) (Raychaudhuri et al., 2009) was used to examine the 40 genes of the NS-ARID gene-set and identify common cellular process or pathways. This was carried out using a text mining algorithm to derive a set of statistically significant keywords describing relationship between the 40 NS-ARID genes. Using the a priori gene-set GRAIL can be used to identify a subset of genes that are more related than chance as well as assign statistically significant keywords suggesting a pathway or system that unites the members of the gene-set. Importantly this metric is derived without the use of the phenotype, meaning that potentially biased ideas about which pathways or biological functions influence the phenotype cannot dominate the analysis. Additionally, undocumented or distant relationships between the members of the gene-set can be derived. Each of these abstracts was converted into a vector of word counts and for each gene a vector consisting of averaged word counts is derived. The relationship between any pair of genes is derived as the correlation between the two vectors of averaged word counts. This means that if two

genes are described using the same words they will receive a high similarity score however, they do not need to be mentioned in the same abstract in order to be classed as similar. After the relationship between the members of the gene-set has been examined keywords are derived. These keywords are defined as those that have the greatest weight across all of the text vectors for the genes of the gene-set. Keywords are restricted to those that appear in >500 abstracts and contain >3 letters and no numbers.

These keywords were derived using a database of 259,638 abstracts taken from PubMed before December 2006. This date was selected as it is prior to the mainstream application of GWAS, as abstracts detailing the regions identified by GWAS would be expected to confound the analysis by describing the NS-ARID gene set as being associated with NS-ARID. The GRAIL parameters applied were as follows release 22 / HG 18; HapMap population: CEU; Functional Data source PubMed Text (December 2006); Gene size Correction on; Gene lists; All human genes within the database.

Following the generation of the keywords, Gene Ontology (GO) (Ashburner et al., 2000) was mined. Here the keywords derived by GRAIL to suggest pathways or systems common to the NS-ARID gene-set were used as search terms in GO. All gene-sets with at least five human genes were extracted and examined using INRICH to determine if these showed significant overlap with the intervals generated from the GWAS data. As multiple gene-sets are being tested in this section of the study the p-value generated for each gene-set will need to be corrected for the number of tests made. As the gene-sets are not independent corrections such as the Bonferroni or false discovery rate will yield an overly conservative estimate of significance (Holmans et al., 2009) and so bootstrap approach was used. Firstly, one of the 10,000 permuted interval sets was selected at random to serve as the observed interval set. Secondly, the statistical significance for the interval set serving as the observed data was derived as before by generating intervals across the genome and comparing the overlap with the gene-sets. Finally, the proportion of bootstrapped samples where the minimum gene p-value over all the gene-sets is at least as significant as the p-value for the

gene-set being corrected for forms the corrected p-value. The hypothesis tested here was that the genes responsible for NS-ARID are found in pathways where common SNP variation can explain variation in intelligence.

7.3.1 Results

7.3.2 Single marker analysis

Single marker analysis indicated that no single SNP in either the fluid ability or crystallised cognitive phenotypes was statistically significant using the alpha threshold of 9.194557e-06. See Table 7.2 and Figure 7.2.

7.3.3 Gene-based analysis

In order to examine the contribution each gene in the NS-ARID gene-set made to both fluid and crystal ability VEGAS (Liu et al., 2010)) was used. No single gene-based statistic was significant at the adjusted alpha level of 0.00125 (see Table 7.3). With three nominally significant genes for *gf* and three in crystallised ability, these results are consistent with what would be expected under the null hypothesis.

7.3.4 Gene-set analysis

In order to conduct a gene-set analysis of the NS-ARID set using INRICH (Lee et al., 2012a), a series of LD independent genomic intervals were created. Using the clump function for the fluid phenotype, 407 genomic intervals were created, of which 248 overlapped within 20kb of a known gene. Overlapping intervals were then merged leaving 176 LD independent intervals to be analysed for enrichment. For the crystallised ability, 403 intervals were produced with 221 overlapping known genes and the 20kb boundary. Once overlapping intervals had been merged for the crystallised ability phenotype, 166 non-overlapping intervals were created and tested for an enriched association with the NS-ARID gene set. 10,000 permutations were used to assess statistical significance.

For *gf,* one member of the NS-ARID gene set, *TTI2*, overlapped with one of the LD independent genomic intervals on chromosome 8 spreading from 33398369 to 33503864. This overlap was not significant, p = 0.520. For crystallised ability none of the most significant regions in the GWAS overlapped with the NS-ARID gene set, p=1.

7.3.5 Gene-set analysis of gene-sets functionally related to the NS-ARID set

In order to assemble a list of gene sets that were most likely to be involved in variation in intelligence GRAIL was used to derive 18 keywords describing the relationship between the 40 NS-ARID genes, see Table 7.4. These keywords were then used as search terms to mine Gene Ontology, producing 180 gene sets which were then tested for an enriched association with *gf* and crystallised ability. Table 7.5 shows the most significant pathways for fluid ability. The overlap between the most significant LD regions in the GWAS and GO:0006814, sodium ion transporters, was statistically significant after controlling for multiple tests. Table 7.6 shows the results for crystallised ability. Whilst the overlap with the 180 gene sets produced using GRAIL and Gene Ontology did not survive multiple correction, GO:0006354 is nominally significant in both *gf* and crystallised ability and this is partly due to the same genes, *POLR2B* on chromosome 5 and *POLR2E* on chromosome 19 being tagged by the LD independent intervals for both the fluid and the crystallised phenotypes.

Table 7.2. The top 20 SNPs for both fluid ability (*gf*) and crystallised ability are shown below.

	Fluid ability				Crystallised ability				
Gene	SNP	Beta	Std Beta	P-value	Gene	SNP	Beta	Std Beta	P-value
TUSC3	rs2604364	-0.0671	0.0216	0.0019	*GRIK2*	rs2579931	0.1169	0.0378	0.0020
GRIK2	rs1465169	0.0645	0.0210	0.0021	*ST3GAL3*	rs2906457	-0.0804	0.0264	0.0023
GRIK2	rs2579924	-0.0645	0.0210	0.0021	*ELP2*	rs17739785	-0.1708	0.0563	0.0024
CRADD	rs3858606	0.0651	0.0220	0.0030	*ST3GAL3*	rs6665014	-0.085	0.0283	0.0027
PRSS12	rs10016452	0.1030	0.0351	0.0033	*GRIK2*	rs2852515	-0.1141	0.038	0.0027
RGS7	rs7545872	0.7330	0.2532	0.0038	*GRIK2*	rs1856307	-0.1149	0.0384	0.0028
TTI2	rs7833337	-0.2105	0.0727	0.0038	*TRAPPC9*	rs6999767	0.0736	0.0249	0.0032
RGS7	rs12064291	0.7323	0.2532	0.0038	*ST3GAL3*	rs803675	0.0837	0.0284	0.0032
PRSS12	rs6850687	0.0662	0.0229	0.0038	*ST3GAL3*	rs803679	0.0837	0.0284	0.0032
GRIK2	rs2787554	-0.0619	0.0214	0.0039	*ST3GAL3*	rs2527776	0.0777	0.0265	0.0034
GRIK2	rs6914311	0.0621	0.0216	0.0040	*ST3GAL3*	rs4660261	0.0751	0.0259	0.0037
GRIK2	rs9404105	0.0620	0.0216	0.0040	*ST3GAL3*	rs2906458	0.0778	0.027	0.0039
RGS7	rs7548485	0.7296	0.2538	0.0040	*ST3GAL3*	rs6429638	-0.0742	0.0257	0.0040
RGS7	rs7548577	0.7296	0.2538	0.0040	*ST3GAL3*	rs4660260	-0.073	0.0254	0.0040
RGS7	rs7550902	0.7232	0.2536	0.0044	*CRADD*	rs12825691	0.0735	0.0256	0.0041
RGS7	rs7556401	-0.7232	0.2536	0.0044	*CRADD*	rs11107212	0.1892	0.0659	0.0041
RGS7	rs7548582	0.7225	0.2538	0.0044	*CRADD*	rs11107211	0.1892	0.0659	0.0041
ELP2	rs17739652	-0.0778	0.0275	0.0047	*ST3GAL3*	rs2485996	0.077	0.027	0.0043
GRIK2	rs1465168	-0.0600	0.0214	0.0049	*ST3GAL3*	rs3791041	-0.0733	0.0257	0.0044
TRAPPC9	rs4736144	0.0723	0.0258	0.0051	*ST3GAL3*	rs304303	-0.0725	0.0255	0.0044

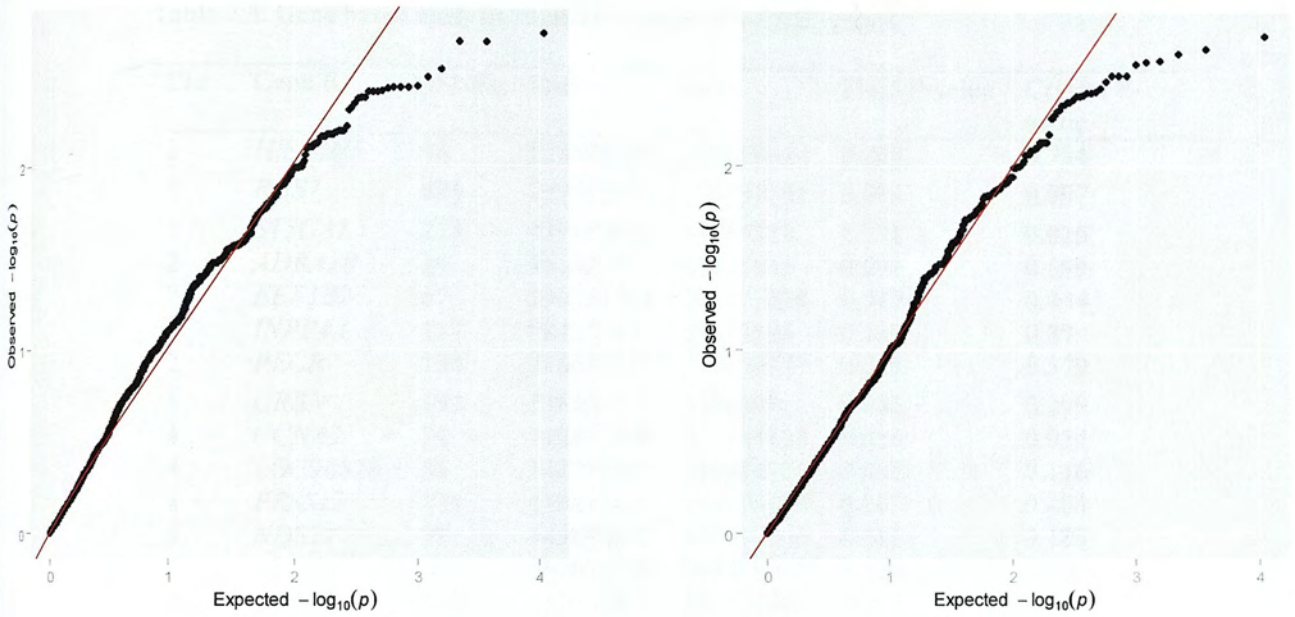

Figure 7.2. These qq plots show the full complement of 5,438 SNPs in both fluid ability (left) and crystallised ability (right). These plots indicate that, for both phenotypes, there is not an increase in the amount of low p-values in the NS-ARID gene set.

Table 7.3. Gene based analysis results for the 40 NS-ARID genes.

Chr	Gene ID	nSNPs	Start	Stop	Fluid P-value	Crystal P-value
1	*HIST3H3*	48	226679168	226679649	0.203	0.734
1	*RGS7*	804	239005439	239587101	0.983	0.997
1	*ST3GAL3*	233	43945804	44169418	0.378	**0.025**
2	*ADRA2B*	29	96142349	96145615	0.091	0.599
2	*EEF1B2*	67	206732562	206735898	0.747	0.444
2	*INPP4A*	117	98427844	98570598	0.138	0.89
2	*PECR*	130	216611355	216654777	0.221	0.379
3	*CRBN*	132	3166695	3196390	0.883	0.299
4	*CCNA2*	74	122957048	122964538	**0.016**	0.054
4	*LOC90826*	55	148778982	148824730	0.482	0.116
4	*PRSS12*	134	119421864	119493370	0.06	0.404
5	*NDST1*	99	149880622	149917966	0.661	0.185
6	*ASCC3*	404	101063328	101435945	0.128	0.575
6	*GRIK2*	868	101953625	102624651	0.435	0.775
6	*MED23*	104	131936798	131991056	0.982	0.598
7	*CASP2*	69	142695523	142714907	0.885	0.502
8	*C8orf41*	109	33475777	33490245	**0.014**	0.313
8	*NIBP*	738	140811769	141537860	0.732	0.851
8	*TUSC3*	490	15442100	15666366	0.105	0.441
9	*C9orf86*	57	138822201	138855460	0.979	0.822
9	*MAN1B1*	41	139101199	139123460	0.947	0.809
9	*RALGDS*	104	134962927	135014409	0.893	0.995
10	*ADK*	376	75580970	76139066	0.983	0.312
10	*ENTPD1*	220	97461525	97627013	0.642	0.455
11	*C11orf46*	110	30301224	30315741	0.680	0.663
12	*ASCL1*	11	101875581	101878424	0.950	0.05
12	*COQ5*	66	119425464	119451347	0.553	0.397
12	*CRADD*	239	92595281	92768662	0.485	0.239
12	*KIAA1033*	159	104025621	104087036	0.567	0.265
12	*ZCCHC8*	41	121523387	121551471	0.549	0.204
14	*UBR7*	91	92743153	92765314	0.751	0.886
14	*ZC3H14*	71	88099066	88149606	0.904	0.292
15	*SCAPER*	298	74427591	74963247	0.298	0.051
16	*PRRT2*	17	29730909	29734703	0.393	0.471
17	*FASN*	52	77629502	77649395	0.514	0.276
18	*ELP2*	144	31963884	32008605	**0.044**	0.123
19	*CC2D1A*	35	13878051	13902692	0.657	0.722
19	*GPSN2*	68	14501381	14537792	0.074	0.223
19	*TRMT1*	32	13076714	13088332	0.633	0.811
19	*ZNF526*	16	47416331	47424193	0.509	0.595

Three genes were nominally associated with fluid ability and one was nominally associated with crystalised ability. Start and end positions do not include the ±50 kb boundary. Bold indicates nominally significant ($p < 0.05$) genes.

Table 7.4. Shows the statistically significant keywords describing the shared biological functions of the NS-ARID genes. This set was ascertained through an automatic literature search implemented in GRAIL. Note two keywords were removed as they yielded 0 gene-sets when used as search terms in Gene Ontology.

Keywords	Gene symbols
Synthase (2 genes)	*ST3GAL3, ELP2*
Reductase (3 genes)	*FASN, ENTPD1, ADK*
Mitochondrial (7 genes)	*CASP2, ECR, CRADD, RABL6, TECR, ADK, FASN*
Apoptosis (7 genes)	*RABL6, ELP2, FASN, ENTPD1, HIST3H3, MED23, ADK*
Methyltransferase (1 gene)	*HIST3H3*
Elegans (11 genes)	*CASP2, TRMT1, TUSC3, SCAPER, RABL6, ASCC3, TRAPPC9, ADK, PRSS12, CRADD, EEF1B2*
Complex (12 genes)	*MED23, EEF1B2, CRADD, HIST3H3, CASP2, SCAPER, TRAPPC9, TECR, ASCC3, MAN1B1, RALGDS, TTI2*
Death (4 genes)	*TRAPPC9, FASN, ENTPD1, NDST1*
Genome (10 genes)	*TRAPPC9, ELP2, TECR, RABL6, TRMT1, COQ5, EEF1B2, PRSS12, TUSC3, MED23*
Histone (2 genes)	*CC2D1A, MED23*
Enzyme (12 genes)	*ADK, TUSC3, ST3GAL3, PECR, PRSS12, MAN1B1, FASN, ENTPD1, CASP2, TECR, SCAPER, HIST3H3*
Trna (3 genes)	*ELP2, TUSC3, TECR*
Adenosine (1 gene)	*NDST1*
Elongation (5 genes)	*ELP2, PECR, MED23, RALGDS, TUSC3*
Fatty (3 genes)	*PECR, ADK, CASP2*
Saccharomyces (9 genes)	*ELP2, ASCC3, MAN1B1, COQ5, EEF1B2, TECR, ADK, NDST1, MED23*
Cerevisiae (9 genes)	*TRMT1, ASCC3, MAN1B1, COQ5, EEF1B2, TECR, ADK, NDST1, MED23*
Yeast (13 genes)	*COQ5, EEF1B2, TRMT1, ASCC3, RABL6, TECR, MAN1B1, MED23, RALGDS, SCAPER, HIST3H3, TRAPPC9, NDST1*

Abbreviation: NS-ARID, Non-syndromic autosomal recessive intellectual disability. Trna, Transfer Ribonucleic acid

Table 7.5. Displaying the five most significant Gene Ontology gene sets for the functional gene group analysis and their association with fluid ability.

GO term	Name	Number of genes		P-value		Genes in LD independent intervals
		Total	N hit	Enrichment	Corrected	
GO:0006814	Sodium ion transport	165	11	$7.9e^{-5}$	0.014	*SLC10A7, SLC8A1, SLC5A1, SLC4A5, SLC4A10, ACCN1, SLC9A10, SLC9A9, SLC17A8, NEDD4L, SLC34A2*
GO:0055029	Nuclear DNA-directed RNA polymerase complex	97	4	0.015	0.685	*SUPT3H, POLR2E, POLR2B, POLR3F*
GO:0030880	RNA polymerase complex	98	4	0.015	0.695	*SUPT3H, POLR2E, POLR2B, POLR3F*
GO:0006354	DNA-templated transcription, elongation	85	3	0.023	0.810	*POLR2E, POLR2B, POLR3F*
GO:0016591	DNA-directed RNA polymerase II, holoenzyme	86	3	0.059	0.958	*POLR2E, POLR2B, POLR3F*

Number of genes total pertains to the full number of genes in the gene set. Number of genes N hit indicates how many of the independent intervals overlapped with the genes of the gene set. Abbreviations: GO, Gene Ontology

Table 7.6. Displaying the five most significant Gene Ontology gene sets for the functional gene group analysis and their association with Crystallised ability.

