The Genomics of Cerebral Palsy
Are Copy Number Variants associated with Cerebral Palsy?

A thesis submitted for the degree of Master of Philosophy to the University of Adelaide

By

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Statement of Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the test.

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Gai Lisette McMichael

November 2011

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HUGO Gene Nomenclature gene symbol and gene name.

ABCC1- ATP-binding cassette, sub-family C (CFTR/MRP), member 1

ADAMTS13 - ADAM metallopeptidase with thrombospondin type 1 motif, 13

AGRN - agrin

CACNA1H - calcium channel, voltage-dependent, T type, alpha 1H subunit

CHRNA4 - cholinergic receptor, nicotinic, alpha 4

CHAT - choline O-acetyltransferase

CNTN1 - contactin 1

CNTNAP3 - contactin associated protein-like 3

COPS3 - COP9 constitutive photomorphogenic homolog subunit 3

CTNND2 - catenin (cadherin-associated protein)

C16orf62 - chromosome 16 open reading frame 62

DHCR7 - 7-dehydrocholesterol reductase

DLGAP2 - discs, large (Drosophila) homolog-associated protein 2

FLRT3 - fibronectin leucine rich transmembrane protein

FSCB - fibrous sheath CABYR binding protein

GABRD - gamma-aminobutyric acid (GABA) A receptor, delta

INPP5E – inositol polyphosphate-5-phosphatase, 72 kDa

KCNMB3 – potassium large conductance calcium-activated channel

KCNQ2 - potassium voltage-gated channel

MACROD2 - MACRO domain containing 2

MC2R - melanocortin 2 receptor (adrenocorticotropic hormone)

MCPH1 - microcephalin 1

HUGO Gene Nomenclature Committee approved gene symbol and gene name

MPV17L – MPV17 mitochondrial membrane protein-like

MYO5B - myosin VB

HUGO Gene Nomenclature (continued)

NBEA – neurobeachin

NCOR2 - nuclear receptor corepressor 2

NF1 - neurofibromin 1

NIPA1 - non imprinted in Prader-Willi/Angelman syndrome 1

NOS3 - nitric oxide synthase 3 (endothelial cell)

NPHP1 – nephronophthisis 1 (juvenile)

PAK2 – p21 protein (Cdc42/Rac)-activated kinase 2

PARK2 – parkinson protein 2, E3 ubiquitin protein ligase (parkin)

PCDH11X - protocadherin 11 X-linked

PNKP – polynucleotide kinase 3'-phosphatase

PRAME - preferentially expressed antigen in melanoma

PRODH - proline dehydrogenase (oxidase) 1

PTCHD3 - proline dehydrogenase (oxidase) 1

SHANK2 – SH3 and multiple ankyrin repeat domains 2

SHANK3 – SH3 and multiple ankyrin repeat domains 3

SH3GL3 - SH3-domain GRB2-like 3

SLC6A1 – solute carrier family 6 (neurotransmitter transporter, GABA), member 1

SLC6A3 - solute carrier family 6 (neurotransmitter transporter, dopamine), member 3

SLC6A11 – solute carrier family 6 (neurotransmitter transporter, GABA), member 11

SLC25A22 - solute carrier family 25 (mitochondrial carrier: glutamate), member 22

SORCS2 – sortilin-related VPS10 domain containing receptor 2

TBX1 – T-box 1

TSPAN7 – tetraspanin 7

UGT2B - UDP glucuronosyltransferase 2 family, polypeptide B4

UROC1 - urocanase domain containing 1

Abbreviations

CP – Cerebral Palsy

CNVs – Copy number variants

DGV – Database of Genomic Variants

CHOP – Children's Hospital of Philadelphia database

OMIM – Online Mendelian Inheritance of Man

UCSC – University of California Santa Cruz

CMV – Cytomegalovirus

EBV – Epstein Barr Virus

IL-4 – Interleukin – 4

MBL – Mannose Binding Lectin

APOE – Apolipoprotein

SNPs – single nucleotide polymorphisms

bps – base pairs

kb – kilo base

NAHR – non allelic homologous recombination

aCGH- array Comparative Genome Hybridization

HMM – Hidden Markov Model

FISH – fluorescent in situ hybridization

qPCR – quantitative real time polymerase reaction

Au – Austism

ASD – Autism Spectrum Disorders

Ep – Epilepsy

ID – Intellectual Disability

JS – Jourbett Syndrome

PLS – Potocki-Lupski Syndrome

Abbreviations (continued)

SZ – Schizophrenia

SMS – Smith-Magenis syndrome

TS – Tourette Syndrome

VWD –Von Willebrand Disease

IUGR – Intrauterine growth restriction

ACD – acid citrate dextrose

EDTA – ethylenediaminetetraacetic acid

GRA – Genetic Repositories Australia

LCLs – Lymphoblastoid cell lines

URLs

Database of Genomic Variants, http://profjects.tcag.ca/variation/

UCSC Genome Bioinformatics, http://genome.ucsc.edu/cgi-bin/hgGateway

Decipher database, https://**decipher**.sanger.ac.uk/

Online Mendelian Inheritance in Man, www.ncbi.nlm.nih.gov/**omim**.

Abstract

Background Cerebral palsy describes a group of permanent disorders of the development of movement and posture that are attributed to non-progressive disturbances occurring in the developing fetal or infant brain. It is often accompanied by additional features including intellectual disability, autism, epilepsy and visual and hearing impairment. The overall incidence of cerebral palsy has not changed in the last 50 years despite major improvements in perinatal medicine, and remains at around 2-2.5/1,000 deliveries world-wide. Treatment is symptomatic rather than curative. A child under 18 years of age is three times more likely to be diagnosed with cerebral palsy than cancer. There are major social, economic and quality of life issues for both the child with cerebral palsy and their family. In Australia, approximately 600 children are diagnosed with cerebral palsy each year. Several studies have suggested that genetic susceptibility factors and adverse environmental triggers such as perinatal viral infection can act both independently and in combination to contribute to the neuropathology of cerebral palsy. For the majority of cases the exact determinants responsible for injury to the child's developing brain have not been defined.

This thesis hypothesises that cerebral palsy is genetically highly heterogeneous and caused by many diverse and individually rare mutations of large effect in genes involving brain development, the most common of which are copy number variants (CNVs).

Study design To explore the hypothesis that CNVs contribute to the aetiology of cerebral palsy, 50 DNA samples from individuals with cerebral palsy were tested on a

custom-designed 180K chromosomal microarray with targeted plus whole genome coverage. The targeted coverage includes known clinically relevant regions such as microdeletion/duplication syndromes, telomeres and centromeres at a resolution of ~20-50 kb plus exon-level coverage of >1200 genes involved in neurodevelopmental disorders. The whole-genome backbone results in a resolution in unique DNA of ~225kb. These same samples were also separately assessed on a 135K custom designed array with targeted coverage of ~50kb in all genomic hotspots and backbone coverage of 350kb. Combined results were compared with 8,329 adult controls with no known neurological disorders.

Results Three out of 50 cases were identified with a CNV that included candidate genes of special interest for the cerebral palsy phenotype; *CTNND2* (446 kb duplication including the first exon), *MCPH1* (219 kb duplication including exons 1-8) and *COPS* (4 kb deletion including exons 6-8). All three CNVs were shown to be inherited from an unaffected parent. Several additional CNVs of possible interest to the cerebral palsy phenotype were selected from 30 out of 50 cases, including the above three mentioned cases, as they encompassed genes expressed in the brain or were previously recognized in other neurodevelopmental disorders. These included Histone Cluster genes, 7q21 and 12p12.1p12.2, single-gene CNVs across *CNTNAP3, MC2R, FSCB*, *PTCHD3, NPHP1* and *TARP* and intragenic CNVs in *DLGAP2, PARK2*, *NBEA, PAK2*, *MACROD2*, *CNTN1*, *MPV17L, NF1*, NCOR2, NOS3, SH3G13 and TBX1.

Conclusion Copy number changes in cerebral palsy cases have been identified in this largest study to date. Amongst 50 cases there were three potential candidate genes

for cerebral palsy and several additional variants involved in brain developmental genes. The pathogenicity of these rare CNVs is not currently resolved but these preliminary studies justify further evaluation of CNVs in a larger cohort of cerebral palsy families and functional studies. This is currently underway.

Chapter 1 **Literature Review**

1.1 Introduction

1.1.1 Cerebral Palsy

Cerebral palsy is a non-progressive neurodevelopmental disorder which presents in early childhood and continues throughout life. The definition and classification of cerebral palsy has presented a challenge over the years. Until recently the definitions of cerebral palsy by Bax[2] (1964) *"a disorder of movement and posture due to a defect or lesion of the immature brain",* and Mutch[3] (1992) *"an umbrella term covering a group of non-progressive, but often changing, motor impairment syndromes secondary to lesions or anomalies of the brain arising in the early stages of development"* have been the most frequently cited throughout the literature. However, both of these classifications failed to encompass the complex heterogeneity of cerebral palsy. In 2004 an International Workshop on Definition and Classification of cerebral palsy was given the task of updating and redefining the classification of cerebral palsy. The revised definition and reclassification released in 2006 from the International Workshop is as follows: "*Cerebral palsy (CP) describes a group of permanent disorders of the development of movement and posture, causing activity limitation that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour; by epilepsy, and by secondary musculoskeletal problems"*[4].

This definition has more accurately encapsulated the heterogeneous aetiology of cerebral palsy. Emphasis was placed on motor disorder which affects movement and posture at varying degrees of severity, from mild problems with muscle coordination

to severe spasticity of all four limbs[4] and is often the first reason children present for medical diagnosis[4]. However, other neurodevelopmental disorders often accompany motor impairment, such as epilepsy, musculoskeletal dysfunction, intellectual disability, behavioural problems, inability to communicate and sensory loss[4].

1.1.2 Prevalence of Cerebral Palsy

In 2007 there were 33,797 estimated cases of Australians with cerebral palsy. This is an immense social and economic burden with an annual financial cost of $1.47 billion and an additional $2.4 billion in lost wellbeing[5]. Cerebral palsy is the most common physical neurological disability of childhood and is three times more likely to be diagnosed than cancer in a child less than 18 years of age. The prevalence of cerebral palsy throughout the developed world has not changed in the last 50 years and affects 2-2.5/1000 live births[6-9]. In contrast, there have been significant improvements to prenatal care with substantial decreases in both perinatal and maternal mortality[7,10]. A common non-evidence based belief in the past has been that most cases of cerebral palsy are due to fetal distress in labour, birth asphyxia or birth trauma. However, this belief has had to be revised as epidemiological studies indicate that only about 10% of cerebral palsy cases show possible signs of intrapartum fetal compromise at birth and some of these signs may have been a result of chronic pregnancy pathologies[11-13]. Currently, there is no antenatal test for susceptibility to cerebral palsy, no preventable measures in pregnancy and no known cure.

1.1.3 Risk factors for Cerebral Palsy

There are a number of known major epidemiological risk factors for cerebral palsy, including preterm delivery, intrauterine growth restriction, intrauterine infection, antepartum haemorrhage, multiple pregnancy, a sibling with cerebral palsy, gender and perinatal stroke[14-16]. The risk of cerebral palsy is greater in premature infants, occurring 20-30 times more often in very low birth weight infants (<1500 grams)[15]. Low birth weight infants include babies born preterm and/or growth restricted. Infants born <32 week gestation represent 2% of births and also represent 25% of children with cerebral palsy[14] whilst near-term or term babies account for at least half of all diagnosed cases of cerebral palsy[16]. Cerebral palsy has been reported to occur 1.2 – 1.6 times more frequently among boys than girls and the most common type for both girls and boys is spastic cerebral palsy[17, 18].

Recent observational case-control studies have suggested that genetic susceptibility factors and adverse environmental triggers such as perinatal viral infection, can act both independently and in combination to contribute to the neuropathology of cerebral palsy[19-23]. Genetic linkage studies involving whole genome searches have identified two possible loci which may be involved in cerebral palsy in a small number of families, 2q24-25[24] and 9p12-q12[25]. Promising candidate genes for cerebral palsy include cytokine[23, 26-28], thrombophilic[19] and Apolipoprotein E (APOE)[22] genes.

An earlier study performed by the South Australian Cerebral Palsy Research Group, comprising of blood spots from newborn screening cards of 443 cerebral palsy cases and 883 matched controls demonstrated associations between specific SNPs and

cerebral palsy and in some cases this was potentiated with exposure to a viral infection[19-21, 26, 27]. Following on from this a national prospective study comprising of buccal swabs from 587 cerebral palsy mother/child pairs and 1,154 control mother/child pairs was recently completed to further examine these findings[29]. Genotyping results were linked with extensive epidemiological data collected from individual state perinatal outcomes statistic units and completed questionnaires regarding mother's health during pregnancy. Carriage of Prothrombin gene mutation in hemiplegia cerebral palsy cases and several epidemiological factors including preterm and multiple birth, intrauterine growth restriction, perinatal infection can increase the risk factor for cerebral palsy[30, 31]. As part of our ongoing cerebral palsy work we extended our viral research to include approximately 1000 additional dried blood spots from newborn screening cards. Viral detection included cytomegalovirus (CMV), herpes simplex viruses 1 and 2, Epstein-Barr virus (EBV), human herpes viruses 6, 7 and 8, varicella zoster virus and parvovirus. This work occurred in parallel to our National Cerebral Palsy study and is further contributing to our understanding of cerebral palsy and its causation.

1.1.4 Intrauterine Infection

Intrauterine infection has been associated with an increased risk for cerebral palsy. It has been long established that fever during labour and/or a diagnosis of chorioamnionitis contributes to and increases the risk of cerebral palsy[32-35]. Chorioamnionitis is a condition where the membranes that surround the fetus, the chorion and amnion, and the amniotic fluid are subject to infection[36]. There is limited information in relation to viral infection and cerebral palsy. Some viruses are

able to cross the placenta and infect the fetus[37]. It is postulated that if the virus crosses the blood-brain barrier, the infection is then able to directly damage the developing neuronal tissue[20]. This may be due to the immaturity of the blood brain barrier and/or the response of the immune system involving cytokine polymorphisms.

Our own investigations of perinatal viral infection have identified CMV and EBV as possible contributors to the development of cerebral palsy[20], (McMichael *et al.* in press). Results found a correlation between potentially neurotropic viruses and cerebral palsy both directly and in conjunction with genetic mutations[20, 22, 23, 28].

1.1.5 Cytokines

Inflammation is one of the main responses of the immune system to infection. It is clinically recognised by pain, redness, heat and/or swelling caused by increased blood flow to infected tissue. Inflammation is physiologically precipitated by cytokines, interleukins and other inflammatory mediators, which are released by the infected cell. Cytokines are the main agents that mediate the fetal inflammatory response and may be released from either the fetal or maternal immune system[38]. Cytokines are either anti-inflammatory or pro-inflammatory[39] and in the event of a cytokine genetic mutation the fetal inflammatory response may be altered. It is unclear whether an abnormal excessive cytokine response to infection or inflammation ("cytokine storm") or the direct effects of a neurotropic infection is responsible for the white matter damage that causes cerebral palsy. Pro-inflammatory cytokines may be neurotropic when over expressed, whereas anti-inflammatory cytokines

when under expressed can compromise the immune system, allowing an infection to proceed unopposed[40].

Nelson[41] *et al*. reported a significant association between Lymphotoxin-α and increased risk for cerebral palsy in preterm babies[41]. A large case-control study examining cytokine single nucleotide polymorphisms and cerebral palsy has found significant associations between Tumour Necrosis Factor-α, Mannose Binding Lectin (MBL)[26, 28], Interleukin-6 and Interleukin-4 (IL-4)[23] and cerebral palsy. Both IL-4 and MBL were associated with cerebral palsy in combination with perinatal viral infection[23, 26]. This is an important finding as it highlights possible gene-environment interactions. Inflammation and coagulation mutations have also been suggested to interact, producing an additive increased risk of cerebral palsy[40].

1.1.6 Thrombophilia

Both inherited and acquired thrombophilia mutations may increase the risk of blood clotting in the mother and/or the fetus[42]. Clots that form in the placenta can travel directly to the fetal brain via the foramen ovale, leading to possible fetal stroke. A HuGE review[29] reported that the majority of studies examining inherited thrombophilias and cerebral palsy have been small, with limited statistical power, and did not demonstrate any associations[7, 43-46]. However, the largest case-control study that review reported a complex and heterogeneous relationship between inherited thrombophilias and subtypes of cerebral palsy, at different gestational ages[19]. Thrombophilia gene mutations including factor V Leiden, prothrombin G20210A and methylenetetrahydrofolate reductase polymorphisms C677T and A1298C were examined. This study found the risk of cerebral palsy doubled with carriage of one

clotting mutation and a five-fold increased risk was demonstrated with carriage of two or more clotting mutations[19].

1.1.7 Apolipoprotein E

The possible involvement of apolipoprotein E (APOE) in cerebral palsy causation is not yet fully researched, but as APOE plays a significant role in the growth and repair of injured neurons, it is postulated that an APOE mutation affects the recovery of a brain insult or injury[47]. APOE is polymorphic, with three alleles (ε2, ε3, ε4), ε3 being the most common. The protein from APOE is produced abundantly in the liver and the brain[48, 49]. Meirelles Kalil Pessoa[50] *et al.* and Kuroda[51] *et al.* were the first to report, in two separate observational studies, a possible association between carriage of the ε4 allele and an elevated risk for cerebral palsy (>3-fold and >4-fold, respectively) compared to matched controls. The same study by Kuroda[51] *et al*. observed a 12 fold increase risk for cerebral palsy with carriage of the ε2 allele. More recently two larger studies reported a >2-fold[22] and >3-fold[52] increased risk for cerebral palsy with carriage of the ε2 allele. McMichael[22] *et al*. found that this risk was further potentiated in term babies when exposed to a viral infection. A study by Blackman[47] *et al*. found a trend towards significance with carriage of at least one copy of the ε4 allele and the lower severity cerebral palsy group, suggesting the ε4 allele has a protective effect.

1.1.8 Genomic Progress

For decades chromosomal quantity and structural changes large enough to be viewed under a microscope have been identified. These include aneuploidies, which were the first reported chromosomal anomalies[53, 54] and fragile sites[55]. Aneuploidies, such as Klinefelter syndrome have an additional X chromosome added to the male karyotype[56], or Down syndrome (trisomy 21) which has part of or all of a third copy of chromosome 21[54]. Fragile sites are regions of the chromosome where gaps and breaks occur[57]. Common fragile sites are normally stable, however, Fragile XA is well documented as a common inherited cause of mental impairment because of expansion of triplet repeat[58, 59]. These large chromosome anomalies span several megabases of DNA, often resulting in a notable clinical phenotype, but account for only a small fraction of the pathogenic variation found in the human genome[60,61]. Since the advent of molecular biology and the completion of the human genome project smaller submicroscopic alterations such as individual single nucleotide polymorphisms (SNPs) have identified genomic markers for complex and common diseases. There are at least 10 million documented (www.ncbi.nlm.nih.gov/sites/entrez) SNPs within the human population and until recently it was thought that these small-scale variants were the most abundant source of variation in the human genome[60]. However, more recently larger submicroscopic structural variants are revealing more variation than SNPs[1].

1.1.9 Copy number variants

The rapid development of array technologies has revealed DNA variations involving segments smaller than those able to be recognised microscopically but much larger than those previously detected by conventional sequence analysis[60]. These submicrosopic variants include both deletions and duplications, collectively known as copy number variations and are proving to be the most prevalent type of variation found in the human genome. Copy number variations are segments of DNA from a few hundred to several million base pairs (bps) which are present in variable copy numbers in comparison with a human reference genome.

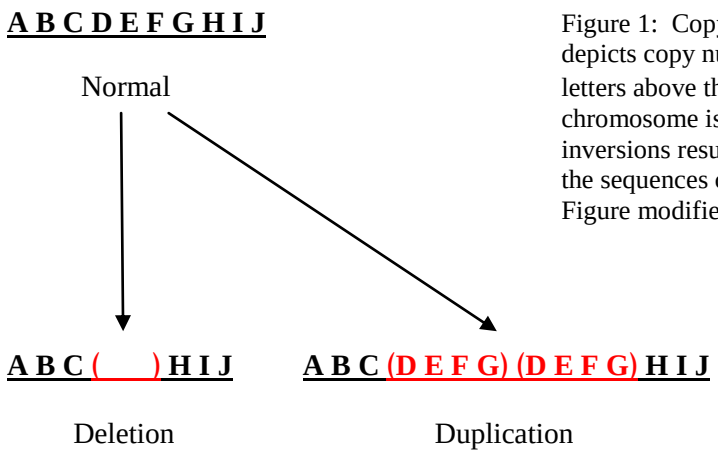


Figure 1: Copy Number Variants. This figure depicts copy number variation. Horizontal lines with letters above them represent the genome. Only one chromosome is shown. Deletions, duplications and inversions result in variations in the copy number in the sequences contained within the rearrangements. Figure modified from **Lupski,[1] *et al.* (2007)**

CNVs can disrupt parts of genes, entire genes or several genes, affecting gene dosage and gene distribution[60, 62, 63]. Current research suggests that the involvement of entire genes or several genes have important effects on biological function that substantially contribute to disease susceptibility[62, 63].

Two models for CNV-phenotype associations have been described in the literature[64]. The first model, common copy number polymorphisms (CNPs), describes variants that

are polymorphic within a population with a frequency that exceeds 1%[64]. CNP exist in multiple allelic states and are involved in biological functions such as immunity and drug responses. The second model involves CNVs that are truly rare within a population with a frequency of 0.1 - 1%. This model is representative of rare CNV events which are highly penetrant and short-lived in a population. They are more likely to be rare *de novo* events (absent in both parents' genome) or rare inherited (present in one or both parents) persisting for only a few generations within a pedigree[64]. A proportion of these are recurrent, found in clusters of subgroups of samples sharing common regions of the genome presenting a constant alteration[65]. These CNV events are more likely to be pathogenic harbouring disease critical genes[65] with a large proportion arising from nonallelic homologous recombination (NAHR)[64]. Non-allelic homologous recombination is due to the repair of double-stranded DNA breaks, which results in recombination between non-allelic paralogous segmental duplications. They are concentrated in areas with high recombination rates known as chromosome 'hotspots'[66].

Rearrangement hotspots are defined as regions of the genome from 50kb to 10Mb flanked by large segmental duplications that share a high level of sequence identity (≥ 10 kb, ≥ 95% identity) but are not allelic[67-69]. During meiosis these flanking duplications are susceptible to rearrangements by NAHR, thus predisposing the region to novel deletion/duplication events[64]. Segmental duplications promote unequal crossing over during meiosis resulting in gametes with gained or lost copies of genomic regions. These segmental duplications have been associated with both

normal and disease variation. Rearrangement hotspots represent good candidate sites of recurrent rearrangements that may be linked with novel genomic disorders[69]. A large number of hotspot regions associated with known genomic disorders have been found on chromosomes 7, 15, 16, 17 and 22[70, 71].

Information regarding rare CNVs and common copy number polymorphisms can be found in the Database of Genomic Variants (DGV)[67] which incorporates the Children's Hospital of Philadelphia (CHOP) database[72]. The DGV represents structural variants found in healthy individuals[67].

CNVs are providing new insight into the aetiology of disease phenotypes involved in complex genetic inheritance. Understanding the effect of CNVs, in particular those that are truly rare or unique and how they influence phenotypes requires further analysis in both diseased and normal individuals. The impact of rare CNVs can be assessed in two ways, either in terms of an individual's genome wide burden, or by specific loci which are significantly associated with disease[73].

1.1.10 Platforms for assessing copy number variations

Microarray technology has had a significant impact on CNV detection due to its high-throughput capabilities[74]. However, one of the main challenges has been to reliably interpret the enormous volume of data produced from the variety of genotyping platforms and analysis software programs available. CNVs are not always reproducible between the different genotyping platforms and analysis programs and

this has the potential to contribute to inconsistent results. In addition, analysis parameters also vary between different genotyping platforms and software programs. A gold standard approach for interpreting these data has not yet been adopted[61,75].

Generally CNVs are detected by array comparative genome hybridization (aCGH) or SNP genotyping arrays. Array comparative hybridization detects CNVs by co-hybridizing differential fluorescent labelled test and reference DNA samples to several thousand probes (DNA sequences), from coding and non-coding regions of the genome. Detection of copy number changes are determined by measuring the fluorescence ratio between the test and reference samples[75].

SNP genotyping arrays were initially employed to detect single base pair changes across the genome and have been widely utilised in genome wide association studies. More recently SNP arrays have been used as a proxy for CNV detection[60]. The principle is similar to aCGH, involving hybridization of fluorescently labelled test samples to allele specific probes. The main advantage of this platform is the two applications for collecting data, SNP genotyping and CNV detection. However, at present SNP arrays only target non-repetitive regions of the genome and consequently significant CNV regions may be missed as many are found in areas susceptible to high recombination events[76]. As SNP arrays were not initially designed to detect CNVs, specific software programs, from basic analyses parameters to complex statistical modelling, have been designed to compare the signal intensities of the test samples with the reference samples, determining the relative copy number per locus[75]. Data

from both aCGH and SNP arrays are prone to high signal noise (unwanted signal) leading to either false positive or false negative results. The choice of analysis software does not resolve this but helps identify spurious data that needs to be removed[75, 76]. It is recommended to validate CNVs, in particular those that are *de novo* with another method such as quantitative PCR (qPCR) or fluorescent in situ hybridization (FISH). There is a large amount of published literature available with guidelines and recommendations for accurately assessing CNVs in the human genome[61, 76, 77]. To date, the application of these technologies has successfully facilitated the detection of clinically relevant CNVs in patients with neurologic and psychiatric disorders such as autism, schizophrenia, mental retardation and epilepsy [78-81].

1.1.11 Autism

Autism and autism spectrum disorders (ASDs) are a group of highly heritable neurodevelopmental disorders involving difficulties with non verbal and verbal communication, social deficits and repetitive behaviours[82-85]. The prevalence of ASDs is approximately 1 in 166 children and is usually diagnosed within the first three years of life[79]. A plausible hypothesis is that several susceptibility genes interact together with a complex mode of inheritance contributing to the aetiology of autism and ASDs. Many genetic regions have been implicated but no specific genes have been unequivocally identified. Through the last decade, whole genome screening, association studies and cytogenetic studies have provided information to help unravel the genetics of autism. The development and advancement of array technologies has

facilitated the detection of CNV regions in autism and ASDs, giving new insights into possible causation of this disorder.

There have been several prevalent CNV regions enriched with functional genes involving neurodevelopmental and synaptic pathways reported in autism and ASDs [86-92]. Of particular interest are recurrent and large deletions located in hotspot regions such as 15q11.2[93], 15q13.3[94], 15q24.1[95] and 16p11.2[79, 91, 96]. Marshall[91] *et al.* and Weiss[96] *et al*. also reported a reciprocal duplication on chromosome 16p11.2. These recurrent and large events are more likely to harbour disease critical genes. Recurrent CNV events are clusters of subgroups of samples that share regions of the genome that present a constant alteration[65].

1.1.12 Schizophrenia

Schizophrenia is a highly heritable severe neuropsychiatric disorder where symptoms include cognitive deficits, hallucinations and delusions. It usually manifests in late adolescence or early adulthood and has a worldwide frequency of approximately 1%. The genetic involvement is complex and not well understood, further compounded by possible environmental influences[73, 81]. Until recently the accepted hypothesis for genetic influences on schizophrenia was based on the "common-disease-common allele" model (common disease-causing alleles will be found in all people who manifest a given disease), however Walsh[81] *et al.* proposed a model that rare, highly penetrant mutations may also be associated with schizophrenia. Again the development of microarray based methods revealed smaller structural variations that until now have gone undetected.

There have been several studies where a significant difference in the number of CNV events was found between schizophrenia cases and controls[73,97-100], with the majority of these variations involving deletions and occasionally duplications. Recurrent and large *de novo* CNV events were reported in hotspot regions involving deletions on 1q21.1[98, 101], 15q11.2[98] and 15q13.3[98]. Both deletions and duplications were reported on 3p29[73,81] and 16p.12[73]. A gain or loss in the same CNV region may be representative of genes being dose sensitive. The majority of CNVs were largely found in gene enriched regions involving neurodevelopmental and synaptic pathways [73, 81, 88, 102].

1.1.13 Intellectual disability

Intellectual disability affecting ~2-3% of the population[80] can be mild to severe, involving motor and language skills with failure to develop intellectually[103]. Severe intellectual disability is defined as an intelligence quotient (IQ) of <50 affects ~0.3-0.4% of the population[80]. There are several known causes including chromosome abnormalities, genetic and metabolic disorders, intrauterine exposure to toxins and infection present at birth[103]. Trisomy 21, the most common chromosome abnormality, accounts for 5-15% of intellectual disability cases and cytogenetic analyses detect approximately another 5% of cases[80]. The development of microarray technology has made it possible to scan the whole genome and this has revealed copy number changes in 10-15% of mental retardation cases [104], explaining other possible

causes for intellectual disability. There is a wide spectrum of phenotypic features associated with intellectual disability adding to its genetic complexity.

Recurrent and large deletions were reported on 1q21.1[101], 15q13.3[105, 106], 15q24.1[95] and 17q21.31[106, 107]. Several well-characterised intellectual disability syndromes have been defined in the Online Mendelian Inheritance in man (OMIM) database and the most frequently recorded CNVs in OMIM were deletions on chromosomes 1p36[108], 17q21.31[108] and deletions and reciprocal duplications on chromosome 22q11.2[108]. Girirajan *et al.* found a 16p12.1 microdeletion to be a significant risk factor for developmental delay and intellectual disability[109]. This same study suggested that when in conjunction with another CNV event, a single base pair mutation or an environmental risk factor, more severe neurological sequelae was observed, in line with a double jeopardy model[109]. Several other CNV events in gene enriched regions involving neurodevelopmental and synaptic pathways have also been reported for intellectual disabillity [87, 88, 105, 106, 110-113]. A recent study examining a cohort of 15,767 cases with a general diagnosis of developmental delay and/or intellectual disability identified 940 candidate dose-sensitive genes. Controls were comprised of 8,329 unaffected adults from nine previous multiple genome-wide associations studies[114].

1.1.14 Epilepsy

Epilepsy is a complex brain disorder characterised by seizures. It is one of the most common neurological disorders with a frequency of 1% and a lifelong incidence of 3%[115]. Several candidate genes have been identified[116] but despite this the genetic mechanism of epilepsy is not well understood[115]. More recently, CNVs in previously

known genomic hotspot regions at 15q11.2[115, 117], 15q13.3[115, 118, 119] and 16p13.11[115, 117] were observed in epilepsy cases. The deletion on locus 15q13.3 is found in 1% of epilepsy cases and represents the largest known genetic risk factor for epilepsy[119]. Furthermore, deletions were observed on 7q34-q32, a non-hotspot region, involving CNTNAP2, as a potential candidate gene[115].

Many of the CNVs found in autism, ASDs, schizophrenia, mental retardation and epilepsy are not distinct for each individual neurological disorder. In fact, nearly all CNVs overlap with at least one other neurological disorder and locus 15q13.3 has been associated with all of the above mentioned neurological disorders. This suggests that there is a more general risk factor for neuropsychiatric or central nervous system disorders. CNVs enriched with many genes involving the neurodevelopmental pathways result in the expression of different neurodevelopmental phenotypes[81]. Resulting phenotypes could also be due to the 'two hit' hypothesis, which postulates the additive effects of two or more genetic abnormalities in the affected individual [120].

1.1.15 Cerebral Palsy and copy number variants

To date, only one study has examined cerebral palsy and copy number variants in a consanguineous family. They identified a homozygous deletion in the AP4E1 sub unit which is part of the adaptor protein-4 (AP4) complex and plays an important role in brain development[121]. Previous research has identified both genetic components and environmental triggers as increased risk factors for cerebral palsy. Several possible candidate genes have been identified but no specific genes have been directly linked

to cerebral palsy[19, 20, 22, 23, 26, 27]. There is very little evidence to suggest a strong hereditary component, however family history of cerebral palsy is a moderate increased risk factor in subsequent children[122]. Literature is rapidly emerging on links between both rare *de novo* and some inherited CNVs and neurological disorders, such as autism, schizophrenia, mental retardation and epilepsy. There is overwhelming evidence that rare CNVs have a significant role in the aetiology of both common and more complex neurological disorders. We hypothesise that this can be extended to the neuropathology of cerebral palsy and that individually rare and/or unique CNVs contribute to the genetic aetiology of cerebral palsy. CNVs may either predispose an individual to cerebral palsy or exacerbate the effect of another mutation or environmental factor.

Both genetic and clinical considerations must be taken into account when examining associations between CNVs and cerebral palsy. From a genetic perspective, factors which need to be considered include: determining the size and frequency of the CNV, whether it is inherited or a *de novo* event requiring screening of both parents, the positioning of the CNV on the genome (e.g. located in a known hotspot region) and which genes and biological pathways are interrupted by the CNV. From a clinical perspective knowledge of family and pregnancy history will assist in identifying possible epidemiological factors which may influence the genetic aetiology of cerebral palsy. Phenotypic characteristics, such as type of cerebral palsy, gestational age and intrauterine growth restriction (IUGR) may be particularly relevant with respect to the CNV origin (inherited or *de novo*). The major clinical or pathogenicity consideration is

to ascertain the influence the CNV region will have on future intervention strategies. Identifying CNVs in neurological functional areas of the genome as genetic susceptibility mutations for cerebral palsy will go a long way towards understanding the genetic contribution to cerebral palsy. This will facilitate future preventative intervention strategies which could range from better genetic counselling, either-pre pregnancy or antenatal diagnosis, avoidance of environmental triggers in the genetically susceptible to gene silencing.

1.1.16 Conclusion

Cerebral palsy is a non-progressive neurodevelopmental disorder which presents in early childhood. Several recent studies have suggested that genetic susceptibility factors and adverse environmental triggers such as perinatal viral infection, can act both independently and in combination to contribute to the neuropathology of cerebral palsy[19-23]. Copy number variations are proving to be the most prevalent type of variation found in the human genome providing new insights into neurodevelopmental disorders and human disease. CNVs can disrupt parts of genes, entire genes or several genes, affecting gene dosage and gene distribution[60, 62, 63]. Clinically known CNVs have been identified in at least 10% of patients with neurologic and psychiatric disorders such as autism, schizophrenia, mental retardation and epilepsy [78]. This has led to the hypothesis that individually rare and/or unique CNVs of large effect will contribute to the neuropathology and genetic aetiology of cerebral palsy. This study is designed to examine this hypothesis. The evidence for rare CNVs having a significant role in the aetiology of some of the common and more complex

neurological disorders is increasingly becoming clear and will be the focus of research in the coming years.

1.2 Hypothesis and Aims

1.2.1 Hypothesis

Cerebral palsy is genetically highly heterogeneous and can be caused by individually rare/unique mutations of large effect, either de novo or inherited, in genes associated with brain development, the most common of which to date are copy number variants (CNVs).