GO term	Name	Number of genes		P-value		Genes in LD independent intervals
		Total	N hit	Enrichment	Corrected	
GO:0032781	Positive regulation of ATPase activity	19	3	0.002	0.174	*TPM1, RYR2, UHRF1*
GO:0006353	DNA-templated transcription, termination	82	4	0.003	0.218	*DHX38, CCNH, POLR3B, POLR2E*
GO:0006354	DNA-templated transcription, elongation	85	4	0.004	0.299	*CCNH, POLR3B, POLR2E, POLR2B*
GO:0043462	Regulation of ATPase activity	29	3	0.004	0.302	*TPM1, RYR2, UHRF1*
GO:0006368	Transcription elongation from RNA polymerase II promoter	65	3	0.012	0.569	*CCNH, POLR2E, POLR2B*

Number of genes total pertains to the full number of genes in the gene set. Number of genes N hit indicates how many of the independent intervals overlapped with the genes of the gene set. Abbreviations: GO, Gene Ontology

7.3.6 Replication

In order to try and replicate the overrepresentation of the genes from the Sodium ion transport gene-set, the performance IQ phenotype from the BATS cohort was used. The same data processing pipeline was used to assemble independent intervals. Observed intervals from the BATS cohort overlapped with three genes from the Sodium ion transport gene-set, *SLC6A5*, *SLC6A6,* and *SLC9A9.* This overlap was not statistically significant p = 0.551. None of the other nominally significant gene-sets contained genes which overlapped with the intervals of the BATS cohort and so no p-value could be derived.

7.4 Discussion

Four analysis strategies were used to examine whether genes involved in large deficits of cognitive ability are also involved in the small fluctuations which characterise the

normal range of intelligence. The first test examined each SNP using single marker analysis and found no evidence for a role in intelligence for any single SNP examined. The second strategy adopted was to sum the effect of multiple SNPs into a gene-based statistic using VEGAS (Liu et al., 2010). Here, none of the 40 genes tested withstood correction for multiple comparisons. The third test examined the NS-ARID gene-set as the statistical unit of association. INRICH was used for this test to show that the most significant hits in the GWAS did not overlap with the genes found in the NS-ARID set more than would be expected by chance. This does not provide evidence that the genes involved in major deleterious deviations in cognitive ability also account for intelligence differences.

The lack of association between the NS-ARID SNPs, genes and gene-set raises the possibility that intellectual disability is genetically distinct from the normal variation of intelligence differences. Evidence to support this comes from a study conducted examining the siblings of children affected by severe mental retardation, classified as those whose IQ was <50, and those with mild mental retardation, IQ 50-69 (Nichols, 1984). It was found that the siblings of those affected by severe mental retardation had an average IQ when compared to the population (mean = 103.4, SD = 12.1) and none of the siblings were suffering with any form of mental retardation. This contrasts with the siblings of children with mild mental retardation whose mean level fell below that of the population (mean = 84.8, SD = 18.1). In addition, 20.7% of these siblings also suffered with mental retardation. Whilst the sample size used to test this hypothesis (Nichols, 1984) was small (n=58) this trend has been replicated in much larger samples Plomin, Cederlöf, & Lichtenstein (unpublished conference talk, ISIR 2013) showed data drawn from 370,000 sibling pairs and 9000 twin pairs who, as part of their military conscription, had undergone cognitive testing. By linking these individuals to the Swedish National Patient Register 813 were identified as having a sibling with mild mental retardation (or an IQ 50-70) and they themselves were shown to have a lower IQ than the population average. In addition to this, 308 individuals were identified as having siblings who had severe mental retardation. However, despite having a sibling with

severe mental retardation these individuals did not have a significantly different mean IQ from the population average of 100. Whilst the cut offs in IQ used to define the NS-ARID gene-set used here were <70 and so should include variants encompassing the mild mental retardation range used in the studies by Nichols (1984) and Plomin, Cederlöf, and Lichtenstein (2013) should include the variants that could contribute toward variation in the normal range, it would also include genes responsible for major retardation introducing noise into the analyses. Whilst this remains plausible it should also be noted in the study by Nichols (1984) 72% of those with severe mental retardation also had central nervous system pathology (including Down's syndrome, central nervous system malformation, cerebral palsy and epilepsy), which was only present in 1.2% of those with mild mental retardation. As the gene set considered here included only genes resulting in non-syndromic intellectual disability, the contamination of the NS-ARID gene-set by genes responsible for these maladies should be minimal. Future work could perform the same analysis but omit genes where variants have been shown to be associated with an IQ below 50.

Future research using this method or genes associated with cognitive impairment may benefit from examining males and females separately. As males are at greater risk from intellectual disabilities as children and adults (Maulik, Mascarenhas, Mathers, Dua, & Saxena, 2011) as well as from mild cognitive impairment in old age (Roberts et al., 2012) it may be that genes responsible for large deviations primarily in males also exert small effects more often in males than females.

The fourth analysis was conducted using GRAIL (Raychaudhuri et al., 2009) to quantify the relationship between the genes of the NS-ARID set with the goal of using this knowledge to examine the systems and pathways that reflect these processes to find a gene-set associated with intelligence. Whilst the genes of the NS-ARID gene-set (Musante & Ropers, 2014) may not be directly involved in the normal range of intelligence differences they may reflect hub genes and should variation occur here it may have deleterious consequences for any system that they are a part of. The goal of the GRAIL analysis was to

identify the systems and process they are a part of as these may be more tolerant of functional variation and so may be involved in intelligence differences. The results of GRAIL identified 180 systems and processes and gene-sets were assembled from Gene Ontology (Ashburner et al., 2000) and examined for overrepresentation. One gene-set, Sodium ion transport, GO:0006814 remained significant for fluid ability after correction for the 180 gene-sets examined. This gene-set is involved in the directed movement of sodium ions across the boundary of a cell by means of a transporter or a pore (Ashburner et al., 2000). Such actions are found in the nervous system in the form of the sodium-potassium pumps of the neuron. These pumps are responsible in establishing the resting potential of neurons where they serve to keep the concentration of sodium within the neuron low by moving against the gradient of electrostatic pressure. By the same means, but involving a greater degree of metabolic effort, they also re-establish this gradient following depolarisation. This indicates a role for genetic variation of the neuron being involved in fluid cognitive abilities. However, this association failed to replicate in the BATS cohort indicating that that the initial significant result may be a type 1 error. It is also possible that due to winner's curse (Ioannidis, 2008) the replication sample lacked the statistical power needed for replication.

Another finding was that the DNA-templated transcription, elongation gene-set (GO:0006354) was nominally significant in both the fluid and the crystallised phenotypes. Whilst gene set analysis does not require for the same genes to show an effect across two phenotypes for significance of the set to be established in this instance two genes *POLR2B* on chromosome 5 and *POLR2E* on chromosome 19, were found to tag SNPs with low p-values indicated by their presence in the LD independent intervals. The DNA-templated transcription, elongation gene-set is described by Gene Ontology as being involved in the extension of the RNA molecule following the initiation of transcription and promoter clearance at DNA dependent RNA polymerase promoters through the inclusion of ribonucleotides catalyzed by an RNA polymerase. Whilst this may indicate that the

mechanisms involved in transcription, particularly elongation, are involved in cognitive abilities. It should be noted that in the BATS sample none of the genes from the DNA-templated transcription, elongation gene-set overlapped with any of the LD independent intervals.

The limitations of this study include the use of Gene Ontology. Whilst mining Gene Ontology for gene-sets using GRAIL represents a method to eliminate a number of the gene-sets found in gene ontology it could be omitting potential causal pathways as unknown relationships cannot be considered. In addition in using INRICH a boundary is placed around the gene in order to capture regulatory regions. Whilst 95% of common genetic variation that can effect transcription is found within these region (Veyrieras et al., 2008) this is likely to vary by gene.

In conclusion this study found no evidence that the genetic architecture of NS-ARID involves the same genes as those responsible for the normal range of intelligence differences indicating they may be genetically distinct. In addition there was tentative evidence that the sodium ion transporters may underlie genetic variation in fluid ability. Future studies should involve an increase of statistical power in order to overcome the winner's curse in order to establish if the failure to replicate is indeed a lack of power or if the initial significant finding is best explained as a type one error.

Chapter 8: General discussion

The aim of this thesis was to make a contribution to understanding the molecular genetic basis of human intelligence. This chapter will begin by summarising the main findings of the thesis before moving on to discuss the strengths and limitations of the studies, and the broader implications of the work carried out. The final section will outline additional work that can be conducted to build upon the current findings with the continued aim of identifying genetic variants responsible for human intelligence differences.

8.1 Overview of findings

The work presented in this thesis focused on the use of gene-set analysis to examine the joint effect of multiple genes and their role in intelligence differences. A number of new results were found.

Firstly, in chapter 4, proteomic data on human and animal cortical tissue was used to derive 5 gene sets, two of which detailed the molecular constituents of the postsynaptic density (Bayes et al., 2010) and three sets contained the genes in the AMPA-RC, NMDA-RC, and mGlu5-RC (Collins et al., 2006). These sets were examined for enrichment in fluid cognitive ability, crystallised ability, memory, and mental speed where the NMDA-RC was found to be enriched for fluid ability in sample of 3,403 older individuals. This finding was replicated using a performance IQ phenotype in 2,062 young Australians (mean age = 16.6, SD = 1.5). This finding coupled with the lack of significant SNP or gene-based statistics is consistent with the notion that phenotypic intelligence arises as a result of a large number of variants being involved, each of which has only a small effect (Plomin, 1999). This explains why the association between fluid ability and the NMDA-RC has not been found before as, by combining the signal from groups of SNPs rather than examining each SNP separately, as in candidate gene studies and GWA studies, provides the power required. The association

between the NMDA-RC and fluid ability also indicates that the function of the NMDA-RC, be it synaptic plasticity or neuronal death, plays a role in creating these differences.

In chapter 5 a novel method of grouping genes for analysis, horizontal or functional gene-group analysis, was combined with a self-contained test (Lips et al., submitted) in an attempt to replicate the finding that the genes which express the heterotrimeric g proteins are associated with differences in cognitive ability (Ruano et al., 2010). Horizontal grouping describes gene-sets assembled to include genes which are involved in the same cellular process such as heterotrimeric g protein genes. This grouping strategy exploits the fact that some proteins act across pathways meaning that they can exert an effect in multiple systems, such as the glutamatergic and dopaminergic systems. The original study used self-contained testing and phenotype permutation to show that, in a sample of 627 children with attention deficit hyper activity disorder (ADHD) (mean age in years = 11, SD = 2.7, 13.3% female), a significant association existed between 359 of the SNPs found in 25 of the 27 genes which express the heterotrimeric g proteins and IQ (Ruano et al., 2010). This was subsequently replicated in the same paper using a sample of 1507 non-ADHD children drawn from the Avon Longitudinal Study of Parents and Children (ALSPAC) (Golding, Pembrey, & Jones, 2001). Whilst differences in genotyping platform led to only 265 SNPs from 23 genes being tested the replication was statistically significant. However, this result did not replicate in this thesis where it was sought using the 5 samples of the CAGES consortium using both fluid ability and crystallised ability. In addition, replication was sought using the age 11 IQ scores of both LBC1921 and LBC1936 where again no significant association was found. These results highlight two important issues regarding the use of self-contained tests.

Firstly, it is well known that self-contained tests are more susceptible to the effects of population stratification than competitive tests. This is because all of the SNP based statistics can be become inflated in the presence of stratification and each of these incremental effects will be summed along with the signal from each SNP. As self-contained tests test against 0, or no association, this can lead to false positives and large gene-sets are at increased risk of

this. Competitive tests, whilst still affected by stratification, ameliorate the issue somewhat as they test against the average level of association found in the GWAS data set, meaning that if population stratification has the same effect across gene-sets then by testing against the average level of association found, the effect would be cancelled out. However, there may be instances where genes in the set are highly conserved, meaning there may be very little difference in allele frequency between any two populations. This contrasts with gene-sets including much less conserved variants which may have very different allele frequencies between populations (Fridley & Biernacka, 2011). This known effect of stratification inflating the alpha level, particularly for self-contained tests, can explain the replication in the ALSPAC sample (Ruano et al., 2010) as no method to control for the effects of stratification were employed in contrast to other GWAS performed on the ALSPAC sample (Benyamin et al., 2013; Spycher et al., 2012). In addition to this issue self-contained tests should be followed up with competitive tests if a gene-set is found to be significantly associated with a trait. The reason for this is that in a GWAS data set there are already a greater proportion of low p-values than would be expected under the null hypothesis of no association leading to an inflation of the type 1 error rate. The findings from chapter 5 show that there is no evidence for an association between the genes of the heterotrimeric g protein gene-set and cognitive abilities and highlight the importance of using competitive tests with GWAS data sets.

In chapter 6 functional SNPs, defined as those that can affect gene expression or function, were examined for a greater level of association with fluid cognitive ability than the background level found in a GWAS using two complementary statistical procedures. A variant of the Simes test (Li et al., 2011) was used to determine if any of the sets contained a small number of SNPs with effects larger than those they were grouped with, and a sum of squares test to detect many SNPs of small effect. A total of 10 SNP-sets were tested and corresponded to the functional categories of SNPs found in promoter regions (Tahira et al., 2005), SNPs found in micro-RNA seed sites (Richardson et al., 2011), SNPs associated with

methylation in the frontal lobes, the temporal lobes, the pons, and the cerebellum (Gibbs et al., 2010), and a SNP-set associated with mRNA levels in the same four cortical regions as the methylation SNP-set. Using the data processing pipeline of Bacanu et al. (2013) the results indicated that there was no significant enrichment for fluid ability found in any of the SNP- sets tested using either the Simes test or the sum of squares test. This indicates that any signal that may be coming from these sets is too small to detect with the sample size used here. Alternatively, these sets may not represent the underlying biology of intelligence and may include many SNPs that should not be included in the set due to type 1 errors or do not contain crucial variants, type 2 errors.

Chapter 7 examined genes that have previously been associated with non-syndromic autosomal recessive intellectual disability (NS-ARID) (Musante & Ropers, 2014) for an enriched association with fluid ability. In addition these genes were analysed using the text-mining software Gene Relationships Across Implicated Loci (GRAIL) (Raychaudhuri et al., 2009) in order to determine what processes these genes were involved in. This analysis produced a set of statistically significant keywords describing the relationship between the members of the NS-ARID gene set. Each of these words was used as a search term in Gene Ontology (Ashburner et al., 2000) and gene-sets corresponding to these sets were retained and tested for enrichment. One gene-set consisting of 165 genes corresponding to the sodium ion transport (GO:0006814) remained statistically significant after corrections were made for the 180 gene-sets tested. This sodium ion transport gene-set is involved in the directed movement of sodium ions across the boundary of a cell by means of a transporter or a pore (Ashburner et al., 2000). This finding, as with the finding of the NMDA-RC being significantly enriched for fluid ability, indicates a role for the genetic variation of the synapse being involved in intelligence differences. Sodium ion transportation can be seen in the nervous system where sodium potassium pumps are used to maintain the low concentration of sodium within the neuron by moving sodium ions against the gradient of electrostatic pressure. Unlike the result in chapter 4, the finding of an enriched association between the

sodium ion transport gene-set and fluid ability did not replicate in the BATS sample. This raises the possibility that the initial finding of association between fluid ability and the sodium ion transport reported in the CAGES sample was inflated. The use of p-value cut off to define significance in conjunction with an underpowered discovery sample is known to result in an inflated estimate of a true association (Ioannidis, 2008). The consequence of having an inflated estimate of the effect is that should replication fail it will be attributable to the initial discovery being a type 1 error rather than larger sample sizes being sought in the replication sample. It is also possible that the initial discovery of enrichment for the sodium ion transport was a type one error but replication in a larger sample would be help to differentiate between these two possibilities.

8.2. Implications for Theory

8.2.1 Non-random distribution of causal variants

Gene-set analysis is predicated on the notion that the underlying cause of phenotypic variance is, at least partly, the result of variance in an underlying biological system (Pedroso & Breen, 2011; Wang et al., 2007a). The goal of gene-set analysis is to test sets of genes grouped by the biological function they perform in order to capture the collective variance of the system they are a part of, rather than to test each component separately. The results of this thesis indicate that causal variants are not distributed randomly across the genome, but rather cluster in genes that are part of a greater system such as the NMDA-RC. This indicates that gene-set analysis could be used to elucidate other systems like the NMDA-RC, where genetic variation is linked to phenotypic variation in intelligence.

8.2.2 The use of Animal models for cognitive ability

The NMDA-RC gene set was sourced from proteomic studies of mice (Collins et al., 2006). The genes found in the NMDA-RC, notably *DLG2, DLG3* and *DLG4* have been shown to be involved in the cognitive abilities of mice (Migaud et al., 1998; Nithianantharajah

et al., 2013) with copy number variations in *DLG2* in humans producing similar deficit on tests of attention, visuo-spatial learning and memory as knock-out mutations in mice. This coupled with the high degree of similarity in the composition of the human and mouse PSD where 70% of the proteins in the human postsynaptic density genes are also present in the mouse postsynaptic density (Bayés et al., 2012) led to the hypothesis that these genes and, more broadly, the PSD and the associated complexes may be involved in human intelligence differences. The success here of showing that the mouse derived NMDA-RC was enriched for fluid ability in humans indicates that future work using mouse models could be fruitful. As general cognitive ability has been shown to exist in mice (Matzel et al., 2011) and that it is heritable (Galsworthy et al., 2005) future work using mouse models could utilise this general factor rather than specific cognitive abilities (Migaud et al., 1998; Nithianantharajah et al., 2013), the greater similarity between the phenotypes, particularly if the same or highly similar tasks are used (Nithianantharajah et al., 2013), would assist in making comparisons between species.

8.2.3 Use of GWAS data to indicate biological process involved in intelligence differences.

Gene-set analysis, when compared with single SNP or single gene methods, has the advantage of being able to provide ground for a biological explanation of any significant results found as the functional relationship between the genes in the set is typically known (Subramanian et al., 2005; Wang et al., 2010). Whilst some applications of gene-set analysis do not utilise genes grouped according to their role in biology, such as chapter 7 where genes previously associated with NS-ARID were used, such studies are in the minority. This known relationship between the unit of statistical association, the gene-set, and its function allows for hypotheses regarding the biological mechanisms involved in intelligence to be tested. Chapter 4 examined the postsynaptic density and its associated components provide an example of this as these gene-sets contain genes known to be involved in synaptic plasticity. Additional,

mechanistic theories of intelligence, such as speed of processing, could be examined by looking at gene sets containing genes known to be involved in the expression of proteins underlying myelin formation and/or integrity can be tested with gene set analysis.

8.3 Strengths

The strengths of this thesis include the combined use of statistical genetic techniques of GWAS and to incorporate them with findings from proteomics to help elucidate possible biological mechanisms underlying variation in intelligence as found in chapters 4 and 5. The methods used in chapter 4 where gene-based statistics were combined with gene-set analysis to interrogate the postsynaptic density has now begun to be used by other groups exploring different phenotypes (Föcking et al., 2014). In addition to this for any significant findings an attempt to replicate in an independent sample was made. This is an important point as attempts to find variants associated with intelligence have often been met with failure (Houlihan et al., 2009). Indeed, the basis of chapter 5 was to investigate a previous association between the genes of the heterotrimeric proteins and intelligence (Ruano et al., 2010). Using 5 separate samples and intelligence data gathered in youth and old age the data presented in this thesis indicates that this initial finding was likely to be spurious or at least inflated in the original discovery cohort used by Ruano et al. (2010). Other strengths include the examination of non-coding regions of the genome for an association with intelligence extending gene-set analysis to regions outside of genes. In addition the genetic links between intellectual disabilities and intelligence in the normal range was explored. Here, evidence was found that the genes involved in intellectual disabilities are found in the same systems, the sodium ion transporters, which may influence intelligence differences although this did not replicate.

8.4 Limitations

8.4.2 Common SNPs in protein coding regions

One limitation to the use of gene-set analysis is that it is limited to protein coding genes and the areas around them. As many GWAS hits fall outside of protein coding regions (Edwards, Beesley, French, & Dunning, 2013) these variants would not be included even though they may have an effect on protein expression. Future work should aim to incorporate such variants into gene-set analysis as measures based on distance (such as VEGAS (Liu et al., 2010)) or linkage disequilibrium may miss them. Rare variants have also been examined using gene-set analysis (Fromer et al., 2014; Kirov et al., 2012; Purcell et al., 2014). Future work could examine the gene and SNP-sets examined here but examine the role of rare variants and copy number variants in these regions.

8.4.3 Sample size

A second limitation is that the sample sizes are modest when compared with large scale psychiatric GWAS on disorders such as schizophrenia where 36,989 cases and 113,075 control participants was used to identify 108 associated loci (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). As the GWAS performed in this thesis contained no genome wide significant hits statistical power is lacking. Indeed, should intelligence have a similar genetic architecture to schizophrenia then relatively few associations will be found before a sample size of 20,000 is reached. Although, there were significant finding of enrichment for the NMDA-RC in chapter 4 and the sodium ion transport in chapter 7 the addition of a well powered sample would mean a more accurate measure of the effects of each SNP, and in turn would mean greater accuracy in establishing an association for any gene set. Indeed once large numbers of SNPs are found to be reliably

associated with intelligence the problem of establishing how these genes operate will still exist and this problem can also be tackled by examining genes grouped by a common function (Subramanian et al., 2005).

8.4.4 The focus on common SNPs

The work carried out in this thesis focused on aggregating the effects of common SNPs as the combined effect of many SNPs has been shown to capture variants responsible for intelligence differences (Davies et al., 2011). However, rare variants are also thought to play a role (Penke, Denissen, & Miller, 2007). Although the total burden or rare variants in protein coding genes does not show association with cognitive ability (Marioni et al., 2014b), their position in the genome may be more important than the total quantity of rare variants. Future work should examine gene-sets for burden to examine this hypothesis.

8.5 Future directions

The results of this thesis have indicated that gene-set analysis can help in the search for genetic variants involved in cognitive abilities. The finding that variants are non-randomly distributed across the genome indicates that future work using gene-set analysis methods can be a fruitful method for the additional analysis of GWAS data sets. This next section explores ideas for the future application of gene-set analysis to find additional variants involved in intelligence.

8.5.2 Further investigation of synaptic plasticity.

As the NMDA-RC has shown significant enrichment for fluid ability this raises the question of whether other processes also linked with synaptic plasticity show enrichment. This would also help to address the issue of whether the enrichment found for the NMDA-RC was due to its role in plasticity rather than its involvement with neuronal death. The activity-

regulated cytoskeleton-associated (ARC) protein, has also been associated with long term potentiation (Guzowski et al., 2000) as well as long term depression (Waung et al., 2008). Following plasticity inducing activity ARC mRNA is transported to these active synaptic regions via the dendritic spine where it is then translated and serves to modulate AMPA trafficking (Chowdhury et al., 2006). In addition to being involved in plasticity, variation in ARC has been shown to be associated with psychiatric phenotypes in humans where *de novo* mutations in the ARC protein have been implicated in schizophrenia (Kirov et al., 2012), a disease in part predicted by a low premorbid cognitive ability (Khandaker, Barnett, White, & Jones, 2011), which may be due to a shared genetic component between the two traits (Toulopoulou et al., 2010). High quality gene-sets, comparable to the NMDA-RC, PSD, AMPA, or mGlu5-RC, detailing the genes of the ARC are not yet available. As the ARC set currently available is likely to contain both genes which do not belong in the set (type 1 errors) and be missing genes that should be included (type 2 errors) the meaning of finding enrichment in the ARC set for intelligence is unclear.

The role that genetic variation in other synaptic components, particularly those that can modulate NMDA activity can also be examined for enrichment with cognitive abilities. In addition to the components of the glutamatergic system, the gamma-aminobutyric acid receptors (GABA) can contribute toward synaptic plasticity. For the NMDA receptors to allow the entry of Ca^{2+}, critical to the initiation of synaptic plasticity, a sufficiently large depolarisation of the postsynaptic region must co-occur with glutamate release from the presynaptic neuron. Following GABA release from the presynaptic neuron the postsynaptic neuron becomes hyperpolarised, which serves to intensify the voltage dependent Mg^{2+} block preventing Ca^{2+} intake (Dingledine, Hynes, & King, 1986). This role for GABA in synaptic plasticity also makes it a viable candidate gene-set to study for being enriched in intelligence.

Stimulation of the muscarinic acetylcholine receptors (mAChRs) are also known to result in an increase in the level of NMDA receptor activity (Markram & Segal, 1990). One

way in which mAChRs facilitate NMDA activity, and with it synaptic plasticity, is by depolarizing the postsynaptic neuron by inhibiting K^+ channels. This indicates that synaptic plasticity can be modulated indirectly by altering the activity of non-glutamatergic transmission. Whether common genetic differences in these systems result in variation in synaptic plasticity or intelligence has yet to be shown.

Non-neuronal cells can also mediate synaptic plasticity (Han et al., 2013) and so should also be considered as potential targets to test the notion that genetic variability in the systems that can modulate synaptic plasticity also are responsible for intelligence differences. Human astrocytes, a subtype of glial cell, have been shown to be structurally and functionally different from those of non-primate mammals as they are larger, more complex, and produce a more rapid calcium signalling (Oberheim et al., 2009). Han et al. (2013) experimentally tested the idea that this increase in the complexity of human astrocytes contributed towards greater cognitive abilities by producing mice with a chimeric central nervous system (Windrem et al., 2008) by engrafting them neonatally with human glial progenitor cells. As the mouse developed these human glia cells integrated themselves into the brain of the mice and upon reaching adulthood, a large proportion of the forebrain of the mice was replaced with human glial cells. This had neuro-physiological effects in that long term potentiation was increased in these mice and this was not through modulation of activity of the NMDA receptors. This increase in long term potentiation was accompanied by an increased ability to perform cognitive tasks including auditory fear conditioning, where a tone is paired with a painful electrical shock to the feet, and a spatial learning task where the mice had to successfully navigate a maze, and tests of recognising a familiar object in a novel location. These findings indicate that the astrocytes contribute toward species differences in cognitive abilities and further work utilising gene-set analysis could be performed to determine if they are also involved in individual differences within species.

8.5.3 Gene-set analysis and white matter

Intelligence has been associated with a number of neurological correlates such as increased brain size (McDaniel, 2005), but its association with white matter tract integrity makes for a particularly appealing target for gene-set analysis. This is because of the functional role white matter integrity appears to play in intelligence through its role in processing speed (Penke et al., 2012). A sample of 420 individuals taken from the Lothian Birth Cohort of 1936 were subjected to diffusion tensor magnetic resonance imagery as well as cognitive testing (Penke et al., 2012). Three complementary imaging methods were used to provide a measure of white matter integrity and a latent component for each was derived using a principal component analysis carried out on 12 white matter tracts, with the first unrotated component being extracted. Participants' scores on each of these principal components were found to predict their level of intelligence. This link, however, was broken by adding a latent component for information processing speed. Together this indicates that white matter integrity, through processing speed, underlies a portion of the variance in intelligence that is measureable with the sample sizes currently available. Studies have also shown that white matter integrity is heritable (Jahanshad et al., 2013). A viable strategy to build on the results of this thesis would be to use existing gene- sets containing genes for oligodendrocytes, the cells responsible for the myelination of the central nervous system, and examine them for enrichment using white matter phenotypes as well as both processing speed and general cognitive ability. By examining white matter integrity, processing speed and intelligence using gene-set analysis there is the potential to find genes involved in intelligence and to understand one of the mechanisms responsible for variation in intelligence.

8.6 Summary

The results of this thesis have indicated that gene-set analysis can be included in an

analysis pipeline to complement single SNP and single gene analysis. This can be done in order to test a specific group of genes as carried out in chapters 4 and 5 or as in the case of chapter 7 gene-sets can be constructed from genes showing association to a potentially related phenotype. In chapter 6 groups of SNPS formed the unit of association and represent another variant of the gene-set analysis approach. The collection of studies presented as the body of this thesis support the existing notion of the genetic architecture of intelligence being highly polygenic. This is extended by indicating the causal variants involved in intelligence cluster in genes, which themselves work together as components in biological systems as indicated by the significant enrichment of the NMDA-RC in chapter 4 and to a lesser extent the significant enrichment of the sodium ion transporter (GO:0006814) in chapter 7. Genome wide analysis in conjunction with GCTA has expanded our understanding of the genetic architecture of human intelligence by showing that common variants tag causal variants. Genome wide analysis in conjunction with gene-set analysis has the potential to both identify these common variants and connect them with a known biological process. By capitalising on the ever increasing sample sizes available in modern GWA studies along with bioinformatics resources such the Allen Brain Atlas (Hawrylycz et al., 2012) or proteomic work on the human synapse conducted by the genes to cognition group (https://www.genes2cognition.org/) gene-set analysis will continue to make contributions to the field of intelligence differences.

Appendices

Appendix A.

Details the full set of genes for the postsynaptic density and its associated components

PSD Full total genes = 1433

AAK1, AARS, AASDHPPT, ABCB8, ABCD1, ABCD3, ABCF3, BAT5, ABI1, ABI2, ABLIM1, ABLIM2, ABLIM3, ABR, ACAA1, ACACA, ACAT1, ACBD5, ACLY, ACO2, THEM2, ACOT7, ACOT8, ACP1, ACSL3, ACSL4, ACSL6, ACTB, ACTN1, ACTN2, ACTN3, ACTN4, ACTR1A, ACTR1B, ACTR2, ACTR3, ACYP2, ADAM22, ADAM23, ADD1, ADD2, ADD3, ADRBK1, AFG3L2, CENTG2, CENTG1, CENTG3, AGK, AGL, AGPAT5, AHCY, AHCYL1, AHNAK, AHSA1, AIFM3, AIP, AK1, AK3, AK5, AKAP12, AKR1A1, AKR1C1, AKR1C2, AKR7A2, ALDH16A1, ALDH1L1, ALDH2, ALDH3A2, ALDH4A1, ALDH5A1, ALDH6A1, ALDH7A1, ALDOA, ALDOC, AMPD2, AMPH, ANK1, ANK2, ANK3, ANKFY1, ANKRD24, ANKS1B, ANXA1, ANXA11, ANXA2, ANXA5, ANXA6, ANXA7, AP1B1, AP1G1, AP1M1, AP1S1, AP2A1, AP2A2, AP2B1, AP2M1, AP2S1, AP3B1, AP3B2, AP3D1, AP3M2, AP3S1, AP3S2, APC, APOD, APOE, APOL2, APOOL, APPL1, APPL2, AQP1, AQP4, ARCN1, ARF5, ARFGAP2, ARFGEF2, ARHGAP1, ARHGAP21, ARHGAP23, ARHGAP26, KIAA1688, RICH2, ARHGDIA, ARHGEF2, ARHGEF7, ARPC2, ARPC3, ARPC4, ARRB1, ATAD1, ATAD3A, C6orf134, ATIC, SPG3A, ARL6IP2, ATP12A, ATP13A1, ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B1, ATP1B2, ATP2A1, ATP2A2, ATP2B1, ATP2B2, ATP2B3, ATP2B4, ATP4A, ATP5A1, ATP5B, ATP5C1, ATP5D, ATP5F1, ATP5I, ATP5J2, ATP5L, ATP5O, ATP6V0A1, ATP6V0D1, ATP6V1A, ATP6V1B2, ATP6V1C1, ATP6V1D, ATP6V1E1, ATP6V1E2, ATP6V1G1, ATP6V1G2, ATP6V1H, ATP8A1, ATP8A2, ATXN10, AUH, B3GAT3, BAG3, BAG5, BAT3, BAI1, BAIAP2, BASP1, BCAN, BCAS1, BCKDK, BCR, BEGAIN, BIN1, BLVRB, BRSK1, BSG, BSN, LGALS3BP, C10orf35, C11orf2, C11orf41, C14orf156, C14orf159, C17orf61, C1QBP, C1QC, C1orf198, C1orf95, C22orf28, C2CD4C, C2orf55, C2orf72, C3, C6orf154, C8orf55, C9orf46, C9orf5, CA1, CA2, CA4, CACNA2D1, CACNA2D2, CACNB1, CACNB3, CACNB4, CACYBP, CAD, CADPS, CALCOCO1, CAMK2A, CAMK2B, CAMK2D, CAMK2G, CAMKK1, CAMKK2, CAMKV, CAND1, CAND2, CAP1, CAP2, CAPG, CAPN1, CAPN5, CAPZA2, CAPZB, CASK, CASKIN1, CBR1, CBR3, CC2D1A, CCDC124, CCDC127, CCDC22, CCDC93, CCNY, CCT2, CCT3, CCT4, CCT5, CCT6A, CCT6B, CCT7, CCT8, CD59, CD9, CDC42, CDC42BPA, CDC42BPB, CDC42EP4, CDH10, CDH13, CDH2, CDH4, PCTK3, CDK5, CDK5RAP3, CDKL5, CEND1, CFL1, CFL2, CHCHD3, CHCHD6, CHL1, CHMP1A, CHMP4B, CISD1, CIT, CKAP4, CKAP5, CKB, CKMT1B, CLASP1, CLASP2, CLIP2, CLIP3, CLTA, CLTB, CLTC, CLU, CMC1, CMPK1, CNDP2, CNP, CNTN1, CNTN2, CNTNAP1, CNTNAP2, COASY, COG3, COPA, COPG, COQ10B, CORO1A, CORO1B, CORO1C, CORO2A, CORO2B, COX4I1, COX5A, COX5B, COX6B1, COX6C, COX7A2L, CPNE5, CPNE6, CPT1A, CRAT, CRIP2, CRKL, CRMP1, CRTAC1, CRYAB, CRYM, CRYZ, CS, CSE1L, CSNK1D, CSNK1E, CSNK2A1, CSNK2A2, CSRP1, CST3, CTBP1, CTNNA1, CTNNA2, CTNNB1, CTNND1, CTNND2, CYB5R1, CYBRD1, CYCS, CYFIP1, CYFIP2, CYLD, PSCD2, PSCD3, DAAM1, DAAM2, DAB2IP, DAD1, DARC, DARS, DBN1, DBNL, DBT, DCAKD, DCLK1, DCLK2, DCTN1, DCTN2, DCTN3, DCTN4, DCX, DDAH1, DDOST, C20orf116, DDX1, DDX17, DDX3X, DDX6, DECR1, DECR2, DES, DGKB, DHX30, DIP2B, DIRAS2, DLAT, DLD, DLG1, DLG2, DLG3, DLG4, DLGAP1, DLGAP2, DLGAP3, DLGAP4, DLST, DMWD,