1.2.2 Aims/Objectives of the project

1. To identify either rare *de novo* or rare inherited CNV events of large effect in cases of cerebral palsy.
2. To identify potential candidate genes for cerebral palsy involved with brain development.
3. To correlate CNV results with epidemiological data, such as type of cerebral palsy, gestation, IUGR and any other co-morbidities in order to understand the specific determinants associated with the heterogeneity of cerebral palsy.
4. To compare two separate microarray platforms to determine the most efficient method of identifying CNVs in cerebral palsy.

Chapter 2 **Cohort Demographics**

2.1 Cohort Demographics

2.1.1 Study Cohort

This project is a pilot study and is the first of its kind. It examined the associations between genomic structural variations and cerebral palsy in a cohort of 50 family trios; mother, father and affected cerebral palsy child who were of Caucasian origin. The sample was a convenience sample of unselected children with cerebral palsy who were attending Hospital for procedures under general anaesthetic (mostly Botox injections). This allowed blood sampling with consent during the anaesthesia. Their recruitment is described in the methodology section. Whole blood was collected from each family trio along with extensive clinical data using a maternal questionnaire. Linkage to the South Australia Cerebral Palsy register, or contacting specialist clinicians if linkage was not possible, determined the type of cerebral palsy for each individual. Signed parental consent and ethics approval from the Children's Youth and Women's Health Service Human Research Committee (REC 1946/4/10) was obtained for this project.

2.1.2 Cerebral Palsy Cohort Clinical Characteristics

Clinical characteristics for this study included:

1. Type of cerebral palsy
2. Gestational age
3. IUGR
4. Other co-morbidities Autism (Au), Intellectual disability (ID), Epilepsy (EP)
5. Gender

2.1.3 Cerebral palsy subtype

Three classifications describe the different motor impairments of cerebral palsy:

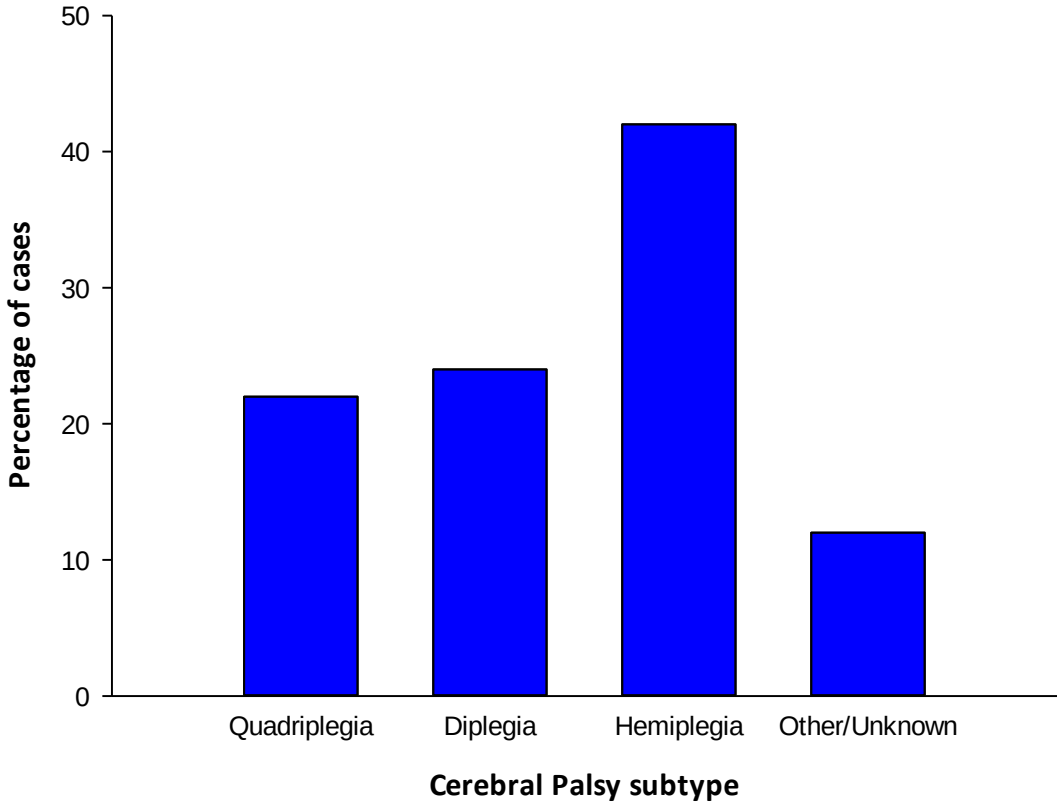
1. Spastic cerebral palsy is classified by the region of the body affected i.e. hemiplegia (one side), diplegia (lower limbs), triplegia (three limbs) and quadriplegia (four limbs). Affected individuals with spastic cerebral palsy are normally hypertonic (increased muscle tension).
2. Ataxic cerebral palsy in most cases is characterised by hypotonia (low muscle tone).
3. Dyskinetic cerebral palsy is characterised by a mixed muscle tone of hypertonia and hypotonia.

Amongst spastic cerebral palsy cases hemiplegia is the most prevalent type of cerebral palsy in term babies and diplegia is the most prevalent in preterm infants[123]. Table 2.1 and Figure 2.1 illustrate the distribution of cerebral palsy subtypes for the individuals in our cerebral palsy cohort.

Table 2.1 Type of cerebral palsy.

Type of CP	Number	%
Quadriplegia	11	22
Triplegia	2	4
Diplegia	12	24
Hemiplegia	21	42
Dyskinetic	2	4
Unknown	2	4
Total	50	100

Figure 2.1 Type of cerebral palsy in our cohort of 50 cases.



2.1.4 Gestational Age

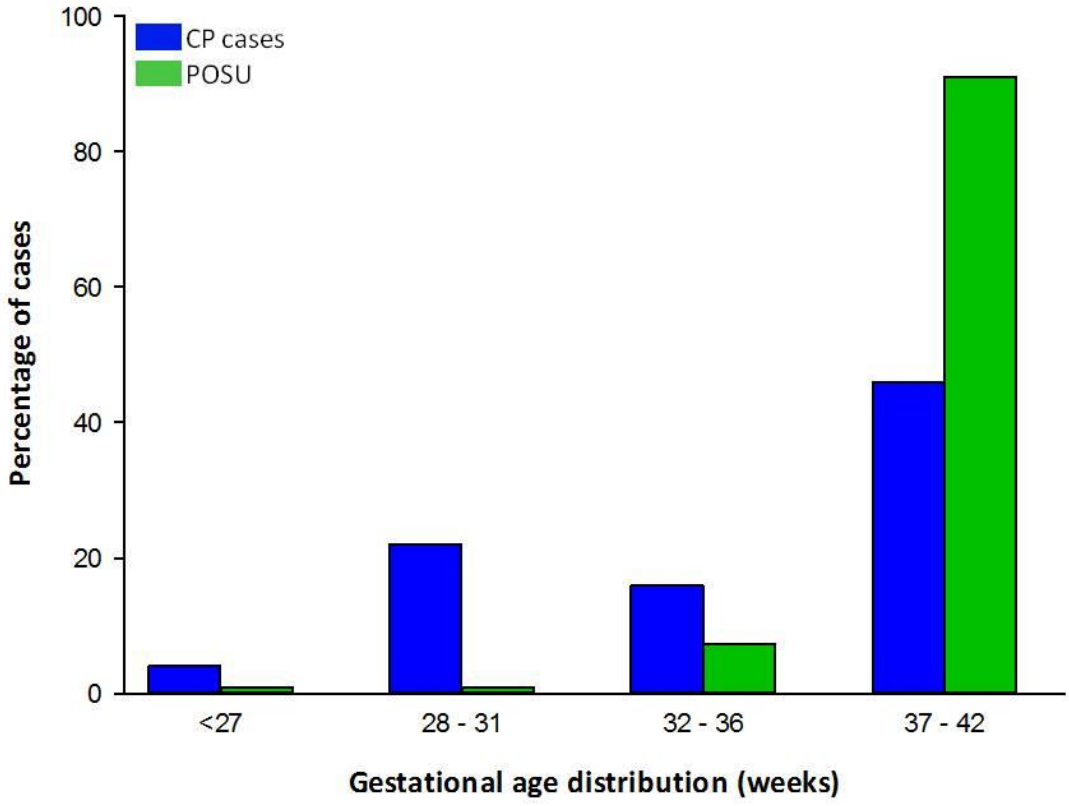
The risk of cerebral palsy increases with decreasing gestational age. Very preterm infants 24 - 26 weeks and 27 – 32 weeks represent 20% and 4% of diagnosed cases of cerebral palsy respectively, with term infants accounting for half of all diagnosed cases[124]. A more recent study based on our Australian cohort found that babies born between 32 – 36 weeks were at an increased risk for cerebral palsy (OR 5.0) and even more so if born before 32 weeks (OR 59.2) compared to term infants[30]. There appears to be a direct correlation between increased rates of cerebral palsy and decline in mortality rate amongst preterm babies[125]. Approximately half of the cohort in this thesis (46%) was born at term, 12% were born between 33 and 36 weeks and the remaining (30%) fell into the very premature category of less than 32 weeks. (Table 2.2 and Figure 2.2). In comparison, 90% of all South Australian Births in 2009 were term born[126]

Table 2.2 Gestational age distribution of cerebral palsy cohort.

Weeks GA	Number	% CP cohort	% POSU*
≥37	23	46	91
32 - 36	6	12	7.2
28 - 31	13	26	0.9
<27	2	4	0.9
Unknown	6	12	0
Total	50	100	100

*POSU - Pregnancy Outcome in South Australia 2009

Figure 2.2 Gestational age distribution of babies with cerebral palsy compared with Pregnancy Outcome Unit South Australia data (POSU).



2.1.5 Birth Weight Distribution

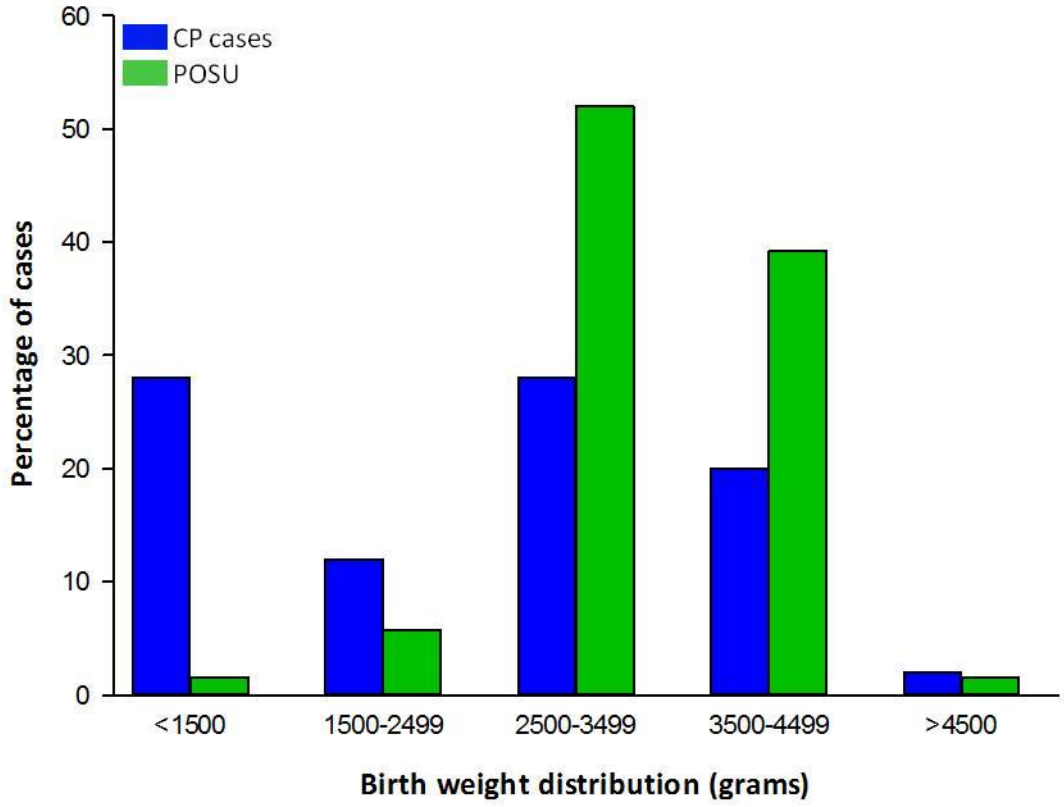
The risk of cerebral palsy increases amongst low birth weight babies compared with normal birth weight babies. Babies born less than 2500 grams accounted for approximately 50% of cerebral palsy cases[127]. Over the last few decades the incidence of low birth weight babies amongst cerebral palsy cases has risen from 33% to 50% and, as with gestational age, this is largely due to increased survival rate[127]. The mean birth weight for our cerebral palsy cohort was 2461 grams (680 – 4750 grams). In comparison the mean weight of all South Australian births in 2009 was 3328 grams (55 – 5930 grams)[126] (Table 2.3 and Figure 2.3)

Table 2.3 Birth weight distribution of cerebral palsy cohort.

Birth weight (grams)	Number	% CP Cohort	% POSU*
<1500	14	28	1.6
1500-2499	6	12	5.7
2500-3499	14	28	52
3500-4499	10	20	39.2
≥4500	1	2	1.5
Unknown	5	10	0
Total	50	100	100

*POSU - Pregnancy Outcome in South Australia 2009

Figure 2.3 Birth weight distribution of babies with cerebral palsy compared with Pregnancy Outcome Unit South Australia data (POSU).



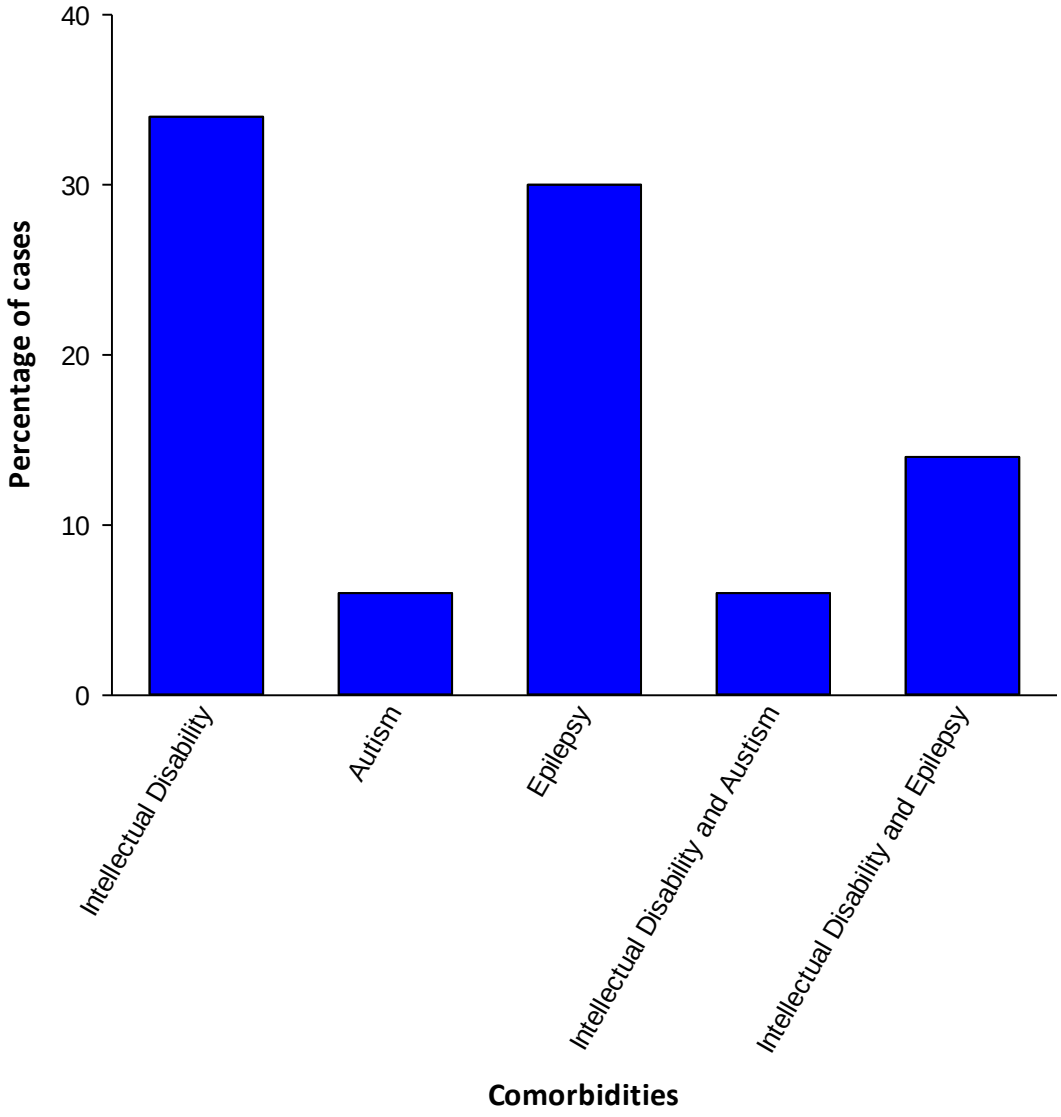
2.1.6 Co-morbidities distribution

Cerebral palsy is often accompanied by other neurodevelopmental disorders including Intellectual disability (ID), autism (au) and epilepsy (ep). Spastic hemiplegia cerebral palsy has the highest reported incidence of additional co-morbidities, approximately 42%[127]. Nearly half of our cohort (48%) had one or more of these co-morbidities Table 2.4 and Figure 2.4. Intellectual disability, epilepsy and autism have been extensively examined for CNVs, all of which have demonstrated an increased overall CNV burden compared to a healthy population. Both *de novo* and rare inherited CNVs containing genes involved in neurodevelopmental pathways have been associated with the above disorders in 5 – 21 % of cases[79, 115, 128-130].

Table 2.4 Co-morbidities distribution of cerebral palsy cohort.

Co-morbidities	Number	%
Intellectual disability (ID)	17	34
Autism (Au)	3	6
Epilepsy (EP)	15	30
ID and Au	3	6
ID and EP	7	14
Au and EP	0	0

Figure 2.4 Co-morbidities distribution of cerebral palsy cohort.



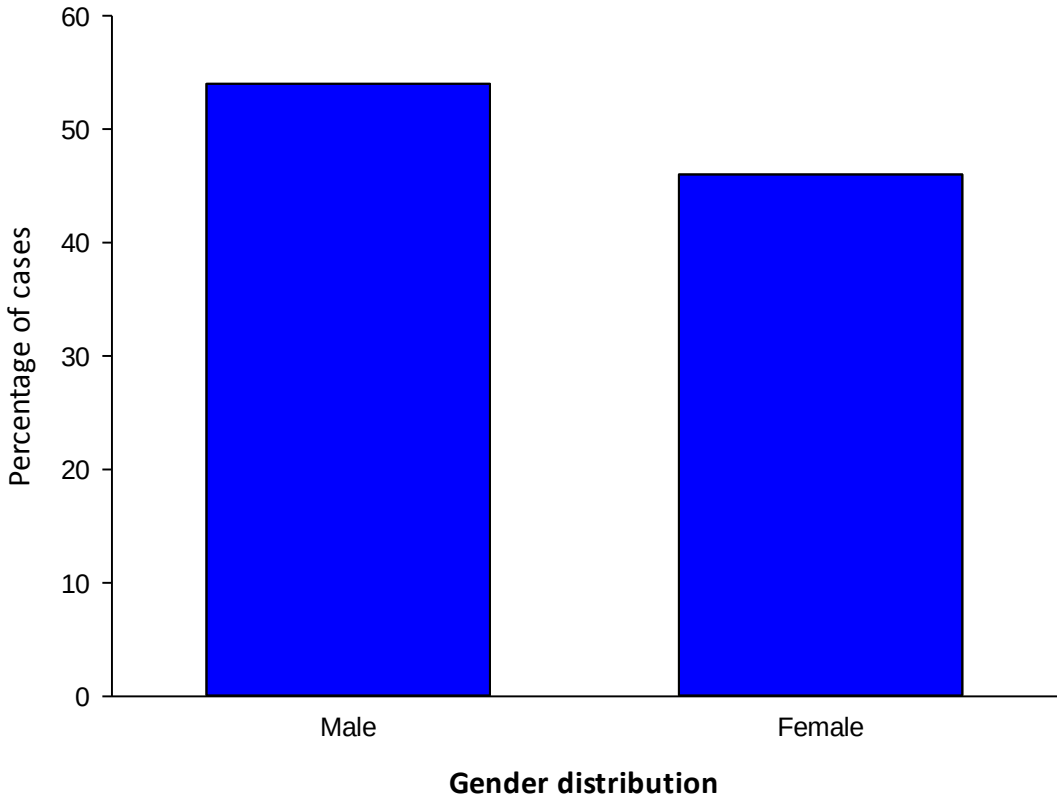
2.1.7 Gender Distribution

Table 2.5 and Figure 2.5 illustrate the gender distribution of our cerebral palsy cohort. Cerebral palsy has been found to be more prevalent in males compared with females.

Table 2.5 Gender distribution of cerebral palsy cohort.

Gender	Number	%
Male	27	54
Female	23	46
Total	50	100

Figure 2.5 Gender distribution of cerebral palsy cohort.



2.1.8 Controls

To determine those CNVs which were rare (<1% population frequency) results were compared to 8,329 adult control samples with no known neurological disorders. The control cohort was largely of Caucasian origin (81.2%) with the remaining of African, African-American and mixed ancestry. DNA was derived from cell lines and whole blood as part of the Illumina SNP profiles from nine previous genome wide SNP microarray studies of neurologically normal adult individuals. Detailed information on this control cohort can be found in the supplementary data of the published article[114]. In summary, the cohort of 8,329 individuals is made up of 984[64, 131] ethnically diverse samples from the Human Hapmap study, 441[64, 132] and 227[64, 132] Caucasian neurologically normal individuals, 936[133], 534[134] and 232[134] individuals of European decent with moderately high cholesterol levels, 1,430[114] ethnically diverse post-menopausal women, 695[135, 136] and 2,090[137] Caucasian blood donors. Due to the increased probe coverage (most controls were assayed using arrays with >555,000 probes) the control data set had a higher detection power and level of resolution reducing the potential for false CNV enrichments within cases.

Well characterised public databases such as the Database of Genomic Variants (DGV) hosted by the Hospital for Sick Children in Toronto[67] which incorporates the Children's Hospital of Philadelphia (CHOP) database of 2,026 healthy controls[72] assisted when determining the frequency of CNVs discovered in our cerebral palsy cohort (see URLs ppxii). The DGV represents structural variation of normal individuals and has been designed to provide control data which is suitable for studies when comparing

genomic structural variation, thereby assisting researchers in determining pathogenic variants in their disease cohort. To date, results from 42 separate studies, consisting of a total of 14,000 controls have been submitted to the DGV which includes 66,741 CNV entries and a total of 15,963 CNV loci.

To assist in further characterisation of potential CNVs for the cerebral palsy phenotype, we identified previously reported chromosomal imbalances utilising the Wellcome Trust Sanger Institute's Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (Decipher) and Online Mendelian Inheritance in Man (OMIM) (see URLs ppxii). Both databases contain comprehensive information on genetic mutations correlated with phenotypic information. Decipher is specifically designed to assist in determining the pathogenicity of chromosomal imbalances by identifying patients who share the same genetic mutation and similar phenotypes. OMIM consists of a comprehensive collection of human genes and genetic phenotypes reporting on all known mendelian disorders and consisting of over 12,000 genes. As with Decipher, OMIM also focuses on the relationship between genotype and phenotype.

Chapter 3 **Materials and Methods**

3.1 Methodology

3.1.1 Study Design

This was a population-based pilot study involving 50 South Australian family trios (mother, father, 27 affected males and 23 affected females). We tested the hypothesis that cerebral palsy is genetically highly heterogeneous and is caused by many diverse and individually rare genetic abnormalities, the most common of which, are copy number variants. All cerebral palsy cases were isolated with no known previous family history of cerebral palsy. No selection criteria were applied to this cohort. The extensive clinical data collected for this cohort considered the type of cerebral palsy, gestational age, gender and other co-morbidities such as autism, intellectual disability and epilepsy to assist in determining the pathogenicity of CNVs found in cerebral palsy cases. Respective parents of individuals carrying a CNV region of potential interest for the cerebral palsy phenotype were tested to determine if the CNV event is *de novo* or inherited. Genotyping results were manually correlated with public control databases to ascertain the population frequency of the CNV of plausible neurological dysfunction for the cerebral palsy phenotype.

3.1.2 Recruitment of cerebral palsy families

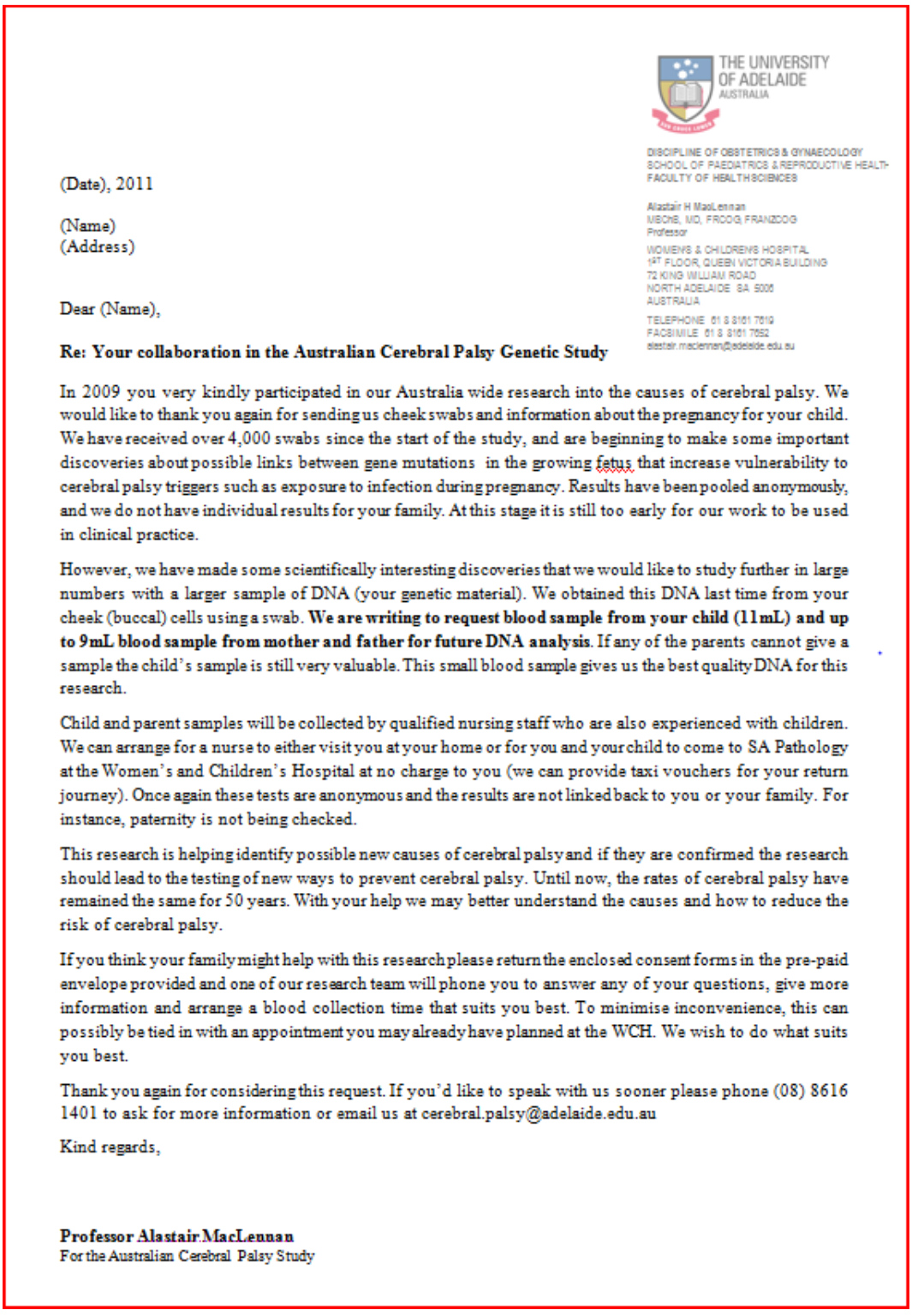
Recruitment for this pilot study coincided with the development of a National lymphocyte cell line and blood derived DNA biobank of cerebral palsy family trios (mother, father and affected child). A cerebral palsy DNA biobank is an essential resource for the continuance of copy number variation research and for new cost effective genomic methodologies as they become available. Participating South

Australian cerebral palsy families from our existing database (99% agreed to be recontacted for future research) were invited to take part in this current study and the development of a cerebral palsy DNA biobank. New families were also sought through ongoing recruitment and contact with the Paediatric Rehabilitation Department at the Women's and Children's Hospital, Adelaide, South Australia. For the purpose of the cerebral palsy DNA biobank, collaborations have been established with the paediatric rehabilitation department of the Princess Margaret Hospital, Perth, Western Australia. Collaborations are planned to include New South Wales and Tasmania. Participant recruitment was initially established in South Australia before extending to Western Australia and therefore this pilot study consisted of only South Australian participating families. The age distribution ranged between 4 years and 25 years with a mean age of 11 years. All participants were of Caucasian decent with a confirmed specialist diagnosis of cerebral palsy and, where possible, confirmation by the South Australian State Cerebral Palsy Register.

Invited families were sent a study kit containing a cover letter (Figure 3.1) informing them about the study, University of Adelaide study information sheet (Figure 3.2) and consent form (Figure 3.3), Genetic Repositories Australia participant information sheet (Figure 3.4) and consent form (Figure 3.5), a detailed maternal questionnaire requesting pregnancy, labour and delivery details of their participating child and a pre-paid envelope. Participants wishing to be involved in this research were asked to sign and return the enclosed consent forms in the provided pre-paid envelope which allowed the research coordinator to phone to discuss the project further, answer any

questions the family may have and arrange a suitable arranging a suitable and convenient time for blood collection.

Figure 3.1 Letter of invitation for Cerebral Palsy Research project.



THE UNIVERSITY OF ADELAIDE
AUSTRALIA

DISCIPLINE OF OBSTETRICS & GYNAECOLOGY
SCHOOL OF PAEDIATRICS & REPRODUCTIVE HEALTH
FACULTY OF HEALTH SCIENCES

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alastair.maclennan@adelaide.edu.au

(Date), 2011

(Name)
(Address)

Dear (Name),

Re: Your collaboration in the Australian Cerebral Palsy Genetic Study

In 2009 you very kindly participated in our Australia wide research into the causes of cerebral palsy. We would like to thank you again for sending us cheek swabs and information about the pregnancy for your child. We have received over 4,000 swabs since the start of the study, and are beginning to make some important discoveries about possible links between gene mutations in the growing fetus that increase vulnerability to cerebral palsy triggers such as exposure to infection during pregnancy. Results have been pooled anonymously, and we do not have individual results for your family. At this stage it is still too early for our work to be used in clinical practice.

However, we have made some scientifically interesting discoveries that we would like to study further in large numbers with a larger sample of DNA (your genetic material). We obtained this DNA last time from your cheek (buccal) cells using a swab. **We are writing to request blood sample from your child (11mL) and up to 9mL blood sample from mother and father for future DNA analysis.** If any of the parents cannot give a sample the child's sample is still very valuable. This small blood sample gives us the best quality DNA for this research.

Child and parent samples will be collected by qualified nursing staff who are also experienced with children. We can arrange for a nurse to either visit you at your home or for you and your child to come to SA Pathology at the Women's and Children's Hospital at no charge to you (we can provide taxi vouchers for your return journey). Once again these tests are anonymous and the results are not linked back to you or your family. For instance, paternity is not being checked.

This research is helping identify possible new causes of cerebral palsy and if they are confirmed the research should lead to the testing of new ways to prevent cerebral palsy. Until now, the rates of cerebral palsy have remained the same for 50 years. With your help we may better understand the causes and how to reduce the risk of cerebral palsy.

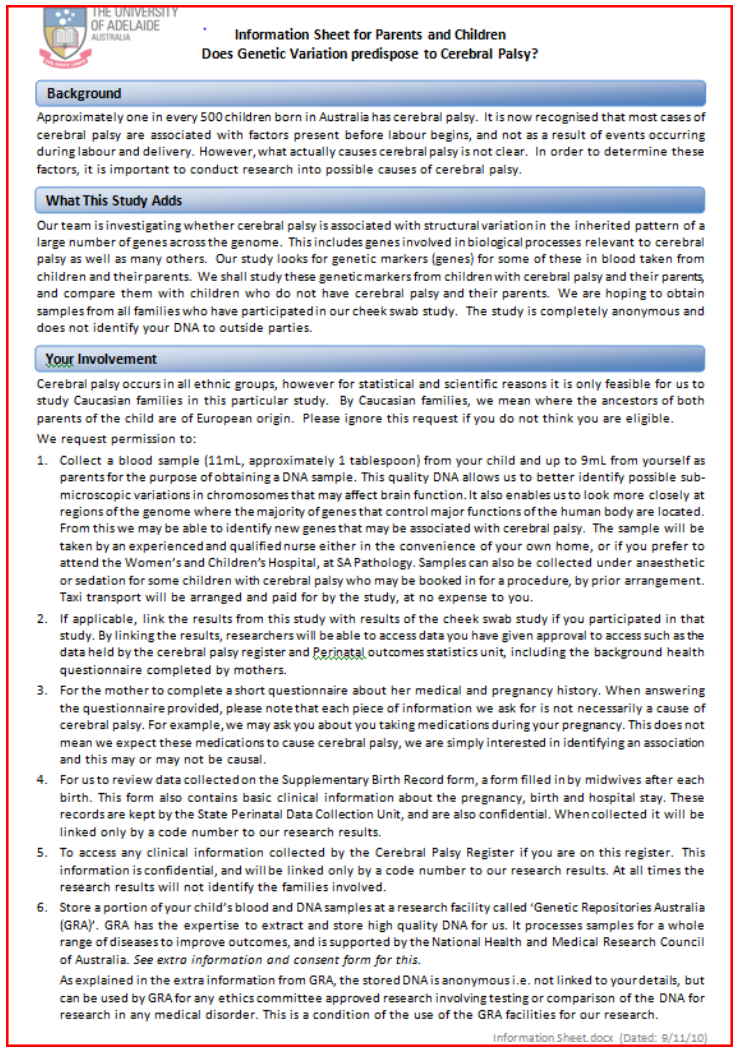
If you think your family might help with this research please return the enclosed consent forms in the pre-paid envelope provided and one of our research team will phone you to answer any of your questions, give more information and arrange a blood collection time that suits you best. To minimise inconvenience, this can possibly be tied in with an appointment you may already have planned at the WCH. We wish to do what suits you best.

Thank you again for considering this request. If you'd like to speak with us sooner please phone (08) 8616 1401 to ask for more information or email us at cerebral.palsy@adelaide.edu.au

Kind regards,

Professor Alastair MacLennan
For the Australian Cerebral Palsy Study

Figure 3.2 University of Adelaide Information Sheet.



THE UNIVERSITY OF ADELAIDE AUSTRALIA

Information Sheet for Parents and Children
Does Genetic Variation predispose to Cerebral Palsy?