DMXL2, DNAJA1, DNAJA2, DNAJA3, DNAJB1, DNAJB2, DNAJB4, DNAJB6, DNAJC11, DNAJC13, DNAJC19, DNAJC6, DNM1, DNM1L, DNM2, DNM3, DOCK1, DOCK10, DOCK2, DOCK3, DOCK4, DOCK5, DOCK9, DPM1, DPP6, DPYSL2, DPYSL3, DPYSL4, DSP, DST, DSTN, DTNA, DUSP3, DYNC1H1, DYNC1I1, DYNC1I2, DYNC1LI1, DYNC1LI2, DYNLL1, DYNLL2, DYNLRB1, DYNLRB2, PECI, EDARADD, EEA1, EEF1A1, EEF1A2, EEF1D, EEF1G, EEF2, EFHD2, EFR3A, EFR3B, EHD1, EHD3, EHD4, EIF2AK2, EIF2S1, EIF3C, EIF4A2, EIF4G1, ELFN2, ELMO2, ENO1, ENO2, ENO3, ENPP6, EPB41, EPB41L1, EPB41L2, EPB41L3, EPB42, EPB49, EPHA4, EPPK1, EPRS, EPS15L1, EPX, ERBB2IP, ERC1, ERC2, ERLIN1, ERLIN2, FAM62A, FAM62B, ETFB, EVL, EXOC1, EXOC2, EXOC3, EXOC4, EXOC5, EXOC6, EXOC6B, EXOC7, EXOC8, EZR, FAAH, FABP3, FABP7, FAM107A, FAM123A, FAM171A1, FAM18A, FAM49A, FAM49B, FAM82A2, FARP1, FARSA, FARSB, FASN, FBXO2, FBXO41, FDPS, FGB, FGF2, FGG, FH, FIS1, FKBP15, FKBP1B, FKBP4, FKBP8, FLII, FLNA, FLOT1, FLOT2, FMN2, FMNL1, FMNL2, FMNL3, FN3K, FRYL, FSCN1, FSD1, FTH1, FXR2, FYN, G6PD, GABARAPL2, GABBR1, GABBR2, GABRA1, GABRA4, GAK, GAP43, GAPDH, GAPVD1, GAS7, GBAS, GDAP1, GDAP1L1, GDI1, GDI2, GFAP, GGA3, GIPC1, GIT1, GJA1, GK, GLG1, C9orf19, GLOD4, GLS, GLUD1, GLUL, GNA11, GNA13, GNA14, GNAI1, GNAI2, GNAI3, GNAO1, GNAQ, GNAS, GNAZ, GNB1, GNB2, GNB4, GNB5, GNG12, GNG2, GNG3, GNG7, GNL1, GNPAT, C10orf132, GOLGB1, GOT1, GOT2, GPC1, GPD2, GPHN, GPI, GPR158, GPRC5B, GPRIN1, GPSM1, GPX1, GPX4, GRHPR, GRIA1, GRIA2, GRIA3, GRIA4, GRIN1, GRIN2A, GRIN2B, GRIN2D, GRLF1, GRM2, GRM5, GSK3B, GSN, GSTK1, GSTM2, GSTM3, GSTO1, GSTP1, GUK1, HADH, HADHA, HADHB, HAPLN1, HAPLN2, HARS2, HCK, HDLBP, HECW1, HECW2, HGS, HIBCH, HIGD1A, HIP1, HIP1R, HK1, HMOX2, HNRNPA1, HNRNPK, HNRNPM, HOMER1, HOMER2, HOOK3, HPCA, HSD17B12, HSD17B4, HSDL1, HSDL2, HSP90AA1, HSP90AB1, HSP90B1, HSPA12A, HSPA2, HSPA4, HSPA4L, HSPA5, HSPA8, HSPA9, HSPB1, HSPB8, HSPD1, HSPH1, HTT, IARS, ICAM5, IDH2, IDH3A, IGHM, IGSF21, IGSF8, IMMT, IMPA1, INA, INF2, IPO5, IPO7, IQGAP1, IQSEC1, IQSEC2, IQSEC3, IRGQ, ITSN1, ITSN2, JUP, KALRN, KANK2, KARS, KBTBD11, KCNAB2, KCNQ2, KCTD12, KCTD16, KCTD8, KIAA0090, KIAA0174, KIAA0196, KIAA0284, KIAA0408, KIAA0513, KIAA0528, KIAA0776, KIAA1033, KIAA1045, KIAA1468, KIAA1543, KIAA1549, KIAA1598, KIF1A, MPHOSPH1, KIF21A, KIF2A, KIF2B, KIF2C, KIF3A, KIF5A, KIF5B, KIF5C, KLC1, KLC2, CCDC128, KPNA1, KPNB1, KRAS, KTN1, L1CAM, C11orf59, LANCL1, LANCL2, LAP3, LASP1, LCP1, LDHA, LDHB, LETM1, LGALS8, LGI1, LIMA1, LIMCH1, LIN7A, LIN7B, LIN7C, LINGO1, LIPE, LLGL1, LMNB2, LMO7, LMTK2, LMTK3, LONP1, LPHN1, LPHN3, LRP1, LRPPRC, LRRC40, LRRC47, LRRC57, LRRC59, LRRC7, LRRC8A, LRSAM1, LSAMP, LY6H, LYN, LYNX1, MACF1, MACROD1, MADD, MAG, MAGI2, MAOA, MAOB, MAP1A, MAP1B, MAP1LC3A, MAP2, MAP2K1, MAP2K2, MAP4, MAP6, MAP6D1, MAP7D1, MAPK1, MAPK3, MAPK8IP3, MAPRE1, MAPRE2, MAPRE3, MAPT, MARCKSL1, MARK1, MARK2, MARS, MBP, MCCC2, MDH1, MDH2, MFF, MFN2, MINK1, MLLT4, MOG, GCS1, MOSC2, MPHOSPH1, MPO, MPP2, MPP6, MPP7, MPRIP, MRAS, MSN, MT-CO2, MTCH1, MTCH2, MTDH, MTHFD1, MTHFD1L, MTX1, MVP, MYCBP2, MYH10, MYH11, MYH14, MYH9, MRLC2, MYL6, MYL6B, MYO18A, MYO1B, MYO1C, MYO1D, MYO1E, MYO1F, MYO5A, MYO6, NAP1L4, NAPA, NAPB, NAPG, NCALD, NCAM1, NCAM2, NCAN, NCDN, NCKAP1, NCKAP1L, NCKIPSD, NDRG1, NDRG2, NDUFA10, NDUFA12, NDUFA13, NDUFA2, NDUFA4, NDUFA5, NDUFA7, NDUFA9, NDUFB10, NDUFB4, NDUFB6, NDUFB7, NDUFB8, NDUFB9, NDUFS1, NDUFS2, NDUFS3, NDUFS6, NDUFS7, NDUFV1, NDUFV2, NEBL, NEFH, NEFL, NEFM, NEGR1, NEO1, NFASC, NINJ2, NIPSNAP1, NLGN2, NME1, NME3, NNT, NOMO1, NPEPPS, NPM1, NPTN,

NPTX1, NRAS, NRCAM, NRN1, NRXN1, NRXN3, NSF, NSFL1C, NT5C1B, NT5E, HNT, NUDCD2, NUMBL, OGDH, OGDHL, OGT, OLA1, OLFM1, OMG, OPA1, OPCML, OXCT1, OXR1, PABPC1, PABPC4, PACS1, PACSIN1, PACSIN2, PADI2, PAICS, PAK1, PALM, PALM2, CORO7, PARK7, PARP1, PBXIP1, PC, PCBP1, PCBP2, PCCA, PCDH1, PCLO, PCMT1, PDCD6IP, PDE1A, PDE2A, PDE8A, PDHA1, PDHB, PDHX, PDIA3, PDIA6, PDK3, PDPK1, PDXK, PDXP, PEA15, PEBP1, PEX11B, PFKL, PFKM, PFKP, PFN1, PGAM1, PGAM5, PGD, PGK1, PGM2L1, PHACTR1, PHB, PHB2, PHGDH, PHLDB1, PHYHIP, PI4KA, PIN1, PIP4K2A, PIP4K2B, PIP5K1C, PITPNA, PKM2, PKN1, PKP4, PLCB1, PLCB4, PLCD1, PLCD3, PLCG1, PLD3, PLEC1, PLEKHA1, PLEKHA5, PLEKHA6, PLLP, PLP1, PLXNA1, PLXNA2, PLXNA3, PMVK, PNKD, PNPLA6, POR, PPAP2B, PPFIA1, PPFIA2, PPFIA3, PPFIA4, PPIA, PPIB, PPID, PPIL1, PPP1CA, PPP1CB, PPP1CC, PPP1R12A, PPP1R7, PPP1R9A, PPP1R9B, PPP2R1A, PPP2R5D, PPP3CA, PPP3CB, PRAF2, PRDX1, PRDX2, PRDX5, PRDX6, PREX1, PRKACB, PRKAR1A, PRKAR2A, PRKAR2B, PRKCA, PRKCB1, PRKCE, PRKCG, PRKDC, PRKRA, PRMT5, PRODH, PROSC, PRPH, PRPS1, PRR7, PRRT1, PSD, PSD3, PSMB6, PSMC1, PSMD11, PSMD14, PSMD2, PTK2, PTK2B, PTPLAD1, PTPN11, PTPN23, PTPRD, PTPRF, PTPRS, PTPRZ1, PURA, PURB, PYCRL, PYGB, PYGM, QARS, QDPR, RAB10, RAB11B, RAB11FIP5, RAB13, RAB14, RAB15, RAB18, RAB1A, RAB1B, RAB21, RAB35, RAB3A, RAB3C, RAB3GAP1, RAB3GAP2, RAB4B, RAB5A, RAB5B, RAB5C, RAB6A, RAB6B, RAB7A, RAB8B, RAC1, RALA, GARNL1, RANGAP1, RAP1A, RAP1GAP, RAP1GDS1, RAP2C, RAPGEF2, RAPGEF4, RAPH1, RARS, RASAL1, RASAL2, RASGRF2, RBX1, RDX, REEP5, REPS2, RFTN1, RGS7, RHOA, RHOB, RHOG, RHOT1, RHOT2, RIC8A, RIMBP2, RIMS1, RIN1, ROCK1, ROCK2, ROGDI, RPH3A, RPL10A, RPL12, RPL13, RPL13A, RPL18A, RPL24, RPL3, RPL30, RPL35, RPL36, RPL38, RPL4, RPL6, RPL7, RPL7A, RPL8, RPL9, RPLP0, RPN1, RPN2, RPS11, RPS13, RPS14, RPS15A, RPS16, RPS17-2, RPS18, RPS19, RPS25, RPS27, RPS3, RPS3A, RPS5, RPS8, RRBP1, RTN1, RTN3, RTN4, RTN4RL2, RUFY3, RYR2, SACM1L, SACS, SAMM50, SAR1A, SARS, SBF1, SBF2, C9orf126, SCCPDH, SCFD1, SCIN, SCRIB, SCRN1, SDCBP, SEC13, SEC14L2, SEC22B, SEC23A, SEC24C, SEC31A, SEPT10, SEPT11, SEPT2, SEPT3, SEPT4, SEPT5, SEPT6, SEPT7, SEPT8, SEPT9, SESTD1, SFN, SFXN1, SFXN3, SFXN5, SGCD, SGIP1, SGSM1, SH3GL1, SH3GL2, SH3GL3, SH3GLB2, SH3PXD2A, SHANK1, SHANK2, SHANK3, SHISA7, SHMT2, SIPA1L1, SIRPA, SIRT2, SKIV2L, SLC12A2, SLC12A5, SLC17A7, SLC1A2, SLC1A3, SLC25A1, SLC25A11, SLC25A12, SLC25A13, SLC25A18, SLC25A22, SLC25A26, SLC25A3, SLC25A31, SLC25A4, SLC25A46, SLC25A5, SLC27A1, SLC27A4, SLC2A1, SLC3A2, SLC4A1, SLC4A4, SLC8A2, SLC9A3R2, SLK, SNAP25, SNAP91, SND1, SNPH, SNTA1, SNTB1, SNTB2, SNX1, SNX12, SNX2, SNX27, SNX3, SNX4, SNX5, SNX6, SNX9, SORBS1, SORBS2, SPECC1, SPIRE1, SPTAN1, SPTB, SPTBN1, SPTBN2, SPTBN4, SRC, SNIP, SRGAP3, SRI, SRPK2, SRPR, SRPRB, SSBP1, STAT1, STIP1, STK32C, STK38L, STK39, STOM, STOML2, STRAP, STRN4, STUB1, STX12, STX1A, STX1B, STX4, STX7, STXBP1, STXBP3, STXBP5, SUCLA2, SUCLG1, SV2A, SYN1, SYN2, SYN3, SYNCRIP, SYNE1, SYNGAP1, SYNGR3, SYNJ1, DMN, SYNPO, SYP, SYT1, SYT5, SYT7, TACC1, TAGLN2, TAGLN3, TALDO1, TAOK1, TAOK2, TARSL2, TBC1D17, TBC1D24, TBCB, TBCD, TBK1, TCP1, TCP11L1, TFAM, TIMM50, TJP1, TJP2, TKT, TLN1, TLN2, TMEM126A, TMEM85, TMOD1, TMOD2, TNC, TNPO1, TNR, TOLLIP, TOM1L2, TOMM20, TOMM34, TOMM40L, TOMM70A, TPI1, TPM1, TPM3, TPM4, TPP1, TPPP, TRAP1, TRAPPC3, NIBP, TRIM2, TRIM3, TRIO, TSC2, TSC22D4, TSG101, TTC35, TTC37, TTC7B, TUBA1A, TUBA1B, TUBA4A, TUBB2A, TUBB2B, TUBB2C, TUBB3, TUBB4, TUFM, TWF1, TXNL1, UBA1, UBC, UBE2M, UBE2N, UBE2V2, UBE3C, UBE4A, UBL4A, UBR4, UBXD1, UCHL1, UGP2, UNC13A, UPF1, UQCRB, UQCRC1, UQCRC2,

UQCRFS1, USMG5, USO1, USP14, USP15, USP5, USP9X, VAC14, VAMP2, VAMP3, VAPA, VAPB, VARS, VAT1, VCAN, VCL, VCP, VCPIP1, VDAC1, VDAC2, VDAC3, VGF, VIM, VPS11, VPS16, VPS18, VPS29, VPS33A, VPS35, VPS39, VPS45, VPS4A, VPS52, VPS53, VPS8, VSNL1, VTA1, WASF1, WASF2, WASF3, WASL, WDR1, WDR37, WDR47, WDR48, WDR7, WDR91, WFS1, WIPF2, WNK1, XPO1, XPO7, YARS, YES1, YWHAB, YWHAE, YWHAG, YWHAH, YWHAQ, YWHAZ.

PSD consensus, total genes = 737,

AAK1, AARS, ABCD3, ABI1, ABI2, ABLIM1, ABR, ACBD5, ACLY, ACO2, ACP1, ACSL3, ACSL6, ACTB, ACTN1, ACTN2, ACTN4, ACTR1A, ACTR1B, ACTR2, ACTR3, ADD1, ADD2, ADD3, AFG3L2, CENTG1, AHCYL1, AHNAK, AK1, AK5, AKR1A1, AKR7A2, ALDH2, ALDH6A1, ALDH7A1, ALDOA, ALDOC, AMPH, ANK1, ANK2, ANK3, ANKFY1, ANKS1B, ANXA2, ANXA5, ANXA6, AP1B1, AP1S1, AP2A1, AP2A2, AP2B1, AP2M1, AP2S1, AP3B2, APOD, APOE, APOL2, AQP4, ARF5, ARHGAP21, ARHGDIA, ARHGEF2, ARPC2, ARPC3, ATAD3A, C6orf134, ATIC, SPG3A, ATP1A1, ATP1A2, ATP1A3, ATP1B1, ATP1B2, ATP2A2, ATP2B1, ATP2B2, ATP2B3, ATP2B4, ATP5A1, ATP5B, ATP5D, ATP5I, ATP5O, ATP6V0A1, ATP6V0D1, ATP6V1A, ATP6V1B2, ATP6V1C1, ATP6V1D, ATP6V1E1, ATP6V1G2, ATP6V1H, ATP8A1, BAG3, BAIAP2, BASP1, BCAN, BCAS1, BIN1, BLVRB, BRSK1, BSN, C10orf35, C11orf41, C1QC, CA2, CA4, CACNA2D1, CACNB4, CACYBP, CADPS, CAMK2A, CAMK2B, CAMK2D, CAMK2G, CAMKK1, CAMKV, CAND1, CAP1, CAP2, CAPG, CAPN5, CAPZA2, CAPZB, CASK, CASKIN1, CBR1, CBR3, CC2D1A, CCT2, CCT3, CCT4, CCT5, CCT6A, CCT7, CCT8, CDC42BPA, CDC42BPB, CDH13, CDH2, CDK5, CEND1, CFL1, CHCHD3, CHCHD6, CHMP4B, CISD1, CIT, CKAP4, CKAP5, CKB, CKMT1B, CLASP2, CLIP2, CLTC, CLU, CNDP2, CNP, CNTN1, CNTN2, CNTNAP1, CORO1A, CORO1C, CORO2B, COX5A, COX5B, COX6B1, COX6C, COX7A2L, CPNE5, CRIP2, CRMP1, CRTAC1, CRYAB, CRYM, CS, CSE1L, CST3, CTNNA1, CTNNA2, CTNNB1, CTNND1, CTNND2, CYBRD1, CYFIP1, CYFIP2, PSCD2, PSCD3, DARS, DBN1, DBNL, DCLK1, DCTN1, DCTN2, DDAH1, DDX1, DDX3X, DIP2B, DLAT, DLD, DLG1, DLG2, DLG3, DLG4, DLGAP1, DLGAP2, DLGAP3, DLST, DMXL2, DNAJA1, DNAJB4, DNAJB6, DNAJC11, DNAJC13, DNM1, DNM1L, DNM2, DNM3, DOCK10, DOCK9, DPYSL2, DPYSL3, DPYSL4, DST, DSTN, DYNC1H1, DYNC1I1, DYNC1LI2, DYNLL1, DYNLL2, DYNLRB1, DYNLRB2, EEF1A1, EEF1D, EEF1G, EFHD2, EHD1, EHD3, EIF4A2, ELMO2, ENO1, ENO2, ENO3, ENPP6, EPB41, EPB41L1, EPB41L3, EPB49, EPHA4, EPPK1, EPRS, EPS15L1, ERC1, ERC2, FAM62A, EVL, EXOC1, EXOC2, EXOC3, EXOC4, EXOC8, EZR, FAM18A, FAM49A, FAM82A2, FARP1, FARSA, FARSB, FASN, FBXO41, FKBP8, FLNA, FLOT1, FLOT2, FMN2, FMNL2, FMNL3, FSCN1, FSD1, FTH1, FYN, GABBR2, GABRA1, GAP43, GAPDH, GBAS, GDI2, GFAP, GIT1, GJA1, GLUD1, C9orf19, GLUD1, GLUL, GNA13, GNAI2, GNAO1, GNAS, GNAZ, GNB1, GNB2, GNB4, GNL1, GNPAT, C10orf132, GOT1, GPHN, GPI, GPR158, GPRC5B, GPRIN1, GRIA1, GRIA2, GRIA3, GRIN1, GRIN2B, GRIN2D, GSN, GSTM3, HADHA, HADHB, HAPLN2, HCK, HK1, HOMER1, HSD17B4, HSP90AA1, HSP90AB1, HSPA12A, HSPA2, HSPA4, HSPA4L, HSPA5, HSPA8, HSPA9, HSPB1, HSPD1, HSPH1, ICAM5, IDH2, IGSF21, IGSF8, IMMT, INA, IPO5, IQSEC1, IQSEC2, IRGQ, ITSN1, KBTBD11, KCTD12, KCTD8, KIAA0284, KIAA0408, KIAA0528, KIAA1045, KIAA1543, KIF1A, KIF21A, KIF2A, KIF5A, KIF5B, KIF5C, KLC1, KLC2, KPNB1, KRAS, L1CAM,

C11orf59, LANCL2, LDHA, LDHB, LIMCH1, LIN7B, LIN7C, LLGL1, LMO7, LMTK3, LPHN1, LRPPRC, LRRC47, LRRC7, LSAMP, LYN, LYNX1, MACF1, MAOA, MAOB, MAP1A, MAP1B, MAP1LC3A, MAP2, MAP2K1, MAP4, MAP6, MAP6D1, MAP7D1, MAPK3, MAPRE2, MAPRE3, MAPT, MARK2, MBP, MDH1, MDH2, MINK1, MLLT4, MOG, MPP2, MSN, MT-CO2, MTCH2, MTDH, MTHFD1, MYH10, MYH11, MYH14, MYH9, MRLC2, MYL6, MYL6B, MYO18A, MYO1D, MYO5A, MYO6, NAPA, NAPB, NAPG, NCAM1, NCAM2, NCAN, NCDN, NCKAP1, NCKIPSD, NDUFA13, NDUFA2, NDUFA4, NDUFA7, NDUFB10, NDUFB6, NDUFB7, NDUFS1, NDUFS2, NDUFS3, NDUFS7, NDUFV2, NEFH, NEFL, NEFM, NEGR1, NFASC, NIPSNAP1, NLGN2, NME1, NNT, NPEPPS, NPTN, NRCAM, NRXN1, NSF, NSFL1C, HNT, OGDH, OGT, OMG, OPA1, OPCML, OXCT1, OXR1, PABPC1, PACS1, PACSIN1, PAICS, PAK1, PALM, PALM2, PBXIP1, PCBP2, PCLO, PCMT1, PDCD6IP, PDE2A, PDHA1, PDHB, PEA15, PEBP1, PFKL, PFKM, PFKP, PGAM1, PGD, PGK1, PHB, PHB2, PHGDH, PI4KA, PIN1, PIP4K2A, PIP4K2B, PKM2, PKP4, PLCB1, PLD3, PLEC1, PLP1, PLXNA1, PPFIA1, PPFIA2, PPFIA3, PPFIA4, PPIA, PPP1CA, PPP1CB, PPP1R12A, PPP1R7, PPP1R9B, PPP2R1A, PPP3CA, PPP3CB, PRDX1, PRDX2, PRDX5, PRKAR1A, PRKAR2A, PRKAR2B, PRKCG, PRKDC, PRPH, PSD3, PTK2B, PTPN11, PTPN23, PTPRD, PTPRS, PTPRZ1, PURA, PYCRL, PYGB, QDPR, RAB10, RAB13, RAB15, RAB1A, RAB1B, RAB35, RAB3A, RAB3GAP2, RAB5B, RAB5C, RAB6A, RAB6B, RAB7A, RAC1, RALA, RAP1A, RAP1GDS1, RAPGEF2, RAPGEF4, RASAL1, RBX1, RGS7, RHOG, RIMBP2, RIMS1, ROCK2, RPH3A, RPL12, RPL38, RPL7, RPL8, RPLP0, RPN1, RPS13, RPS14, RPS18, RPS19, RPS25, RPS27, RPS3, RTN1, RTN4, RUFY3, RYR2, SACM1L, SAMM50, SBF1, C9orf126, SCCPDH, SCFD1, SCRIB, SCRN1, SEC22B, SEPT10, SEPT11, SEPT2, SEPT3, SEPT4, SEPT5, SEPT6, SEPT7, SEPT8, SEPT9, SFN, SFXN1, SFXN3, SFXN5, SGIP1, SH3GL1, SH3GL2, SH3GLB2, SHANK1, SHANK2, SHANK3, SIRPA, SLC1A3, SLC25A11, SLC25A12, SLC25A13, SLC25A18, SLC25A22, SLC25A26, SLC25A3, SLC25A4, SLC25A5, SLC27A4, SNAP25, SNAP91, SND1, SNTA1, SNTB2, SNX27, SNX4, SORBS1, SORBS2, SPECC1, SPIRE1, SPTAN1, SPTB, SPTBN1, SPTBN2, SPTBN4, SRC, SNIP, SRI, SRPR, STIP1, STOM, STX1A, STX1B, STX7, STXBP1, STXBP3, SUCLA2, SYN1, SYN2, SYN3, SYNGAP1, SYNGR3, SYNJ1, SYNPO, SYP, SYT1, SYT5, SYT7, TAGLN3, TAOK1, TAOK2, TBC1D24, TBCB, TCP1, TFAM, TJP1, TJP2, TLN1, TLN2, TMOD1, TMOD2, TNC, TNR, TOLLIP, TOM1L2, TOMM20, TOMM70A, TPI1, TPM1, TPM3, TPM4, TPPP, TRAP1, TRAPPC3, NIBP, TRIM2, TRIM3, TTC7B, TUBA1A, TUBA1B, TUBA4A, TUBB2A, TUBB2B, TUBB2C, TUBB3, TUBB4, TUFM, TXNL1, UBA1, UBC, UBE2N, UBE2V2, UCHL1, UNC13A, UQCRC1, UQCRC2, VAMP2, VAPA, VAPB, VCAN, VCL, VCP, VDAC1, VDAC2, VDAC3, VIM, VPS11, VPS35, VPS52, VSNL1, WASF1, WASF3, WASL, WDR7, YES1, YWHAB, YWHAE, YWHAG, YWHAH, YWHAQ, YWHAZ.

NMDA RC, total genes = 181

ABLIM1, ACO2, ACTN2, ACTN3, ACTN4, ADAM22, AKAP5, AKAP9, AKT2, ALDOC, APPL1, ARC, ARF3, ARNT, ARPC2, ARPC3, ARPC4, ATG16L1, ATP1A1, ATP1A3, ATP2B4, ATP5A1, ATP5C1, ATP6V0D1, ATP6V1A, BAD, BSN, CACNG2, CALB2, CALM1, CAMK2A, CAMK2B, CAPZA2, CAPZB, CDH2, CIT, CLTC, CSE1L, CTNNB1, CTTN, DBN1, DLAT, DLG1, DLG2, DLG3, DLG4, DLGAP1, DNM1, DPYSL2, DSG1, DUSP4,

EHMT2, FABP5, FAM171A1, FGD4, FLNC, FUS, GAP43, GAPDH, C21orf66, GLUL, GNAS, GNB1, GNB2, GNB2L1, GNB4, GOT2, GRB2, GRIK2, GRIN1, GRIN2A, GRIN2B, GRM1, GRM5, GSK3B, GSN, HOMER1, HRAS, HSPA1B, INA, IRS1, KALRN, KLC2, L1CAM, LCA5, LDHA, LDHB, LGI1, LIN7A, LMNB1, MAP2, MAP2K1, MAP2K2, MAP2K3, MAP2K7, MAPK1, MAPK10, MAPK3, MAPK8IP1, MBP, MOG, MPP2, MPP3, MYH10, MYH11, MYH6, MYH9, MYL6, ROCK1, MYO1B, MYO5A, NDUFV2, NEFL, NEFM, NF1, NOS1, NSF, PDPK1, PFKL, PHB2, PIK3CA, PKLR, PLA2G4A, PLCB1, PLCG1, PLP1, PPP1CC, PPP2CA, PPP2R1A, PPP3CA, PPP5C, PRDX1, PRDX2, PRDX6, PRKACB, PRKAR2B, PRKCB1, PRKCE, PRKCG, PTK2B, PTPN11, PTPN5, RAB2A, RAB37, RAB3A, RAB6A, RAC1, RAF1, RALA, RAN, RAP2A, RPL13, RPL13A, RPL7, RPS6KA3, RTN1, SERPINA3, SHANK1, SHANK2, SLC1A2, SLC25A12, SLC25A22, SLC25A4, SLC25A5, SLMAP, SNAP25, SPINK5, SPTBN1, SRC, STK39, STX1A, STXBP1, SYNGAP1, SYNGR1, SYT1, TJP1, TP53BP1, TPI1, TUBA1A, VDAC1, VDAC2, VEGFA, YWHAE, YWHAG, YWHAH, YWHAZ.

mGluR5, total genes = 50,

SEPT5, SEPT7, ACAN, ADD1, ADD2, ALDOA, ALDOC, AP2B1, ATP1A1, ATP1A2, ATP1A3, ATP1B1, ATP2B1, BSN, CACNA1A, CALB2, CALM1, CIT, CLTC, CSNK2A1, DNM1, DNM2, GDI1, GNAO1, GNAQ, GRIN2A, GRIP1, GRM5, HOMER1, ITPR1, L1CAM, MAP1A, MAP1B, MAP2, NSF, PLCB1, RAB10, RAB3A, RPH3A, SHANK1, SLC12A5, SLC4A4, SP140, SYNGR1, SYNJ1, SYNPO, VAMP2, YWHAB, YWHAG, YWHAH, YWHAQ, YWHAZ.

AMPA RC, total genes = 7,

ATP1A1, DSP, GRIA1, GRIA2, GRIA3, GRIA4, PLP1, STXBP1, TUBA1A

References

1000 Genomes Project Consortium. (2010). A map of human genome variation from population-scale sequencing. *Nature, 467*(7319), 1061-1073.

Abecasis, G. R., Cherny, S. S., Cookson, W. O., & Cardon, Lon R. (2002). Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nature Genetics, 30*(1), 97-101. doi: 10.1038/ng786

Abraham, C., & Cho, J. (2009a). Interleukin-23/Th17 pathways and inflammatory bowel disease. *Inflammatory bowel diseases, 15*(7), 1090-1100.

Abraham, C., & Cho, J. H. (2009b). IL-23 and autoimmunity: new insights into the pathogenesis of inflammatory bowel disease. *Annual review of medicine, 60*, 97-110.

Adrian, E. (1928). *The basis of sensation, the action of the sense organs.* New York: Norton.

Alberts, B. (1998). The cell as a collection of protein machines: preparing the next generation of molecular biologists. *Cell, 92*(3), 291-294.

Allen, H. L., Estrada, K., Lettre, G., Berndt, S. I., Weedon, M. N., Rivadeneira, F., . . . Hayward, C. (2010). Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature Genetics, 467*(7317), 832-838.

Altshuler, D., Hirschhorn, J. N., Klannemark, M., Lindgren, C. M., Vohl, M. C., Nemesh, J., . . . Lander, E. S. (2000). The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nature genetics, 26*(1), 76-80.

American Psychiatric Association. (2000). Diagnostic and Statistic Manual of Mental Disorders (4th Revision: DSM-IV-TR).

Anggono, V., & Huganir, R. L. (2012). Regulation of AMPA receptor trafficking and synaptic plasticity. *Curr Opin Neurobiol, 22*(3), 461-469. doi: 10.1016/j.conb.2011.12.006

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., . . . Sherlock, G. (2000). Gene Ontology: tool for the unification of biology. *Nature genetics, 25*(1), 4.

Askland, Kathleen, Read, Cynthia, & Moore, Jason. (2009). Pathways-based analyses of whole-genome association study data in bipolar disorder reveal genes mediating ion channel activity and synaptic neurotransmission. *Human genetics, 125*(1), 63-79.

Attucci, S., Carla, V., Mannaioni, G., & Moroni, F. (2001). Activation of type 5 metabotropic glutamate receptors enhances NMDA responses in mice cortical wedges. *Br J Pharmacol, 132*(4), 799-806. doi: 10.1038/sj.bjp.0703904

Aulchenko, Y. S., Ripke, S., Isaacs, A., & van Duijn, C. M. (2007). GenABEL: an R library for genome-wide association analysis. *Bioinformatics, 23*(10), 1294-1296. doi: 10.1093/bioinformatics/btm108

Bacanu, S. A., Chen, J., Sun, J., Richardson, K., Lai, C. Q., Zhao, Z., O'Donovan, M. C., . . . Chen, X. (2013). Functional SNPs are enriched for schizophrenia association signals. *Molecular psychiatry.*

Baker, D.W. , Parker, R.M. , Williams, M.V., & Clark, W.S. (1998). Health literacy and the risk of hospital admission. *J Gen Intern Med, 13*, 791–798.

Bakken, T. E., Roddey, J. C., Djurovic, S., Akshoomoff, N., Amaral, D. G., Bloss, C. S., . . . Dale, A. M. (2012). Association of common genetic variants in GPCPD1 with scaling of

visual cortical surface area in humans. *Proceedings of the National Academy of Sciences, 109*(10), 3985-3990.

Banks, G. C., Batchelor, J. H., & McDaniel, M. A. (2010). Smarter people are (a bit) more symmetrical: A meta-analysis of the relationship between intelligence and fluctuating asymmetry. *Intelligence, 38*(4), 393-401.

Barnett, J. H., Scoriels, L., & Munafò, M. R. (2008). Meta-Analysis of the Cognitive Effects of the Catechol-O-Methyltransferase Gene Val158/108Met Polymorphism. *Biological psychiatry, 64*(2), 137-144.

Bartel, D. P. (2009). MicroRNAs: target recognition and regulatory functions. *Cell, 136*(2), 17.

Basel-Vanagaite, L., Attia, R., Yahav, M., Ferland, R. J., Anteki, L., Walsh, C. A., . . . Shohat, M. (2006). The CC2D1A, a member of a new gene family with C2 domains, is involved in autosomal recessive non-syndromic mental retardation. *Journal of medical genetics, 43*(43), 203-210.

Bashi, J. (1977). Effects of inbreeding on cognitive performance. *Nature, 266*, 440 - 442.

Batty, G. D., Wennerstad, K. M., Smith, G. D., Gunnell, D., Deary, I. J., Tynelius, P., & Rasmussen, F. (2009). IQ in early adulthood and mortality by middle age: cohort study of 1 million Swedish men. *Epidemiology, 20*(1), 100-109.

Batty, G.D. , Deary, I., & Macintyre, S. (2006). Childhood IQ and life course socioeconomic position in relation to alcohol induced hangovers in adulthood: the Aberdeen children of the 1950s study. *J Epidemiol Community Health, 60*(10), 872–874.

Bayés, À., Collins, M. O., Croning, M. D., van de Lagemaat, L. N., Choudhary, J. S., & Grant, S. G. (2012). Comparative study of human and mouse postsynaptic proteomes finds high compositional conservation and abundance differences for key synaptic proteins. *PloS one, 7*(10), e46683.

Bayes, A., van de Lagemaat, L. N., Collins, M. O., Croning, M. D., Whittle, I. R., Choudhary, J. S., & Grant, S. G. (2010). Characterization of the proteome, diseases and evolution of the human postsynaptic density. *Nat. Neurosci., 14*(1), 19-21. doi: 10.1038/nn.2719

Benyamin, B., Perola, M., Cornes, B. K., Madden, P. A., Palotie, A., Nyholt, D. R., . . . Visscher, P. M. (2008). Within-family outliers: segregating alleles or environmental effects? A linkage analysis of height from 5815 sibling pairs. *European Journal of Human Genetics, 16*(4), 516-524.

Benyamin, B., Pourcain, B., Davis, O. S., Davies, G., Hansell, N. K., Brion, M. J., . . . Visscher, P. M. (2013). Childhood intelligence is heritable, highly polygenic and associated with FNBP1L. *Mol. Psychiatry, 19*, 253–258. doi: 10.1038/mp.2012.184

Betel, D., Wilson, M., Gabow, A., Marks, D. S., & Sander, C. . (2008). The microRNA. org resource: targets and expression. *Nucleic acids research, 36*, 149-153.

Binet, A. (1905). New methods for the diagnosis of the intellectual level of subnormals. *L'Annee Psychologique, 12* 191–244.

Bittles, A.H. (2001). Consanguinity and its relevance to clinical genetics. *Clinical genetics, 60*(2), 89-98.

Bliss, T. V., & Lømo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *The Journal of physiology, 232*(2), 331-356.

Bouchard, T. J., Lykken, D. T., McGue, M., Segal, N. L., & Tellegen, A. (1990). Sources of human psychological differences: The Minnesota study of twins reared apart. *Science, 250*(4978), 223-228.

Bouchard, T. J., & McGue, M. (1981). Familial studies of intelligence: A review. *Science, 212*(4498), 1055-1059.

Brennecke, J., Stark, A., Russell, R. B., & Cohen, S. M. . (2005). Principles of microRNA–target recognition. *PLoS biology, 3*, e85.

Burt, C. (1917). *The distribution and relations of educational abilities*. London: Darling & Son.

Burt, C. (1966). The genetic determination of differences in intelligence. *British Journal of Psychology, 57*(1-2), 137–153.

Çalışkan, M., Chong, J. X., Uricchio, L., Anderson, R., Chen, P., Sougnez, C., . . . Ober, C. (2011). Exome sequencing reveals a novel mutation for autosomal recessive nonsyndromic mental retardation in the TECR gene on chromosome 19p13. *Human molecular genetics, 20*(7), 1285-1289.

Calvin, C. M., Deary, I. J., Fenton, C., Roberts, B. A., Der, G., Leckenby, N., & Batty, G. D. (2011). Intelligence in youth and all-cause-mortality: systematic review with meta-analysis. *International journal of epidemiology, 40*(3), 626-644.