Background

Approximately one in every 500 children born in Australia has cerebral palsy. It is now recognised that most cases of cerebral palsy are associated with factors present before labour begins, and not as a result of events occurring during labour and delivery. However, what actually causes cerebral palsy is not clear. In order to determine these factors, it is important to conduct research into possible causes of cerebral palsy.

What This Study Adds

Our team is investigating whether cerebral palsy is associated with structural variation in the inherited pattern of a large number of genes across the genome. This includes genes involved in biological processes relevant to cerebral palsy as well as many others. Our study looks for genetic markers (genes) for some of these in blood taken from children and their parents. We shall study these genetic markers from children with cerebral palsy and their parents, and compare them with children who do not have cerebral palsy and their parents. We are hoping to obtain samples from all families who have participated in our cheek swab study. The study is completely anonymous and does not identify your DNA to outside parties.

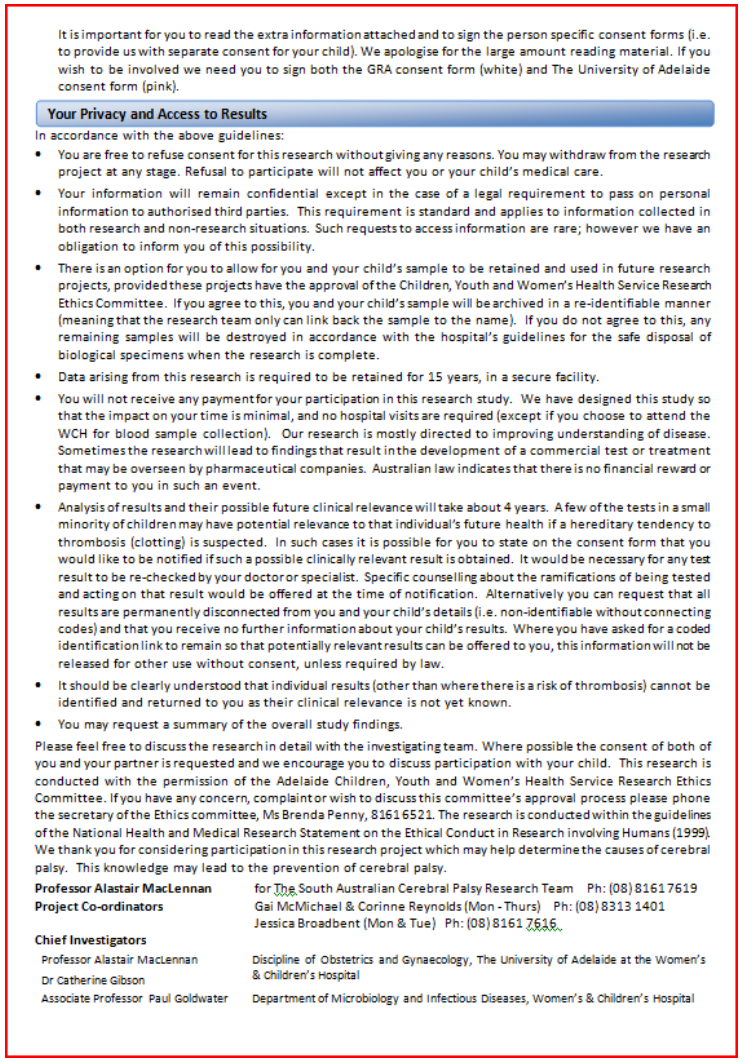
Your Involvement

Cerebral palsy occurs in all ethnic groups, however for statistical and scientific reasons it is only feasible for us to study Caucasian families in this particular study. By Caucasian families, we mean where the ancestors of both parents of the child are of European origin. Please ignore this request if you do not think you are eligible.

We request permission to:

1. Collect a blood sample (11mL, approximately 1 tablespoon) from your child and up to 9mL from yourself as parents for the purpose of obtaining a DNA sample. This quality DNA allows us to better identify possible sub-microscopic variations in chromosomes that may affect brain function. It also enables us to look more closely at regions of the genome where the majority of genes that control major functions of the human body are located. From this we may be able to identify new genes that may be associated with cerebral palsy. The sample will be taken by an experienced and qualified nurse either in the convenience of your own home, or if you prefer to attend the Women's and Children's Hospital, at SA Pathology. Samples can also be collected under anaesthetic or sedation for some children with cerebral palsy who may be booked in for a procedure, by prior arrangement. Taxi transport will be arranged and paid for by the study, at no expense to you.
2. If applicable, link the results from this study with results of the cheek swab study if you participated in that study. By linking the results, researchers will be able to access data you have given approval to access such as the data held by the cerebral palsy register and Perinatal outcomes statistics unit, including the background health questionnaire completed by mothers.
3. For the mother to complete a short questionnaire about her medical and pregnancy history. When answering the questionnaire provided, please note that each piece of information we ask for is not necessarily a cause of cerebral palsy. For example, we may ask you about you taking medications during your pregnancy. This does not mean we expect these medications to cause cerebral palsy, we are simply interested in identifying an association and this may or may not be causal.
4. For us to review data collected on the Supplementary Birth Record form, a form filled in by midwives after each birth. This form also contains basic clinical information about the pregnancy, birth and hospital stay. These records are kept by the State Perinatal Data Collection Unit, and are also confidential. When collected it will be linked only by a code number to our research results.
5. To access any clinical information collected by the Cerebral Palsy Register if you are on this register. This information is confidential, and will be linked only by a code number to our research results. At all times the research results will not identify the families involved.
6. Store a portion of your child's blood and DNA samples at a research facility called 'Genetic Repositories Australia (GRA)'. GRA has the expertise to extract and store high quality DNA for us. It processes samples for a whole range of diseases to improve outcomes, and is supported by the National Health and Medical Research Council of Australia. *See extra information and consent form for this.*

   As explained in the extra information from GRA, the stored DNA is anonymous i.e. not linked to your details, but can be used by GRA for any ethics committee approved research involving testing or comparison of the DNA for research in any medical disorder. This is a condition of the use of the GRA facilities for our research.



It is important for you to read the extra information attached and to sign the person specific consent forms (i.e. to provide us with separate consent for your child). We apologise for the large amount reading material. If you wish to be involved we need you to sign both the GRA consent form (white) and The University of Adelaide consent form (pink).

Your Privacy and Access to Results

In accordance with the above guidelines:

- You are free to refuse consent for this research without giving any reasons. You may withdraw from the research project at any stage. Refusal to participate will not affect you or your child's medical care.
- Your information will remain confidential except in the case of a legal requirement to pass on personal information to authorised third parties. This requirement is standard and applies to information collected in both research and non-research situations. Such requests to access information are rare; however we have an obligation to inform you of this possibility.
- There is an option for you to allow for you and your child's sample to be retained and used in future research projects, provided these projects have the approval of the Children, Youth and Women's Health Service Research Ethics Committee. If you agree to this, you and your child's sample will be archived in a re-identifiable manner (meaning that the research team only can link back the sample to the name). If you do not agree to this, any remaining samples will be destroyed in accordance with the hospital's guidelines for the safe disposal of biological specimens when the research is complete.
- Data arising from this research is required to be retained for 15 years, in a secure facility.
- You will not receive any payment for your participation in this research study. We have designed this study so that the impact on your time is minimal, and no hospital visits are required (except if you choose to attend the WCH for blood sample collection). Our research is mostly directed to improving understanding of disease. Sometimes the research will lead to findings that result in the development of a commercial test or treatment that may be overseen by pharmaceutical companies. Australian law indicates that there is no financial reward or payment to you in such an event.
- Analysis of results and their possible future clinical relevance will take about 4 years. A few of the tests in a small minority of children may have potential relevance to that individual's future health if a hereditary tendency to thrombosis (clotting) is suspected. In such cases it is possible for you to state on the consent form that you would like to be notified if such a possible clinically relevant result is obtained. It would be necessary for any test result to be re-checked by your doctor or specialist. Specific counselling about the ramifications of being tested and acting on that result would be offered at the time of notification. Alternatively you can request that all results are permanently disconnected from you and your child's details (i.e. non-identifiable without connecting codes) and that you receive no further information about your child's results. Where you have asked for a coded identification link to remain so that potentially relevant results can be offered to you, this information will not be released for other use without consent, unless required by law.
- It should be clearly understood that individual results (other than where there is a risk of thrombosis) cannot be identified and returned to you as their clinical relevance is not yet known.
- You may request a summary of the overall study findings.

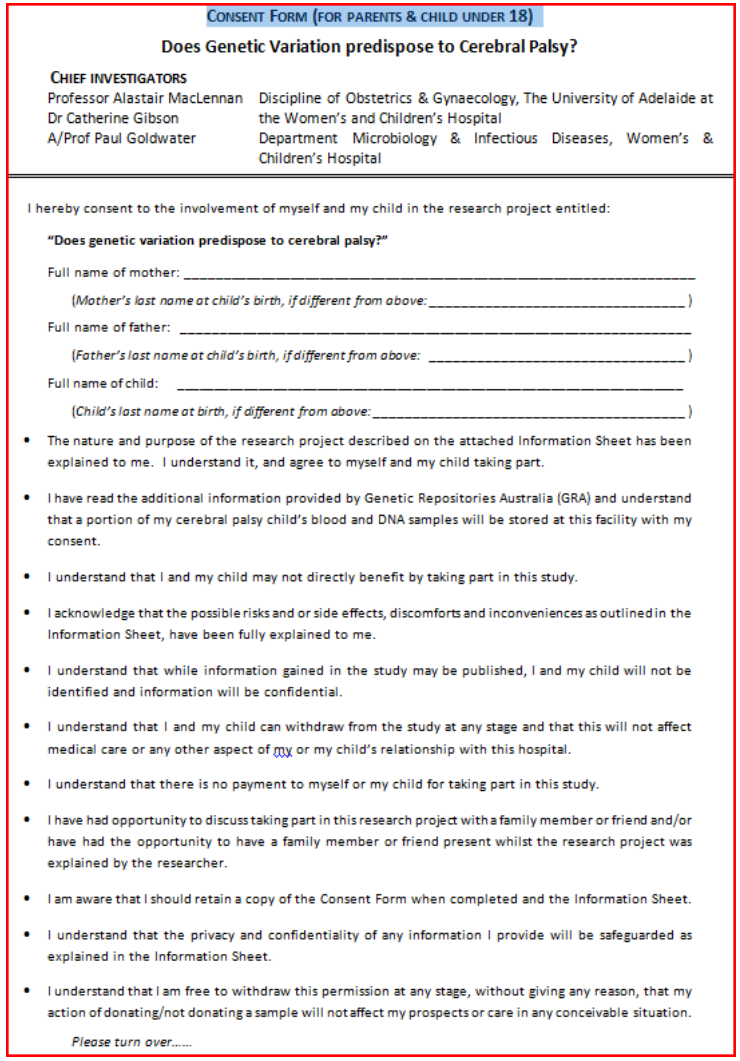
Please feel free to discuss the research in detail with the investigating team. Where possible the consent of both of you and your partner is requested and we encourage you to discuss participation with your child. This research is conducted with the permission of the Adelaide Children, Youth and Women's Health Service Research Ethics Committee. If you have any concern, complaint or wish to discuss this committee's approval process please phone the secretary of the Ethics committee, Ms Brenda Penny, 8161 6521. The research is conducted within the guidelines of the National Health and Medical Research Statement on the Ethical Conduct in Research involving Humans (1999). We thank you for considering participation in this research project which may help determine the causes of cerebral palsy. This knowledge may lead to the prevention of cerebral palsy.

Professor Alastair MacLennan for The South Australian Cerebral Palsy Research Team Ph: (08) 8161 7619
Project Co-ordinators Gai McMichael & Corinne Reynolds (Mon - Thurs) Ph: (08) 8313 1401
Jessica Broadbent (Mon & Tue) Ph: (08) 8161 7616

Chief Investigators

Professor Alastair MacLennan	Discipline of Obstetrics and Gynaecology, The University of Adelaide at the Women's & Children's Hospital
Dr Catherine Gibson	
Associate Professor Paul Goldwater	Department of Microbiology and Infectious Diseases, Women's & Children's Hospital

Figure 3.3 University of Adelaide consent form.



CONSENT FORM (FOR PARENTS & CHILD UNDER 18)

Does Genetic Variation predispose to Cerebral Palsy?

CHIEF INVESTIGATORS

Professor Alastair MacLennan — Discipline of Obstetrics & Gynaecology, The University of Adelaide at the Women's and Children's Hospital
Dr Catherine Gibson
A/Prof Paul Goldwater — Department Microbiology & Infectious Diseases, Women's & Children's Hospital

I hereby consent to the involvement of myself and my child in the research project entitled:

"Does genetic variation predispose to cerebral palsy?"

Full name of mother: _____

(*Mother's last name at child's birth, if different from above:* _____)

Full name of father: _____

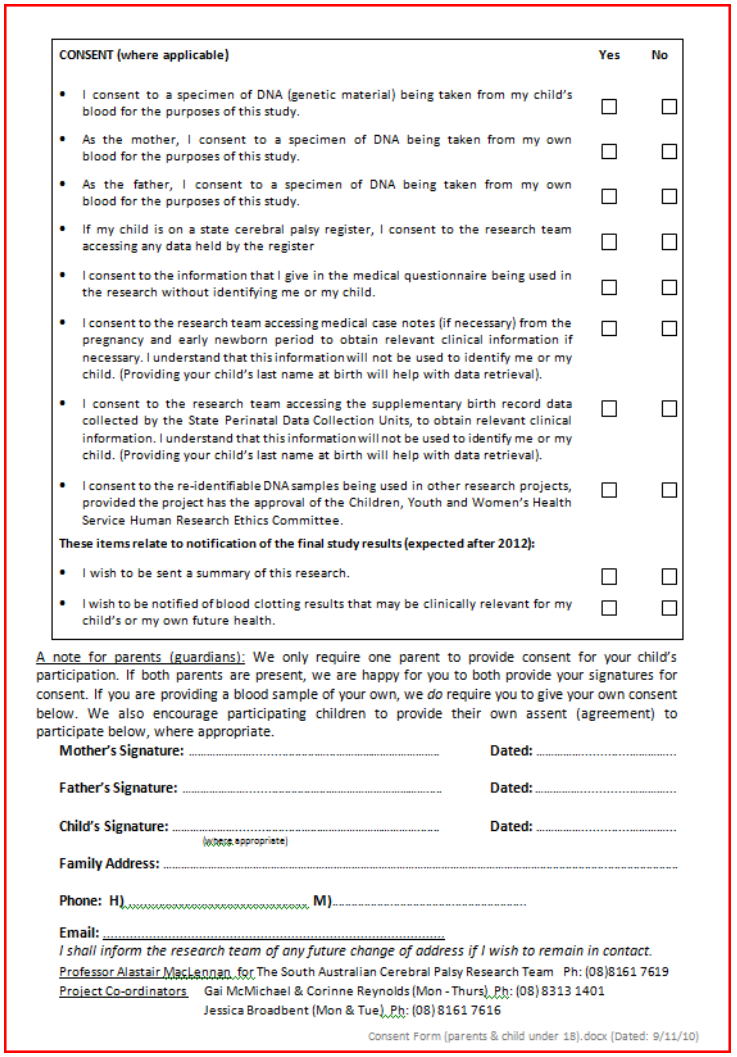
(*Father's last name at child's birth, if different from above:* _____)

Full name of child: _____

(*Child's last name at birth, if different from above:* _____)

- The nature and purpose of the research project described on the attached Information Sheet has been explained to me. I understand it, and agree to myself and my child taking part.
- I have read the additional information provided by Genetic Repositories Australia (GRA) and understand that a portion of my cerebral palsy child's blood and DNA samples will be stored at this facility with my consent.
- I understand that I and my child may not directly benefit by taking part in this study.
- I acknowledge that the possible risks and or side effects, discomforts and inconveniences as outlined in the Information Sheet, have been fully explained to me.
- I understand that while information gained in the study may be published, I and my child will not be identified and information will be confidential.
- I understand that I and my child can withdraw from the study at any stage and that this will not affect medical care or any other aspect of my or my child's relationship with this hospital.
- I understand that there is no payment to myself or my child for taking part in this study.
- I have had opportunity to discuss taking part in this research project with a family member or friend and/or have had the opportunity to have a family member or friend present whilst the research project was explained by the researcher.
- I am aware that I should retain a copy of the Consent Form when completed and the Information Sheet.
- I understand that the privacy and confidentiality of any information I provide will be safeguarded as explained in the Information Sheet.
- I understand that I am free to withdraw this permission at any stage, without giving any reason, that my action of donating/not donating a sample will not affect my prospects or care in any conceivable situation.

Please turn over……



CONSENT (where applicable)	**Yes**	**No**
• I consent to a specimen of DNA (genetic material) being taken from my child's blood for the purposes of this study.	☐	☐
• As the mother, I consent to a specimen of DNA being taken from my own blood for the purposes of this study.	☐	☐
• As the father, I consent to a specimen of DNA being taken from my own blood for the purposes of this study.	☐	☐
• If my child is on a state cerebral palsy register, I consent to the research team accessing any data held by the register	☐	☐
• I consent to the information that I give in the medical questionnaire being used in the research without identifying me or my child.	☐	☐
• I consent to the research team accessing medical case notes (if necessary) from the pregnancy and early newborn period to obtain relevant clinical information if necessary. I understand that this information will not be used to identify me or my child. (Providing your child's last name at birth will help with data retrieval).	☐	☐
• I consent to the research team accessing the supplementary birth record data collected by the State Perinatal Data Collection Units, to obtain relevant clinical information. I understand that this information will not be used to identify me or my child. (Providing your child's last name at birth will help with data retrieval).	☐	☐
• I consent to the re-identifiable DNA samples being used in other research projects, provided the project has the approval of the Children, Youth and Women's Health Service Human Research Ethics Committee.	☐	☐
These items relate to notification of the final study results (expected after 2012):		
• I wish to be sent a summary of this research.	☐	☐
• I wish to be notified of blood clotting results that may be clinically relevant for my child's or my own future health.	☐	☐

A note for parents (guardians): We only require one parent to provide consent for your child's participation. If both parents are present, we are happy for you to both provide your signatures for consent. If you are providing a blood sample of your own, we *do* require you to give your own consent below. We also encourage participating children to provide their own assent (agreement) to participate below, where appropriate.

Mother's Signature: ..... **Dated:** .....

Father's Signature: ..... **Dated:** .....

Child's Signature: ..... **Dated:** .....
(where appropriate)

Family Address: .....

Phone: H) ..... **M)** .....

Email: .....

I shall inform the research team of any future change of address if I wish to remain in contact.

Professor Alastair MacLennan for The South Australian Cerebral Palsy Research Team Ph: (08)8161 7619
Project Co-ordinators Gai McMichael & Corinne Reynolds (Mon - Thurs) Ph: (08) 8313 1401
Jessica Broadbent (Mon & Tue) Ph: (08) 8161 7616

Figure 3.4 Genetic Repositories Australia information sheet.

GRA ID Number UNSW HREC Approval No 06323

Neuroscience Research Australia. Discover. Conquer. Cure. GRA GENETIC REPOSITORIES AUSTRALIA UNSW THE UNIVERSITY OF NEW SOUTH WALES

Genetic Repositories Australia (GRA)

Combined Participant and Parental (or Guardian) Information Statement

Samples and clinical data for submission to Genetic Repositories Australia.

You and your child are invited to participate in a research initiative that will provide a source of de-identified material, with descriptors of their disease, as well as your family to facilitate genetic analyses for medical research. If you agree for you and your child to participate in the study you will each be asked to provide a sample of blood (up to 15ml), saliva or a cheek swab that will be submitted to Genetic Repositories Australia (GRA), a research resource supported by the National Health and Medical Research Council of Australia, for subsequent processing. GRA provides Australian medical researchers with a national facility for the processing, long-term secure storage and distribution to qualified investigators of human genetic material (DNA and cell lines) collected from patients and healthy participants from studies on a range of diseases and their outcomes.

What is DNA and how are genes inherited?

Deoxyribonucleic acid (DNA) is the molecule that stores the genetic code responsible for the shape and make up of an organism. The segment of DNA that determines the shape and makeup is known as a gene. Genes are the units of inheritance and are responsible for the hereditary characteristics passed on from one generation to the next. Errors in the DNA that make up a gene are called mutations and may lead to disease. By studying DNA, researchers aim to identify the genes involved in disease. When researchers understand how a gene goes wrong they have a better chance of designing better methods to diagnose, treat or cure that disease.

Why should you consider submitting your child's samples?

If you agree for yourself and your child to participate, submission of your blood/saliva/cheek swab samples to the Repository may give scientists material that can help them to develop new understanding about the cause of disease, new diagnostic tests, new treatments, and new ways to prevent diseases. To participate, you must sign and date the attached "Participant Consent Form". To permit your child to participate, you as the parent or guardian, must also sign and date the attached "Parental (or Guardian) Consent Form".

Arrangements for protection of you and your child's identity and other private information?

The Repository will take full measures to protect the privacy of all participants. The specimen will be given a unique identification number to protect you and your child's privacy. Genetic Repositories Australia will not give out any personal information that can identify you or your child to the scientists who are approved to receive the samples. Some information, such as age, disease status and gender, will be made available to enable the medical research to proceed. Any information that is obtained in connection with you or your child's sample and that can be identified with you or that of your child will remain confidential and will be disclosed only with your permission or except as required by law. The researchers that will use your samples may publish or present results in scientific journals or scientific meetings. In any publication, information will be provided in such a way that you or your child cannot be identified.

How are GRA samples used?

GRA collects, stores, and distributes DNA samples and cell lines which are derived from lymphocytes (white blood cells) from healthy people, people with many kinds of disorders as well as from unaffected family members. All researchers wishing to access the genetic material stored by GRA must first have approval from the relevant Human Research Ethics Committee for their project. Those researchers must then apply in writing to the GRA Management Committee, describing the intended use of the samples for approval for access to the samples. This approval will be ratified by the UNSW Human Research Ethics Committee. Access will be given only for the stated use. It is not possible to foresee all the potential projects that may use the genetic material, now or in the future. Researchers may be from non-profit research institutes, universities, etc or from commercial organisations from Australia or overseas.

Researchers using samples obtained from GRA may make discoveries which may be subject to patent applications or commercialisation. However, in this regard, such discoveries only arise from the analysis of many samples, and any individual sample will not be of benefit. No payment, compensation, royalty or any other financial benefit will be made to you or your child which may result from the research facilitated by GRA. Note that GRA makes no claim on intellectual property arising from research undertaken on samples distributed by GRA. The storage and use of all genetic material will be conducted in accordance with the National Health & Medical Research Council's *Guidelines for Genetic Registers and Associated Genetic Material (1999), the Human Tissue Act (1984, amendments 2003), the National Statement on Ethical Conduct in Human Research (2007)* and relevant Australian Legislation.

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GRA ID Number UNSW HREC Approval No 06323

What happens to you and your child's blood/tissue sample?

The blood/saliva/cheek swab samples are sent to the facility, located at Neuroscience Research Australia, Sydney, Australia. Genetic material will be extracted from you and your child's blood, saliva or cheek swab. In order to ensure an ongoing source of DNA, white blood cells (lymphocytes) are cultured and kept growing in the laboratory, as a cell line, which allows a source of genetic material without having to obtain another blood sample. These cell lines can be stored indefinitely. In rare cases, a second sample may be requested if there are technical difficulties in establishing a cell line with the first sample.

What happens to you and your child's samples if GRA closes?

The genetic material stored by GRA is a precious and scientifically valuable resource that should not be lost to future medical research, however in the event GRA closes, attempts will be made initially to transfer you and your child's samples to a third party willing to undertake the responsibility for managing GRA. This would only occur if the third party adhered to the current guidelines set out by the GRA Scientific Advisory Committee and *the National Statement on Ethical Conduct in Human Research (2007)*. Should no third party exist, or if funding for this initiative ceased, then GRA will no longer operate and all genetic samples and accompanying data will be destroyed.

What are the risks to you and your child in providing a sample?

The medical risks of providing these specimens are minimal. The risk for venipuncture may include minor transient pain, a slight possibility of infection or some bruising that may be present for a few days. During a blood draw, you or your child may experience some discomfort or transient pain at the site of needle entry into the vein as you might during any routine blood test. There is a remote risk of fainting.

Will you or your child be provided with any results?

Since the results generated from you and your child's samples are for research purposes only, no results will be provided to either of you. However, in the event that information of clinical significance (i.e. one which has a significant probability of impacting on the health of you or that of your child) is identified by the researchers using samples sourced from GRA, every effort will be made to contact you through the Chief investigator of the study in which you and your child is a participant.

What happens if you decide you want to withdraw you or your child's sample from the GRA repository collection?

Your decision to permit you and your child's donation is entirely voluntary and if you choose not to participate there will be no penalty or loss of benefits should you withdraw. If you wish to withdraw you or your child's sample from GRA, this can be done by notifying the Chief Investigator of the study in which you and your child is a participant. If you withdraw you or your child from the recruiting study, GRA will follow the directions of the relevant Chief Investigator to destroy your child's sample or to allow their sample to continue to be used as detailed in the attached revocation of consent form.

What are the benefits of participating in GRA's repository collection?

If you agree to participate, you or your child will not directly benefit from the research conducted using you and your child's genetic material, nor will you or your child receive either now or in the future any payment, compensation, royalty or any other financial benefit which may result from the research, but your child's sample may benefit the community at large or some particular group. We thank you and your child for your participation – your assistance will help advance medical research.

Whom to contact with questions?

If you have any questions or complications relating to collection of this specimen, you should contact the Collection Center. If you have any questions relating to the study, you should contact "*CHIEF INVESTIGATOR & CONTACT DETAILS*" If you have any questions about GRA, you should contact Mr. Steve Turner, Facility Manager, "Genetic Repositories Australia", Neuroscience Research Australia, Barker Street, Randwick, NSW, 2031. Sydney, Australia. (Telephone: 02 9399 1068).

Whom to contact with issues of complaint?

Complaints may be directed to the Ethics Secretariat, The University of New South Wales, Sydney 2052 Australia (Telephone 02 9385 4234, Facsimile 02 9385 6648, Email ethics.sec@unsw.edu.au). Any complaint you make will be treated in confidence and investigated, and you will be informed of the outcome. You will be given a copy of this document. Please keep it for your reference in case you want to read it again.

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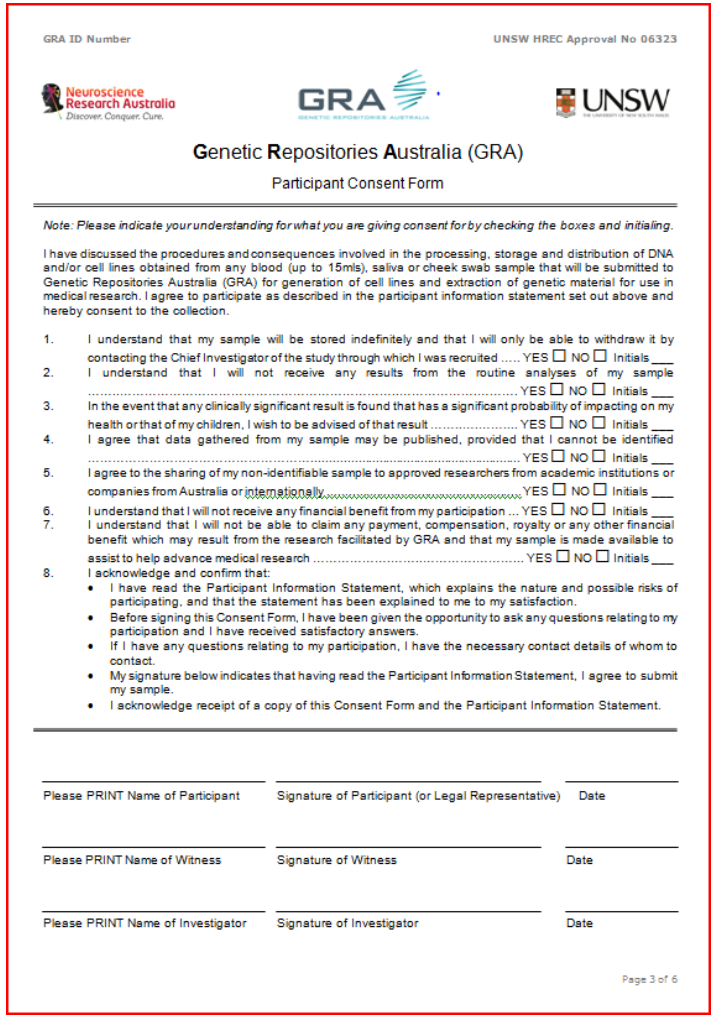
Figure 3.5 Genetic Repositories Australia Consent Forms.



GRA ID Number UNSW HREC Approval No 06323

Neuroscience Research Australia. Discover. Conquer. Cure. GRA GENETIC REPOSITORIES AUSTRALIA UNSW

Genetic Repositories Australia (GRA)

Participant Consent Form

Note: Please indicate your understanding for what you are giving consent for by checking the boxes and initialing.

I have discussed the procedures and consequences involved in the processing, storage and distribution of DNA and/or cell lines obtained from any blood (up to 15mls), saliva or cheek swab sample that will be submitted to Genetic Repositories Australia (GRA) for generation of cell lines and extraction of genetic material for use in medical research. I agree to participate as described in the participant information statement set out above and hereby consent to the collection.

1. I understand that my sample will be stored indefinitely and that I will only be able to withdraw it by contacting the Chief Investigator of the study through which I was recruited ..... YES ☐ NO ☐ Initials ___
2. I understand that I will not receive any results from the routine analyses of my sample ..... YES ☐ NO ☐ Initials ___
3. In the event that any clinically significant result is found that has a significant probability of impacting on my health or that of my children, I wish to be advised of that result ..... YES ☐ NO ☐ Initials ___
4. I agree that data gathered from my sample may be published, provided that I cannot be identified ..... YES ☐ NO ☐ Initials ___
5. I agree to the sharing of my non-identifiable sample to approved researchers from academic institutions or companies from Australia or internationally ..... YES ☐ NO ☐ Initials ___
6. I understand that I will not receive any financial benefit from my participation ... YES ☐ NO ☐ Initials ___
7. I understand that I will not be able to claim any payment, compensation, royalty or any other financial benefit which may result from the research facilitated by GRA and that my sample is made available to assist to help advance medical research ..... YES ☐ NO ☐ Initials ___
8. I acknowledge and confirm that:
   - I have read the Participant Information Statement, which explains the nature and possible risks of participating, and that the statement has been explained to me to my satisfaction.
   - Before signing this Consent Form, I have been given the opportunity to ask any questions relating to my participation and I have received satisfactory answers.
   - If I have any questions relating to my participation, I have the necessary contact details of whom to contact.
   - My signature below indicates that having read the Participant Information Statement, I agree to submit my sample.
   - I acknowledge receipt of a copy of this Consent Form and the Participant Information Statement.

Please PRINT Name of Participant	Signature of Participant (or Legal Representative)	Date
Please PRINT Name of Witness	Signature of Witness	Date
Please PRINT Name of Investigator	Signature of Investigator	Date

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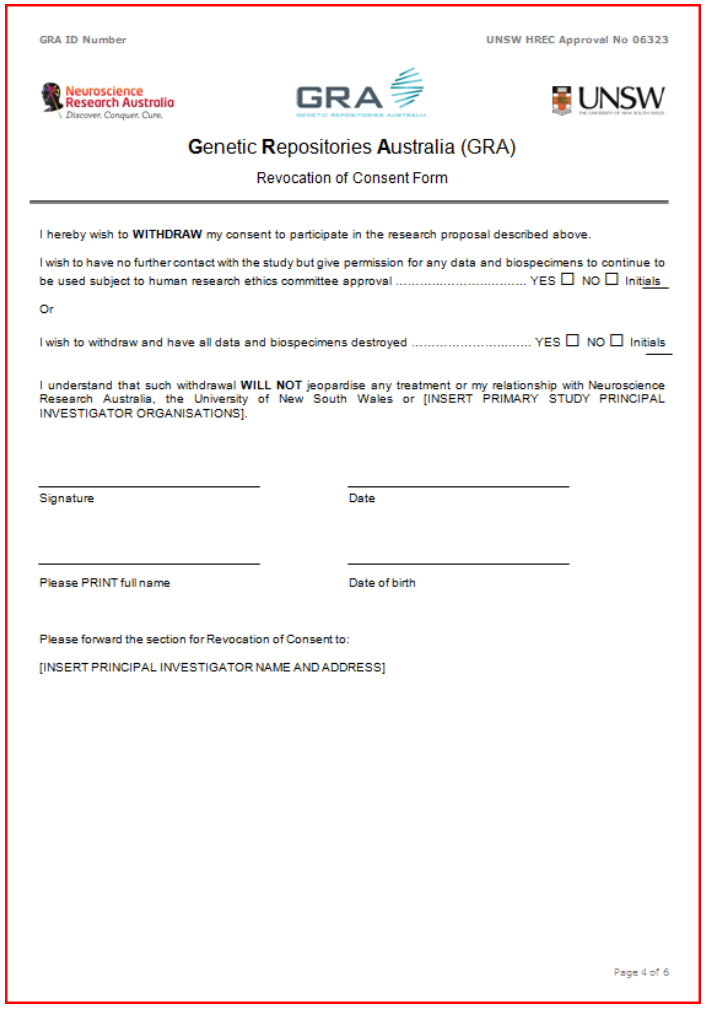

GRA ID Number UNSW HREC Approval No 06323

Neuroscience Research Australia. Discover. Conquer. Cure. GRA GENETIC REPOSITORIES AUSTRALIA UNSW

Genetic Repositories Australia (GRA)

Revocation of Consent Form

I hereby wish to **WITHDRAW** my consent to participate in the research proposal described above.

I wish to have no further contact with the study but give permission for any data and biospecimens to continue to be used subject to human research ethics committee approval ..... YES ☐ NO ☐ Initials ___

Or

I wish to withdraw and have all data and biospecimens destroyed ..... YES ☐ NO ☐ Initials ___

I understand that such withdrawal **WILL NOT** jeopardise any treatment or my relationship with Neuroscience Research Australia, the University of New South Wales or [INSERT PRIMARY STUDY PRINCIPAL INVESTIGATOR ORGANISATIONS].

Signature	Date
Please PRINT full name	Date of birth

Please forward the section for Revocation of Consent to:

[INSERT PRINCIPAL INVESTIGATOR NAME AND ADDRESS]

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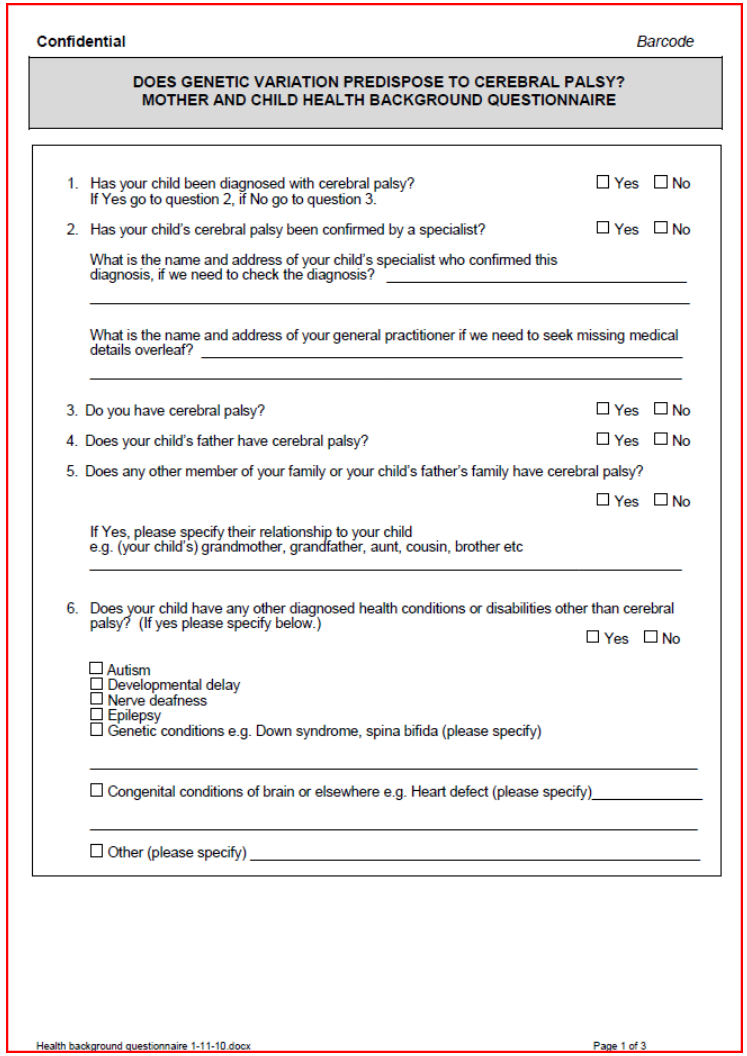
Figure 3.6 Participant Health Background Questionnaire



Confidential *Barcode*

DOES GENETIC VARIATION PREDISPOSE TO CEREBRAL PALSY?
MOTHER AND CHILD HEALTH BACKGROUND QUESTIONNAIRE

1. Has your child been diagnosed with cerebral palsy? ☐ Yes ☐ No
   If Yes go to question 2, if No go to question 3.
2. Has your child's cerebral palsy been confirmed by a specialist? ☐ Yes ☐ No

   What is the name and address of your child's specialist who confirmed this diagnosis, if we need to check the diagnosis? _____

   _____

   What is the name and address of your general practitioner if we need to seek missing medical details overleaf? _____

   _____

3. Do you have cerebral palsy? ☐ Yes ☐ No
4. Does your child's father have cerebral palsy? ☐ Yes ☐ No
5. Does any other member of your family or your child's father's family have cerebral palsy? ☐ Yes ☐ No

   If Yes, please specify their relationship to your child
   e.g. (your child's) grandmother, grandfather, aunt, cousin, brother etc

   _____

6. Does your child have any other diagnosed health conditions or disabilities other than cerebral palsy? (If yes please specify below.) ☐ Yes ☐ No

   ☐ Autism
   ☐ Developmental delay
   ☐ Nerve deafness
   ☐ Epilepsy
   ☐ Genetic conditions e.g. Down syndrome, spina bifida (please specify)

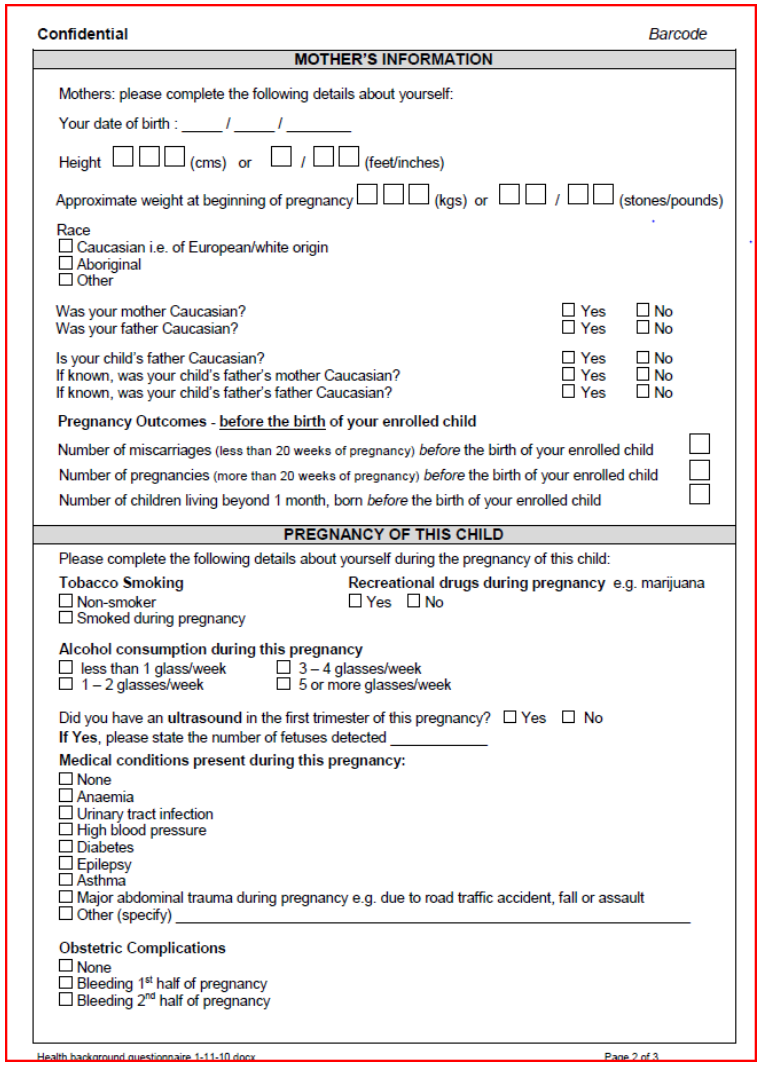
   _____

   ☐ Congenital conditions of brain or elsewhere e.g. Heart defect (please specify)_____

   _____

   ☐ Other (please specify) _____

Health background questionnaire 1-11-10.docx Page 1 of 3



Confidential *Barcode*

MOTHER'S INFORMATION

Mothers: please complete the following details about yourself:

Your date of birth : ____ / ____ / _____

Height ☐☐☐ (cms) or ☐ / ☐☐ (feet/inches)

Approximate weight at beginning of pregnancy ☐☐☐ (kgs) or ☐☐ / ☐☐ (stones/pounds)

Race
☐ Caucasian i.e. of European/white origin
☐ Aboriginal
☐ Other

Was your mother Caucasian? ☐ Yes ☐ No
Was your father Caucasian? ☐ Yes ☐ No

Is your child's father Caucasian? ☐ Yes ☐ No
If known, was your child's father's mother Caucasian? ☐ Yes ☐ No
If known, was your child's father's father Caucasian? ☐ Yes ☐ No

Pregnancy Outcomes - before the birth of your enrolled child

Number of miscarriages (less than 20 weeks of pregnancy) *before* the birth of your enrolled child ☐
Number of pregnancies (more than 20 weeks of pregnancy) *before* the birth of your enrolled child ☐
Number of children living beyond 1 month, born *before* the birth of your enrolled child ☐

PREGNANCY OF THIS CHILD

Please complete the following details about yourself during the pregnancy of this child:

Tobacco Smoking
☐ Non-smoker
☐ Smoked during pregnancy

Recreational drugs during pregnancy e.g. marijuana
☐ Yes ☐ No

Alcohol consumption during this pregnancy
☐ less than 1 glass/week ☐ 3 – 4 glasses/week
☐ 1 – 2 glasses/week ☐ 5 or more glasses/week

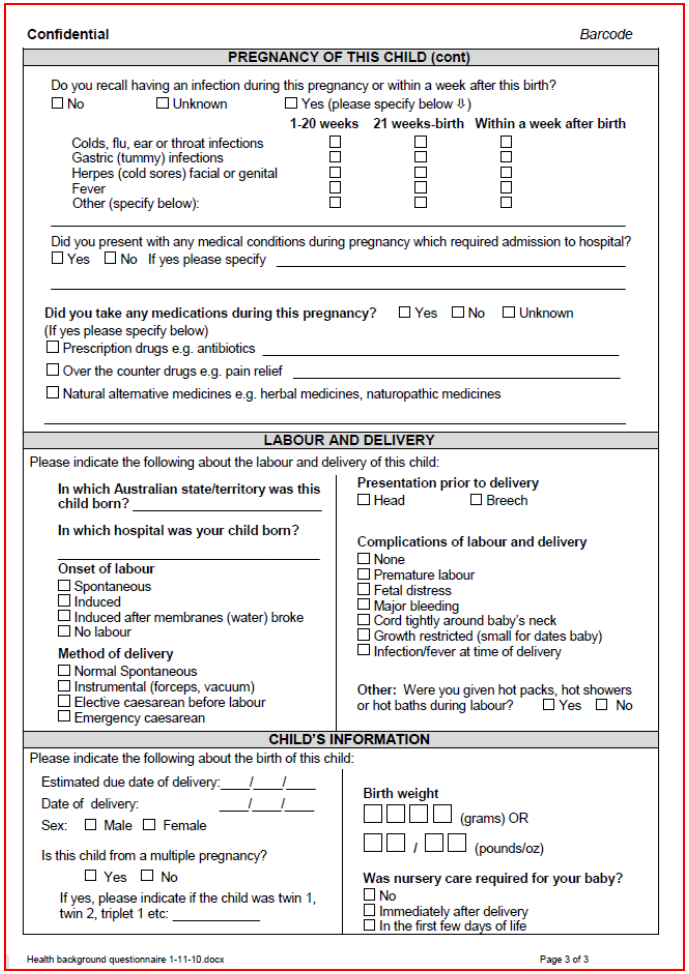
Did you have an **ultrasound** in the first trimester of this pregnancy? ☐ Yes ☐ No
If Yes, please state the number of fetuses detected _____

Medical conditions present during this pregnancy:
☐ None
☐ Anaemia
☐ Urinary tract infection
☐ High blood pressure
☐ Diabetes
☐ Epilepsy
☐ Asthma
☐ Major abdominal trauma during pregnancy e.g. due to road traffic accident, fall or assault
☐ Other (specify) _____

Obstetric Complications
☐ None
☐ Bleeding 1st half of pregnancy
☐ Bleeding 2nd half of pregnancy

Health background questionnaire 1-11-10.docx Page 2 of 3

Figure 3.6 (continued)



Confidential *Barcode*

PREGNANCY OF THIS CHILD (cont)

Do you recall having an infection during this pregnancy or within a week after this birth?
☐ No ☐ Unknown ☐ Yes (please specify below ⇩)

	1-20 weeks	21 weeks-birth	Within a week after birth
Colds, flu, ear or throat infections	☐	☐	☐
Gastric (tummy) infections	☐	☐	☐
Herpes (cold sores) facial or genital	☐	☐	☐
Fever	☐	☐	☐
Other (specify below):	☐	☐	☐

Did you present with any medical conditions during pregnancy which required admission to hospital?
☐ Yes ☐ No If yes please specify _____

Did you take any medications during this pregnancy? ☐ Yes ☐ No ☐ Unknown
(If yes please specify below)
☐ Prescription drugs e.g. antibiotics _____
☐ Over the counter drugs e.g. pain relief _____
☐ Natural alternative medicines e.g. herbal medicines, naturopathic medicines

LABOUR AND DELIVERY

Please indicate the following about the labour and delivery of this child:

In which Australian state/territory was this child born? _____

In which hospital was your child born?

_____

Onset of labour
☐ Spontaneous
☐ Induced
☐ Induced after membranes (water) broke
☐ No labour

Method of delivery
☐ Normal Spontaneous
☐ Instrumental (forceps, vacuum)
☐ Elective caesarean before labour
☐ Emergency caesarean

Presentation prior to delivery
☐ Head ☐ Breech

Complications of labour and delivery
☐ None
☐ Premature labour
☐ Fetal distress
☐ Major bleeding
☐ Cord tightly around baby's neck
☐ Growth restricted (small for dates baby)
☐ Infection/fever at time of delivery

Other: Were you given hot packs, hot showers or hot baths during labour? ☐ Yes ☐ No

CHILD'S INFORMATION

Please indicate the following about the birth of this child:

Estimated due date of delivery: ___/___/___
Date of delivery: ___/___/___
Sex: ☐ Male ☐ Female

Is this child from a multiple pregnancy?
☐ Yes ☐ No
If yes, please indicate if the child was twin 1, twin 2, triplet 1 etc: _____

Birth weight
☐☐☐☐ (grams) OR
☐☐ / ☐☐ (pounds/oz)

Was nursery care required for your baby?
☐ No
☐ Immediately after delivery
☐ In the first few days of life

Health background questionnaire 1-11-10.docx Page 3 of 3

3.1.3 Blood collection

Whole blood (11mL) was collected (9mL in an acid citrate dextrose (ACD) collection tube and 2mL in an Ethylenediaminetetraacetic acid (EDTA) collection tube) from the affected child and 9mL of whole blood was collected from each parent in an EDTA collection tube. To minimise any inconvenience to the participating families, a number of ways to collect blood were offered. This included a pre-arranged home visit by a registered nurse, collection via a blood pathology centre or at SA Pathology at the Women's and Children's Hospital. In each case all child and parental samples were collected from a qualified phlebotomist experienced in blood collection from children. A number of children with cerebral palsy have Botox therapy under general anaesthetic (GA). In these instances, visits were coordinated with Paediatric Rehabilitation and Anaesthesiology Departments at the Women's and Children's Hospital to collect the child's blood during this time, with no discomfort to the child under GA.

3.1.4 Lymphocyte cell line development

Genetic Repositories Australia (GRA) are a National Health and Medical Research Council supported facility that develops, stores and distributes de-identified DNA samples and lymphoblastoid cell lines (LCLs) to facilitate genetic medical research. This ensures an ongoing source of DNA and cell lines for all ethically approved medical research. Importantly, it negates the inconvenience of recontacting families each time new genetic technologies become available, as well as saving valuable time for the researcher in project recruitment.

The technique applied by GRA to develop Epstein Barr Viral (EBV) transformed LCLs involves collection of fresh whole blood into 9ml citrated (ACD) blood collection tubes. Fresh human B lymphocytes are isolated via density gradient separation, washed, counted and inoculated with EBV viral supernatant (B95-8 Monkey Marmoset cell line). Cells are then transferred to a $25cm^2$ tissue culture flask containing transformation cell culture media (RPMI 1640, supplemented with fetal bovine serum, L-glutamine and Phytohaemaglutinin MN) and cultured at 37°C with 10% $C0_2$ in a humidity controlled incubator. Developing cultures are routinely checked microscopically for signs of microbial contamination and cell line transformation. Following transformation, cells are washed, counted and cryopreserved in cryovials at 1×10^7 in freeze down media containing 10% dimethyl sulfoxide, cooled at a controlled rate of -1°C/minute prior to transferring to a vapour phase liquid nitrogen tank for long term storage.

The first 50 blood samples from the South Australian cohort to complete this process were nominated for this pilot study. This included both families from our existing database (n = 35) and new families through the Paediatric Rehabilitation Department at the Women's and Children's Hospital, Adelaide (n = 15).

3.1.5 DNA extraction

DNA extraction from LCLs was performed at GRA on a fully automated large volume nucleic acid purification system (Qiagen Autopure LS) (Qiagen, Stanford, CA) which ensures high quality DNA from EBV transformed LCLs. The remaining 2mL of the

affected child's blood and 9mL of the parent's blood was extracted in our laboratories using a QIAamp DNA Blood Mini Kit and QIAamp DNA Blood Maxi Kit (Qiagen, Stanford, CA) respectively, following the manufacturer's instructions. The resulting purified DNA has been catalogued and securely stored in -80°C freezers at University of Adelaide facilities.

3.1.6 DNA quantification from LCLs and blood

DNA samples derived from LCLs were quantified at GRA facilities and blood derived DNA samples were quantified at our laboratories, in each case with a Nanodrop spectrophotometer (Thermo Scentific, MA., USA).

3.1.7 Array Comparative Genome Hybridization (array-CGH)

This project was in collaboration with the University of Washington, Seattle, Washington, US led by Professor Evan Eichler and Emory University, Atlanta, Georgia, US led by Professor Christa Martin. Both research groups have extensive experience in the use of microarray analysis for detecting CNVs. Prepared DNA samples were sent to both laboratories for CNV profiling on two individual microarray platforms; the first a custom-design 180K microarray (Agilent Technologies, CA, US) and a custom-designed 135K microarray (Roche NimbleGen, Madison, WI, US). This maximised detection of potentially pathogenic CNVs for cerebral palsy. Both arrays were custom-designed and developed and validated for CNV detection in individuals with intellectual disability, autism spectrum disorders and multiple congenital anomalies. Since the design of the arrays over five years ago analyses has been successfully

carried out on a broad range of neurological disorders in greater than 5000 individuals. The design and resolution of these arrays combined with the CNV analysis algorithms has shown a very high rate of CNV calling accuracy (99%) and both arrays are utilised for research and diagnostic purposes. The candidate was responsible for the analyses and interpretation of the raw data with all aspects of wet-lab procedures performed by technical staff in the Seattle and Atlanta laboratories respectively. However, the candidate travelled to both laboratories to gain a better understanding of the wet-lab chemistry involved.

Array-CGH genotyping methodologies are presented below as Part A- Custom-design 180K microarray and Part B – Custom-design 135K microarray.

3.2 Part A – Custom-design 180K microarray

3.2.1 CNV discovery

Initially the 50 proband samples were tested on a custom-designed 180K chromosomal microarray with targeted (a minimum of 10 probes per gene or region) plus whole genome coverage (probes spaced on average every 75 kb) according to the standard protocols (Agilent Technologies, CA, US). The targeted coverage included known clinically relevant regions such as microdeletion/duplication syndromes, telomeres and centromeres at a resolution of ~20-50 kb plus exon-level coverage of >1200 genes involved in neurodevelopmental disorders. The whole-genome backbone results in a resolution in unique DNA of ~225kb.

3.2.2 Sample labelling and hybridization

Microarray labelling and hybridization experiments were according to the manufacturer's recommendations with no modifications (Agilent Technologies, CA, US).

3.2.3 Analysis/Filtering

Feature Extraction (version 10.5.1.1) and DNA Analytics (Version 4.0.81) software (Agilent Technologies) was used to perform data analysis. The March 2006 human genome reference NCBI36/hg18 assembly produced by the International Human Genome Sequencing Consortium was used for this project. CNVs were manually inspected in the University of California Santa Cruz (UCSC) database (see URLs ppxii).

3.3 Part B – Custom-design 135K microarray

3.3.1 CNV discovery

The same 50 DNA samples were separately assessed on a custom-designed 135K array comprising of 135,000 probes with one probe every 10 kb covering the duplication architecture of the human genome, recurrent events and genomic hotspots (regions flanked by segmental duplications) and 20 - 30 kb density in the genomic backbone. An empirical detection resolution of >50 kb within the hotspots and >350 kb in the genomic backbone have been determined for this array.

3.3.2 Sample labelling and hybridization

All microarray hybridisation experiments were performed as described previously [138], using a single unaffected male (GM15724 from Coriell) as reference. DNA was randomly fragmented by sonication to a size range of 500–2,000 bp. For labelling, each DNA sample (1 µg) was denatured at 98°C in the presence of 1 O.D. of 50-Cy3- or 50-Cy5-labeled random nonamer (TriLink Biotechnologies, San Diego, CA) in 62.5 mM Tris-HCl, pH 7.5, 6.25 mM MgCl2, and 0.0875% b-mercaptoethanol. Denatured samples were chilled on ice, prior to incubation with 100 units (exo-) Klenow fragment (NEB, Ipswich, MA) and dNTP mix (6 mM each in Tris, EDTA (TE), Invitrogen, Carlsbad, CA) for 2 hr at 37°C. Reactions were terminated by addition of 0.5 M EDTA (pH 8.0); the end products were precipitated with isopropanol and resuspended in water. Fifty-fold amplification was typically achieved. The Cy-labelled test samples (Cy3) and reference samples (Cy5) were combined (15 µg each) and dried down by vacuum centrifugation. The sample was rehydrated in 40 µl of NimbleGen Hybridization Buffer (NimbleGen Systems, Inc.), denatured at 95°C for 5 min, and then cooled to 42°C. Hybridizations were carried out for 18 hr at 42°C. The arrays were washed using a NimbleGen Wash Buffer System (NimbleGen Systems, Inc.) and immediately dried down by centrifugation. Arrays were scanned at 5-µm resolution using the GenePix4000B scanner (Axon Instruments, Molecular Devices Corp., Sunnyvale, CA). Data were extracted from scanned images using NimbleScan 2.0 extraction software (NimbleGen Systems, Inc.), which allows for automated grid alignment, extraction, and generation of data files[138].

3.3.3 Analyses/Filtering

All microarrays were analysed by mapping probe coordinates to the human genome assembly Build 36 (hg18). Using chromosome-specific means and standard deviations, normalized log intensity ratios samples were transformed into z-scores. These z-scores were classified as "increased", "normal" or "decreased" in copy-number using a three state Hidden Markov Model (HMM). The HMM was applied using HMMSeg [139]. For each sample, HMM state assignments of probes were merged into segments if consecutive probes of the same state less than 50kb apart were merged. If two segments of the same state were separated by an intervening sequence of ≤5 probes and ≤10kb, both segments and intervening sequence were called as a single variant. Subsequently, putative CNVs were automatically filtered and divided based on size, z-scores, and probe counts. These filtering criteria enabled the HMM outputs for CNV events to be thoroughly scanned. The validity of each call was manually checked by examining the normalized log intensity ratios across a chromosome. To minimise false positives, copy number deletions and duplications were determined by a minimum of three consecutive probes beyond a mean significance Log 2 ratio of -0.3 and 0.3 respectively[140]. All CNV calls were manually inspected in the UCSC data base (see URLs ppxii).

3.3.4 Determining candidate regions for cerebral palsy from both array-CGH platforms

To address our hypothesis that CNV events in cerebral palsy cases are individually rare, we firstly identified those that were unique to one individual in our cohort followed by identification of all CNVs which disrupted genes involved in brain

development. To confirm if a CNV region was truly rare (<1% population frequency) all CNVs were manually inspected in the DGV[67] and only those CNVs with less than 50% reciprocal overlap to previously reported events were considered for further analysis. The same CNVs were re-examined in a control data set from the Signature Genomics Initiative consisting of 8,329 individuals from multiple studies of neurologically normal adult individuals[114]. For further confirmation of population frequency and to help resolve the potential pathogenicity of CNVs for cerebral palsy, remaining CNVs were manually inspected in both OMIM and Decipher databases. The recently published morbidity map of developmental delay which consists of 15,767 cases as part of the Signature Genomics initiative[114] also contributed towards determining the pathogenicity of potential CNVs for cerebral palsy. Where a CNV was nominated to be of potential interest for the cerebral palsy phenotype, parental samples were tested to ascertain if the CNV was *de novo* or inherited. Final genotyping results were correlated with epidemiological data, including type of cerebral palsy, gestational age, IUGR, any other co-morbidities and gender.

3.3.5 CNV validation and mode of transmission by qPCR for both array-CGH platforms

Parental samples for three cases with potential candidate gene regions for cerebral palsy were assayed on the custom-designed 180K microarray platform. The following TaqMan Copy Number Assays were utilised to confirm and determine the mode of transmission of CNVs which may contribute to cerebral palsy outcome. Six pre-designed assays [hs01663667 (12p12.1p12.2), hs02958873 (FSCB), hs05327322 (SH3GL3), hs01835833 (7q21), hs00913691 (PTCHD3) and hs07530615 (TARP)] were

individually run simultaneously with a TaqMan Copy Number Reference Assay (RNaseP) in a duplex real-time polymerase chain reaction (PCR) on a 7500 Real-Time PCR System (ABI, Life Technologies, Carlsbad, CA). Each sample was run in triplicate with a known control of two copies and a no template control in a 20 µl reaction which contained 10 µl of 2X TaqMan genotyping master mix, 1 µl of TaqMan Copy number assay 20X working stock, 1 µl of TaqMan Copy number reference assay, 4µl of nuclease-free water and 5µl of template DNA (5ng/µl) as per manufacturer's instructions. Amplification conditions were hold 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. The delta-delta-CT method for quantitative real time-PCR (qPCR) was employed for data analysis[141].

Chapter 4 **Results**

4.1 Results

4.1.1 DNA quantification from LCLs and blood

The mean quantity of DNA from EBV transformed LCLs was 375ng/µl (range 166 – 679ng/µl) (Supplementary data Table 8.1). Microarray genotyping currently requires approximately 200ng/µl. A small number of samples (n = 4) fell just below this gold standard amount but were still considered to be within an acceptable range for this work. The mean quantity of DNA from whole blood using a DNA blood maxi kit was 332ng/µl (range 234.81 – 452.22ng/µl) (Supplementary data Table 8.2). The mean quantity for the DNA blood mini kit was 48.45ng/µl (range 27.2 – 67.88ng/µl) (Supplementary data Table 8.3). The DNA blood maxi kit provided an adequate amount of DNA to screen parents on either a microarray platform or a TaqMan copy number assay system. DNA extracted from the DNA blood mini kit was substantially less than other methods, but was still considered to be more than adequate for validation using the TaqMan copy number system.

The samples were interrogated for CNV under two different design purposes. The 180K Agilent array was focussed on discovering events that have a high prior, namely, known microdeletion and microduplication syndrome regions and ~1200 known candidate genes for neurodevelopmental disorders (Part A). Alternatively, we utilized the duplication architecture of the human genome to identify variants that are recurrent and associated accounting for about 15% of neuropsychiatric disease burden[142] (Part B).

4.2 Part A – Custom-designed 180K microarray

To determine the most plausible CNVs for cerebral palsy, stringent filtering parameters were applied, focusing the final analyses on *de novo* and rare inherited CNVs (<1% population frequency) which disrupted genes involved in neurodevelopment. After filtering, final downstream analyses consisted of 69 gene-locus regions comprised of 36 deletions and 33 duplications. These ranged in size from 4kb to 2.1 MB with the majority of CNVs, 31 (45%) ranging between 100 kb and 500kb (Table 4.1). Of these, 11 (22%) were unique to one individual (Table 4.1 and Table 4.2). Three cases were identified with a CNV that included interesting candidate genes for the cerebral palsy phenotype: CTNND2, catenin delta-2 (446 kb duplication including the first exon; MCPH1, microcephalin (219 kb duplication including exons 1-8); and COPS3, COP9 signalosome complex subunit 3 (4 kb deletion including exons 6-8). The probe coverage for the CNV regions encompassing CTNND2, MCPH1 and COPS3 was 14, 66 and 13 respectively. All three CNVs were shown to be inherited from an unaffected parent. Each of these CNV regions are extremely rare (<1% population frequency) with no overlapping CNVs identified in the control data set or in the DGV database (Table 4.3). Several other rare CNV regions in 20 out of 50 cases, including the above mentioned three cases, were identified including 12p12.1p12.2, as well as single-gene CNVs across *CNTNAP3*, *MC2R*, and *FSCB* and intragenic CNVs in *DLGAP2*, *PARK2*, *NBEA*, *PAK2*, *MACROD2*, *CNTN1*, *MPV17L* and *NF1* (Table 4.3).

Table 4.1 Summary of CNVs from custom design 180K microarray.

Size	Total	Deletions	Duplications
Summary of CNVs			
Number of CNVs in gene-locus regions	69	36	33
Number of CNVs unique to one individual	11	7	4
Size distribution	n (%)	n (%)	n (%)
> 1 MB	1 (1)	1 (3)	0 (0)
> 500 kb - < 1 MB	10 (14)	2 (5)	8 (24)
> 100 kb - < 500 kb	16 (24)	4 (11)	12 (37)
< 100 kb - > 50 kb	15 (22)	10 (28)	5 (15)
< 50 kb	27 (39)	19 (53)	8 (24)

Table 4.2 CNVs unique to one individual in CP cohort (custom-designed 180K array).

Sample	Location	Start	Stop	Size (kb)	CNV	Region - Gene	Inheritance
CP36	5p15.2	11956988	12403682	446	dup	CTNND2 (includes the first exon)	**Maternal**
CP25	8p23.2.p23.1	6072234	6291658	219	dup	MCPH1 (includes exons 1-8)	**Maternal**
CP42	9p13.1	39062229	39278299	216	del	CNTNAP3 (entire gene)	Unknown
CP34	12q12	39673307	39694367	21	del	CNTN1 (intragenic)	Unknown
CP03	12p12.1p12.2	20908843	21295433	386	del	12p12.1p12.2	**Paternal**
CP17	14q21.3	43617568	44369205	751	del	FSCB (entire gene)	**Maternal**
CP25	15q11.2	20316992	20851728	534	dup	15q11.2 (BP1 - BP2)	Unknown
CP18	16p13.11	15397501	15409849	12	del	MPV17L (intragenic)	Unknown
CP20	17p11.2	17104399	17108906	4	del	COPS3 (includes exons 6-8)	**Paternal**
CP53	18p11.21	13861404	13925908	64	dup	MC2R (entire gene)	Unknown
CP42	20p12.1	14266199	14422174	155	del	MACROD2 (intragenic)	Unknown

Unknown – Mode of inheritance not determined as parental samples have not been tested at this time.

4.2.1 Figures 4.1, 4.2 and 4.3

The following three figures represent a Chromosome snapshot of the three CNV regions of potential interest for cerebral palsy. The centre line (0) represents normal copy number and probe coverage for normal copy number is represented by the black dots. Deletions to the left (-) are represented by the green dots and duplications to the right (+) are represented by the red dots. The shaded boxes represent the position of the CNV.

Figure 4.1 CTNND2 Snapshot of gene location on Chr 5 including probe coverage (14).

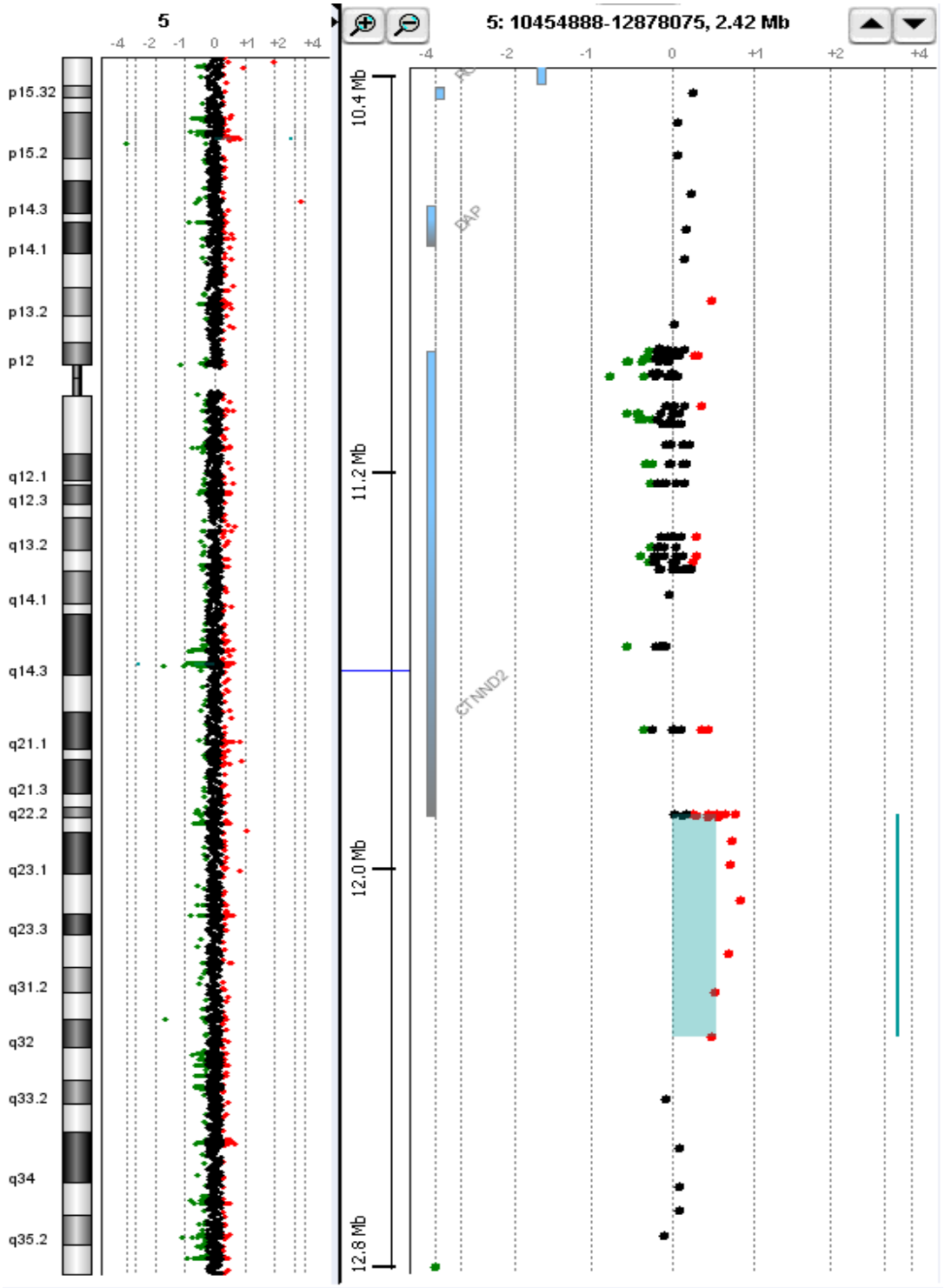


Figure 4.2 MCPH1 Snapshot of gene location on Chr 8 including probe coverage (66).