Carroll, John B. (1993). *Human cognitive abilities: A survey of factor-analytic studies*. New York, NY, USA: Cambridge University Press.

Cattell, R. B. (1963). Theory of fluid and crystallized intelligence: A critical experiment. *Journal of educational psychology, 54*(1), 1-22.

Cattell, R. B. (1971). *Abilities: Their Structure, Growth, and Action*: Houghton Mifflin Company.

Cattell, R. B. (1987). *Intelligence: Its Structure, Growth and Action: Its Structure, Growth and Action*: Elsevier.

Cattell, R.B., & Cattell, A.K.S. (1960). *The individual or group Culture Fair Intelligence Test*. Champaign, Ill: IPAT.

Chabris, C.F., Hebert, B.M., Benjamin, D.J., Beauchamp, J.P., Cesarini, D., van der Loos, M.J.H.M., . . . Laibson, D. I. (2011). Most reported genetic associations with general intelligence are probably false positives. *Psychol. Sci., 23* (11), 1314-1323.

Chowdhury, S., Shepherd, J. D., Okuno, H., Lyford, G., Petralia, R. S., Plath, N., . . . Worley, P. F. (2006). Arc/Arg3. 1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. . *Neuron, 52*(3), 14.

Collingridge, G.L. (1985). Long-term potentiation in the hippocampus - mechanisms of initiation and modulation by neurotransmitters. *Trends in Pharmacological Sciences, 6*, 4.

Collins, M. O., Husi, H., Yu, L., Brandon, J. M., Anderson, C. N., Blackstock, W. P., . . . Grant, S. G. (2006). Molecular characterization and comparison of the components and multiprotein complexes in the postsynaptic proteome. *J. Neurochem., 97*(1), 16-23. doi: 10.1111/j.1471-4159.2005.03507.x

Comijs, H. C., Kriegsman, D. M., Dik, M. G., Deeg, D. J., Jonker, C., & Stalman, W. A. (2009). Somatic chronic diseases and 6-year change in cognitive functioning among older persons. *Archives of gerontology and geriatrics, 48*(2), 191-196.

Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G., . . . Pericak-Vance, M. A. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science, 261*(5123), 921-923.

Cornes, B. K., Medland, S. E., Ferreira, M. A., Morley, K. I., Duffy, D. L., Heijmans, B. T., . . . Martin, N. G. . (2005). Sex-limited genome-wide linkage scan for body mass index in an unselected sample of 933 Australian twin families. *Twin Research and Human Genetics, 8*(06), 616-632.

Davies, G., Tenesa, A., Payton, A., Yang, J., Harris, S. E., Liewald, D., . . . Deary, I. J. (2011). Genome-wide association studies establish that human intelligence is highly heritable and polygenic. *Molecular Psychiatry, 16*(10), 996-1005. doi: 10.1038/mp.2011.85

de Zubicaray, G. I., Chiang, M. C., McMahon, K. L., Shattuck, D. W., Toga, A. W., Martin, N. G., . . . Thompson, P. M. (2008). Meeting the Challenges of Neuroimaging Genetics. *Brain Imaging Behav, 2*(4), 258-263. doi: 10.1007/s11682-008-9029-0

Deary, I. J. (2012). Looking for 'System Integrity'in Cognitive Epidemiology. *Gerontology, 58*(6), 545-553.

Deary, I. J., & Der, G. (2005). Reaction time explains IQ's association with death. *Psychological Science, 16*(1), 64-69.

Deary, I. J., Gow, A. J., Pattie, A., & Starr, J. M. (2012). Cohort profile: the Lothian Birth Cohorts of 1921 and 1936. *Int J Epidemiol, 41*(6), 1576-1584. doi: 10.1093/ije/dyr197

Deary, I. J., Strand, S., Smith, P., & Fernandes, C. (2007a). Intelligence and educational achievement. *Intelligence, 35*(1), 13-21.

Deary, I. J., Taylor, M. D., Hart, C. L., Wilson, V., Smith, G. D., Blane, D., & Starr, J. M. (2005). Intergenerational social mobility and mid-life status attainment: Influences of childhood intelligence, childhood social factors, and education. *Intelligence, 33*(5), 455-472.

Deary, I. J., Whiteman, M. C., Starr, J. M., Whalley, L. J., & Fox, H. C. (2004). The impact of childhood intelligence on later life: Following up the Scottish Mental Surveys of 1932 and 1947. *Journal of Personality and Social Psychology, 86*(1), 130-147. doi: 10.1037/0022-3514.86.1.130

Deary, I.J., Gow, A. J., Taylor, M. D., Corley, J., Brett, C., Wilson, V., . . . Starr, J. M. (2007b). The Lothian Birth Cohort 1936: a study to examine influences on cognitive ageing from age 11 to age 70 and beyond. *BMC Geriatr., 7*, 28.

Deary, I.J., Whalley, L.J., & Starr, J.M. (2009). *A Lifetime of Intelligence, Follow-up Studies of the Scottish Mental Surveys of 1932 and 1947*. Washington, DC: American Psychological Association.

Devlin, B., & Roeder, K. (1999). Genomic control for association studies. *Biometrics, 55*(4), 997-1004.

Dingledine, R. , Hynes, M. A., & King, G. L. (1986). Involvement of N-methyl-D-aspartate receptors in epileptiform bursting in the rat hippocampal slice. *The Journal of physiology, 380*(1), 175-189.

Dong, C. (2008). TH17 cells in development: an updated view of their molecular identity and genetic programming. *Nature Reviews Immunology, 8*(5), 337-348.

Edmonds, C. J., Isaacs, E. B., Visscher, P. M., Rogers, M., Lanigan, J., Singhal, A., & Deary, I. J. (2008). Inspection time and cognitive abilities in twins aged 7 to 17 years: Age-related changes, heritability and genetic covariance. *Intelligence, 36*(3), 210-225.

Edwards, S. L., Beesley, J., French, J. D., & Dunning, A. M. (2013). Beyond GWASs: illuminating the dark road from association to function. *The American Journal of Human Genetics, 93*(5), 779-797.

Egger, G., Liang, G., Aparicio, A., & Jones, P. A. (2004). Epigenetics in human disease and prospects for epigenetic therapy. *Nature, 429*(6990), 6.

Emes, R. D., & Grant, S. G. (2012). Evolution of synapse complexity and diversity. *Annu Rev Neurosci, 35*, 111-131. doi: 10.1146/annurev-neuro-062111-150433

Emes, R. D., Pocklington, A. J., Anderson, C. N., Bayes, A., Collins, M. O., Vickers, C. A., . . . Grant, S. G. (2008). Evolutionary expansion and anatomical specialization of synapse proteome complexity. *Nature Neuroscience, 11*(7), 799-806. doi: 10.1038/nn.2135

Ersland, K. M., Christoforou, A., Stansberg, C., Espeseth, T., Mattheisen, M., Mattingsdal, M., . . . Le Hellard, S. (2012). Gene-based analysis of regionally enriched cortical

genes in GWAS data sets of cognitive traits and psychiatric disorders. *PLoS One, 7*(2), e31687. doi: 10.1371/journal.pone.0031687

Espeseth, T., Christoforou, A., Lundervold, A. J., Steen, V. M., Le Hellard, S., & Reinvang, I. (2012). Imaging and cognitive genetics: the Norwegian Cognitive NeuroGenetics sample. *Twin Res Hum Genet, 15*(3), 442-452. doi: 10.1017/thg.2012.8

Falconer, D. S., & MacKay, T. F. C. (1996). *Introduction to Quantatative Genetics*. UK: Longman.

Farr, C. D., Gafken, P. R., Norbeck, A. D., Doneanu, C. E., Stapels, M. D., Barofsky, D. F., . . . Saugstad, J. A. (2004). Proteomic analysis of native metabotropic glutamate receptor 5 protein complexes reveals novel molecular constituents. *J Neurochem, 91*(2), 438-450. doi: 10.1111/j.1471-4159.2004.02735.x

Fernandez, E., Collins, M. O., Uren, R. T., Kopanitsa, M. V., Komiyama, N. H., Croning, M. D., . . . Grant, S. G. (2009). Targeted tandem affinity purification of PSD-95 recovers core postsynaptic complexes and schizophrenia susceptibility proteins. *Mol Syst Biol, 5*, 269. doi: 10.1038/msb.2009.27

Fisher, R. A. (1919). The Correlation between Relatives on the Supposition of Mendelian Inheritance. *Transactions of the Royal Society of Edinburgh, 52*(02), 399-433.

Flint, J., & Munafò, M. R. (2007). The endophenotype concept in psychiatric genetics. *Psychological medicine, 37*(02), 163-180.

Flynn, J. R. (1999). Searching for justice: the discovery of IQ gains over time. *American Psychologist, 54*(1), 5-20.

Föcking, M., Lopez, L. M., English, J. A., Dicker, P., Wolff, A., Brindley, E., . . . Cotter, D. R. (2014). Proteomic and genomic evidence implicates the postsynaptic density in schizophrenia. *Molecular psychiatry*, 1-9.

Frank, R. A., McRae, A. F., Pocklington, A. J., van de Lagemaat, L. N., Navarro, P., Croning, M. D., . . . Grant, S. G. (2011). Clustered coding variants in the glutamate receptor complexes of individuals with schizophrenia and bipolar disorder. *PLoS One, 6*(4), e19011. doi: 10.1371/journal.pone.0019011

Fridley, B. L., & Biernacka, J. M. (2011). Gene set analysis of SNP data: benefits, challenges, and future directions. *European Journal of Human Genetics, 19*(8), 837-843.

Friedman, R. C., Farh, K. K. H., Burge, C. B., & Bartel, D. P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome research, 19*(1), 92-105.

Fromer, M., Pocklington, A. J., Kavanagh, D. H., Williams, H. J., Dwyer, S., Gormley, P., . . . O'Donovan, M. C. (2014). De novo mutations in schizophrenia implicate synaptic networks. *Nature, 506*(7487), 179–184.

Galsworthy, M. J., Paya-Cano, J. L., Liu, L., Monleón, S., Gregoryan, G., Fernandes, C., . . . Plomin, R. . (2005). Assessing reliability, heritability and general cognitive ability in a battery of cognitive tasks for laboratory mice. *Behavior genetics, 35*(5), 675-692.

Galton, F. (1892). *Hereditary Genius*: Macmillan and Company.

Galton, F. (1883). Inquiries into human faculty. London: Dent.

Gardner, H. (1983). *Frames of Mind: The theory of multiple Intelligences*. New York: Basic Books.

Garshasbi, M., Hadavi, V., Habibi, H., Kahrizi, K., Kariminejad, R., Behjati, F., . . . Kuss, A. W. (2008). A Defect in the *TUSC3* Gene Is Associated with Autosomal Recessive Mental Retardation. *The American Journal of Human Genetics, 82*(5), 1158-1164.

Gibbs, J. Raphael, Brug, Marcel P. van der, Hernandez, Dena G., Traynor, Bryan J., Nalls, Michael A., Lai, Shiao-Lin, . . . Singleton, Andrew B. (2010). Abundant quantitative trait loci exist for DNA methylation and gene expression in human brain. . *PLoS genetics, 6*(5).

Goeman, J. J., & Bühlmann, P. (2007). Analyzing gene expression data in terms of gene sets: methodological issues. *Bioinformatics, 23*(8), 980-987.

Golding, J., Pembrey, M., & Jones, R. (2001). ALSPAC--the Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatric and perinatal epidemiology, 15*(1), 74-87.

Gottfredson, L. S. (1997). Why g matters: The complexity of everyday life. *Intelligence, 24*(1), 79-132.

Goudriaan, A., de Leeuw, C., Ripke, S., Hultman, C. M., Sklar, P., Sullivan, P. F., . . . Verheijen, M. H. (2013). Specific glial functions contribute to schizophrenia susceptibility. *Schizophrenia bulletin, sbt109*.

Gould, S. J. (1981). *The mismeasure of man*. New York: W. W. Norton.

Grant, S. G. (2012). Synaptopathies: diseases of the synaptome. *Current opinion in neurobiology, 22*(3), 522-529.

Guildford, J.P., Christensen, P.R., Merrifield, P.R, & Wilson, J, C. (1978). *Alternate uses: manual of instructions and interpretation* Orange, Calif: Sheridan Psychological Service.

Guzowski, J. F., Lyford, G. L., Stevenson, G. D., Houston, F. P., McGaugh, J. L., Worley, P. F., & Barnes, C. A. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *The Journal of neuroscience, 20*(11), 8.

Han, X., Chen, M., Wang, F., Windrem, M., Wang, S., Shanz, S., . . . Nedergaard, M. . (2013). Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. *Cell Stem Cell, 12*(3), 342-353.

Hashimoto, S., Boissel, S., Zarhrate, M., Rio, M., Munnich, A., Egly, J. M., & Colleaux, L. (2011). MED23 mutation links intellectual disability to dysregulation of immediate early gene expression. *Science, 333*(6046), 1161-1163.

Haworth, C. M. A., Wright, M. J., Luciano, M., Martin, N. G., De Geus, E. J. C., Van Beijsterveldt, C. E. M., . . . Plomin, R. (2010). The heritability of general cognitive ability increases linearly from childhood to young adulthood. *Molecular psychiatry, 15*(11), 1112-1120.

Hawrylycz, M. J., Lein, E. S., Guillozet-Bongaarts, A. L., Shen, E. H., Ng, L., Miller, J. A., . . . Royall, J. J. (2012). An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature, 489*(7416), 391-399.

Hebb, D. O. . (1949). *The organization of behavior: A neuropsychological theory*. New York: Wiley.

Heim, A. (1970). *AH 4 Group Test of General Intelligence Manual, .* Windsor, UK: NFER Publishing Company.

Hermans, E., & Challiss, R. A. (2001). Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors. *Biochem J, 359*(Pt 3), 465-484.

Higgins, J. J., Pucilowska, J., Lombardi, R. Q., & Rooney, J. P. (2004). A mutation in a novel ATP-dependent Lon protease gene in a kindred with mild mental retardation. *Neurology, 63*(10), 1927-1931.

Hill, W. D., Davies, G., van de Lagemaat, L. N., Christoforou, A., Marioni, R.E., Fernandes, C.P.D., . . . Deary, I. J. (2014a). Human cognitive ability is influenced by genetic variation in components of postsynaptic signalling complexes assembled by NMDA receptors and MAGUK proteins. *Transl. Psychiatry, 4*(1), e341.

Hill, W. D., de Leeuw, C., Davies, G. , Liewald, D.C.M., Payton, A., Craig, L.C., . . . Deary, I. J. (2014b). Functional Gene Group Analysis Indicates No Role for Heterotrimeric G Proteins in Cognitive Ability. *PloS one, 9*(3).

Hoekstra, R. A., Bartels, M., & Boomsma, D. I. (2007). Longitudinal genetic study of verbal and nonverbal IQ from early childhood to young adulthood. *Learning and Individual Differences, 17*(2), 97-114.

Holmans, P., Green, E. K., Pahwa, J. S., Ferreira, M. A., Purcell, S. M., Sklar, P., . . . Craddock, N. (2009). Gene ontology analysis of GWA study data sets provides insights into the biology of bipolar disorder. *The American Journal of Human Genetics, 85*(1), 13-24.

Horn, J. L. (1985). Remodeleling old models of intelligence. In B. B. Wolman (Ed.), *Handbook of intelligence: Theories, measurements, and applications.* (pp. 267-300). New York: Wiley.

Horn, J. L. (1994). Theory of fluid and crystallised intelligence. In Robert J. Sternberg (Ed.), *Encyclopedia of human intelligence*. New York: Macmillan.

Houlihan, L. M., Harris, S. E., Luciano, M., Gow, A. J., Starr, J. M., Visscher, P. M., & Deary, I. J. (2009). Replication study of candidate genes for cognitive abilities: the Lothian Birth Cohort 1936. *Genes, Brain and Behavior, 8*(2), 238-247.

Hu, H., Eggers, K., Chen, W., Garshasbi, M., Motazacker, M. M., Wrogemann, K., . . . Kuss, A. W. (2011). *ST3GAL3* Mutations Impair the Development of Higher Cognitive Functions. *The American Journal of Human Genetics, 89*(3), 407-414.

Husi, H., & Grant, S. G. (2001). Isolation of 2000-kDa complexes of N-methyl-D-aspartate receptor and postsynaptic density 95 from mouse brain. *Journal of neurochemistry, 77*(1), 281-291.

Husi, H., Ward, M. A., Choudhary, J. S., Blackstock, W. P., & Grant, S. G. (2000). Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nature Neuroscience, 3*(7), 661-669. doi: 10.1038/76615

Ioannidis, J. P. (2008). Why most discovered true associations are inflated. *Epidemiology, 19*(5), 640-648.

Jackson, Douglas N. (1984). *Multidimensional Aptitude Battery manual.* (Vol. 1). Port Huron, MI: Research Psychologists Press.

Jacobs, N, van Os, J, Derom, C, & Thiery, E. (2007). Heritability of intelligence. Twin Research and Human Genetics. *10, S1*(11-14).

Jahanshad, N., Kochunov, P. V., Sprooten, E., Mandl, R. C., Nichols, T. E., Almasy, L., . . . Glahn, D. C. (2013). Multi-site genetic analysis of diffusion images and voxelwise heritability analysis: A pilot project of the ENIGMA–DTI working group. *Neuroimage, 81*(455-469).

Jencks, C. (1979). *Who gets ahead?: The determinants of economic success in America*. New York, NY: Basic Books.

Jensen, A. R. (1998). *The g factor: The science of mental ability.* Westport: CT: Praeger.

Jia, Z., Lu, Y., Henderson, J., Taverna, F., Romano, C., Abramow-Newerly, W., . . . Roder, J. (1998). Selective abolition of the NMDA component of long-term potentiation in mice lacking mGluR5. *Learn Mem, 5*(4-5), 331-343.