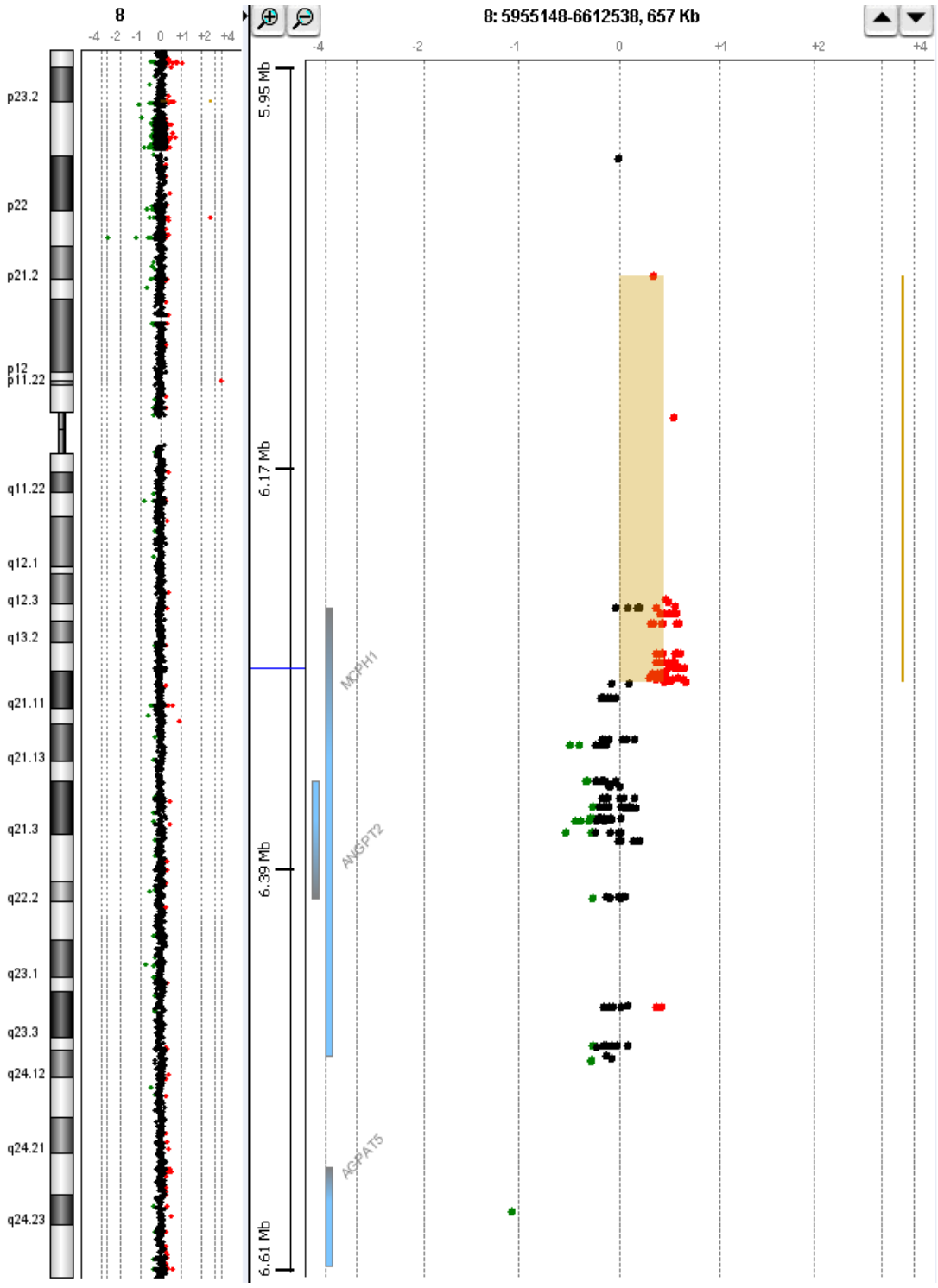


Figure 4.3 COPS3 Snapshot of gene location on Chr 17 including probe coverage (13).

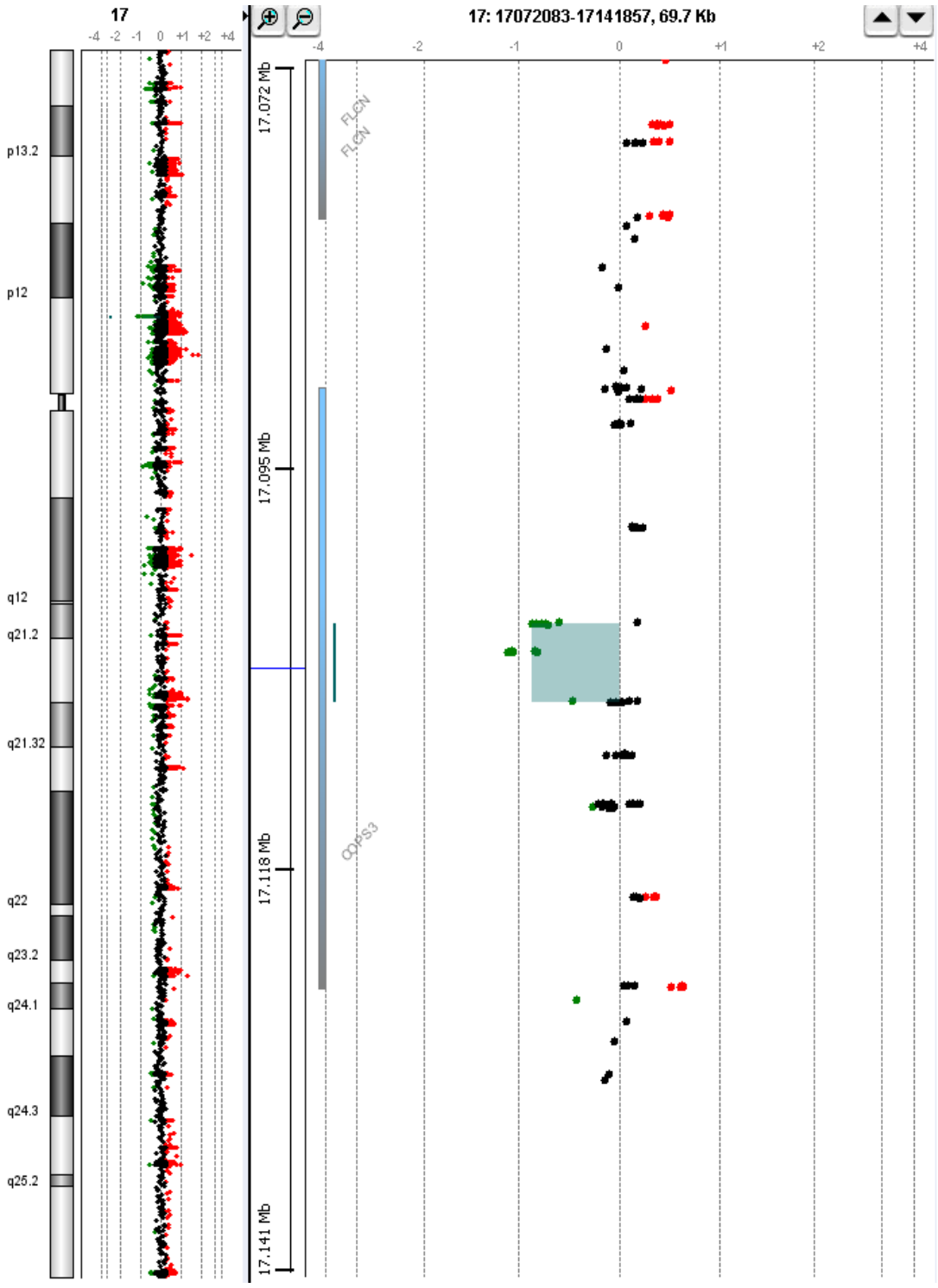


Table 4.3 CNVs in Gene - Locus regions (custom designed 180K microarray).

Location	Start	Stop	Size (kb)	Gene - Locus	Cases (n = 50)		Controls (n =8,329)		DGV (n=~14,000)		Decipher	
					Gain	**Loss**	**Gain**	**Loss**	**Gain**	**Loss**	**Gain**	**Loss**
2p11.2	89009521 89009521	91129998 89871328	2120 861	centromeric	0 0	1 1	0	0	27 28	97 19	14	17
3q29	197993965 197993893 197993893	198042740 198041421 198040466	48 47 46	PAK2	2 1 2	1 1 0	0 0 0	0 0 0	8	11	4	3
5p15.2	11956988	12403682	446	**CTNND2**	1	0	0	0	0	0	2	2
6q26	162314385	162395160	80	PARK2	0	8	0	0	1	0	0	2
8p23.2.p23.1	6072234	6291658	219	**MCPH1**	1	0	0	0	0	0	3	2
8p23.3	1436950	1484791	47	DLGAP2	0	12	0	0	0	20	4	5
9p13.1	39062229	39278299	216	CNTNAP3	0	1	0	2	0	0	0	0
12q12	39673307	39694367	21	CNTN1	0	1	0	0	0	0	0	0
12p12.1p12.2	20908843	21295433	386	12p12.1p12.2	0	1	0	2	0	2	1	2
13q13.2	34522405 34528146 34570433 34570433	34595707 34595707 34614425 34595707	73 67 43 25	NBEA	1 2 0 2	0 0 1 0	0 0 0 0	0 0 0 0	11	2	1	0
14q21.3	43617568	44369205	751	FSCB	0	1	0	1	0	0	0	1
15q11.2	20316992	20851728	534	15q11.2 BP1- BP2,	1	0	0	19	221	59	13	8
16p13.11	15397501	15409849	12	MPV17L	0	1	0	0	10	0	6	7

Table 4.3 (continued)

Location	Start	Stop	Size (Kb)	Gene - Locus	Cases (n = 50)		Controls (n = 8,329)		DGV (n=~14,000)		Decipher	
					Gain	Loss	Gain	Loss	Gain	Loss	Gain	Loss
17q11.2	26577764 26574587 26577764	26646057 26616479 26616414	68 41 38	NF1	1 0 1	0 1 0	0 0 0	0 0 0	0	0	1	2
17p11.2	17104399	17108906	4	**COPS3**	0	1	0	0	0	0	9	8
18p11.21	13861404	13925908	64	MC2R	1	0	0	0	0	0	2	4
20p12.1	14266199	14422174	155	MACROD2	0	1	0	2	0	0	1	1
22q11.22	20747679 20669985 20747679 20747679 21004976 21342013 21427178 21427178 21482416 21236042	21571664 21289729 21289729 21230019 21318823 21570286 21571664 21570286 21571664 21289729	823 619 542 482 313 228 144 143 89 53	22q11.22	1 1 5 1 0 1 1 7 0 0	0 0 0 0 1 0 0 0 1 1	3 3 3 3 3 3 3 3 3 0	16 16 16 16 16 60 60 60 60 60	8	92	17	18

4.3 Part B- Custom-designed 135K microarray

In total, 2,529 CNV calls were identified. When filtered on the basis of three or more consecutive probes, 1,965 CNV calls remained and 1,530 CNV calls were left after removing CNV calls which fell between 0.3 and -0.3 (Supplementary data Table 8.4). Following manual inspection of the DGV to determine rare CNVs (<1% population frequency) or CNVs in genomic regions involved in brain developmental pathways, the final downstream analyses consisted of 121 gene-locus regions. These comprised of 70 deletions and 51 duplications, ranging in size from 18 kb to 1 MB with the majority falling between 100 kb and 500 kb (Table 4.4). Of these, 23 (19%) were unique to one individual in our cerebral palsy cohort (Table 4.4 and Table 4.5). Results from the custom-designed 135K microarray confirmed CNV regions for *CTNND2*, *FSCB* and 12p12.1-p12.2 found on the custom-designed 180K microarray. CNV regions were also identified in 19 out of 50 cases, including the above mentioned regions, involving Histone cluster genes and 7q21 as well as single-gene CNVs for *PTCHD3*, *NPHP1* and *TARP* and intragenic CNVs in *NCOR2*, *SH3GL3*, *NOS3* and *TBX1* (Table 4.6).

Table 4.4 Summary of CNVs from custom-designed 135K microarray.

Size	Total	Duplications	Deletions
Summary of CNVs			
Number of CNVs in gene-locus regions	121	51	70
Number of CNVs unique to one individual	23	12	11
Size distribution	n (%)	n (%)	n (%)
> 1 MB	12 (10)	2 (04)	10 (14)
> 500 kb - < 1 MB	12 (9)	6 (12)	6 (9)
> 100 kb - <500 kb	54 (44)	16 (31)	38 (54)
< 100 kb - > 50 kb	28 (24)	14 (28)	14 (20)
< 50 kb	15 (13)	13 (25)	2 (3)

Table 4.5 CNVs unique to one individual in CP cohort (custom-designed 135K mircroarray).

Sample	Location	Start	End	Size(kp)	CNV	Genes/Locus	Inheritance
CP56	1q21.1	147359369	148393750	1034	del	Histone cluster genes	unknown
CP56	3q21.2	127495226	127774136	278	dup	UROC1	unknown
CP28	3q26.32q26.33	180461080	180763349	302	del	KCNMB3	unknown
CP36	5p15.2	11729847	12368479	638	dup	CTNND2	**Maternal**
CP57	7p14.1	38232522	38322177	89	del	TARP	**Paternal**
CP03	7q21	88216082	89767697	1551	dup	7q21	**Maternal**
CP56	9q34.2	135241231	135376125	134	dup	ADAMTS13	unknown
CP08	9q34.3	138337067	139547101	1210	del	INPP5E	unknown
CP37	10p12.1	27651566	27745676	94	del	PTCHD3	**Paternal**
CP56	10q11.23	50489357	50540778	51	dup	CHAT	unknown
CP08	11p15.5	583577	2212038	1628	del	SLC25A22/DRD4	unknown
CP03	12p12.1p12.2	20908843	21295433	386	dup	12p12.1p12.2	**Paternal**
CP31	12q24.31	123077344	123481132	403	dup	NCOR2	unknown
CP17	14q21.3	44032315	44406005	373	del	FSCB	**Maternal**
CP20	15q25.2	81850715	82009135	158	del	SH3GL3	**Maternal**
CP14	16p12.3	19507692	19573177	65	dup	C16orf62	unknown
CP32	16p13.11	16069514	16112701	43	dup	ABCC1	unknown
CP10	16p13.3	305046	1516494	1211	del	CACNA1H	Unknown
CP02	19q13.33	55053686	55094066	40	del	PNKP	unknown
CP05	22q11.21	17258338	17387467	129	del	PRODH	unknown
CP17	22q13.33	49338574	49542684	204	del	SHANK3	unknown
CP39	Xp11.4	38378381	38512481	134	dup	TSPAN7	unknown
CP07	Xq21.31	88358618	92293681	3935	dup	PCDH11X	unknown

Unknown – Mode of inheritance not determined. as parental samples have not been tested at this time due to test restrictions.

Table 4.6 CNVs in gene-locus regions (custom-designed 135K microarray).

Location	Start	Stop	Size (kb)	Gene-locus	Cases (n = 50)		Controls (n = 8,329)		DGV (n ~14,000)		Decipher	
					Gain	Loss	Gain	Loss	Gain	Loss	Gain	Loss
1p36.33	844092	1361016	516	AGRN	0	4	0	20	13	0	2	13
1p36.33	2681642 2681642 1964452 1420541 1213233	3590041 3590041 2605548 1624843 1361016	908 908 641 204 147	1p36 microdeletion syndrome	2 0 1 0 0	0 1 0 1 1	0 0 0 0 0	0 0 0 0 1	0	1	3	12
1p36.33	1420541 1796419 1830001 1830001 1420451	2502209 2605548 2605548 2605548 2132404	1185 1081 809 775 711	GABRD	0 0 1 1 0	2 2 0 0 1	0 0 0 0 0	0 0 0 0 0	11	0	2	12
1q21.1	147359369	148393750	1034	Histone cluster genes	0	1	0	0	52	75	11	8
1q44	246754089	246861619	107	Olfactory receptors	0	16	0	52	12	371	8	7
2q13	110182395 110198803 97078280	110339339 110338694 97210556	156 139 132	NPHP1	2 0 0	0 1 1	32 0 0	0 31 1	38	42	3	1
3p25.3	11032469 11035214	11054028 11054028	21 18	SLC6A1	1 1	0 0	0	0	0	0	0	3
3p25.3	10777751 10930815	10856344 10991998	78 61	SLC6A11	1 1	0 0	0	0	0	0	0	2
3q21.2	127495226	127774136	278	UROC1	1	0	0	0	0	0	0	2
3q26.32q26.33	180461080	180763349	302	KCNMB3	0	1	0	0	0	0	1	1

Table 4.6 CNVs in gene-locus regions (custom-designed 135K microarray) (continued)

Location	Start	Stop	Size (kb)	Gene-locus	Cases (n = 50)		Controls (n = 8,329)		DGV (n ~ 14,000)		Decipher	
					Gain	Loss	Gain	Loss	Gain	Loss	Gain	Loss
4p16.1	7440933 7169227 7290362 7298191 7288454 7466692	7799216 7366340 7369799 7354674 7333652 7508384	358 197 79 56 45 41	SORCS2	1 1 0 0 0 2	0 0 1 1 1 0	1 0 0 0 0 0	0 0 0 0 0 0	0	7	3	6
4q13.2	70142221 70153568 70152674 70165721 70165721 70167978 70187284	70261759 70268156 70261759 70261759 70260690 70261759 70261759	119 114 109 96 94 93 74	UGT2B	0 0 0 0 0 0 0	1 1 2 1 1 1 2	0 0 0 0 0 0 0	18 20 20 29 29 29 48	139	699	2	6
5p15.2	11729847	12368479	638	CTNND2	1	0	0	0	0	0	2	2
5p15.33	1432486 1443341 1474769	1570459 1545647 1546556	137 102 71	SLC6A3	0 1 1	1 0 0	0 0 0	0 0 0	0	0	3	0
6p12	66973399	67448096	474	no genes, near EYS	0	1	0	3	0	0	0	0
7q11.23	73018866	73121421	102	7q11.23 duplication	1	0	0	0	0	0	7	3
7q21	88216082	89767697	1551	7q21 duplication	1	0	4	0	4	0	5	3
7p14.1	38232522	38322177	89	TARP	0	1	0	12	9	14	7	11

Table 4.6 CNVs in gene-locus regions (custom-designed 135K microarray) (continued)

Location	Start	Stop	Size (kb)	Gene-locus	Cases (n = 50)		Controls (n = 8,329)		DGV (n ~ 14,000)		Decipher	
					Gain	Loss	Gain	Loss	Gain	Loss	Gain	Loss
7q36.1	150314843 150314843 150326044 150327326 150314843 150341274 150326044	150461710 150456631 150456631 150456631 150411765 150411765 150394729	146 141 130 129 96 70 68	NOS3	1 2 1 1 1 1 1	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0 0 0 0 0 0 0	0	6	0	7
9q34.2	135241231	135376125	134	ADAMTS13	1	0	0	0	0	0	0	0
9q34.3	138337067	139547101	1210	INPP5E	0	1	0	0	0	1	1	3
10p12.1	27651566	27745676	94	PTCHD3	0	1	1	56	0	30	1	3
10q11.23	50489357	50540778	51	CHAT	1	0	0	0	0	0	0	1
11p15.5	583577	2212038	1628	SLC25A22	0	1	0	0	0	0	1	1
11q13.3	70570849 70571781 70570849	70613144 70613144 70589988	42 41 19	SHANK2	1 3 1	0 0 0	0 0 0	0 0 0	1	0	1	0
11q13.4	70784604 70779258	70871807 70829878	87 50	DHCR7	2 1	0 0	0 0	0 0	0	0	1	0
12p12.1p12.2	20908843	21295433	386	12p12.1p12.2	0	1	0	2	0	2	1	2
12q24.31	123077344	123481132	403	NCOR2	1	0	1	0	0	0	1	0
14q21.3	44032315	44406005	373	FSCB	0	1	0	0	0	2	0	1
15q11.2	20635093 20636614	20834571 20795812	199 159	NIPA1	0 0	1 1	0 0	0 0	14	52	11	5
15q25.2	81850715	82009135	158	SH3GL3	0	1	0	0	0	0	0	0

Table 4.6 CNVs in gene-locus regions (custom-designed 135K microarray) (continued)

Location	Start	Stop	Size (kb)	Gene-locus	Cases (n = 50)		Controls (n = 8,329)		DGV (n ~14,000)		Decipher	
					Gain	Loss	Gain	Loss	Gain	Loss	Gain	Loss
16p12.3	19507692	19573177	65	C16orf62	1	0	0	0	0	0	0	1
16p13.11	16069514	16112701	43	ABCC1	1	0	0	0	0	13	15	13
16p13.3	305046	1516494	1211	CACNA1H	0	1	0	0	1	0	4	2
18q11.2	22490922 22490922	22591419 22557684	100 66	LOC728606	0 0	1 5	0	0	0	0	1	0
18q21.1	45849713 45883516	45950607 45950607	100 67	MYO5B	1 1	0 0	0	0	0	0	1	0
19q13.33	55053686	55094066	40	PNKP	0	1	0	0	0	33	1	2
20q13.33	61137963 61171503	62418379 62418379	1280 1246	KCNQ2, CHRNA4	0 0	1 1	0	0	0	0	6	2
22q11.21	18074502 18087632	18135835 18135835	61 48	TBX1	2 3	0 0	0	0	12	4	8	7
22q11.21	17258338	17387467	129	PRODH	0	1	0	23	87	23	4	5
22q11.22	21081688	21562882	481	PRAME	0	3	0	74	12	91	16	20
22q13.33	49338574	49542684	204	SHANK3	0	1	0	0	0	0	6	10
Xp11.4	38378381	38512481	134	TSPAN7	1	0	0	0	2	0	1	9
Xq21.31	88358618	92293681	3935	PCDH11X	1	0	0	0	0	0	0	15

4.3.1 CNV burden analyses for cerebral palsy

A comparison of CNV burden between the 50 individuals with cerebral palsy and 337 controls from the National Institute of Mental Health (NIMH) showed no increase in the large CNV frequency (Figure 4.4). A broader comparison in cerebral palsy to CNV data from individuals with ID, autism, dyslexia, and controls also showed no increase in large CNV frequency (Figure 4.5). Fisher's exact test ($p>0.05$) at 1 Mb. This might reflect the lack of statistical power in detecting larger, rare events in our set of cerebral palsy cases.

4.3.2 CNV burden analysis for cerebral palsy

Figure 4.4 CNV burden analysis for cerebral palsy shows the population frequency of the largest CNVs (as a survivor function) in individuals with cerebral palsy compared to 337 controls from the National Institute of Mental Health (NIMH) cohort[140]. The frequency of the large CNVs is not vastly different between the two groups.

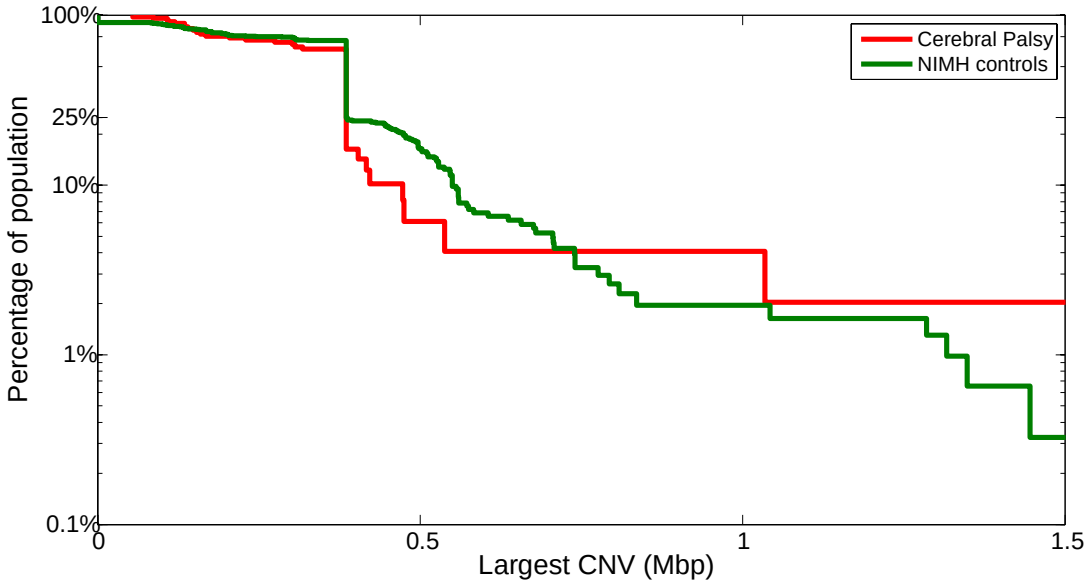
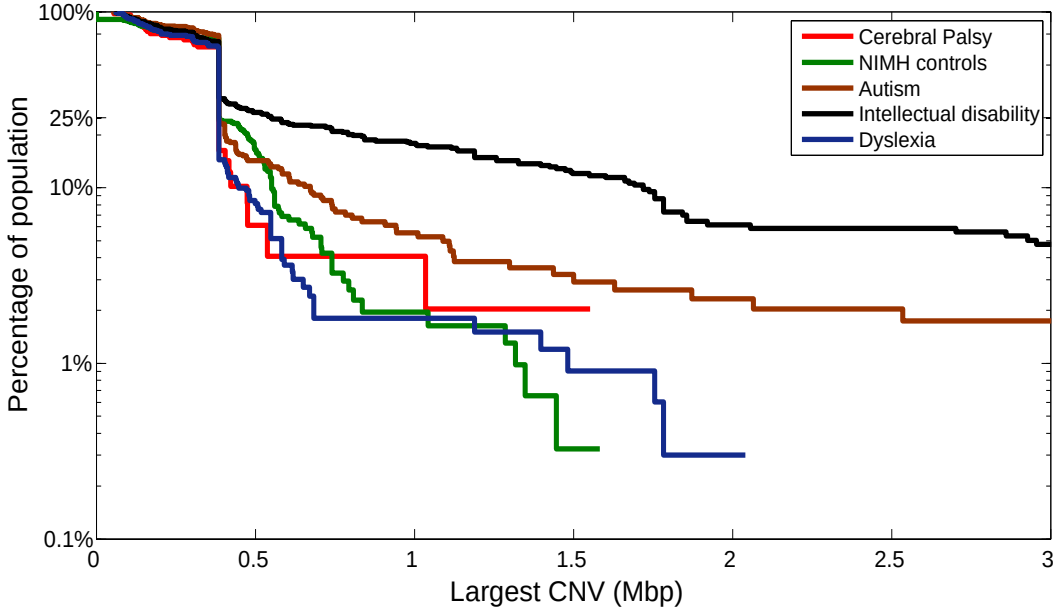


Figure 4.5 A broader comparison in cerebral palsy to CNV data from individuals with ID, autism, dyslexia, and controls[140]. Note that all the samples were analysed using the same hotspot chip.



4.3.3 Results from Taqman assays

To date on the basis of gene content and availability of parental DNA, six additional gene-locus regions have been validated, including parental testing, to determine the mode of inheritance. Each Taqman assay concurred with the microarray data. The CNVs included; one maternal duplication in 7q.21 chromosome region, two maternal deletions in 14q21.3 and 15q25.2 chromosome regions and three paternal deletions in 7p14.1, 10p12.1 and 12p12.1p12.2 chromosome region (Table 4.7). No *de novo* CNVs were identified but whole-genome screening will be performed on all parents to determine the involvement of *de novo* CNVs in this cohort.

Table 4.7 Results of Taqman assays.

Proband	Location	Gene – Locus	Size (kb)	Probe ID	Type
P03 F03 M03	12p12.1p12.2	12p12.1p12.2	386	hs01663667	**Loss** **Loss** Normal
P17 F17 M17	14q21.3	FSCB	757	hs02958873	**Loss** Normal **Loss**
P20 F20 M20	15q25.2	SH3GL3	158	hs05327322	**Loss** Normal **Loss**
P03 F03 M03	7q21	7q21	1552	hs01835833	**Gain** Normal **Gain**
P37 F37 M37	10p12.1	PTCHD3	94	hs00913691	**Loss** **Loss** Normal
P57 F57 M57	7p14.1	TARP	89	hs07530615	**Loss** **Loss** Normal

In addition to public control databases, OMIN and Decipher databases were interrogated to assist in deciding which CNVs were more likely to be pathogenic. Where there was available information we reported on the specific role of the gene-region and any known published associations with other neurodevelopmental disorders assisted with this process (Table 4.8). Twenty seven previously reported OMIN disorders and 13 Decipher syndromes were identified. Many of these regions are involved in synapse formation, neurotransmission and DNA repair pathways. Several CNV regions were enriched for candidate genes involved in neurodevelopmental disorders such as autism, intellectual disability, epilepsy and schizophrenia (Table 4.8).

Table 4.9 lists genetic results for each cerebral palsy case correlated with clinical data collected from the patient questionnaire and information from the South Australia Cerebral Palsy register. Clinical information of particular interest included type of cerebral palsy, gestational age, IUGR, gender and other co-morbidities. Final combined results from both microarray platforms reported 187 CNVs in 49 out of 50 cerebral palsy cases, ranging from one to 11 CNVs per individual (Table 4.9). Two samples CP11 and CP30 reported no CNV events following stringent filtering parameters.

Table 4.8 Information on gene-locus regions.

Location	Gene - Locus	OMIM	Decipher	Role	Association
1q21.1	Histone cluster genes			Nucleosome structure, transcription regularity, DNA repair	
1p36.33	AGRN	Myasthenia, limb-girdle, familial (103320)	1p36 microdeletion syndrome	Synapse formation	
1p36.33	1p36 microdeletion syndrome		1p36 microdeletion syndrome		
1p36.33	GABRD	Epilepsy (137163)	1p36 microdeletion syndrome	Neurotransmitter transporter GABA	Ep[143]
1q44	Olfactory receptors				
2q13	NPHP1	Joubert syndrome (607100)		Cell division and adhesion	JS[144]
2p11.**2**	centomeric				
3q21.2	UROC1	Urocanase deficiency (613012)		Histidine catabolism	ID[145]
3q26.32q26.	KCNMB3			Smooth muscle control and neuronal excitability	Ep[146]
3q29	PAK2		3q29 microdeletion syndrome	Axon guidance	SZ[147]
3p25.3	SLC6A1			Neurotransmitter transporter GABA	
3p25.3	SLC6A11			Neurotransmitter transporter GABA	
4q13.2	UGT2B				
4p16.1	SORCS2				
5p15.2	CTNND2	ID, Cri-du-chat Syndrome (604275)	Cri du Chat Syndrome	Synapse function and neurotransmission	Au[92], SZ[148], ID[149]

Table 4.8 Information on gene –locus regions (continued)

Location	Gene - Locus	OMIM	Decipher	Role	Association
5p15.33	SLC6A3	Parkinsonism-dystonia, infantile, ADHD (126455)		Amine transporter	TS[150]
6q26	PARK2	Parkinson disease, juvenile, type 2 (602544)		Synapse function and neurotransmission	Au[92]
6p12	No genes, near E				
7p14.1	TARP				
7q11.23	7q11.23		7q11.23 duplication syndrome Williams-Beuren Syndrome		
7q21	7q21				
7q36.1	NOS3	Alzheimer disease, Pregnancy hypertension, Ischemic stroke (163729)		Neurotransmission	AD[151]
8p23.2.p23.1	**MCPH1**	Microcephaly (607117)		Neurogenesis	Au[152], ID[153]
8p23.3	DLGAP2			Neurotransmission	Au[91, 152, 154]
9p13.1	CNTNAP3			Cell adhesion	
9q34.2	ADAMTS13	Thrombotic thrombo-cytopenic purpura, Von Willebrand factor (604134)		Involved in blood clotting	VWD[155]
9q34.3	INPP5E	Joubert syndrome, Mental retardation (613037)		Regulate Golgi-vesicular trafficking	JS[156], ID[157]
10p12.1	PTCHD3				Au[158] ,ID[158]
10q11.23	CHAT	Myasthenic syndrome (118490)			AD[159]
11p15.5	SLC25A22	Epileptic encephalopathy (609302)		Glutamate transporter	Ep[160]
11q13.3	SHANK2	Autism susceptibility (603290)		Synapse formation	Au[161], ID[161]

Table 4.8 Information on gene-locus regions (continued)

Location	Gene - Locus	OMIM	Decipher	Role	Association
11q13.4	DHCR7	Smith-Lemli-Opitz syndrome (602858)			
12q12	CNTN1	Myopathy		Cell adhesion	
12p12.1p12.	12p12.1p12.2				
12q24.31	NCOR2			Transcriptional repression	
13q13.2	NBEA			Membrane trafficking and signalling	Au[162]
14q21.3	FSCB			Fibrous sheath biogenesis	
15q11.2	15q11.2 BP1- BP				
15q11.2	NIPA1	Spastic paraplegia-6 (608145)	Prader-Willi syndrome, Angelman syndrome	Nervous system development	SZ[163]
15q25.2	SH3GL3				
16p12.3	C16orf62				
16p13.11	MPV17L				
16p13.11	ABCC1		16p13.11 Recurrent microdeletion, MR/MCA susceptibility		
16p13.3	CACNA1H	Epilepsy (607904)		Neurotransmission	Au[164], Ep[165]
17q11.2	NF1		Microdeletion syndrome	Negative regulator of the Ras signal transduction pathway	Au[166]
17p11.2	**COPS3**		Potocki-Lupski syndrome, Smith-Magenis syndrome	Signal transduction involved in cellular and developmental processes	
18q11.2	LOC728606				
18q21.1	MYO5B	Microvillus inclusion disease (606540)		Vesicular trafficking	

Table 4.8 Information on gene-locus regions. (continued)

Location	Gene - Locus	OMIM	Decipher	Role	Association
18p11.21	MC2R	Glucocorticoid deficiency (607397)		Involved in inflammation, immunomodulation, temperature control	
19q13.33	PNKP	Epileptic encephalopathy (605610)	DNA repair	DNA repair	Ep[167], Microcephaly[167]
20q13.33	KCNQ2	Epilepsy (602235)		Neuronal excitability	EP[168]
20q13.33	CHRNA4	Epilepsy (118504)		Signal transmission at synapse	Ep[169]
20p12.1	MACROD2				Au[170]
22q11.21	TBX1	DiGeorge syndrome, Velocardiofacial syndrome (602054)	DiGeorge syndrome, Velocardiofacial syndrome	Regulation of developmental processes	
22q11.21	PRODH	Schizophrenia susceptibility (606810)		Neuromodulation	Au[88], SZ[88], ID[88]
22q11.22	22q11.22				
22q11.22	PRAME		22q11.2 distal deletion syndrome		
22q13.33	SHANK3	22q13 deletion syndrome , Autism , Schizophrenia (606230)	22q13 deletion syndrome	Synapse formation	Au[89, 91], ID[91]
Xp11.4	TSPAN7	Mental retardation (30096)		Neuronal migration and growth	Au[91], ID[113]
Xq21.31	PCDH11X				

Au – autism, ID – Intellectual disability, Ep – Epilepsy, SZ – Schizophrenia, TS – Tourette Syndrome, JS – Joubert Syndrome, VWD – Von Willebrand disease.

Table 4.9 Combined Genetic and Clinical data for each individual CP case.