Johnson, W., & Bouchard Jr, T. J. (2005a). Constructive replication of the visual–perceptual-image rotation model in Thurstone's (1941) battery of 60 tests of mental ability. *Intelligence, 33*(4), 417-430.

Johnson, W., & Bouchard Jr, T. J. (2005b). The structure of human intelligence: It is verbal, perceptual, and image rotation (VPR), not fluid and crystallized. *Intelligence, 33*(4), 393-416.

Johnson, W., Bouchard Jr, T. J., Krueger, R. F., McGue, M., & & Gottesman, I. I. (2004). Just one *g*: consistent results from three test batteries. *Intelligence, 32*(1), 95-107.

Johnson, W., Bouchard Jr, T. J., McGue, M., Segal, N. L., Tellegen, A., Keyes, M., & Gottesman, I. I. (2007). Genetic and environmental influences on the Verbal-

Perceptual-Image Rotation (VPR) model of the structure of mental abilities in the Minnesota study of twins reared apart. *Intelligence, 35*(6), 542-562.

Johnson, W., te Nijenhuis, J., & Bouchard, T. J. (2008). Still just 1 g: Consistent results from five test batteries. *Intelligence, 36*(1), 81-95. doi: Doi 10.1016/J.Intell.2007.06.001

Jung, R. E., & Haier, R. J. (2007). The Parieto-Frontal Integration Theory (P-FIT) of intelligence: converging neuroimaging evidence. *Behavioral and Brain Sciences, 30*(02), 135-154.

Kerem, B. S., Rommens, J. M., Buchanan, J. A., Markiewicz, D., Cox, T. K., Chakravarti, A., . . . Tsui, L. C. . (1989). Identification of the cystic fibrosis gene: genetic analysis. *Science, 245*(4922), 1073-1080.

Khandaker, G. M., Barnett, J. H., White, I. R., & Jones, P. B. (2011). A quantitative meta-analysis of population-based studies of premorbid intelligence and schizophrenia. *Schizophrenia research, 132*(2), 7.

Kim, C. H., Lee, J., Lee, J. Y., & Roche, K. W. (2008). Metabotropic glutamate receptors: phosphorylation and receptor signaling. *J Neurosci Res, 86*(1), 1-10. doi: 10.1002/jnr.21437

Kirov, G., Pocklington, A. J., Holmans, P., Ivanov, D., Ikeda, M., Ruderfer, D., . . . Owen, M. J. (2012). De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. *Molecular Psychiatry, 17*(2), 142-153. doi: 10.1038/mp.2011.154

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kåreholt, I., Winblad, B., . . . Nissinen, A. (2005). Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Archives of neurology, 62*(10), 1556-1560.

Kovas, Y., & Plomin, R. (2006). Generalist genes: implications for the cognitive sciences. *Trends in cognitive sciences, 10*(5), 198-203.

Lee, P. H., O'Dushlaine, C., Thomas, B., & Purcell, S. M. (2012a). INRICH: interval-based enrichment analysis for genome-wide association studies. *Bioinformatics, 28*(13), 2.

Lee, P. H., Perlis, R. H., Jung, J. Y., Byrne, E. M., Rueckert, E., Siburian, R., . . . Smoller, J. W. . (2012b). Multi-locus genome-wide association analysis supports the role of glutamatergic synaptic transmission in the etiology of major depressive disorder. *Translational psychiatry, 2*(10).

Lencz, T., Knowles, E., Davies, G., Guha, S., Liewald, D. C., Starr, J. M., . . . Malhotra, A. K. (2013). Molecular genetic evidence for overlap between general cognitive ability and risk for schizophrenia: a report from the Cognitive Genomics consorTium (COGENT). *Molecular psychiatry*.

Lezak, MD, Howieson, D.B., & Loring, D.W. (2004). *Neuropsychological Assessment* (4 ed.). New York: Oxford University Press, USA.

Li, M. X., Gui, H. S., Kwan, J. S., & Sham, P. C. (2011). GATES: a rapid and powerful gene-based association test using extended Simes procedure. *The American Journal of Human Genetics, 88*(3), 283-293.

Li, Y., Willer, C. J., Ding, J., Scheet, P., & Abecasis, G. R. (2010). MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol, 34*(8), 816-834. doi: 10.1002/gepi.20533

Lips, E. S. , Cornelisse, L. N., Toonen, R. F., Min, J. L., Hultman, C. M., the International Schizophrenia Consortium, . . . Posthuma, D. (2011). Functional gene group analysis identifies synaptic gene groups as risk factor for schizophrenia. *Molecular psychiatry, 17*(10), 10.

Lips, ES., Kooyman, M., de Leeuw, C. , van Bochoven, AE. , & Posthuma, D. . (submitted). JAG: a tool to evaluate the role of gene-sets in complex traits.

Liu, J. Z., McRae, A. F., Nyholt, D. R., Medland, S. E., Wray, N. R., Brown, K. M., . . . Macgregor, S. (2010). A versatile gene-based test for genome-wide association studies. *American Journal of Human Genetics, 87*(1), 139-145. doi: 10.1016/j.ajhg.2010.06.009

Loehlin, J. C. (1989). Partitioning environmental and genetic contributions to behavioral development. *American Psychologist, 44*(10), 1285-1292.

Lopez, L. M., Bastin, M. E., Maniega, S. M., Penke, L., Davies, G., Christoforou, A., . . . Deary, I. J. (2012). A genome-wide search for genetic influences and biological pathways related to the brain's white matter integrity. *Neurobiol Aging, 33*(8), 1847 e1841-1814. doi: 10.1016/j.neurobiolaging.2012.02.003

Maher, B. (2008). The case of the missing heritability. *Nature Genetics, 456*(7218), 18-21.

Major, J. T., Johnson, W., & Deary, I. J. (2012). Comparing models of intelligence in Project TALENT: The VPR model fits better than the CHC and Extended Gf-Gc models. *Intelligence, 40*(6), 543-559.

Manahan-Vaughan, D., Ngomba, R. T., Storto, M., Kulla, A., Catania, M. V., Chiechio, S., . . . Nicoletti, F. (2003). An increased expression of the mGlu5 receptor protein following LTP induction at the perforant path-dentate gyrus synapse in freely moving rats. *Neuropharmacology, 44*(1), 17-25.

Marioni, R. E. , Davies, G., Hayward, C., Liewald, D., Kerr, S. M., Campbell, A., . . . Deary, I. J. (2014a). Molecular genetic contributions to socioeconomic status and intelligence. *Intelligence, 44*, 26-32.

Marioni, R. E., Penke, L., Davies, G., Huffman, J. E., Hayward, C., & Deary, I. J. (2014b). The total burden of rare, non-synonymous exome genetic variants is not associated with childhood or late-life cognitive ability. *Proceedings of the Royal Society B: Biological Sciences, 281*(1781), 20140117.

Markram, H., & Segal, M. (1990). Acetylcholine potentiates responses to N-methyl-D-aspartate in the rat hippocampus. *Neuroscience letters, 113*(1), 62-65.

Martin, L.T., Fitzmaurice, G.M., Kindlon, D.J. , & Buka, S.L. (2004). Cognitive performance in childhood and early adult illness: A prospective cohort study. *J Epidemiol Community Health, 58* 674–679.

Matzel, L. D., Light, K. R., Wass, C., Colas-Zelin, D., Denman-Brice, A., Waddel, A. C., & Kolata, S. (2011). Longitudinal attentional engagement rescues mice from age-related cognitive declines and cognitive inflexibility. *Learning & memory, 18*(5), 345-356.

Maulik, P. K., Mascarenhas, M. N., Mathers, C. D., Dua, T., & Saxena, S. (2011). Prevalence of intellectual disability: a meta-analysis of population-based studies. *Research in developmental disabilities, 32*(2), 419-436.

McCartney, K., Harris, M. J., & Bernieri, F. . (1990). Growing up and growing apart: a developmental meta-analysis of twin studies. *Psychological bulletin, 107*(2), 226-237.

McDaniel, Michael A. (2005). Big-brained people are smarter: A meta-analysis of the relationship between in vivo brain volume and intelligence. *Intelligence, 33*(4), 337-346.

McIntosh, A. M., Gow, A., Luciano, M., Davies, G., Liewald, D. C., Harris, S. E., . . . Deary, I. J. (2013). Polygenic Risk for Schizophrenia Is Associated with Cognitive Change Between Childhood and Old Age. *Biol Psychiatry, 73*(10), 938-943. doi: 10.1016/j.biopsych.2013.01.011

Medland, S. E., Nyholt, D. R., Painter, J. N., McEvoy, B. P., McRae, A. F., Zhu, G., . . . Martin, N. G. (2009). Common variants in the trichohyalin gene are associated with straight

hair in Europeans. *American Journal of Human Genetics, 85*(5), 750-755. doi: 10.1016/j.ajhg.2009.10.009

Mendel, G. (1865). Versuche fiber Pflanzen-Hybriden. *Verh. Naturforsch. Ver. Brünn, 4*(3–47).

Migaud, M., Charlesworth, P., Dempster, M., Webster, L. C., Watabe, A. M., Makhinson, M., . . . Grant, S. G. (1998). Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature, 396*(6710), 433-439.

Mir, A., Kaufman, L., Noor, A., Motazacker, M. M., Jamil, T., Azam, M., . . . Vincent, J. B. (2009). Identification of Mutations in *TRAPPC9*, which Encodes the NIK-and IKK-β-Binding Protein, in Nonsyndromic Autosomal-Recessive Mental Retardation. *The American Journal of Human Genetics, 85*(6), 909-915.

Molinari, F., Rio, M., Meskenaite, V., Encha-Razavi, F., Augé, J., Bacq, D., . . . Colleaux, L. (2002). Truncating neurotrypsin mutation in autosomal recessive nonsyndromic mental retardation. *Science, 298*(5599), 1779-1781.

Mooney, M. A., Nigg, J. T., McWeeney, S. K., & Wilmot, B. (2014). Functional and genomic context in pathway analysis of GWAS data. *Trends in Genetics, 30*(9), 390-400.

Morris, R. G. M., Anderson, E., Lynch, G. A., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature, 319*, 774-776.

Motazacker, M. M., Rost, B. R., Hucho, T., Garshasbi, M., Kahrizi, K., Ullmann, R., . . . Kuss, A. W. (2007). A Defect in the Ionotropic Glutamate Receptor 6 Gene (*GRIK2*) Is Associated with Autosomal Recessive Mental Retardation. *The American Journal of Human Genetics, 81*(4), 792-798.

Mukherjee, S., & Manahan-Vaughan, D. (2013). Role of metabotropic glutamate receptors in persistent forms of hippocampal plasticity and learning. *Neuropharmacology, 66*, 65-81. doi: 10.1016/j.neuropharm.2012.06.005

Munafò, M. R., & Flint, J. (2014). The genetic architecture of psychophysiological phenotypes. *Psychophysiology, 51*(12), 1331-1332.

Musante, L., & Ropers, H. (2014). Genetics of recessive cognitive disorders. *Trends in Genetics, 30*(1), 32-39.

Muthen, B., Asparouhov, T., & Rebollo, I. (2006). Advances in behavioral genetics modeling using Mplus: applications of factor mixture modeling to twin data. *Twin Res Hum Genet, 9*(3), 313-324. doi: 10.1375/183242706777591317

Naisbitt, S., Kim, E., Tu, J. C., Xiao, B., Sala, C., Valtschanoff, J., . . . Sheng, M. . (1999). Shank, a novel family of postsynaptic density proteins that binds to the NMDA receptor/PSD-95/GKAP complex and cortactin. *Neuron, 23*(3), 569-582.

Najmabadi, H., Hu, H., Garshasbi, M., Zemojtel, T., Abedini, S. S., Chen, W., . . . Ropers, H. H. (2011). Deep sequencing reveals 50 novel genes for recessive cognitive disorders. *Nature, 478*(7367), 57-63.

Neale, M. C., Boker, S. M., Xie, G., & Maes, H. H. . (1999). Statistical Modelling. *Richmond, Virginia: Department of Psychiatry*.

Neale, M., & Cardon, L. . (1992). *Methodology for genetic studies of twins and families*: Springer.

Neisser, U., Boodoo, G., Bouchard Jr, T. J., Boykin, A. W., Brody, N., Ceci, S. J., . . . Urbina, S. (1996). Intelligence: Knowns and unknowns. *American psychologist, 51*(2), 77.

Nelson, H. E., & Willison, J. (1991). *National Adult Reading Test (NART) Test Manual* (2nd ed.). Windsor: NFER-Nelson.

Nichols, P. L. (1984). Familial mental retardation. *Behavior genetics, 14*(3), 161-170.

Nichols, R.C. (1978). Twin studies of ability, personality and interests. *Homo 29*(158–173).

Nithianantharajah, J., Komiyama, N. H., McKechanie, A., Johnstone, M., Blackwood, D. H., Clair, D. S., . . . Grant, S. G. (2013). Synaptic scaffold evolution generated components of vertebrate cognitive complexity. *Nature Neuroscience, 16*(1), 16-24. doi: 10.1038/nn.3276

O'Toole, B. I. (1990). Intelligence and behaviour and motor vehicle accident mortality. *Accident Analysis & Prevention, 22*(3), 211–221.

O'Toole, B. I. , & Stankov, L. (1992). Ultimate validity of psychological tests. *Personality and Individual Differences 13*(6), 699–716.

Oberheim, N. A., Takano, T., Han, X., He, W., Lin, J. H., Wang, F., . . . Nedergaard, M. (2009). Uniquely hominid features of adult human astrocytes. *The Journal of Neuroscience, 29*(10), 3276-3287.

Oldham, W. M., & Hamm, H. E. (2008). Heterotrimeric G protein activation by G-protein-coupled receptors. *Nat Rev Mol Cell Biol, 9*(1), 60-71. doi: 10.1038/nrm2299

Pak, C., Garshasbi, M., Kahrizi, K., Gross, C., Apponi, L. H., Noto, J. J., . . . Kuss, A. W. (2011). Mutation of the conserved polyadenosine RNA binding protein, ZC3H14/dNab2, impairs neural function in Drosophila and humans. *Proceedings of the National Academy of Sciences, 108*(30), 12390-12395.

Pastinen, T., & Hudson, T. J. (2004). Cis-acting regulatory variation in the human genome. *Science, 306*(5696), 647-650.

Pedersen, N. L., McClearn, G. E., Plomin, R., & Nesselroade, J. R. (1992). Effects of early rearing environment on twin similarity in the last half of the life span. *British Journal of Developmental Psychology, 10*(3), 255-267.

Pedroso, I., & Breen, G. (2011). Gene set analysis and network analysis for genome-wide association studies. *Cold Spring Harbor Protocols, 9*, pdb-top065581.

Penke, L., Denissen, J. J., & Miller, G. F. (2007). The evolutionary genetics of personality. *European Journal of Personality, 21*(5), 549-587.

Penke, L., Maniega, S. M., Bastin, M. E., Valdes Hernandez, M. C., Murray, C., Royle, N. A., . . . Deary, I. J. (2012). Brain white matter tract integrity as a neural foundation for general intelligence. *Molecular Psychiatry, 17*(10), 1026-1030. doi: 10.1038/mp.2012.66

Petrill, S. A. (1997). Molarity versus modularity of cognitive functioning? A behavioral genetic perspective. *Current Directions in Psychological Science, 6*(4), 96-99.

Plomin, R. (1999). Genetics and general cognitive ability. *Nature, 402*, C25-C29.

Plomin, R., Cederlöf, M. , & Lichtenstein, P. . (2013). *Low IQ and mild mental retardation are heritable but severe mental retardation is not: A genetic analysis of 740 000 siblings and 18 000 twins.* Paper presented at the ISIR, Melbourne, Australia.

Plomin, R., DeFries, J. C., McClearn, G. E., & McGuffin, P. (2001). *Behavioral genetics (4th ed.)*. New York: Worth Publishers.

Plomin, R., DeFries, J. C., McClearn, G. E., & McGuffin, P. (2007). *behavioral genetics*. Unites States of America: Worth Publishers and W.H. Freeman and Company.

Plomin, R., & Kovas, Y. (2005). Generalist Genes and Learning Disabilities. *Psychological Bulletin, 131*(4), 592–617.

Pocklington, A. J., Cumiskey, M., Armstrong, J. D., & Grant, S. G. (2006). The proteomes of neurotransmitter receptor complexes form modular networks with distributed functionality underlying plasticity and behaviour. *Mol Syst Biol, 2*, 2006 0023. doi: 10.1038/msb4100041

Purcell, S. M., Moran, J. L., Fromer, M., Ruderfer, D., Solovieff, N., Roussos, P., . . . Sklar, P. (2014). A polygenic burden of rare disruptive mutations in schizophrenia. *Nature, 506*(7487), 185–190.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., . . . Sham, P. C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics, 81*(3), 559-575. doi: 10.1086/519795

Purcell, Shaun M, Wray, Naomi R, Stone, Jennifer L, Visscher, Peter M, O'Donovan, Michael C, Sullivan, Patrick F, . . . Morris, Derek W. (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature, 460*(7256), 748-752.

Rabbitt, P.M.A., McInnes, L., Diggle, P., Holland, F., Bent, N., Abson, V., . . . Horan, M. (2004). The University of Manchester Longitudinal Study of Cognition in Normal Healthy Old Age, 1983 through 2003. *Aging Neuropsychology and Cognition, 11*, 245-279.

Rafnsson, S. B., Deary, I. J., Smith, F. B., Whiteman, M. C., & Fowkes, F. G. R. (2007). Cardiovascular diseases and decline in cognitive function in an elderly community population: the Edinburgh Artery Study. *Psychosomatic medicine, 69*(5), 425-434.

Raven, J.C. (1965). *The Mill Hill vocabulary scale*. London: H.K. Lewis.

Raven, J.C., Court, J.H., & Raven, J. (1977). *Manual for Raven's Progressive Matrices and Vocabulary Scales*. London: H.K. Lewis.

Raychaudhuri, S., Plenge, R. M., Rossin, E. J., Ng, A. C., Purcell, S. M., Sklar, P., . . . Daly, M. J. . (2009). Identifying relationships among genomic disease regions: predicting genes at pathogenic SNP associations and rare deletions. *PLoS genetics, 5*(6), 15.