Sample	Location	Size (kb)	CNV	Array	Genes – Locus Region	Sex	Type CP	Gestation	IUGR	Co-morbidities
CP01	1p36.33 4q13.2 18q11.2	147 74 66	del del del	135K 135K 135K	1p36 microdeletion syndrome UGT2B LOC728606	Female	Quadriplegia	unknown	No	
CP02	1p36.33 1p36.33 19q13.33	1185 516 40	del del del	135K 135K 135K	GABRD AGRN PNKP	Female	Diplegia	<32 weeks	No	
CP03	7q21 12p12.1p12.2	1551 386	dup dup	135K 135K,180K	7q21 duplication 12p12.1p12.2	Female	Diplegia	32 - 36 weeks	No	ID, Ep,
CP04	1q44 18q11.2	127 66	del del	135K 135K	Olfactory receptors LOC728606	Female	Hemiplegia	≥ 37 weeks	No	
CP05	6p12 22q11.21	474 129	del del	135K 135K	no genes, near EYS PRODH	Female	Hemiplegia	32 - 36 weeks	No	
CP06	2q13	139	del	135K	NPHP1				No	
CP07	1p36.33 18q21.1 Xq21.31	711 100 3935	del dup dup	135K 135K 135K	GABRD MYO5B PCDH11X	Female	Diplegia	32 - 36 weeks	yes	
CP08	1p36.33 1p36.33 1p36.33 9q34.3 20q13.33 4p16.1 5p15.33 11p15.5	908 1185 516 1210 1280 79 1628 137	del del del del del del del del	135K 135K 135K 135K 135K 135K 135K 135K	1p36 microdeletion syndrome GABRD AGRN INPP5E KCNQ2/CHRNA4 SORCS2 SLC6A3 SLC25A22	Male	Tripegia	32 - 36 weeks	No	

Table 4.9 Combined Genetic and Clinical data for each individual CP case. (continued)

Sample	Location	Size (kb)	CNV	Array	Genes – Locus Region	Sex	Type CP	Gestation	IUGR	Co-morbidities
CP09	1q44 1p36.33 1p36.33 20q13.33	84 1081 516 1246	del del del del	135K 135K 135K 135K	Olfactory receptors GABRD AGRN KCNQ2/CHRNA4	Male	Hemiplegia	32 - 36 weeks	No	
CP10	1p36.33 1p36.33 16p13.3 3q29 4q13.2 13q13.2	204 516 1211 48 119 43	del del del del del del	135K 135K 135K 180K 135K 180K	1p36 microdeletion syndrome AGRN CACNA1H PAK2 UGT2B NBEA	Male	Diplegia	≥ 37 weeks	No	Au, ID
CP12	1p36.33 18q11.2	1081 66	del del	135K 135K	GABRD LOC728606	Female	Diplegia	<32 weeks	No	Au, ID
CP13	15q11.2	1994	del	135K	NIPA1	Female	Hemiplegia	≥ 37 weeks	No	ID
CP14	1q44 3q29 15q11.2 16p12.3 18q11.2	107 47 159 65 66	del del del dup del	135K 180K 135K 135K 135K	Olfactory receptors PAK2 NIPA1 C16orf62 LOC728606	Male	Quadriplegia	<32 weeks	No	
CP15	2p11.2 4q13.2 18q11.2	861 74 66	del del del	180K 135K 135K	centromeric UGT2B LOC728606	Male	Hemiplegia	< 32 weeks	No	Ep
CP16	1q44 2p11.2 22q11.22	105 2120 472	del del del	135K 180K 135K	Olfactory receptors centromeric PRAME	Male	Quadriplegia	≥ 37 weeks	No	Ep
CP17	14q21.3 22q13.33	751 204	del del	135K,180K 135K	FSCB SHANK3	Male	Quadriplegia	≥ 37 weeks	No	ID

Table 4.9 Combined Genetic and Clinical data for each individual CP case. (continued)

Sample	Location	Size (kb)	CNV	Array	Genes – Locus Region	Sex	Type CP	Gestation	IUGR	Co-morbidities
CP18	16p13.11	12	del	180K	MPV17L	Female	Dipplegia	<32 weeks	No	
CP19	22q11.22	481	del	135K	PRAME	Male	Diplegia	≥ 37 weeks	No	
CP20	15q25.2 17p11.2	158 4	del del	135K 180K	SH3GL3 **COPS3**	Female	Hemiplegia	≥ 37 weeks	No	
CP21	18q21.1	67	dup	135K	MYO5B	Male	Hemiplegia	≥ 37 weeks	No	
CP22	11q.11	89	del	135K	Olfactory receptors	Male	Quadriplegia	≥ 37 weeks	No	
CP23	4p16.1 22q11.22 22q11.22	56 143 542	del dup dup	135K 180K 180K	SORCS2 22q11.22 22q11.22	Female	Quadriplegia	≥ 37 weeks	No	Ep
CP24	3q29 4q13.2 13q13.2 17q11.2 18q11.2 22q11.22 22q11.22	47 93 67 38 100 143 823	dup del dup dup del dup dup	180K 135K 180K 180K 135K 180K 180K	PAK2 UGT2B NBEA NF1 LOC728606 22q11.22 22q11.22	Female	Hemiplegia	≥ 37 weeks	No	Ep
CP25	1p36.33 1p36.33 7q36.1 8p23.2.p23.1 15q11.2	641 908 68 219 534	dup dup dup dup dup	135K 135K 135K 180K 180K	1p36 microdeletion syndrome 1p36 microdeletion syndrome NOS3 **MCPH1** 15q11.2 (BP1 - BP2)	Male	Hemiplegia	≥ 37 weeks	No	
CP26	1q44 4p16.1 22q11.22 22q11.22	84 45 143 542	del del dup dup	135K 135K 180K 180K	Olfactory receptors SORCS2 22q11.22 22q11.22	Female	Diplegia	≥ 37 weeks	yes	ID

Table 4.9 Combined Genetic and Clinical data for each individual CP case. (continued)

Sample	Location	Size (kb)	CNV	Array	Genes – Locus Region	Sex	Type CP	Gestation	IUGR	Co-morbidities
CP27	1q44 2q13	84 156	del dup	135K 135K	Olfactory receptors NPHP1	Male	Quadriplegia	<32 weeks	No	ID
CP28	3q26.32 3q29 8p23.3 13q13.2 22q11.22 22q11.22	302 46 47 67 144 542	del dup del dup dup dup	135K 180K 180K 180K 180K 180K	KCNMB3 PAK2 DLGAP2 NBEA 22q11.22 22q11.22	Female	Hemiplegia	≥ 37 weeks	No	Ep
CP29	1q44 4q13.2	84 94	del del	135K 135K	Olfactory receptors UGT2B	Male	Hemiplegia	32 – 36 weeks	No	
CP31	1q44 8p23.3 12q24.31 17q11.2	105 47 403 41	del del dup del	135K 180K 135K 180K	Olfactory receptors DLGAP2 NCOR2 NF1	Male	Hemiplegia	<32 weeks	No	
CP32	1q44 4q13.2 6q26 7q36.1 8p23.3 16p13.11	107 114 80 70 47 43	del del del dup del dup	135K 135K 180K 135K 180K 135K	Olfactory receptors UGT2B PARK2 NOS3 DLGAP2 ABCC1	Male	Triplegia	<32 weeks	No	ID
CP33	6q26 8p23.3	80 47	del del	180K 180K	PARK2 DLGAP2	Male	Quadriplegia	≥ 37 weeks	No	ID, Ep
CP34	8p23.3 12q12	47 21	del del	180K 180K	DLGAP2 CNTN1	Male	Hemiplegia	≥ 37 weeks	No	Ep

Table 4.9 Combined Genetic and Clinical data for each individual CP case. (continued)

Sample	Location	Size (kb)	CNV	Array	Genes – Locus Region	Sex	Type CP	Gestation	IUGR	Co-morbidities
CP35	1q44	127	del	135K	Olfactory receptors	Female	Hemiplegia	<32 weeks	No	ID, Ep
	3q29	48	dup	180K	PAK2					
	6q26	80	del	180K	PARK2					
	8p23.3	47	del	180K	DLGAP2					
	13q13.2	73	dup	180K	NBEA					
	17q11.2	68	dup	180K	NF1					
	22q11.22	228	dup	180K	22q11.22					
	22q11.22	619	dup	180K	22q11.22					
CP36	5p15.2	446	dup	135K,180K	**CTNND2**	Female	Hemiplegia	≥ 37 weeks	yes	
	6q26	80	del	180K	PARK2					
	8p23.3	47	del	180K	DLGAP2					
	13q13.2	25	dup	180K	NBEA					
	22q11.22	143	dup	180K	22q11.22					
	22q11.21	48	dup	135K	TBX1					
CP37	3q29	46	dup	180K	PAK2	Female	Hemiplegia	< 32 weeks	No	
	4q13.2	96	del	135K	UGT2B					
	6q26	80	del	180K	PARK2					
	8p23.3	47	del	180K	DLGAP2					
	10p.12.1	94	del	135K	PTCHD3					
	13q13.2	25	dup	180K	NBEA					
	22q11.22	53	dup	180K	22q11.22					
	22q11.22	143	dup	180K	22q11.22					
	22q11.22	482	du	180K	22q11.22					
CP38	1q44	140	del	135K	Olfactory receptors	Male	Hemiplegia	≥ 37 weeks	No	ID, Ep
	4q13.2	109	del	135K	UGT2B					
	8p23.3	47	del	180K	DLGAP2					
	22q11.22	89	del	180K	22q11.22					
	22q11.22	313	del	180K	22q11.22					

Table 4.9 Combined Genetic and Clinical data for each individual CP case. (continued)

Sample	Location	Size (kb)	CNV	Array	Genes – Locus Region	Sex	Type CP	Gestation	IUGR	Co-morbidities
CP39	8p23.3 Xp11.4	47 134	del dup	180K 135K	DLGAP2 TSPAN7	Male	Diplegia	32 – 36 weeks	No	
CP40	1q44 8p23.3	107 47	del del	135K 180K	Olfactory receptors DLGAP2	Male	Quadriplegia	≥ 37 weeks	No	ID, Ep
CP41	2q13 8p23.3 22q11.22 22q11.22	132 47 143 542	del del dup dup	135K 180K 180K 180K	NPHP1 DLGAP2 22q11.22 22q11.22	Female	Hemiplegia	≥ 37 weeks	No	Ep
CP42	4q13.2 9p13.1 20p12.1 22q11.22 22q11.22	109 216 155 143 542	del del del dup dup	135K 80K 180K 180K 180K	UGT2B CNTNAP3 MACROD2 22q11.22 22q11.22	Female	Hemiplegia	< 32 weeks	No	ID, Ep
CP52	6q26 22q11.21	80 48	del dup	180K 135K	PARK2 TBX1	Female	Dyskinetic	≥ 37 weeks	No	Ep
CP53	1q44 2q13 3q29 3p25.3 4p16.1 6q26 7q36.1 11q13.3 18p11.21 22q11.21	106 156 48 18 41 80 141 41 64 61	del dup dup dup dup del dup dup dup dup	135K 180K 180K 135K 135K 135K 135K 135K 135K 180K	Olfactory receptors NPHP1 PAK2 SLC6A1 SORCS2 PARK2 NOS3 SHANK2 MC2R TBX1	Male	Dyskinetic	32 – 36 weeks	No	

Table 4.9 Combined Genetic and Clinical data for each individual. (continued)

Sample	Location	Size (kb)	CNV	Array	Genes – Locus Region	Sex	Type CP	Gestation	IUGR	Co-morbidities
CP54	1q44	107	del	135K	Olfactory receptors	Male	Diplegia	≥ 37 weeks	No	
	3p25.3	21	dup	135K	SLC6A1					
	4p16.1	40	dup	135K	SORCS2					
	7q36.1	141	dup	135K	NOS3					
	11q13.3	41	dup	135K	SHANK2					
	22q11.21	61	dup	135K	TBX1					
CP55	1p36.33	775	dup	135K	GABRD	Female	Hemiplegia	< 32 weeks	No	
	1p36.33	908	dup	135K	1p36 microdeletion syndrome					
	4p16.1	197	dup	135K	SORCS2					
	4p16.1	358	dup	135K	SORCS2					
	5p15.33	102	dup	135K	SLC6A3					
	7q11.23	102	dup	135K	7q11.23 duplication					
	7q36.1	130	dup	135K	NOS3					
	11q13.3	42	dup	135K	SHANK2					
	11q13.4	87	dup	135K	DHCR7					
	22q11.21	48	dup	135K	TBX1					
CP56	1q21.1	1034	del	135K	Histone cluster genes	Male	Diplegia	32 – 36 weeks	yes	
	1p36.33	809	dup	135K	GABRD					
	1q44	107	del	135K	Olfactory receptors					
	3q21.2	278	dup	135K	UROC1					
	3p25.3	61	dup	135K	SLC6A11					
	5p15.33	71	dup	135K	SLC6A3					
	7q36.1	146	dup	135K	NOS3					
	9q34.2	134	dup	135K	ADAMTS13					
	10q11.23	51	dup	135K	CHAT					
	11q13.4	87	dup	135K	DHCR7					
	11q13.3	41	dup	135K	SHANK2					

Table 4.9 Combind Genetic and Clinical data for each individual. (continued)

Sample	Location	Size (kb)	CNV	Array	Genes – Locus Region	Sex	Type CP	Gestation	IUGR	Co-morbidities
CP57	3p25.3 4p16.1 7q36.1 7p14.1	78 41 96 89	dup dup dup del	135K 135K 135K 135K	SLC6A11/LOC285370 SORCS2 NOS3 TARP	Male	Hemiplegia	< 32 weeks	No	ID
CP58	7q36.1 11q13.3 11q13.4	129 19 50	dup dup dup	135K 135K 135K	NOS3 SHANK2 DHCR7	Male	Quadriplegia	≥ 37 weeks	No	ID
CP59	11q.11 22q11.22	92 307	del del	135K 135K	Olfactory receptors PRAME	Male	Diplegia	< 32 weeks	No	

Au – autism, ID – Intellectual disability, Ep – Epilepsy

Chapter 5 **Discussion**

5.1 Discussion

Whole-genome array-CGH was successfully performed for this pilot study of 50 cerebral palsy individuals to discover plausible copy number variations associated with the cerebral palsy phenotype. This pilot study is only the second to examine the contribution of CNVs to cerebral palsy and is the largest to date. The combined results from both array-CGH platforms following stringent filtering parameters detected 187 CNV events. Many of these variants were rare and are found in brain developmental gene-enriched regions, some of which may be important to the aetiology of cerebral palsy. A total of 31 CNVs were unique to one individual and not shared with other affected individuals, demonstrating the rare nature of these variants. These CNVs may be either potentially causative or contribute to the cerebral palsy phenotype. Of particular interest were three cases with CNVs involving potential candidate genes for cerebral palsy phenotype and included CTNND2, MCPH1 and COPS3.

5.1.1 Family CP36 – CTNND2

The affected female harbouring this variant was diagnosed with spastic hemiplegia cerebral palsy with no other reported co-morbidities such as autism, intellectual disability and/or epilepsy. This individual delivered vaginally at 41 weeks gestation weighing 2440 gram and was diagnosed with intrauterine growth restriction. Several variants involving brain developmental genes were found, including PARK2, DLGAP2, NBEA, TBX1 and CTNND2. From this list, CTNND2 unique to this individual was maternally derived and nominated as a potential candidate gene for cerebral palsy for the following reasons. This gene is located on chromosome 5p15.2 and is

implicated in brain development. It encodes an adhesive junction associated protein of the armadillo/beta-catenin superfamily[92]. CTNND2 is highly expressed in the fetal brain and plays important roles in neuronal functioning, cell motility, dendritic branching and neuron cell adhesion[148, 149]. Medina *et al.* demonstrated that CTNND2 was associated with severe intellectual disability in cri-du-chat syndrome, a rare genetic disorder characterised by a cat-like cry in affected children[149]. CTNND2 is also a known autism candidate gene[92, 171], and has previously been associated with schizophrenia[148].

This is an extremely rare variant in the population no overlapping event was seen in the 8,329 individuals from the control dataset or previously reported in DGV which consists of approximately 14,000 normal controls. Four variants, two deletions and two duplications, expanding this region were identified in the Decipher database with phenotypes including intellectual disability, developmental delay and microcephaly. This is the first study to identify this rare variant in cerebral palsy. Given its known and critical role in brain development, it may contribute to the aetiology of cerebral palsy, either independently or in the presence of another genetic or environmental modifier. In this particular case, the IUGR may have been a contributing factor. IUGR has previously been associated with cerebral palsy, however the underlying mechanisms are not clear[30]. One hypothesis is that the resulting low birth weight from IUGR is due to chronic intrauterine hypoxia[172].

5.1.2 Family CP25 – MCPH1

The affected individual diagnosed with spastic hemiplegia cerebral palsy was male, born at term following a normal pregnancy and delivery. No other co-morbidities were detected in this individual. Variants included duplications for 1p36, 15q11.2, NOS3 and MCPH1. MCPH1 an additional candidate gene for cerebral palsy was unique to this individual and maternally inherited. The MCPH1 gene is located on chromosome 8p23.1 and controls human brain size. It is highly expressed in the developing cerebral cortex of the fetal brain and to a lesser extent in other regions of the developing brain[173, 174]. MCPH1 is involved in neurogenesis and regulation of the cerebral cortex size. Importantly the translated protein *microcephalin* encodes a BRCA1 C-terminal domain. BRCT domains are found in several DNA repair proteins and loss of function of DNA repair genes leads to excessive apoptotic cell death during neurogenesis[175]. BRCT domains are present in major proteins controlling the cell cycle and normal cell cycle regulation may be disrupted in the neural progenitors[176].

Mutations of the MCPH1 gene (OMIM 607117, 606858 and 251200) are mainly inherited as autosomal recessive traits for premature chromosome condensation syndrome[177, 178], intellectual disability[178] and microcephaly[153]. More recently this gene was reported as a susceptibility factor for autism spectrum disorders (ASD)[152]. In a study consisting of 54 families with an affected individual diagnosed with ASD, three were identified with copy number changes of MCPH1. These same children were also diagnosed with unspecified intellectual disability[152]. Two children carried a maternally transmitted duplication incorporating the promoter region and exons one

to three and the remaining child carried a *de novo* deletion of the promoter region and exons one to nine. Both maternal parents of the children who carried the transmitted duplication were diagnosed with learning and behavioural problems[152]. In a similar finding Glancy *et al*[179] identified a maternal transmitted duplication of the 8p23.1-8p23.2 region incorporating the MCPH1 gene, the child presented with a phenotype including speech delay, learning difficulties and autism. The mother was diagnosed with epilepsy[179].

In the present study we identified MCPH1 in one individual which was maternally inherited. This is consistent with other studies of intellectual disability and autism. To our knowledge, the mother has no previous documented neurological disorders. CNVs encompassing MCPH1 are extremely rare in the population. No cases were found in the control dataset or in DGV. The Decipher database reports three previous duplications and two losses with phenotypic features including intellectual disability and developmental delay.

5.1.3 Family CP20 – COPS3

The affected female individual was born at term following an unremarkable pregnancy and normal delivery. The child was diagnosed with spastic hemiplegia cerebral palsy with no detected co-morbidities. Two variants were detected in this individual: COPS3 and SH3GL3. COPS3 is another potential candidate gene for the cerebral palsy phenotype seen only in this individual and paternally derived. The protein encoded by COPS3, COP9 signalosome complex subunit 3, is involved in a variety of cellular and developmental processes and in signal transduction[180]. COPS3

is located on chromosome 17p11.2 which is within the Smith-Magenis and Potocki-Lupski syndrome regions. This region is susceptible to an elevated number of low copy number repeats or segmental duplications (regions that share a high level of sequence identity but are not allelic). This promotes unequal crossing over during meiosis, facilitating a higher susceptibility to novel deletion and duplication events[181].

Smith-Magenis syndrome consists of multiple congenital abnormalities which include features of mild to moderate intellectual disability, speech delay and behavioural problems[182]. Approximately 80% of Smith-Magenis cases share a 3.7 Mb recurrent deletion mediated by non-allelic homologous recombination. Potocki-Lupski syndrome, a microduplication syndrome on 17p11.2, is the reciprocal disease of Smith-Magenis syndrome[183]. It is a milder syndrome in comparison with Smith-Magenis syndrome[184]. Autism spectrum disorder is a main feature of this disorder and has been reported in approximately 80% of cases. As with Smith-Magenis syndrome, Protocki-Lupski syndrome is also characterised by several congenital abnormalities and intellectual disability[185]. Clinical manifestations as seen from Smith-Magenis syndrome and Protocki-Lupski syndrome are diverse between deletions and duplications. Approximately 50 genes map to this region and the dose sensitive RA11 gene is considered the most likely candidate for these two syndromes.

The considered candidate region for cerebral palsy is a 4 kb deletion which includes exon 6 – 8 of the COPS3 gene. Deletions of COPS3 are extremely rare with no reported cases in the control dataset or in DGV. Nine duplications and eight deletions encompassing this region have been recorded in the Decipher database and

phenotypes include intellectual disability, developmental delay and microcephaly. Where both deletions and duplications are found in the same location, this suggests that the gene involved is likely to be dose sensitive.

The above three cases each resulted from an unremarkable delivery and, with the exception of CP36 where IUGR was diagnosed, an unremarkable pregnancy. In all three cases spastic hemiplegia was diagnosed with no reported co-morbidities such as autism, intellectual disability or epilepsy. The fact that no other co-morbidities were diagnosed in these three individuals born at term further strengthens the argument that CTNND2, MCPH1 and COPS3 are strong potential candidate genes for cerebral palsy. Each of these CNVs are rare (<1% population frequency), and have been found to overlap with more than one neurodevelopmental disorder suggesting a more general risk factor for central nervous system disorder. In each case, the candidate gene was inherited from an unaffected parent. Another modifier, either genetic or environmental, may be required to determine the specificity of the final outcome.

Several additional rare CNV regions of potential neurological pathogenicity were identified. They included 7q21 and 12p12.1p12.2; 15q11.2 (BP1-BP2); and 22q11.22 as well as single-gene CNVs across CNTNAP3, MC2R, FSCB, PTCHD3, PNKP, NPHP1 and TARP and intragenic CNVs in DLGAP2, PARK2, NBEA, PAK2, MACROD2, MPV17L, NF1, NCOR2 and SH3GL3. To date six of these variants (7q21, 12p12.1p12.2, FSCB, PTCHD3, TARP and SH3GL3) have been validated with qPCR and found to be inherited from an unaffected parent.

Both a 386 kb deletion on the 12p12.1p12.2 chromosome region and 1.5 Mb duplication on the 7q.21 chromosome region were identified in the same sample (CP03). These were the only variants for this individual, and both were inherited from an unaffected parent, paternally and maternally, respectively. The deletion found on the 12p12.1p12.2 chromosome region encompassed three genes; LST-3TM12, SLC01B1 and SLC01B3. Both SLC01B1 and SLC01B3 belong to the organic anion transporter family which facilitates the sodium ion independent transport of compounds including bile acids, bilirubin, thyroid hormones and toxins[186]. LST-3TM12 is a liver specific organic anion transporter associated with serum levels of bilirubin[187]. Little is currently known about this region. Two previous deletions and one gain have been reported in the Decipher database with phenotypes including intellectual disability and developmental delay. Two previous deletions have been observed in the DGV for this region [72].

The duplication in 7q.21 chromosome region contains six genes, ZNF804B, C7orf62, DPY19L2P4, STEAP1, STEAP2 and C7orf63. Four gains encompassing this region have been recorded in normal individuals in the DGV. Variants for both the 7q.21 and 12p12.1p12.2 chromosome regions are rare. Little is known about the genes involved in these two regions, or the pathogenicity of either region, but both events were unique to this individual and not previously identified in other neurodevelopmental disorders. Clinical characteristics for this case differed from those reported earlier. This individual was premature (33 weeks gestation) and had a diagnoses of spastic diplegia cerebral palsy accompanied by intellectual disability and epilepsy.

Also unique to one individual and not shared with other cases in our cohort were CNVs encompassing CNTNAP3, MC2R, PNKP, MACROD2, MPV17L, NCOR2 FSCB, PTCHD3, TARP and SH3GL3. PNKP plays a crucial role in DNA repair and has been associated with developmental delay, seizures and microcephaly[167]. Again little is known about the function of MACROD2 but FLRT3, which is embedded within MACROD2, is a cell adhesion molecule which functions in neuronal development[170]. MACROD2 is considered a possible candidate gene for Kabuki syndrome [188] and has been found to be a risk factor for autism[170] and schizophrenia[189]. Variants which were not unique to one individual but important for brain development included; 12 deletions for DLGAP2, eight deletions for PARK2, five duplications and two deletions for PAK2, five duplications and one deletion for NBEA and two duplications and one deletion for NF1. PARK2 also a risk factor for autism, is involved in synapse function and neurotransmission[92]. PAK2 is highly expressed in the fetal brain, involved in nuclear signalling and possibly neuronal differentiation, and is associated with intellectual disability, autism and more recently schizophrenia[147]. DLGAP2 is associated with autism[91, 154] and is very important for brain development involved in neuronal cell signalling and the molecular organisation of synapses.

Each of the above variants are plausible contributors for the cerebral palsy phenotype. Several of these variants harbour genes found in brain developmental regions affecting a small group of common functional pathways including neurotransmission and synapse formation and maintenance.

To date no *de novo* events have been detected in this cohort. Whilst large *de novo* events are considered more likely to be pathogenic, inherited CNVs are also contributors to disease. They may show variable expressivity, incomplete penetrance or the presence of a point mutation on the other allele in affected children, not identified by the CNV profiling[60, 190]. Several studies in autism spectrum disorder have identified inherited CNVs from an unaffected parent[82, 83, 91]. The mode of inheritance, maternal or paternal, has been shown to influence the outcome of the phenotype [191, 192].

It might have been expected to find potentially pathogenic CNVs in more severe cases of cerebral palsy i.e. spastic quadriplegia but this was not the case. Spastic hemiplegia may be the most commonly diagnosed cerebral palsy subtype in term infants[123] because of the contribution of CNVs to its development. We also noted that cases harbouring several CNV regions, i.e. eight or more, were premature, but the size of this study precludes the drawing of any conclusions.

5.1.4 Microarray platform comparisons

Results for the custom-designed 180K microarray detected a total of 69 CNVs involved in gene-locus regions previously implicated in brain development 11 of these were only seen once, one per individual. The vast majority of CNVs detected from the 180K microarray were less than 50 kb (39%) followed by 24% reported between 100 kb and 500 kb. In comparison, results from the custom-designed 135K array detected 121 CNVs in gene-locus regions, 23 unique to one individual. The majority of these CNVs were between 100 kb and 500 kb (44%) and 13% were less

than 50 kb. The 180K microarray had a much higher resolution in both the targeted ~ 20 – 50 kb and backbone regions ~ 225 kb compared to ~ 50 kb in the genomic hotspot regions and ~ 350 kb in the genomic backbone for the 135K microarray. Both microarrays are well designed and validated for clinical testing of disorders found in high prevalence in the population including intellectual disability, autism and epilepsy.

5.1.5 Limitations of this study

All parental samples have not yet been tested to determine if *de novo* imbalances contribute to the cerebral palsy phenotype which will help resolve the pathogenicity of these rare CNVs. Whilst the size of this study was suitable to determine is CNVs may contribute to cerebral palsy a much larger study is required to resolve this and is currently underway. A lower than expected rate of overlap between the two platforms was seen. This is most likely due to the different array design and content in the targeted and genomic backbone regions. The 135K array had much lower coverage compared to the 180K array. Differences may also be attributed to different human reference DNA samples. The 180K array was much more accurate and reliable at detecting small CNVs < 50kb compared to the 135K microarray. This is extremely important if small CNVs are pathogenic for cerebral palsy. The detection of a 4 kb potential candidate gene (COPS3) from this current study suggests that smaller CNVs may be pathogenic for the cerebral palsy phenotype. Previous CNV studies have mainly focused on large variants as more likely to be pathogenic but the advent of higher resolution array-CGH and SNP arrays are now able to identify smaller CNVs that are pathogenic and important to disease. Several variants of

potential significance to the cerebral palsy phenotype were detected on both microarrays but it is not feasible to utilise both. It is likely that both microarray platforms are underpowered for determining the true CNV association with cerebral palsy.

Array-CGH platforms have been the gold standard for microarray studies providing accurate determination of CNV detection requiring less probe coverage than SNP arrays for comparative specificity. However, recent advances in SNP arrays are providing more flexibility in probe selection and now include the use of non-polymorphic probes, thereby providing increased coverage and more detection accuracy for CNV studies. Signal to noise ratios are also reduced through the use of longer probes[130]. Higher density platforms such as the 2.1 million array from Nimblegen or use of the SNP 1 million array from Illumina will increase the overall CNV discovery including those CNVs < 50 kb for cerebral palsy. Conversely the detection of benign CNVs will also increase from more dense arrays but this can be taken into consideration when manually sorting the raw data.

5.1.6 Future directions

There is substantial evidence from this novel pilot study that copy number variants are involved with cerebral palsy. To better assess this, the design of future studies is extremely important. A more accurate outcome would be achieved with a higher resolution platform than was used in this study. This would enable greater coverage to improve CNV detection as well as identify smaller CNVs <50 kb. Our growing national DNA biobank which currently stands at 200 cerebral family trios (mother,

father and affected child) will help to facilitate this ongoing work. Future mutational screening of our existing 1000 DNA samples from our previous genome-wide association study will assist in determining the pathogenicity of the three potential candidate genes; CTNND2, MCPH1 and COPS3 for cerebral palsy. As these candidate genes were inherited from an unaffected parent and variable expressivity is a possibility, expression studies of both affected and unaffected individuals would further assist in determining the pathogenicity of these candidate genes.

It is likely that cerebral palsy is created by a number of novel individually rare genetic mutations, including CNVs and gene sequence variants acting individually or together. We expect to find increasing numbers of cerebral palsy cases with genetic variations influencing causation and being directly involved in causation. Along with our genetic database we are collecting extensive epidemiological information about each case, the pregnancy and the parents to help identify possible interaction with known cerebral palsy risk factors or environmental triggers. To assess this and possible gene-gene interactions very large numbers are required and collaboration with other international groups is already underway to pool DNA biobanks of cerebral palsy families. Identification of susceptibility genes for cerebral palsy will allow for functional gene studies which could lead to specific therapies and interventions based on the individual underlying genetic profile. The importance of identifying genetic causes for cerebral palsy cannot be overstated as it will affect recurrence risk estimates for the families and is crucial in defining specific individual genetic diagnosis.

Chapter 6 **Conclusions**

6.1 Conclusions

Cerebral palsy is the most common physical disability of childhood, and is often accompanied by intellectual disability, seizures, speech and language deficits, visual or hearing impairments, behavioural problems and autism. The worldwide incidence ranges from 2-3/1,000 live births and has remained unchanged despite major improvements in perinatal care. Contrary to traditional beliefs, few cases of cerebral palsy are due to problems at birth. Cerebral palsy is a clinically heterogeneous disorder and very likely to be genetically heterogeneous. To date, copy number variants are the most common variation found in the human genome.

We have determined possible causative copy number changes in cerebral palsy by identifying 34 unique CNVs out of 50 cerebral palsy cases of potential interest and three candidate genes of special interest. The possibility of additional variants identified in this study cohort, other than the three potential candidate genes, being involved in cerebral palsy needs further study. The majority of the additional regions are involved in brain development. We have yet to find evidence of *de novo* CNV events in this cohort of 50 cerebral palsy individuals. To date all CNVs tested for transmission have been inherited. Whole genome testing of all parental samples will assist in determining if the remaining imbalances are *de novo* or inherited, which will aid in establishing their significance. Whilst large *de novo* events have largely been considered more likely to be pathogenic, inherited CNVs are also contributors to disease but they have variable expressivity or incomplete penetrance. The pathogenicity of these rare CNVs is not currently resolved but these preliminary studies into the contribution of CNVs to cerebral palsy justify further evaluation

incorporating methods to detect small CNVs in a larger study. This is currently underway using samples which have already been collected as part of our ongoing national DNA biobank.

Cerebral Palsy is clinically heterogeneous and early preliminary data from this study suggest that cerebral palsy is also genetically heterogeneous. Results support the involvement of several different genes, either acting independently or in the presence of another modifier. This modifier may be genetic such as the synergistic action of two or more genes or an environmental factor such as IUGR or infection. The majority of candidate CNVs found in this cohort are not disease specific. Instead they overlap with more than one neurological disorder implying shared biological pathways.

Based on the strong evidence for the involvement of CNVs in neurological disorders such as autism, intellectual disability and epilepsy, the results of this study strongly suggest that CNVs may also be associated with cerebral palsy.

Chapter 7 **References**

7.1.1 References

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Chapter 8 **Supplementary Data**

8.1 Supplementary data

Table 8.1 Quantification results for EBV transformed cell lines

Proband	Conc (ng/ul)	DNA 260/280	DNA 260/230	Proband	Conc (ng/ul)	DNA 260/280	DNA 260/230
P001	310	1.97	1.79	P027	409	1.98	1.61
P002	417	1.94	1.90	P028	450	1.94	1.59
P003	178	1.95	1.98	P029	389	1.96	2.21
P004	166	1.97	1.97	P030	347	1.96	2.18
P005	181	1.94	1.59	P031	330	1.96	2.27
P007	361	1.98	1.81	P032	386	1.95	2.29
P008	490	1.94	1.76	P033	368	1.94	2.32
P009	516	1.94	1.79	P034	332	1.94	2.32
P010	449	1.97	1.68	P035	349	2.00	2.28
P011	312	2.00	1.45	P036	316	1.96	2.25
P012	567	1.93	1.93	P037	444	1.95	2.16
P013	526	1.95	1.91	P038	333	2.00	1.62
P014	370	1.95	1.71	P039	391	1.95	1.65
P015	289	1.96	1.88	P040	242	1.96	1.48
P016	313	1.94	1.98	P041	304	1.95	1.64
P017	449	1.96	1.57	P042	246	1.99	1.73
P018	392	1.99	1.58	P049	307	1.96	2.07
P019	679	1.89	1.70	P052	425	1.99	2.00
P020	172	2.04	2.14	P053	509	1.95	2.21
P021	424	1.96	1.64	P054	355	1.99	2.15
P022	306	1.96	1.72	P055	397	1.98	2.08
P023	368	1.94	2.30	P056	349	1.98	2.26
P024	358	1.95	2.22	P057	375	1.99	2.12
P025	408	1.96	1.80	P058	364	1.97	2.23
P026	512	1.95	1.76	P059	517	1.97	2.22

Table 8.2 Quantification results for DNA maxi preps.

Sample	Conc ng/ul	DNA 260/280	DNA 260/230
F020	310.86	1.89	2.43
M020	356.31	1.86	2.45
F025	234.81	1.87	2.58
M025	279.51	1.88	2.57
F036	452.22	1.85	2.4
M036	363.2	1.86	2.42

Table 8.3 Quantification results for DNA mini preps.

Sample	Conc ng/ul	DNA 260/280	DNA 260/230
P020	65.88	1.85	1.89
P03	57.84	1.83	1.42
F03	28.44	1.85	1.70
M03	42.30	1.96	2.28
P017	72.80	1.79	1.24
F017	27.20	1.78	1.34
M017	67.88	1.86	0.95
P037	40.23	1.86	1.72
F037	30.61	1.83	1.50
M037	46.21	1.86	1.72
P057	58.95	1.89	1.68
F057	48.00	1.84	1.75
M057	43.55	1.81	1.30

Table 8.4 All CNV calls.