Reich, D. E., & Lander, E. S. (2001). On the allelic spectrum of human disease. *TRENDS in Genetics, 17*(9), 502-510.

Richardson, K., Lai, C. Q., Parnell, L., Lee, Y. C., & Ordovas, J. . (2011). A genome-wide survey for SNPs altering microRNA seed sites identifies functional candidates in GWAS. . *BMC genomics, 12*(1).

Rietveld, C. A., Medland, S. E., Derringer, J., Yang, J., Esko, T., Martin, N. W., . . . McMahon, G. (2013). GWAS of 126,559 individuals identifies genetic variants associated with educational attainment. *Science, 340*(6139), 1467-1471.

Rijsdijk, F. V., Vernon, P. A., & Boomsma, D. I. . (2002). Application of hierarchical genetic models to Raven and WAIS subtests: a Dutch twin study. *Behavior Genetics, 32*(3), 11.

Ripke, S., Wray, N. R., Lewis, C. M., Hamilton, S. P., Weissman, M. M., Breen, G., . . . Degenhardt, F. (2012). A mega-analysis of genome-wide association studies for major depressive disorder. *Molecular psychiatry, 18*(4), 497-511.

Risch, N., & Merikangas, K. (1996). The future of genetic studies of complex human diseases. *Science, 273*(5281), 1516-1517.

Roberts, R. O., Geda, Y. E., Knopman, D. S., Cha, R. H., Pankratz, V. S., Boeve, B. F., & Petersen, R. C. (2012). The incidence of MCI differs by subtype and is higher in men The Mayo Clinic Study of Aging. *Neurology, 78*(5), 342-351.

Ropers, F., Derivery, E., Hu, H., Garshasbi, M., Karbasiyan, M., Herold, M., . . . Rajab, A. (2011). Identification of a novel candidate gene for non-syndromic autosomal recessive intellectual disability: the WASH complex member SWIP. *Human molecular genetics., 20*(13), 2585-2590.

Ruano, D., Abecasis, G.R., Glaser, B., Lips, E.S., Cornelisse, L.N., de Jong, A.P.H., . . . Posthuma, D. (2010). Functional gene group analysis reveals a role of synaptic heterotrimeric G proteins in cognitive ability. *Am. J. Hum. Genet., 86*(2), 113-125.

Ryan, T. J., Kopanitsa, M. V., Indersmitten, T., Nithianantharajah, J., Afinowi, N. O., Pettit, C., . . . Komiyama, N. H. (2013). Evolution of GluN2A/B cytoplasmic domains diversified

vertebrate synaptic plasticity and behavior. *Nature Neuroscience, 16*(1), 25-32. doi: 10.1038/nn.3277

Sala, C., Piëch, V., Wilson, N. R., Passafaro, M., Liu, G., & Sheng, M. (2001). Regulation of dendritic spine morphology and synaptic function by Shank and Homer. *Neuron, 31*(1), 115-130.

Savage, R.D. (1984). *Alphabet coding task-15*. Perth, W. Australia: Murdock University.

Schadt, E. E. (2009). Molecular networks as sensors and drivers of common human diseases. *Nature, 461*(7261), 218-223. doi: 10.1038/nature08454

Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium. (2011). Genome-wide association study identifies five new schizophrenia loci. *Nature genetics, 43*(10), 969-976.

Schizophrenia Working Group of the Psychiatric Genomics Consortium. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature, 511*(7510), 421-427.

Schmidt, F. L., & Hunter, J. (2004). General mental ability in the world of work: occupational attainment and job performance. *Journal of personality and social psychology, 86*(1), 162 - 173.

Scottish Council for Research in Education. (1933). *The intelligence of Scottish children: A national survey of an age-group*. London: University of London Press.

Scottish Council for Research in Education. (1949). *The Trend of Scottish Intelligence* London: University of London Press.

Sheng, M., & Kim, E. (2011). The postsynaptic organization of synapses. *Cold Spring Harbor perspectives in biology, 3*(12).

Shepherd, J. D., & Huganir, R. L. (2007). The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annu Rev Cell Dev Biol, 23*, 613-643. doi: 10.1146/annurev.cellbio.23.090506.123516

Siepel, A., Bejerano, G., Pedersen, J. S., Hinrichs, A. S., Hou, M., Rosenbloom, K., . . . Haussler, D. (2005). Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome research, 15*(8), 1034-1050.

Sklar, Pamela, Ripke, Stephan, Scott, Laura J, Andreassen, Ole A, Cichon, Sven, Craddock, Nick, . . . Blackwood, Douglas. (2011). Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. *Nat Genet, 43*(10), 977-983.

Smith, G. D., Hart, C., Watt, G., Hole, D., & Hawthorne, V. (1998). Individual social class, area-based deprivation, cardiovascular disease risk factors, and mortality: the Renfrew and Paisley Study. *Journal of Epidemiology and Community Health, 52*(6), 399-405.

Spearman, C. (1904). "General Intelligence," Objectively Determined and Measured. *The American Journal of Psychology, 15*(2), 201-292.

Spearman, C. (1927). *The Abilities of Man: Their Nature and Measurement*. New York: MacMillan and Company.

Spencer, H. (1855). *The Principals of Psychology*. London: Longman, Brown, Green and Longmans.

Spinath, F. M., Ronald, A., Harlaar, N., Price, T. S., & Plomin, R. (2003). Phenotypic *g* early in life: On the etiology of general cognitive ability in a large population sample of twin children aged 2–4 years. *Intelligence, 31*(2), 195-210.

Spycher, B. D., Henderson, J., Granell, R., Evans, D. M., Smith, G. D., Timpson, N. J., & Sterne, J. A. (2012). Genome-wide prediction of childhood asthma and related phenotypes in a longitudinal birth cohort. *Journal of Allergy and Clinical Immunology, 130*(2), 503-509.

Stark, A., Brennecke, J., Russell, R. B., & Cohen, S. M. (2003). Identification of Drosophila microRNA targets. *PLoS biology, 1*(3), e60.

Stäubli, U, Perez, Y , Xu, F B , Rogers, G , Ingvar, M , Stone-Elander, S , & Lynch, G (1994). Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation in vivo. *Proceedings of the National Academy of Sciences of the United States of America, 91*(23), 4.

Stein, J. L., Medland, S. E., Vasquez, A. A., Hibar, D. P., Senstad, R. E., Winkler, A. M., . . . Enhancing Neuro Imaging Genetics through Meta-Analysis, Consortium. (2012). Identification of common variants associated with human hippocampal and intracranial volumes. *Nature Genetics, 44*(5), 552-561. doi: 10.1038/ng.2250

Strenze, T. (2007). Intelligence and socioeconomic success: A meta-analytic review of longitudinal research. *Intelligence, 35*(5), 401-426.

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., . . . Mesirov, J. P. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U S A, 102*(43), 15545-15550. doi: 10.1073/pnas.0506580102

Tahira, T., Baba, S. , Higasa, K., Kukita, Y., Suzuki, Y., Sugano, S., & Hayashi, K. (2005). dbQSNP: A database of SNPs in human promoter regions with allele frequency information determined by single-strand conformation polymorphism-based methods. *Human mutation, 26*(2), 69-77.

Tarpey, P., Parnau, J., Blow, M., Woffendin, H., Bignell, G., Cox, C., . . . Raymond, F. L. (2004). Mutations in the DLG3 gene cause nonsyndromic X-linked mental retardation. *American Journal of Human Genetics, 75*(2), 318-324. doi: 10.1086/422703

Thurstone, L. L. (1938a). A new rotational method in factor analysis. *Psychometrika, 3*(4), 199-218.

Thurstone, L.L. (1938b). *Primary Mental Abilities*. Chicago: University of Chicago Press.

Torkamani, A, Topol, E. J, & Schork, N. J. (2008). Pathway analysis of seven common diseases assessed by genome-wide association. *Genomics, 92*(5), 7.

Toulopoulou, T., Goldberg, T. E., Mesa, I. R., Picchioni, M., Rijsdijk, F., Stahl, D., . . . Murray, R. M. (2010). Impaired intellect and memory: a missing link between genetic risk and schizophrenia? *Arch Gen Psychiatry, 67*(9), 905-913. doi: 10.1001/archgenpsychiatry.2010.99

Trzaskowski, M., Davis, O. S., DeFries, J. C., Yang, J., Visscher, P. M., & Plomin, R. (2013a). DNA evidence for strong genome-wide pleiotropy of cognitive and learning abilities. *Behavior genetics, 43*(4), 267-273.

Trzaskowski, M., Yang, J., Visscher, P. M., & Plomin, R. (2013b). DNA evidence for strong genetic stability and increasing heritability of intelligence from age 7 to 12. *Mol Psychiatry*. doi: 10.1038/mp.2012.191

Tsien, J. Z., Huerta, P. T., & Tonegawa, S. (1996). The essential role of hippocampal CA1 NMDA receptor–dependent synaptic plasticity in spatial memory. *Cell, 87*(7), 1327-1338.

Tsuchihara, K., Suzuki, Y., Wakaguri, H., Irie, T., Tanimoto, K., Hashimoto, S. I., . . . Sugano, S. (2009). Massive transcriptional start site analysis of human genes in hypoxia cells. *Nucleic acids research, 37*(7), 2249-2263.

Tucker-Drob, E. M., & Salthouse, T. A. (2008). Adult age trends in the relations among cognitive abilities. *Psychology and aging, 23*(2), 453-460.

van der Sluis, S., Verhage, M., Posthuma, D., & Dolan, C. V. (2010). Phenotypic complexity, measurement bias, and poor phenotypic resolution contribute to the missing

heritability problem in genetic association studies. *PLoS One, 5*(11), e13929. doi: 10.1371/journal.pone.0013929

van Scheltinga, A. F., Bakker, S. C., van Haren, N. E. M., Derks, E. M., Buizer-Voskamp, J. E., Cahn, W., . . . Kahn, R. S. (2013). Schizophrenia genetic variants are not associated with intelligence. *Psychological medicine*, 8.

Vernon, P. E. (1964). *The structure of human abilities*. London: Methuen.

Vernon, P. E. (1965). Ability factors and environmental influences. *American Psychologist, 20*(9), 723-733.

Veyrieras, J. B., Kudaravalli, S., Kim, S. Y., Dermitzakis, E. T., Gilad, Y., Stephens, M., & Pritchard, J. K. (2008). High-resolution mapping of expression-QTLs yields insight into human gene regulation. *PLoS genetics, 4*(10), 15.

Visscher, P. M., Brown, M. A., McCarthy, M. I., & Yang, J. (2012). Five years of GWAS discovery. *The American Journal of Human Genetics, 90*(1), 17.

Visser, B. A., Ashton, M. C., & Vernon, P. A. (2006). Beyond *g*: Putting multiple intelligences theory to the test. *Intelligence, 34*(5), 487-502.

von Stumm, S., Macintyre, S., Batty, D. G., Clark, H., & Deary, I. J. (2010). Intelligence, social class of origin, childhood behavior disturbance and education as predictors of status attainment in midlife in men: The Aberdeen Children of the 1950s study. *Intelligence, 38*(1), 202-211.

Walsh, T., McClellan, J. M., McCarthy, S. E., Addington, A. M., Pierce, S. B., Cooper, G. M., . . . Sebat, J. (2008). Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science, 320*(5875), 539-543. doi: 10.1126/science.1155174

Wang, K., Li, M. , & Hakonarson, H. (2010). Analysing biological pathways in genome-wide association studies. *Nat. Rev. Genet., 11*, 843 - 8554.

Wang, K., Li, M., & Bucan, M. (2007a). Pathway-based approaches for analysis of genomewide association studies. *Am. J. Hum. Genet., 81*(6), 1278-1283. doi: 10.1086/522374

Wang, K., Li, M., & Bucan, M. (2007b). Pathway-based approaches for analysis of genomewide association studies. *American Journal of Human Genetics, 81*(6), 1278-1283. doi: 10.1086/522374

Waung, M. W., Pfeiffer, B. E., Nosyreva, E. D., Ronesi, J. A., & Huber, K. M. (2008). Rapid translation of Arc/Arg3. 1 selectively mediates mGluR-dependent LTD through persistent increases in AMPAR endocytosis rate. *Neuron, 59*(1), 13.

Wechsler, D. (1981). *Manual for the Wechsler adult intelligence scale – Revised.* New York: The Psychological Corporation.

Wechsler, D. (1987). *Wechsler Memory Scale—Revised*. New York: Psychological Corporation.

Wechsler, D. (1991). *The Wechsler intelligence scale for children—third edition*. San Antonio, TX: The Psychological Corporation.

Wechsler, D. (1998a). *Wechsler Adult Intelligence Scale - third edition*. London: The Psychological Corporation.

Wechsler, D. (1998b). *Wechsler Memory Scale III UK*. London: Psychological Corporation.

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence*. San Antonio: The Psychological corporation.

Whalley, L. J., & Deary, I. J. (2001). Longitudinal cohort study of childhood IQ and survival up to age 76. *BMJ, 322*(7290).

Whalley, L. J., Murray, A. D., Staff, R. T., Starr, J. M., Deary, I. J., Fox, H. C., . . . Crawford, J. R. (2011). How the 1932 and 1947 mental surveys of Aberdeen schoolchildren provide

a framework to explore the childhood origins of late onset disease and disability. *Maturitas, 69*(4), 365-372. doi: 10.1016/j.maturitas.2011.05.010

Whitlock, M. C. (2005a). Combining probability from independent tests: the weighted Z-method is superior to Fisher's approach. *J Evol Biol, 18*(5), 1368-1373. doi: 10.1111/j.1420-9101.2005.00917.x

Whitlock, M. C. (2005b). Combining probability from independent tests: the weighted Z-method is superior to Fisher's approach. *J. Evol. Biol., 18*(5), 1368-1373. doi: 10.1111/j.1420-9101.2005.00917.x

Willer, C. J., Li, Y., & Abecasis, G. R. (2010). METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics, 26*(17), 2190-2191. doi: 10.1093/bioinformatics/btq340

Williams, M. V., Baker, D. W., Parker, R. M., & Nurss, J. R. . (1998). Relationship of functional health literacy to patients' knowledge of their chronic disease: a study of patients with hypertension and diabetes. *Archives of internal medicine, 158*(2), 166-172.

Williams, M. V., Parker, R. M., Baker, D. W., Parikh, N. S., Pitkin, K., Coates, W. C., & Nurss, J. R. . (1995). Inadequate functional health literacy among patients at two public hospitals. *Jama, 274*(21), 1677-1682.

Windrem, M. S., Schanz, S. J., Guo, M., Tian, G. F., Washco, V., Stanwood, N., . . . Goldman, S. A. . (2008). Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse. *Cell stem cell, 2*(6), 553-565.

Wisdom, N. M., Callahan, J. L., & Hawkins, K. A. (2011). The effects of apolipoprotein E on non-impaired cognitive functioning: a meta-analysis. *Neurobiology of aging, 32*(1), 63-74.

Wright, Margaret J., De Geus, E, Ando, J, Luciano, M, Posthuma, Danielle, Ono, Y, . . . Boomsma, D. (2001). Genetics of cognition: outline of a collaborative twin study. *Twin Research, 4*(1), 48-56.

Wright, Margaret J., & Martin, Nicholas, G. (2004). The Brisbane Adolescent Twin Study: outline of study methods and research projects. *Australian Journal of Psychology, 56*(2), 65-78.

Xiao, F., Zuo, Z., Cai, G., Kang, S., Gao, X., & Li, T. (2009). miRecords: an integrated resource for microRNA–target interactions. . *Nucleic acids research, 37*, 5.

Yamashita, R., Sathira, N. P., Kanai, A., Tanimoto, K., Arauchi, T., Tanaka, Y., . . . Suzuki, Y. (2011). Genome-wide characterization of transcriptional start sites in humans by integrative transcriptome analysis. *Genome research, 21*(5), 775-789.

Yamashita, R., Wakaguri, H., Sugano, S., Suzuki, Y., & Nakai, K. (2010). DBTSS provides a tissue specific dynamic view of Transcription Start Sites. *Nucleic Acids Research, 38*, doi:10.1093.

Yang, J., Benyamin, B. , McEvoy, B. P. , Gordon, S. , Henders, A. K. , Nyholt, D. R. , . . . Visscher, P. M. . (2010). Common SNPs explain a large proportion of the heritability for human height. *Nat Genet, 42*(7), 4.

Yang, J., Lee, S. H., Goddard, M. E., & Visscher, P. M. . (2011). GCTA: a tool for genome-wide complex trait analysis. *The American Journal of Human Genetics, 88*(1), 7.

Yang, K., Trepanier, C., Sidhu, B., Xie, Y. F., Li, H., Lei, G., . . . MacDonald, J. F. (2012). Metaplasticity gated through differential regulation of GluN2A versus GluN2B receptors by Src family kinases. *The EMBO journal, 31*(4), 805-816.

Yoshida, H., Nakaya, M., & Miyazaki, Y. (2009). Interleukin 27: a double-edged sword for offense and defense. *Journal of Leukocyte Biology, 86*(6), 1295-1303.

Zanni, G., van Esch, H., Bensalem, A., Saillour, Y., Poirier, K., Castelnau, L., . . . Chelly, J. (2010). A novel mutation in the DLG3 gene encoding the synapse-associated protein 102 (SAP102) causes non-syndromic mental retardation. *Neurogenetics, 11*(2), 251-255. doi: 10.1007/s10048-009-0224-y

Zaykin, D. V. (2011). Optimally weighted Z-test is a powerful method for combining probabilities in meta-analysis. *J. Evol. Biol., 24*(8), 1836-1841. doi: 10.1111/j.1420-9101.2011.02297.x

Zorumski, C. F., & Izumi, Y. (2012). NMDA receptors and metaplasticity: mechanisms and possible roles in neuropsychiatric disorders. *Neurosci Biobehav Rev, 36*(3), 989-1000. doi: 10.1016/j.neubiorev.2011.12.011