Sample	chrom	start	end	**size**	Probe count	meanSig
CP32	chr1	143641851	143644517	2666	14	0.40488
CP30	chr1	145593779	145597963	4184	9	0.393683333
CP04	chr1	47334021	47349297	15276	4	-0.503385
CP04	chr1	47283791	47320509	36718	11	0.411567273
CP56	chr1	39896124	39933193	37069	26	0.332946538
CP25	chr1	246815354	246858095	42741	68	-0.723986029
CP17	chr1	246815354	246861619	46265	75	-0.422596133
CP02	chr1	143719144	143766167	47023	31	-0.334937742
CP41	chr1	246811545	246861082	49537	74	-0.564034324
CP55	chr1	143659233	143725610	66377	28	-0.487567143
CP19	chr1	246798349	246881971	83622	81	-0.511187284
CP27	chr1	246776969	246861619	84650	81	-2.130351111
CP09	chr1	246776969	246861619	84650	81	-0.663422346
CP29	chr1	246776969	246861619	84650	81	-0.617408025
CP26	chr1	246776969	246861619	84650	81	-0.58562284
CP09	chr1	143694072	143783498	89426	61	-0.333467213
CP38	chr1	38352213	38454454	102241	4	0.8332475
Cp16	chr1	246776969	246881971	105002	82	-0.690045244
CP31	chr1	246776969	246881971	105002	82	-0.683860488
CP53	chr1	246755191	246861619	106428	82	-0.716882683
CP56	chr1	246754119	246861250	107131	83	-0.721276386
CP32	chr1	246754119	246861619	107500	84	-0.71851381
CP54	chr1	246754119	246861619	107500	84	-0.657709048
CP40	chr1	246754119	246861619	107500	84	-0.623025357
CP14	chr1	246754089	246861619	107530	85	-0.672968
CP56	chr1	147917903	148041589	123686	4	-0.51428
CP35	chr1	246754119	246881971	127852	85	-0.730567529
CP04	chr1	246754089	246881971	127882	86	-0.634906163
CP28	chr1	72569303	72703469	134166	5	0.59739
CP31	chr1	17060957	17195882	134925	5	0.300662
CP38	chr1	246754119	246894224	140105	86	-0.544631512
CP53	chr1	143820915	143965028	144113	18	-0.331486667
CP01	chr1	1213233	1361016	147783	5	-0.399344
CP54	chr1	143810678	143965028	154350	24	-0.365979167
CP36	chr1	143810678	143970710	160032	25	-0.3533152
CP20	chr1	109992288	110163912	171624	6	0.399248333
CP10	chr1	1420541	1624843	204302	7	-0.448838571
CP40	chr1	143694072	143965028	270956	103	-0.302073495
CP56	chr1	147359369	147656085	296716	9	-0.854635556
CP36	chr1	147359369	147656085	296716	9	-0.433365556
CP03	chr1	147359369	147656085	296716	9	-0.396837778
CP13	chr1	143659233	143970710	311477	106	-0.359930189

Sample	chrom	start	end	size	Probe count	meanSig
CP31	chr1	16679451	16993922	314471	10	0.313927
CP01	chr1	147291143	147656085	364942	11	-0.304889091
CP08	chr1	844092	1361016	516924	16	-0.383094375
CP10	chr1	844092	1361016	516924	16	-0.374879375
CP09	chr1	844092	1361016	516924	16	-0.346846875
CP02	chr1	844092	1361016	516924	16	-0.34274125
CP25	chr1	1964452	2605548	641096	20	0.651688
CP07	chr1	1420541	2132404	711863	22	-0.300343636
CP55	chr1	1830001	2605548	775547	24	0.35972
CP56	chr1	1796419	2605548	809129	25	0.3437936
CP08	chr1	2681642	3590041	908399	28	-0.3246775
CP55	chr1	2681642	3590041	908399	28	0.305888571
CP25	chr1	2681642	3590041	908399	28	0.434177143
CP09	chr1	1420541	2502209	1081668	33	-0.33748
CP12	chr1	1420541	2502209	1081668	33	-0.321892727
CP08	chr1	1420541	2605548	1185007	36	-0.330347222
CP02	chr1	1420541	2605548	1185007	36	-0.310862778
CP12	chr2	110330447	110332152	1705	15	0.439728
CP10	chr2	110330447	110332152	1705	15	0.456396
CP25	chr2	110183551	110185681	2130	8	0.952395
CP21	chr2	96788896	96791613	2717	7	-0.481082857
CP10	chr2	96787018	96791613	4595	9	-0.516694444
Cp22	chr2	96787018	96791613	4595	9	-0.478645556
CP04	chr2	96787018	96791613	4595	9	-0.462806667
CP05	chr2	96787018	96791613	4595	9	-0.448286667
CP18	chr2	96787018	96791613	4595	9	-0.342868889
CP23	chr2	96787018	96792069	5051	11	-0.42311
CP11	chr2	96786483	96792538	6055	13	-0.47559
CP36	chr2	131509614	131515861	6247	11	0.344710909
CP55	chr2	88204233	88212097	7864	18	0.456102222
CP41	chr2	97379359	97387648	8289	5	-0.436234
CP29	chr2	97379359	97387648	8289	5	0.511306
CP38	chr2	131514333	131522676	8343	11	0.421370909
CP20	chr2	131293354	131301738	8384	8	0.5447775
CP19	chr2	131506323	131514815	8492	12	-0.385835833
CP20	chr2	96891061	96900536	9475	9	0.331828889
CP32	chr2	96723228	96732834	9606	12	0.349871667
CP56	chr2	96723228	96732834	9606	12	0.488064167
CP25	chr2	95377059	95386769	9710	10	0.948142
CP56	chr2	96142127	96151980	9853	11	0.509584545
CP58	chr2	96142127	96151980	9853	11	0.530485455
CP53	chr2	131505473	131517473	12000	20	0.368552
CP54	chr2	131504228	131516946	12718	21	0.381526667
CP12	chr2	131507556	131522701	15145	19	-0.406178947

Sample	chrom	start	end	size	Probe count	meanSig
CP08	chr2	131505911	131522701	16790	22	-0.425090455
CP56	chr2	131505707	131522701	16994	23	0.408429565
CP58	chr2	131505707	131522701	16994	23	0.435276087
CP55	chr2	131505473	131522701	17228	24	0.424172917
CP32	chr2	131504426	131523391	18965	26	0.308673846
CP55	chr2	95403381	95423502	20121	16	0.480441875
CP17	chr2	88915580	88938565	22985	35	-0.362896571
CP58	chr2	131293354	131325018	31664	39	0.349048974
CP55	chr2	131293354	131325018	31664	39	0.427900256
CP55	chr2	95040033	95074069	34036	31	0.413971935
CP56	chr2	131288934	131327493	38559	45	0.356619333
CP25	chr2	95304536	95349293	44757	28	0.705339286
CP25	chr2	96868258	96915751	47493	31	0.634544516
CP56	chr2	95040033	95088383	48350	45	0.323973778
CP02	chr2	96867177	96915751	48574	32	-0.402185
CP08	chr2	96867177	96915751	48574	32	-0.36814375
CP58	chr2	96868258	96917031	48773	32	0.42196375
CP56	chr2	95373624	95423502	49878	39	0.313963333
CP58	chr2	88942401	88994159	51758	51	-0.425582941
CP57	chr2	95309775	95362667	52892	39	0.319250769
CP54	chr2	95294660	95361523	66863	50	0.3134152
CP58	chr2	95294660	95361523	66863	50	0.380626
CP56	chr2	95294660	95361523	66863	50	0.4087836
CP55	chr2	95294660	95361523	66863	50	0.4657568
CP25	chr2	88915580	88995742	80162	92	-0.561076957
CP56	chr2	96868258	96958985	90727	56	0.346956429
CP55	chr2	96868258	96958985	90727	56	0.372712143
CP55	chr2	88915580	89011501	95921	104	-0.525004038
CP07	chr2	88915580	89013409	97829	106	-0.40016217
CP56	chr2	88915580	89013747	98167	107	-0.501530748
CP26	chr2	88915580	89013747	98167	107	-0.478913178
CP52	chr2	88915580	89013747	98167	107	-0.439796168
CP14	chr2	88915580	89013767	98187	108	-0.402569537
CP12	chr2	88915580	89013767	98187	108	-0.388769815
CP57	chr2	88915580	89013767	98187	108	-0.384114167
CP32	chr2	88915580	89014754	99174	109	-0.528242569
CP54	chr2	88915580	89014754	99174	109	-0.513463028
CP42	chr2	88915580	89014754	99174	109	-0.423130092
CP08	chr2	96089501	96189110	99609	47	-0.3228
CP01	chr2	34344506	34444914	100408	4	0.7761925
CP15	chr2	34378118	34478750	100632	4	0.6924325
CP13	chr2	90959554	91061616	102062	4	-0.42061
CP55	chr2	96048597	96151980	103383	33	0.415930303
CP13	chr2	91123184	91238000	114816	4	-0.3387375

Sample	chrom	start	end	size	Probe count	meanSig
CP19	chr2	88912887	89030981	118094	113	-0.465724159
CP41	chr2	88915580	89039755	124175	117	-0.37832906
CP37	chr2	88916645	89044838	128193	117	-0.394762906
CP09	chr2	88912887	89044838	131951	119	-0.510624034
CP41	chr2	97078280	97210556	132276	10	-0.322725
CP35	chr2	88915580	89047957	132377	119	-0.593237143
CP33	chr2	88915580	89048451	132871	120	-0.424512083
CP40	chr2	34378118	34512097	133979	5	0.544518
CP10	chr2	34378118	34512097	133979	5	0.829896
CP24	chr2	34344506	34478750	134244	5	0.780958
CP39	chr2	88915580	89050031	134451	121	-0.422704876
CP02	chr2	96048597	96184793	136196	48	-0.340432292
CP06	chr2	110198803	110338694	139891	347	-0.521152997
CP25	chr2	96048597	96189110	140513	49	0.536527551
CP08	chr2	88915580	89056846	141266	125	-0.48446656
CP27	chr2	110182565	110339339	156774	374	0.408743503
CP53	chr2	110182395	110339339	156944	376	0.372633431
CP15	chr2	91123184	91281917	158733	5	-0.375132
CP42	chr2	242526867	242736153	209286	7	-0.617804286
CP35	chr2	89093010	89322044	229034	13	-0.407733846
CP32	chr2	89093010	89322044	229034	13	-0.380642308
Cp16	chr2	34243499	34512097	268598	9	0.410475556
CP41	chr2	89093010	89367181	274171	14	-0.341781429
CP37	chr2	89093010	89400665	307655	15	-0.341397333
CP52	chr2	89093010	89400665	307655	15	-0.317236
CP33	chr2	89083599	89400665	317066	16	-0.304675
CP02	chr2	88912887	89247511	334624	145	-0.547028
CP03	chr2	88916645	89258158	341513	144	-0.507973889
CP27	chr2	88915580	89258158	342578	145	-0.562951379
CP01	chr2	88915580	89258158	342578	145	-0.496973724
CP24	chr2	88915580	89258158	342578	145	-0.475210897
CP29	chr2	88915580	89258158	342578	145	-0.411383586
CP04	chr2	88915580	89258158	342578	145	-0.410579931
Cp22	chr2	88912887	89258158	345271	146	-0.420150274
CP23	chr2	88912122	89258158	346036	147	-0.385621565
Cp16	chr2	89571965	89921718	349753	11	-0.600087273
CP15	chr2	89571965	89921718	349753	11	-0.58971
CP10	chr2	88915580	89282078	366498	146	-0.549286507
CP11	chr2	88915580	89282078	366498	146	-0.336049863
CP28	chr2	88916645	89322044	405399	146	-0.411829315
CP36	chr2	88915580	89322044	406464	147	-0.554584762
CP21	chr2	88915580	89322044	406464	147	-0.455411837
CP06	chr2	88915580	89322044	406464	147	-0.423986259
CP53	chr2	88915580	89367181	451601	148	-0.467368649

Sample	chrom	start	end	size	Probe count	meanSig
CP59	chr2	88915580	89367181	451601	148	-0.381990068
CP20	chr2	88915580	89400665	485085	149	-0.578819463
CP38	chr2	88915580	89400665	485085	149	-0.565637785
CP15	chr2	88915580	89400665	485085	149	-0.514529933
Cp16	chr2	88915580	89400665	485085	149	-0.484096644
CP13	chr2	88915580	89400665	485085	149	-0.481069463
CP05	chr2	88915580	89400665	485085	149	-0.473011074
CP30	chr2	88915580	89400665	485085	149	-0.443577584
CP40	chr2	88915580	89400665	485085	149	-0.397489799
CP18	chr2	88915580	89400665	485085	149	-0.382278792
CP31	chr2	88915580	89400665	485085	149	-0.380668993
CP59	chr3	198537532	198539695	2163	4	0.54758
CP18	chr3	198537532	198539695	2163	4	0.626535
CP14	chr3	198537532	198539695	2163	4	0.6268875
CP37	chr3	198537532	198539695	2163	4	0.62736
CP27	chr3	198537532	198539695	2163	4	0.6546575
CP41	chr3	198537532	198539695	2163	4	0.65769
CP33	chr3	198537532	198539695	2163	4	0.6632525
CP36	chr3	198537532	198539695	2163	4	0.6669575
CP40	chr3	198537532	198539695	2163	4	0.6776325
CP04	chr3	198537532	198539695	2163	4	0.6926275
CP15	chr3	198537532	198539695	2163	4	0.696315
CP39	chr3	198537532	198539695	2163	4	0.6999375
CP35	chr3	198537532	198539695	2163	4	0.7016725
CP52	chr3	198537532	198539695	2163	4	0.7027475
CP05	chr3	198537532	198539695	2163	4	0.7058425
CP20	chr3	198537532	198539695	2163	4	0.711585
CP21	chr3	198537532	198539695	2163	4	0.7166025
CP03	chr3	198537532	198539695	2163	4	0.723345
CP28	chr3	198537532	198539695	2163	4	0.7315775
CP31	chr3	198537532	198539695	2163	4	0.73186
CP32	chr3	198537532	198539695	2163	4	0.751735
CP54	chr3	198537532	198539695	2163	4	0.7601575
CP07	chr3	198537532	198539695	2163	4	0.760685
CP30	chr3	198537532	198539695	2163	4	0.768195
CP53	chr3	198537532	198539695	2163	4	0.8167475
CP42	chr3	198537532	198541447	3915	5	0.556106
CP59	chr3	198791790	198797681	5891	8	-0.6483825
CP06	chr3	196943602	196950898	7296	9	0.434112222
CP40	chr3	130548387	130561376	12989	5	0.512576
CP59	chr3	196943602	196958627	15025	11	0.660096364
CP53	chr3	11035214	11054028	18814	14	0.351082143
CP06	chr3	196912150	196931653	19503	7	-0.363905714
Cp16	chr3	196943602	196964341	20739	12	0.321464167

Sample	chrom	start	end	size	Probe count	meanSig
CP14	chr3	196943602	196964341	20739	12	0.369389167
CP36	chr3	196943602	196964341	20739	12	0.419704167
CP10	chr3	196943602	196964341	20739	12	0.561335
CP54	chr3	11032469	11054028	21559	16	0.33987875
CP20	chr3	130537687	130561376	23689	6	0.407181667
CP53	chr3	130537687	130561376	23689	6	0.499513333
CP20	chr3	128212309	128238005	25696	10	0.319676
CP02	chr3	197847804	197874677	26873	25	-0.3807816
CP17	chr3	196904691	196931653	26962	11	-0.54739
CP28	chr3	196904691	196931653	26962	11	-0.436426364
CP30	chr3	196943602	196978329	34727	24	0.30686375
CP40	chr3	196943602	196978329	34727	24	0.394154167
CP19	chr3	196904691	196943602	38911	12	-0.420088333
CP42	chr3	196975423	197016165	40742	16	0.310928125
CP40	chr3	128196092	128238005	41913	11	0.306161818
CP53	chr3	127303354	127349803	46449	10	0.45377
CP56	chr3	127303354	127349803	46449	10	0.615849
CP55	chr3	127303354	127349803	46449	10	0.661231
CP35	chr3	128190739	128238005	47266	13	0.322375385
CP53	chr3	128190739	128238005	47266	13	0.442399231
CP40	chr3	127282623	127332375	49752	9	0.333828889
CP03	chr3	196881288	196931653	50365	12	-0.703965
CP11	chr3	196881288	196931653	50365	12	-0.6479725
CP55	chr3	196881288	196931653	50365	12	-0.588540833
CP12	chr3	196881288	196931653	50365	12	-0.491854167
CP27	chr3	196904691	196958627	53936	22	0.498119091
CP13	chr3	196975423	197029600	54177	23	0.311397826
CP53	chr3	196943602	197000359	56757	27	0.322342593
CP56	chr3	196943602	197000359	56757	27	0.375465926
CP52	chr3	196943602	197001293	57691	29	0.454390345
CP56	chr3	10930815	10991998	61183	35	0.373704857
CP23	chr3	196881288	196943602	62314	13	-0.613974615
Cp22	chr3	196881288	196943602	62314	13	-0.420366154
CP54	chr3	127282623	127349803	67180	11	0.418093636
CP08	chr3	196881288	196948528	67240	16	-0.51168375
CP39	chr3	127282623	127350256	67633	12	0.337211667
CP57	chr3	10777751	10856344	78593	46	0.31009087
CP54	chr3	128157609	128238005	80396	17	0.3668
CP56	chr3	10350192	10436540	86348	43	0.335226512
CP35	chr3	196910965	197000359	89394	36	0.412214167
CP58	chr3	10348953	10439401	90448	46	0.328627826
CP30	chr3	128161372	128254125	92753	21	0.302435714
CP32	chr3	128157609	128254125	96516	22	0.365408636
CP29	chr3	199320785	199430925	110140	4	-0.44816

Sample	chrom	start	end	size	Probe count	meanSig
CP56	chr3	128190856	128309528	118672	28	0.461422857
CP02	chr3	128190739	128309528	118789	29	-0.389292069
CP08	chr3	128190739	128309528	118789	29	-0.361142069
CP58	chr3	128190739	128309528	118789	29	0.406476552
CP25	chr3	128190739	128309528	118789	29	0.569022414
CP55	chr3	129540378	129661606	121228	25	0.4002552
Cp16	chr3	163983505	164117861	134356	5	0.523496
CP55	chr3	10350192	10493672	143480	69	0.355337826
CP08	chr3	130678249	130824705	146456	35	-0.335703429
CP54	chr3	130678249	130824705	146456	35	0.301642
CP55	chr3	128161372	128309528	148156	32	0.454284375
CP58	chr3	130678249	130827869	149620	36	0.407451944
CP56	chr3	130678249	130827869	149620	36	0.438709444
CP55	chr3	130678249	130827869	149620	36	0.456677778
CP25	chr3	127495416	127653808	158392	29	0.552283793
CP24	chr3	180494961	180763349	268388	9	-0.463288889
Cp16	chr3	180494961	180763349	268388	9	-0.424463333
CP31	chr3	180494961	180763349	268388	9	-0.384107778
CP33	chr3	180494961	180763349	268388	9	-0.337534444
CP20	chr3	180494961	180763349	268388	9	-0.336896667
CP56	chr3	127495226	127774136	278910	67	0.329401493
CP28	chr3	180461080	180763349	302269	10	-0.413912
CP03	chr3	180494961	180897816	402855	13	-0.359570769
CP23	chr3	163983505	164386392	402887	13	0.431033846
CP12	chr3	163813996	164353022	539026	17	0.506554706
CP30	chr4	6731089	6749596	18507	7	0.482274286
CP54	chr4	7468210	7508384	40174	13	0.394218462
CP53	chr4	7466692	7508384	41692	14	0.339648571
CP57	chr4	7466692	7508384	41692	14	0.408977143
CP26	chr4	7288454	7333652	45198	22	-0.340407727
CP02	chr4	5978871	6034691	55820	17	-0.393567647
CP23	chr4	7298191	7354674	56483	19	-0.309090526
CP02	chr4	6354360	6420243	65883	20	-0.381711
CP55	chr4	4859676	4926523	66847	16	0.480965
CP15	chr4	70187309	70261759	74450	42	-0.376489286
CP01	chr4	70187284	70261759	74475	43	-0.414472558
CP08	chr4	7290362	7369799	79437	26	-0.361685
CP08	chr4	6350822	6431315	80493	23	-0.358445217
CP24	chr4	70167978	70261759	93781	48	-0.400294375
CP29	chr4	70165721	70260690	94969	48	-0.331824792
CP37	chr4	70165721	70261759	96038	49	-0.346377755
CP52	chr4	34479159	34580003	100844	4	0.8019225
CP26	chr4	34479159	34580003	100844	4	0.9733375
CP35	chr4	69023608	69128949	105341	4	-0.92236

Sample	chrom	start	end	size	Probe count	meanSig
CP52	chr4	69023608	69128949	105341	4	-0.7162825
CP42	chr4	70152739	70261759	109020	55	-0.341045455
CP38	chr4	70152674	70261759	109085	56	-0.322183214
CP32	chr4	70153568	70268156	114588	54	-0.433486296
CP52	chr4	48771134	48890107	118973	4	-0.642725
CP18	chr4	48813013	48932544	119531	4	-0.6108975
CP10	chr4	70142221	70261759	119538	59	-1.066809831
CP19	chr4	9696865	9831368	134503	5	-0.644744
CP03	chr4	9696865	9831368	134503	5	-0.59333
CP21	chr4	69023608	69162252	138644	5	-0.84762
CP14	chr4	69023608	69162252	138644	5	-0.766518
Cp22	chr4	69023608	69162252	138644	5	-0.654716
CP27	chr4	69023608	69162252	138644	5	-0.558688
CP28	chr4	9696865	9864639	167774	6	-0.61092
CP09	chr4	8284010	8461157	177147	47	-0.33749617
CP55	chr4	7169227	7366340	197113	58	0.312201897
CP14	chr4	70061204	70261759	200555	90	-0.695933556
CP18	chr4	34479159	34680925	201766	7	0.559122857
CP02	chr4	6935800	7169227	233427	65	-0.315591538
CP55	chr4	6288358	6531385	243027	71	0.343536056
CP55	chr4	7440933	7799216	358283	92	0.343356957
CP25	chr4	8036678	8430961	394283	97	0.425204021
CP58	chr4	8036678	8441880	405202	100	0.3021705
CP08	chr4	8045407	8461157	415750	105	-0.312720667
CP02	chr4	8044149	8461157	417008	106	-0.306006698
CP55	chr4	8036678	8461157	424479	109	0.333560459
CP56	chr4	8036678	8472241	435563	112	0.322471161
CP08	chr5	70343025	70343221	196	27	-0.377892963
CP01	chr5	70343025	70343221	196	27	-0.337691852
CP02	chr5	70343010	70343221	211	30	-0.404755667
CP12	chr5	70343005	70343221	216	31	-0.352430323
CP07	chr5	70343005	70343221	216	31	-0.340751613
CP24	chr5	70343000	70343221	221	32	-0.337861875
CP17	chr5	70343607	70344383	776	58	0.415311897
CP09	chr5	70343199	70344383	1184	94	0.564893723
CP10	chr5	70343296	70344691	1395	110	0.526823364
CP11	chr5	70343209	70345397	2188	155	0.349926452
CP29	chr5	70343193	70345397	2204	157	0.439191401
CP30	chr5	70342939	70345392	2453	193	0.383014456
CP42	chr5	70342965	70346088	3123	195	0.317990359
CP05	chr5	176490667	176494478	3811	8	-0.570405
CP39	chr5	70341309	70345397	4088	202	-0.510242723
CP24	chr5	176490667	176494830	4163	9	-0.486465556
CP04	chr5	176490667	176494910	4243	10	-0.487581

Sample	chrom	start	end	size	Probe count	meanSig
CP23	chr5	176490667	176495015	4348	11	-0.393359091
CP14	chr5	70341329	70346067	4738	199	0.333169849
CP06	chr5	70341324	70346067	4743	200	-0.4549911
CP10	chr5	176489709	176494478	4769	10	-0.511746
CP18	chr5	70341319	70346148	4829	216	0.337038102
CP30	chr5	1158690	1168336	9646	12	0.453368333
CP07	chr5	177319315	177330211	10896	13	-0.67027
CP14	chr5	801218	814245	13027	5	0.721542
CP07	chr5	801218	814245	13027	5	0.794248
CP15	chr5	801218	814245	13027	5	0.803376
CP07	chr5	849093	874582	25489	5	-0.603224
CP15	chr5	849093	874582	25489	5	-0.502492
CP35	chr5	70341319	70367613	26294	239	0.620904059
CP58	chr5	1515851	1546556	30705	23	0.37912087
CP10	chr5	714830	745887	31057	5	-0.453534
CP23	chr5	781140	813830	32690	6	0.613031667
CP17	chr5	781140	813830	32690	6	0.624215
CP03	chr5	781140	813830	32690	6	1.112988333
CP11	chr5	781140	814245	33105	7	0.511108571
CP10	chr5	781140	814245	33105	7	0.619395714
CP29	chr5	781140	814245	33105	7	0.630857143
CP42	chr5	781140	814245	33105	7	0.645818571
Cp22	chr5	781140	814245	33105	7	0.707515714
CP04	chr5	781140	814245	33105	7	0.733994286
CP26	chr5	781140	814245	33105	7	0.784885714
CP24	chr5	781140	814245	33105	7	1.268608571
CP07	chr5	1215816	1254579	38763	9	-0.47369
CP24	chr5	1215816	1254579	38763	9	-0.443446667
CP29	chr5	705521	745887	40366	13	-0.330623846
CP58	chr5	175938873	175981171	42298	37	0.351101622
CP01	chr5	1223151	1265455	42304	13	-0.423303077
CP10	chr5	1223151	1269918	46767	16	-0.539493125
CP23	chr5	1215816	1265455	49639	14	-0.336577857
CP55	chr5	175920201	175974634	54433	43	0.413666744
CP55	chr5	517613	579907	62294	32	0.422159375
CP57	chr5	176821349	176887479	66130	41	0.309676098
CP41	chr5	745887	813830	67943	7	0.509717143
CP33	chr5	781140	849093	67953	8	0.60995875
CP05	chr5	781140	849093	67953	8	0.8971
CP21	chr5	781140	849093	67953	8	1.1100475
CP27	chr5	745887	814245	68358	8	0.52951
CP38	chr5	745887	814245	68358	8	0.92190125
CP25	chr5	509903	579907	70004	40	0.5898555
CP01	chr5	515550	587110	71560	39	-0.308992821

Sample	chrom	start	end	size	Probe count	meanSig
CP56	chr5	1474769	1546556	71787	46	0.33949587
CP12	chr5	1082945	1158690	75745	32	-0.348140313
CP01	chr5	1082945	1158690	75745	32	-0.335918438
CP09	chr5	1082945	1166961	84016	39	-0.355381026
CP25	chr5	1082945	1168245	85300	42	0.513028095
CP55	chr5	176785426	176875698	90272	44	0.333574091
CP12	chr5	515550	607149	91599	44	-0.348285227
CP10	chr5	515550	607149	91599	44	-0.346023182
CP58	chr5	506927	599150	92223	48	0.354095208
CP56	chr5	506927	601315	94388	50	0.3484472
CP13	chr5	717372	814245	96873	11	0.480205455
CP54	chr5	717372	814245	96873	11	0.612140909
CP09	chr5	509903	607149	97246	50	-0.3438142
CP56	chr5	176784036	176881916	97880	53	0.331843585
CP20	chr5	714830	814245	99415	12	0.470054167
CP30	chr5	714830	814245	99415	12	0.475096667
CP52	chr5	714830	814245	99415	12	0.515239167
CP53	chr5	714830	814245	99415	12	0.718574167
CP02	chr5	176141643	176243172	101529	60	-0.320281833
CP55	chr5	1443341	1545647	102306	55	0.346870545
CP28	chr5	745887	849093	103206	9	0.8822
CP59	chr5	745887	849093	103206	9	1.005426667
CP06	chr5	745887	849093	103206	9	1.017131111
CP18	chr5	745887	849093	103206	9	1.364592222
CP58	chr5	176140268	176244176	103908	62	0.345570161
CP55	chr5	176140268	176244176	103908	62	0.413366452
CP56	chr5	176136234	176244176	107942	65	0.342282923
CP25	chr5	176136234	176244176	107942	65	0.538569077
CP37	chr5	730866	849093	118227	10	0.924845
CP35	chr5	717372	849093	131721	12	0.585111667
CP40	chr5	717372	849093	131721	12	0.6967125
CP32	chr5	717372	849093	131721	12	0.8897975
CP57	chr5	714830	849093	134263	13	0.500508462
CP55	chr5	714830	849093	134263	13	0.648991538
CP58	chr5	714830	849093	134263	13	0.659484615
CP25	chr5	714830	849093	134263	13	1.200583077
CP31	chr5	712237	849093	136856	14	0.438575714
CP08	chr5	1432486	1570459	137973	73	-0.328213425
CP56	chr5	175924648	176082479	157831	110	0.301721636
CP08	chr5	176693571	176874855	181284	100	-0.3399867
CP02	chr5	176691076	176874619	183543	100	-0.3622917
CP02	chr5	1415611	1618538	202927	111	-0.333617748
CP39	chr5	714830	918787	203957	23	0.494101739
CP36	chr5	714830	918787	203957	23	0.86553

Sample	chrom	start	end	size	Probe count	meanSig
Cp16	chr5	640087	887255	247168	57	0.510122281
CP08	chr5	1082945	1332735	249790	106	-0.366136132
CP02	chr5	1064485	1332735	268250	114	-0.380265965
CP58	chr5	1045342	1314777	269435	116	0.32369569
CP56	chr5	717372	989266	271894	70	0.403535429
CP08	chr5	366985	640087	273102	132	-0.337220833
CP02	chr5	359711	640087	280376	138	-0.37007413
CP55	chr5	1045342	1345078	299736	123	0.344997073
CP56	chr5	1045342	1377859	332517	145	0.365248621
CP36	chr5	11729847	12368479	638632	20	0.3180145
CP01	chr6	167636476	167641281	4805	9	-0.487564444
CP08	chr6	167631900	167641281	9381	18	-0.407297778
CP02	chr6	167631900	167643151	11251	19	-0.409165263
CP58	chr6	167629601	167641281	11680	21	0.303951429
CP01	chr6	32586503	32686930	100427	4	-0.81287
CP15	chr6	32686930	32788195	101265	4	0.6672975
Cp16	chr6	32686930	32788195	101265	4	0.6915875
CP04	chr6	32620142	32754176	134034	5	0.504464
CP42	chr6	32686930	32821471	134541	5	0.366346
CP32	chr6	32720701	32888412	167711	6	0.534978333
CP35	chr6	32653530	32821471	167941	6	0.558193333
CP21	chr6	32620142	32788195	168053	6	0.447533333
CP03	chr6	32620142	32788195	168053	6	0.513215
CP23	chr6	32552371	32788195	235824	8	0.7928775
CP56	chr6	43929299	44332410	403111	13	0.353469231
CP05	chr6	66973399	67448096	474697	15	-0.432490667
CP58	chr6	33665026	34169244	504218	16	0.357434375
Cp16	chr7	75502067	75503238	1171	7	-0.587101429
CP27	chr7	75502067	75503238	1171	7	-0.538337143
CP28	chr7	36090287	36091509	1222	4	0.63872
CP30	chr7	64348080	64350401	2321	6	0.434555
CP54	chr7	64347696	64350401	2705	9	0.447294444
CP30	chr7	64364352	64369627	5275	10	0.379853
CP35	chr7	36082349	36091509	9160	7	0.439664286
CP04	chr7	51338584	51353022	14438	6	-0.436485
CP56	chr7	149727518	149743152	15634	12	0.522693333
CP36	chr7	36082349	36098016	15667	8	0.4043
CP33	chr7	75192013	75208395	16382	13	0.422920769
CP41	chr7	75192013	75208395	16382	13	0.423216923
CP54	chr7	149727518	149744109	16591	13	0.438687692
CP57	chr7	149727518	149744109	16591	13	0.466832308
CP32	chr7	151112127	151128720	16593	13	0.359926923
CP53	chr7	75190192	75208395	18203	14	0.360402143
CP54	chr7	150503173	150534094	30921	12	0.389716667

Sample	chrom	start	end	size	Probe count	meanSig
CP56	chr7	150503173	150534094	30921	12	0.512439167
CP58	chr7	150503173	150534094	30921	12	0.526178333
CP55	chr7	150503173	150534094	30921	12	0.562304167
CP25	chr7	150503173	150534094	30921	12	0.7969825
CP17	chr7	51137254	51172048	34794	9	0.491972222
CP40	chr7	44243776	44289666	45890	11	0.330127273
CP32	chr7	31055198	31110490	55292	23	0.314872609
CP30	chr7	150351496	150410797	59301	16	0.376796875
CP53	chr7	44222685	44289666	66981	15	0.311789333
CP56	chr7	44222685	44289666	66981	15	0.480502667
CP25	chr7	150326044	150394729	68685	20	0.781987
CP32	chr7	150341274	150411765	70491	20	0.4019595
CP57	chr7	30859522	30933182	73660	22	0.386096818
CP55	chr7	30859522	30933182	73660	22	0.471766364
CP33	chr7	54020944	54105104	84160	5	-0.514734
CP58	chr7	31023279	31112618	89339	30	0.473549
CP57	chr7	38232522	38322177	89655	10	-0.582974
CP57	chr7	31019754	31112618	92864	32	0.362269688
CP54	chr7	31019754	31114683	94929	33	0.359730303
CP55	chr7	31019754	31114683	94929	33	0.455949394
CP56	chr7	31019754	31114683	94929	33	0.466040606
CP57	chr7	150314843	150411765	96922	26	0.396526154
CP55	chr7	73018866	73121421	102555	52	0.368358462
CP58	chr7	150327326	150456631	129305	43	0.380747442
CP55	chr7	150326044	150456631	130587	44	0.440297955
CP53	chr7	150314843	150456631	141788	45	0.302764444
CP54	chr7	150314843	150456631	141788	45	0.308153333
CP56	chr7	150314843	150461710	146867	46	0.41272587
CP03	chr7	88216082	89767697	1551615	47	0.369424681
CP30	chr8	2246685	2250456	3771	4	-0.93424
CP26	chr8	2246685	2250456	3771	4	-0.9049175
CP23	chr8	11283380	11289023	5643	4	-1.036385
CP18	chr8	11272169	11284257	12088	5	0.494032
CP42	chr8	11272169	11284257	12088	5	0.56448
CP35	chr8	2234567	2250456	15889	5	-0.758534
CP29	chr8	2234567	2250456	15889	5	-0.678656
CP28	chr8	11266999	11284257	17258	7	-0.74045
CP25	chr8	10819646	10853116	33470	11	0.763371818
CP39	chr8	10813557	10853116	39559	12	0.368809167
CP55	chr8	10798075	10872820	74745	24	0.4535175
CP56	chr8	10781168	10873846	92678	35	0.396667429
CP58	chr8	10786693	10884816	98123	32	0.387688438
CP21	chr8	39376538	39477280	100742	4	-1.5577525
CP15	chr8	39376538	39477280	100742	4	-1.44036

Sample	chrom	start	end	size	Probe count	meanSig
CP32	chr8	39376538	39477280	100742	4	-1.2179825
CP40	chr8	39376538	39477280	100742	4	-1.11395
CP42	chr8	39376538	39477280	100742	4	-1.0460725
CP59	chr8	39376538	39477280	100742	4	-0.866385
CP02	chr8	7200845	7319031	118186	4	-0.5535475
CP36	chr8	7200845	7319031	118186	4	-0.5084725
CP31	chr8	7200845	7319031	118186	4	-0.492835
CP39	chr8	7200845	7319031	118186	4	-0.47652
CP40	chr8	7200845	7319031	118186	4	-0.3927875
CP10	chr8	7200845	7319031	118186	4	-0.3833475
CP33	chr8	7200845	7319031	118186	4	0.7662175
Cp22	chr8	39342827	39477280	134453	5	-1.12714
CP41	chr8	5763263	6003970	240707	8	-0.61991375
CP36	chr8	39173156	39477280	304124	10	-0.660369
CP56	chr8	47136885	47472903	336018	11	0.485882727
CP25	chr8	143576535	143979794	403259	13	0.647356923
CP25	chr8	144106444	144780211	673767	21	0.453922381
CP58	chr8	142400504	143979794	1579290	48	0.314631875
CP56	chr8	142299813	143979794	1679981	51	0.327287647
CP55	chr8	142299813	143979794	1679981	51	0.373211176
CP11	chr9	66576907	66576982	75	9	-0.495264444
Cp16	chr9	67902664	67902839	175	7	0.436445714
CP56	chr9	67902854	67903199	345	13	0.466689231
CP35	chr9	67909050	67909410	360	27	0.337491481
CP57	chr9	67902777	67903199	422	17	0.415203529
CP12	chr9	66601580	66602091	511	20	-0.495272
CP21	chr9	67910177	67910779	602	22	-0.305004091
CP42	chr9	67910182	67910789	607	23	-0.32679
CP58	chr9	67902854	67903482	628	14	0.545593571
CP36	chr9	67908705	67909400	695	29	0.308055517
CP06	chr9	67908705	67909410	705	31	-0.346659677
Cp16	chr9	67908705	67909425	720	34	0.313196176
Cp22	chr9	67908705	67909425	720	34	0.395096176
CP57	chr9	67910356	67911079	723	23	-0.372742609
CP33	chr9	67908705	67909430	725	35	0.360023143
CP27	chr9	67908705	67909430	725	35	0.394517143
CP26	chr9	67903004	67903825	821	12	-0.452925833
CP29	chr9	67908525	67909410	885	33	0.421815152
CP13	chr9	67910192	67911079	887	29	-0.334233793
CP12	chr9	68288434	68289328	894	26	-0.430661154
CP57	chr9	67906762	67907667	905	39	-0.40163641
CP58	chr9	67906757	67907672	915	41	-0.521969268
CP53	chr9	67906762	67907677	915	41	-0.308157317
CP26	chr9	67906984	67907908	924	45	0.384152222

Sample	chrom	start	end	size	Probe count	meanSig
CP23	chr9	66576907	66577836	929	10	-0.43726
CP41	chr9	66576907	66577836	929	10	-0.423651
CP12	chr9	66576907	66577841	934	11	-0.721313636
CP01	chr9	66576907	66577841	934	11	-0.60823
CP02	chr9	66576907	66577841	934	11	-0.596541818
CP09	chr9	66576907	66577841	934	11	-0.574171818
CP24	chr9	66576907	66577841	934	11	-0.53038
CP07	chr9	66576907	66577841	934	11	-0.415262727
CP05	chr9	66576907	66577841	934	11	-0.405702727
CP37	chr9	66576907	66577841	934	11	-0.383929091
CP04	chr9	66576907	66577841	934	11	-0.305938182
CP29	chr9	67902859	67903825	966	14	-0.337810714
CP58	chr9	67909888	67910913	1025	66	-0.393619242
CP56	chr9	67909888	67910913	1025	66	-0.388155758
CP56	chr9	67906757	67907908	1151	49	-0.440039388
CP11	chr9	67903004	67904238	1234	21	-0.331822381
CP55	chr9	67909883	67911139	1256	70	-0.432203
CP08	chr9	67902859	67904233	1374	22	-0.560665
CP03	chr9	67902859	67904238	1379	23	-0.30312087
CP12	chr9	67902839	67904238	1399	27	-0.440703704
Cp22	chr9	67902839	67904238	1399	27	-0.419487778
CP23	chr9	67902839	67904238	1399	27	-0.347457407
CP19	chr9	67902777	67904208	1431	22	-0.396023636
CP01	chr9	67902839	67904322	1483	28	-0.411724286
CP09	chr9	67902839	67904327	1488	29	-0.545335172
CP02	chr9	67902839	67904327	1488	29	-0.495148621
CP24	chr9	67902839	67904327	1488	29	-0.378718621
CP12	chr9	67915094	67916685	1591	50	-0.3807882
CP55	chr9	67901954	67903825	1871	30	0.397526667
CP58	chr9	67911927	67913899	1972	52	-0.344149038
CP56	chr9	66600779	66603055	2276	35	0.339223714
CP25	chr9	66600779	66603055	2276	35	0.691357143
CP55	chr9	68289790	68292134	2344	60	0.367574167
CP38	chr9	67921585	67924181	2596	13	-0.385441538
CP54	chr9	67904198	67907677	3479	68	-0.380521029
CP55	chr9	67904198	67907908	3710	75	-0.472262667
CP12	chr9	67813932	67817659	3727	8	-0.65854125
CP10	chr9	67813927	67817659	3732	9	-0.52457
CP25	chr9	67813927	67817659	3732	9	0.98281
CP13	chr9	67903825	67907672	3847	68	-0.374686324
CP10	chr9	67902859	67907181	4322	59	-0.333037119
CP38	chr9	67903199	67907914	4715	79	-0.313750253
CP56	chr9	68286313	68292129	5816	115	0.323205826
CP25	chr9	68285683	68292134	6451	122	0.715175

Sample	chrom	start	end	size	Probe count	meanSig
CP54	chr9	67921364	67928731	7367	18	-0.396268889
CP59	chr9	97518832	97527872	9040	8	-0.40332
CP08	chr9	67936282	67945670	9388	9	-0.581564444
CP13	chr9	67918374	67928731	10357	22	-0.356486818
CP42	chr9	66576907	66588429	11522	15	-0.354569333
CP19	chr9	66205558	66217352	11794	11	0.390375455
CP32	chr9	68741827	68754670	12843	11	-0.323812727
CP08	chr9	67799375	67817659	18284	14	-0.542467857
CP07	chr9	67799375	67817659	18284	14	-0.369154286
CP28	chr9	67799375	67817659	18284	14	0.335905714
CP32	chr9	68620577	68640295	19718	4	-0.46665
CP09	chr9	37016401	37037329	20928	16	-0.464018125
CP08	chr9	36156792	36181557	24765	11	-0.553383636
CP12	chr9	66233920	66262216	28296	13	-0.531197692
CP09	chr9	67914738	67945670	30932	101	-0.368459109
CP10	chr9	67914738	67945670	30932	101	-0.313107525
CP08	chr9	36997461	37028491	31030	17	-0.467230588
CP07	chr9	36997461	37028491	31030	17	-0.342155294
CP02	chr9	36997461	37032363	34902	19	-0.466002632
Cp22	chr9	68754540	68790929	36389	9	-0.456284444
CP23	chr9	66537344	66575184	37840	7	0.321321429
CP05	chr9	66537344	66575184	37840	7	0.329781429
CP07	chr9	66537344	66575184	37840	7	0.334522857
CP58	chr9	34536618	34580795	44177	17	0.461430588
CP01	chr9	66233920	66278423	44503	25	-0.435612
CP10	chr9	66233920	66278423	44503	25	-0.4188312
CP54	chr9	34536618	34595010	58392	19	0.314561579
CP56	chr9	34484035	34580795	96760	31	0.357437742
CP24	chr9	44099515	44204901	105386	4	0.38442
CP04	chr9	44099515	44204901	105386	4	0.4142375
CP04	chr9	44666969	44782199	115230	4	0.332355
CP02	chr9	37896535	38017586	121051	42	-0.371587143
CP56	chr9	135241231	135376125	134894	5	0.31763
CP04	chr9	43522781	43676912	154131	6	0.401808333
CP06	chr9	43522781	43676912	154131	6	0.407506667
CP24	chr9	43522781	43676912	154131	6	0.533433333
CP38	chr9	137609295	137913320	304025	10	0.374232
CP26	chr9	11360149	11798823	438674	14	0.411522143
CP56	chr9	135443068	136015354	572286	18	0.334319444
CP08	chr9	138337067	139547101	1210034	37	-0.368716216
CP40	chr10	84492451	84494946	2495	4	0.5424075
CP52	chr10	84492451	84494946	2495	4	0.59503
Cp16	chr10	84492451	84494946	2495	4	0.6004
CP21	chr10	84492451	84494946	2495	4	0.63353

Sample	chrom	start	end	size	Probe count	meanSig
CP36	chr10	84492451	84494946	2495	4	0.6346775
CP03	chr10	84492451	84494946	2495	4	0.63484
CP04	chr10	84492451	84494946	2495	4	0.6368025
CP35	chr10	84492451	84494946	2495	4	0.669255
CP28	chr10	84488307	84494946	6639	5	0.60199
CP52	chr10	50613724	50621872	8148	4	0.6012075
CP55	chr10	50616064	50624241	8177	6	0.82889
CP32	chr10	50613724	50624241	10517	7	0.493767143
CP53	chr10	50613724	50624241	10517	7	0.552258571
CP02	chr10	48028155	48039365	11210	13	-0.488743846
CP55	chr10	50165846	50184446	18600	17	0.533312353
CP08	chr10	50520181	50540778	20597	14	-0.488964286
CP56	chr10	50489357	50540778	51421	21	0.470378571
CP37	chr10	27651566	27745676	94110	38	-0.540394737
CP32	chr10	47058971	47155613	96642	20	0.314481
CP07	chr10	38750258	38851156	100898	4	-0.52747
CP59	chr10	47006155	47112372	106217	15	-0.447752
CP21	chr10	81511539	81635196	123657	4	-0.475085
CP35	chr10	81511539	81635196	123657	4	-0.430505
CP41	chr10	46401730	46526112	124382	50	-0.32445
CP56	chr10	47006155	47155613	149458	22	0.351925455
CP15	chr10	47006155	47173904	167749	27	0.334135185
CP07	chr10	47006155	47173904	167749	27	0.356544444
CP18	chr10	47006155	47173904	167749	27	0.439812593
CP14	chr10	46391937	46560519	168582	60	-0.307420833
CP28	chr10	46391937	46560519	168582	60	0.3885715
CP56	chr10	46391937	46560519	168582	60	0.422511
CP38	chr10	46391937	46560519	168582	60	0.451179167
CP52	chr10	38678696	38851156	172460	6	-0.338958333
CP13	chr10	46368595	46560519	191924	61	0.475491803
CP53	chr10	46368595	46560519	191924	61	0.695976721
CP38	chr10	47006155	47209095	202940	28	0.451316071
CP53	chr10	47006155	47209095	202940	28	0.588306429
CP39	chr10	47006155	47209095	202940	28	0.790277143
CP13	chr10	38645197	38851156	205959	7	-0.395491429
CP56	chr10	87884909	88105768	220859	62	0.35049
CP42	chr10	135093066	135349139	256073	8	0.39218125
CP36	chr10	38909313	39185454	276141	9	-0.315758889
CP18	chr10	38541629	38851156	309527	10	-0.329825
CP02	chr10	134522236	135059695	537459	17	-0.454804706
CP25	chr11	70826568	70837456	10888	6	1.10372
CP15	chr11	69644461	69661365	16904	12	-0.332456667
CP58	chr11	70570849	70589988	19139	12	0.53229
CP53	chr11	70571781	70613144	41363	19	0.342264737

Sample	chrom	start	end	size	Probe count	meanSig
CP54	chr11	70571781	70613144	41363	19	0.345776316
CP56	chr11	70571781	70613144	41363	19	0.444863684
CP08	chr11	68617247	68659329	42082	13	-0.521757692
CP55	chr11	70570849	70613144	42295	20	0.4818075
CP55	chr11	49668955	49713568	44613	15	-0.619814667
CP35	chr11	49668955	49713568	44613	15	-0.585748
CP39	chr11	49668955	49713568	44613	15	-0.419750667
CP03	chr11	49668955	49713568	44613	15	-0.363926667
CP27	chr11	49668955	49713568	44613	15	-0.362267333
CP21	chr11	49668955	49715365	46410	16	-0.53704625
CP25	chr11	68617247	68664900	47653	14	0.796895714
CP38	chr11	49664450	49713568	49118	16	-0.35090625
CP58	chr11	70779258	70829878	50620	23	0.414919565
CP08	chr11	67930294	67984771	54477	15	-0.496379333
CP25	chr11	67907386	67970520	63134	14	0.863056429
CP42	chr11	49663867	49728307	64440	20	-0.3986755
CP55	chr11	67894962	67970520	75558	17	0.473883529
CP55	chr11	69626656	69711484	84828	38	0.390748947
CP12	chr11	55123517	55209462	85945	73	-2.223133699
CP02	chr11	55123517	55209462	85945	73	-1.964349041
CP30	chr11	55123517	55209462	85945	73	-0.734210411
CP20	chr11	55123517	55209462	85945	73	-0.70812137
CP56	chr11	55123517	55209462	85945	73	-0.707665205
CP32	chr11	55123517	55209462	85945	73	-0.695329863
CP36	chr11	55123517	55209462	85945	73	-0.68081
CP31	chr11	55123517	55209462	85945	73	-0.669819315
CP10	chr11	55123517	55209462	85945	73	-0.652181918
CP26	chr11	55123517	55209462	85945	73	-0.625273288
CP53	chr11	55123517	55209462	85945	73	-0.622752329
CP07	chr11	55123517	55209462	85945	73	-0.614233973
CP05	chr11	55123517	55209462	85945	73	-0.612351781
Cp22	chr11	55123517	55209462	85945	73	-0.590077671
CP57	chr11	55123517	55209462	85945	73	-0.557919863
CP56	chr11	70784604	70871807	87203	43	0.400956047
CP55	chr11	70784604	70871807	87203	43	0.404911395
CP29	chr11	55123517	55216262	92745	74	-0.620587703
CP59	chr11	55123517	55216262	92745	74	-0.558966216
CP17	chr11	55123517	55216262	92745	74	-0.458063514
CP28	chr11	55110761	55209462	98701	75	-0.653015867
CP52	chr11	55117355	55216262	98907	75	-0.632762667
CP21	chr11	55097312	55209462	112150	77	-0.730108052
CP58	chr11	68617287	68733299	116012	32	0.398855313
CP57	chr11	68613532	68733299	119767	34	0.343498529
CP54	chr11	22889935	23023989	134054	5	-0.979176

Sample	chrom	start	end	size	Probe count	meanSig
CP08	chr11	69139439	69315520	176081	67	-0.31623806
CP55	chr11	68546194	68733299	187105	41	0.383880976
CP54	chr11	64010313	64413126	402813	13	0.408646154
CP53	chr11	55256305	55672776	416471	122	-0.618242787
CP56	chr11	69932592	70372511	439919	169	0.314739467
CP56	chr11	68518830	69049276	530446	140	0.313505429
CP08	chr11	583577	2212038	1628461	49	-0.332887755
CP05	chr12	34163274	34163289	15	4	-0.7239075
CP13	chr12	34163274	34163289	15	4	-0.705875
CP20	chr12	34163274	34163289	15	4	-0.684605
CP11	chr12	34163274	34163289	15	4	-0.6521325
CP03	chr12	34163274	34163289	15	4	-0.6452225
CP01	chr12	34163274	34163289	15	4	-0.635755
CP07	chr12	34163274	34163289	15	4	-0.6353775
CP37	chr12	34163274	34163289	15	4	-0.616655
CP12	chr12	34163274	34163289	15	4	-0.606265
CP41	chr12	34163274	34163289	15	4	-0.516765
CP35	chr12	34163274	34163289	15	4	-0.500085
CP37	chr12	36926934	36927222	288	14	-0.331322857
CP02	chr12	34162544	34163289	745	5	-0.509352
CP57	chr12	36830281	36831431	1150	15	0.403601333
CP42	chr12	34163274	34164585	1311	7	-0.514534286
CP52	chr12	34163274	34164739	1465	8	-0.4680075
CP28	chr12	34163274	34164744	1470	9	-0.455615556
CP36	chr12	34162544	34164744	2200	10	-0.454309
CP19	chr12	34155300	34158043	2743	11	0.432197273
CP19	chr12	34147955	34150712	2757	11	0.461735455
CP30	chr12	36764625	36768791	4166	16	0.329976875
CP54	chr12	36763493	36767695	4202	17	0.355214118
CP58	chr12	34149947	34154248	4301	18	0.782415556
CP25	chr12	34149947	34154248	4301	18	0.920905556
CP26	chr12	34163274	34168026	4752	15	-0.388678667
CP18	chr12	34163274	34168026	4752	15	-0.312328
CP58	chr12	36763493	36768791	5298	19	0.431484737
CP55	chr12	34149947	34155305	5358	20	0.860942
CP26	chr12	36728991	36734533	5542	26	-0.322932308
CP55	chr12	36763493	36769200	5707	20	0.4273775
CP53	chr12	36763007	36768791	5784	21	0.302985238
CP56	chr12	36762512	36768791	6279	22	0.389895909
CP38	chr12	34147955	34154248	6293	20	0.5612015
CP25	chr12	36762120	36769200	7080	29	0.730865517
CP39	chr12	34147955	34155300	7345	21	0.669473333
CP56	chr12	34147955	34155310	7355	23	0.735688261
CP57	chr12	34147955	34155410	7455	24	0.64493375

Sample	chrom	start	end	size	Probe count	meanSig
CP53	chr12	34147955	34156411	8456	26	0.609786154
CP33	chr12	34147955	34156533	8578	27	0.479972222
CP30	chr12	34147955	34156533	8578	27	0.573137407
CP54	chr12	34147955	34156533	8578	27	0.586269259
CP40	chr12	34147955	34157337	9382	28	0.510778571
CP56	chr12	34641237	34650743	9506	12	0.538006667
CP58	chr12	34641237	34650743	9506	12	0.556213333
CP29	chr12	34147955	34157484	9529	29	0.362512069
CP06	chr12	34147955	34157829	9874	30	0.469441667
CP14	chr12	34147955	34158043	10088	31	0.399996774
CP27	chr12	34147955	34158068	10113	32	0.420147813
CP36	chr12	34147955	34158068	10113	32	0.51433125
CP02	chr12	36830140	36841620	11480	22	-0.410554091
CP53	chr12	36830140	36841620	11480	22	0.377753182
CP13	chr12	34149241	34162544	13303	32	0.416755313
CP11	chr12	34147955	34162544	14589	33	0.373292424
CP18	chr12	34147955	34162544	14589	33	0.381041818
CP20	chr12	34147955	34162544	14589	33	0.410094545
CP41	chr12	34147955	34162544	14589	33	0.425295758
CP59	chr12	34147955	34162544	14589	33	0.431474242
CP31	chr12	34147955	34162544	14589	33	0.448317273
CP03	chr12	34147955	34162544	14589	33	0.453897879
CP26	chr12	34147955	34162544	14589	33	0.460369394
CP24	chr12	34147955	34162544	14589	33	0.468669091
CP21	chr12	34147955	34162544	14589	33	0.474548485
CP37	chr12	34147955	34162544	14589	33	0.480860909
CP05	chr12	34147955	34162544	14589	33	0.481976061
Cp16	chr12	34147955	34162544	14589	33	0.488011212
CP32	chr12	34147955	34162544	14589	33	0.520141818
CP28	chr12	34147955	34162544	14589	33	0.531807273
CP35	chr12	34147955	34162544	14589	33	0.536926364
CP09	chr12	34143328	34158068	14740	34	0.413218235
CP02	chr12	34143328	34158068	14740	34	0.464277941
CP54	chr12	36830140	36846618	16478	23	0.328344348
CP58	chr12	36830140	36846618	16478	23	0.395474783
CP56	chr12	36830140	36846618	16478	23	0.485448261
CP25	chr12	36830140	36846618	16478	23	0.547924783
CP07	chr12	34143545	34162544	18999	34	0.362210294
CP42	chr12	34143545	34162544	18999	34	0.418182647
CP23	chr12	34143545	34162544	18999	34	0.426593235
CP01	chr12	34143328	34162544	19216	35	0.359858286
CP12	chr12	34143328	34162544	19216	35	0.408376286
CP10	chr12	34143328	34162544	19216	35	0.418313714
CP15	chr12	34142847	34162544	19697	36	0.406626944

Sample	chrom	start	end	size	Probe count	meanSig
CP26	chr12	36762130	36782091	19961	34	-0.314894412
CP12	chr12	34380234	34435333	55099	18	-0.413714444
CP09	chr12	34380234	34437003	56769	19	-0.408230526
CP08	chr12	34380234	34437003	56769	19	-0.386673684
CP02	chr12	34362256	34437003	74747	33	-0.393501515
CP58	chr12	34349151	34437003	87852	44	0.403775682
CP24	chr12	130878305	130979533	101228	4	-0.874655
Cp22	chr12	130878305	130979533	101228	4	-0.72224
CP04	chr12	130878305	130979533	101228	4	-0.6640425
CP29	chr12	9526797	9631909	105112	4	0.734335
Cp22	chr12	9526797	9631909	105112	4	0.739185
CP02	chr12	9526797	9631909	105112	4	1.208965
CP55	chr12	34322098	34437003	114905	50	0.4014876
CP56	chr12	34319309	34437003	117694	73	0.33725726
CP05	chr12	130844439	130979533	135094	5	-0.589692
CP54	chr12	34330524	34471636	141112	49	0.30098
CP52	chr12	9452116	9598564	146448	5	0.631124
CP37	chr12	9452116	9598564	146448	5	0.63177
CP13	chr12	9452116	9598564	146448	5	0.701088
CP59	chr12	9452116	9598564	146448	5	0.733852
CP28	chr12	9435862	9598564	162702	6	0.596966667
CP04	chr12	9435862	9598564	162702	6	0.605753333
CP42	chr12	9435862	9598564	162702	6	0.751723333
CP35	chr12	9435655	9598564	162909	7	0.499838571
CP32	chr12	9435655	9598564	162909	7	0.535571429
CP31	chr12	9435655	9598564	162909	7	0.727064286
CP26	chr12	36554896	36726062	171166	10	0.452241
CP30	chr12	9424499	9598564	174065	20	0.402187
CP15	chr12	9435655	9631909	196254	8	0.513205
CP09	chr12	131317106	131518278	201172	7	-0.387887143
CP01	chr12	130710579	130979533	268954	9	-0.47102
CP07	chr12	130710579	130979533	268954	9	-0.462745556
Cp16	chr12	130710579	130979533	268954	9	-0.416694444
CP10	chr12	131317106	131619592	302486	10	-0.311604
CP15	chr12	130674894	130979533	304639	10	-0.383996
CP08	chr12	131317106	131652653	335547	11	-0.399532727
CP10	chr12	130878305	131248687	370382	12	-0.422755833
CP31	chr12	123077344	123481132	403788	13	0.411486923
CP54	chr12	112763093	113267140	504047	16	0.331484375
CP09	chr12	130710579	131248687	538108	17	-0.423310588
CP54	chr13	83645908	83746386	100478	4	-0.86311
CP54	chr13	113474038	113641965	167927	6	0.388466667
CP25	chr13	113758056	114094145	336089	11	0.481366364
CP02	chr13	18219586	18670917	451331	14	0.554482143

Sample	chrom	start	end	size	Probe count	meanSig
CP42	chr14	105842808	105977953	135145	5	-0.8883
Cp16	chr14	105808863	105944389	135526	5	-0.585272
CP20	chr14	34699089	34867273	168184	6	0.554613333
CP23	chr14	105842808	106011748	168940	6	-0.81247
CP01	chr14	105842808	106011748	168940	6	-0.763106667
CP02	chr14	105842808	106011748	168940	6	-0.756933333
CP26	chr14	105842808	106011748	168940	6	-0.6165
CP07	chr14	105842808	106011748	168940	6	-0.612316667
CP41	chr14	105842808	106011748	168940	6	-0.539133333
CP54	chr14	19265972	19467508	201536	7	-0.603037143
CP31	chr14	19265972	19467508	201536	7	-0.599284286
CP52	chr14	19265972	19467508	201536	7	-0.599057143
CP30	chr14	19265972	19467508	201536	7	-0.56389
CP13	chr14	19265972	19467508	201536	7	-0.540131429
CP27	chr14	19265972	19467508	201536	7	-0.511841429
CP42	chr14	19265972	19467508	201536	7	-0.500048571
CP41	chr14	19265972	19467508	201536	7	-0.497485714
CP40	chr14	19265972	19467508	201536	7	-0.484687143
CP37	chr14	19265972	19467508	201536	7	-0.449601429
CP24	chr14	105842808	106045374	202566	7	-1.137318571
CP12	chr14	105842808	106045374	202566	7	-0.849575714
CP05	chr14	105842808	106045374	202566	7	-0.787471429
CP29	chr14	105842808	106045374	202566	7	-0.686398571
CP52	chr14	105808863	106011748	202885	7	-0.572744286
CP06	chr14	105808863	106011748	202885	7	-0.56823
CP27	chr14	105808863	106011748	202885	7	-0.558147143
CP57	chr14	105877182	106112559	235377	8	-0.6168675
CP59	chr14	105775701	106011748	236047	8	-0.395025
CP13	chr14	105842808	106079293	236485	8	-0.76623
CP39	chr14	19265972	19502662	236690	8	-0.4946225
CP32	chr14	19265972	19502662	236690	8	-0.4925425
CP53	chr14	105156090	105395338	239248	8	0.42053875
CP30	chr14	105842808	106112559	269751	9	-0.58519
CP20	chr14	105842808	106112559	269751	9	-0.568737778
CP36	chr14	105842808	106112559	269751	9	-0.563264444
CP40	chr14	105842808	106112559	269751	9	-0.452707778
CP03	chr14	42920232	43256199	335967	11	0.423631818
CP53	chr14	105775701	106112559	336858	11	-0.46532
CP28	chr14	105808863	106180022	371159	12	-0.51258
Cp22	chr14	105808863	106180022	371159	12	-0.426001667
CP17	chr14	44032315	44406005	373690	12	-0.466995833
CP56	chr14	105877182	106347948	470766	15	-0.487019333
CP54	chr14	105877182	106347948	470766	15	-0.390379333
CP21	chr14	105842808	106347948	505140	16	-0.43896625

Sample	chrom	start	end	size	Probe count	meanSig
CP32	chr14	105842808	106347948	505140	16	-0.392158125
CP15	chr14	105842808	106347948	505140	16	-0.357424375
CP09	chr14	105842808	106347948	505140	16	-0.355758125
CP31	chr14	105842808	106347948	505140	16	-0.33481375
CP18	chr14	105842808	106347948	505140	16	-0.30558375
CP11	chr14	105808863	106347948	539085	17	-0.451388235
CP39	chr14	105808863	106347948	539085	17	-0.365308824
CP19	chr14	105808863	106347948	539085	17	-0.364946471
CP14	chr14	105808863	106347948	539085	17	-0.350725882
CP35	chr14	105808863	106347948	539085	17	-0.347751765
CP37	chr14	105775701	106347948	572247	18	-0.322351667
CP04	chr14	105428622	106347948	919326	28	-0.300319643
Cp22	chr15	20636614	20638111	1497	6	-0.703856667
CP23	chr15	20636614	20638111	1497	6	-0.608936667
CP33	chr15	82112750	82115784	3034	6	0.477765
CP37	chr15	82112750	82115784	3034	6	0.570303333
CP35	chr15	82112750	82115784	3034	6	0.596176667
CP57	chr15	82112750	82115784	3034	6	0.83381
CP58	chr15	82112750	82115784	3034	6	0.852081667
CP56	chr15	82112750	82115784	3034	6	0.897521667
CP59	chr15	19840521	19843644	3123	5	-0.702084
CP14	chr15	19840521	19843644	3123	5	-0.530586
CP11	chr15	75118847	75122240	3393	4	0.963785
CP41	chr15	75118847	75122240	3393	4	1.348715
CP24	chr15	75118847	75122240	3393	4	1.418215
CP31	chr15	82112750	82116171	3421	8	0.44517375
CP38	chr15	82112750	82116171	3421	8	0.48469375
CP30	chr15	82112750	82116171	3421	8	0.614175
CP53	chr15	82112750	82116171	3421	8	0.63226625
CP52	chr15	82111785	82115784	3999	7	0.503647143
CP55	chr15	82112750	82116854	4104	9	0.799605556
Cp22	chr15	75118847	75123061	4214	5	0.721644
CP15	chr15	75118847	75123061	4214	5	0.992702
CP02	chr15	75118847	75123061	4214	5	1.064078
CP23	chr15	75118847	75123061	4214	5	1.07933
CP18	chr15	75118847	75123061	4214	5	1.273876
CP08	chr15	75118847	75123061	4214	5	1.35177
CP39	chr15	82111785	82116141	4356	8	0.55146375
CP42	chr15	82111785	82116171	4386	9	0.416978889
CP36	chr15	82112750	82117222	4472	10	0.469065
CP54	chr15	81125383	81132106	6723	11	0.482860909
CP57	chr15	81125383	81132106	6723	11	0.580939091
CP54	chr15	82108562	82115784	7222	10	0.610552
CP58	chr15	81125383	81132679	7296	12	0.582778333

Sample	chrom	start	end	size	Probe count	meanSig
CP20	chr15	82108562	82116171	7609	12	0.326401667
CP41	chr15	82108482	82116171	7689	13	0.331651538
CP01	chr15	23734324	23742127	7803	4	0.89755
CP40	chr15	82108110	82116171	8061	14	0.437058571
CP41	chr15	19840521	19849055	8534	7	-0.497448571
CP04	chr15	20636614	20645361	8747	7	-0.611144286
CP10	chr15	20636614	20645498	8884	8	-0.7063425
CP05	chr15	20636614	20645498	8884	8	-0.56922875
CP03	chr15	20636614	20645498	8884	8	-0.4993125
CP15	chr15	19875550	19884597	9047	9	-0.37131
CP59	chr15	75112876	75122240	9364	5	0.702168
CP38	chr15	75112876	75122240	9364	5	0.97036
CP59	chr15	21795272	21805610	10338	4	-0.6536225
CP21	chr15	20635093	20645498	10405	9	-0.48961
CP25	chr15	20536602	20547269	10667	16	0.884706875
CP12	chr15	19875550	19886398	10848	10	-0.54819
CP40	chr15	82334626	82348362	13736	4	-0.6156875
CP28	chr15	75105249	75119170	13921	8	0.673625
CP37	chr15	75105249	75119170	13921	8	0.67883625
CP54	chr15	75894979	75909248	14269	11	0.553512727
CP01	chr15	75690956	75706568	15612	11	-0.466800909
CP06	chr15	75105249	75122240	16991	10	0.36339
CP55	chr15	22850224	22867599	17375	11	0.657219091
CP13	chr15	75099837	75119170	19333	10	0.676839
CP55	chr15	19926975	19947883	20908	38	0.547376053
CP53	chr15	75099837	75121371	21534	11	0.726409091
CP56	chr15	81111948	81133728	21780	20	0.505468
CP33	chr15	75099837	75123061	23224	13	0.515900769
Cp22	chr15	19840521	19865039	24518	9	-0.724012222
CP06	chr15	19818575	19843644	25069	12	-0.6068125
CP13	chr15	19818575	19843644	25069	12	-0.374234167
CP52	chr15	75093495	75121371	27876	14	0.532747857
CP35	chr15	75093495	75121371	27876	14	0.635130714
CP39	chr15	75088303	75119170	30867	16	0.55014125
CP54	chr15	75090997	75122240	31243	16	0.66201375
CP08	chr15	19774581	19808053	33472	4	0.3884725
CP01	chr15	19774581	19808053	33472	4	0.39141
CP07	chr15	75570999	75604664	33665	24	0.467497083
CP32	chr15	75088303	75122240	33937	18	0.547342222
CP40	chr15	75570999	75604944	33945	25	0.3894368
CP36	chr15	19875550	19909508	33958	11	-0.547811818
CP30	chr15	75088130	75122240	34110	19	0.558035263
CP08	chr15	72673945	72712651	38706	27	-0.433464444
CP58	chr15	19926975	19969914	42939	43	0.408327442

Sample	chrom	start	end	size	Probe count	meanSig
CP13	chr15	19926975	19969914	42939	43	0.464662093
CP56	chr15	19926975	19969914	42939	43	0.520852326
CP55	chr15	19875550	19922896	47346	12	-0.753546667
CP11	chr15	19799652	19849055	49403	17	-0.462668235
CP57	chr15	19774581	19834505	59924	11	0.441926364
CP55	chr15	22977443	23037677	60234	8	0.84230875
CP59	chr15	19774581	19835414	60833	12	0.341265
CP54	chr15	19817024	19882042	65018	28	-0.527529643
CP42	chr15	19820444	19886398	65954	28	-0.332338929
CP08	chr15	19927822	19994339	66517	55	0.324819091
CP56	chr15	75625322	75695021	69699	69	0.372367971
CP07	chr15	19774581	19846703	72122	18	0.322351667
CP39	chr15	75425900	75510049	84149	26	-0.334988077
CP27	chr15	19817024	19909508	92484	31	-0.359537419
CP15	chr15	19774581	19869176	94595	24	0.51038125
CP30	chr15	19818575	19922896	104321	31	-0.48563871
CP31	chr15	19817024	19922896	105872	32	-0.498271563
CP26	chr15	19817024	19922896	105872	32	-0.416715938
CP35	chr15	32536274	32644465	108191	4	-0.8991425
CP12	chr15	19355899	19465053	109154	4	0.8219275
CP38	chr15	19846703	19969914	123211	62	0.328827742
CP39	chr15	19926975	20052598	125623	71	0.357018028
CP41	chr15	19922896	20048840	125944	70	0.325856857
CP32	chr15	19926975	20053812	126837	72	0.434876389
CP36	chr15	19922896	20055216	132320	75	0.336498933
CP03	chr15	19922896	20055216	132320	75	0.358056
CP33	chr15	19926975	20059853	132878	77	0.35526026
CP37	chr15	19926975	20061403	134428	79	0.307976203
CP07	chr15	19926975	20061403	134428	79	0.545407089
CP23	chr15	19922896	20061403	138507	80	0.325144
CP10	chr15	32503075	32644465	141390	5	-1.232756
CP07	chr15	19322398	19465053	142655	5	0.603988
CP40	chr15	19926975	20076089	149114	80	0.328065375
CP19	chr15	19926995	20080161	153166	81	0.331814938
CP20	chr15	81850715	82009135	158420	77	-0.717199351
CP14	chr15	20636614	20795812	159198	15	-0.324523333
CP15	chr15	18686172	18863527	177355	6	0.48061
CP01	chr15	19869176	20057200	188024	89	0.304333933
CP15	chr15	19886398	20080161	193763	85	0.579797059
CP57	chr15	19884597	20080161	195564	86	0.762057209
CP13	chr15	20635093	20834571	199478	17	-0.351662941
CP03	chr15	18652514	18863527	211013	7	0.432714286
Cp22	chr15	18652514	18863527	211013	7	0.514144286
CP18	chr15	18652514	18863527	211013	7	0.531861429

Sample	chrom	start	end	size	Probe count	meanSig
CP24	chr15	18652514	18863527	211013	7	0.537682857
Cp16	chr15	18652514	18863527	211013	7	0.627081429
CP04	chr15	18652514	18863527	211013	7	0.638568571
CP02	chr15	18652514	18863527	211013	7	0.768537143
Cp22	chr15	19865594	20077257	211663	96	0.308621979
CP14	chr15	19846703	20076089	229386	99	0.303061414
CP59	chr15	19846703	20080161	233458	101	0.313446634
CP41	chr15	18609504	18863527	254023	8	0.3078
CP59	chr15	18609504	18863527	254023	8	0.34520875
CP40	chr15	18609504	18863527	254023	8	0.3456425
CP27	chr15	18609504	18863527	254023	8	0.346715
CP36	chr15	18609504	18863527	254023	8	0.454235
CP57	chr15	18609504	18863527	254023	8	0.48224875
CP35	chr15	18609504	18863527	254023	8	0.54681875
CP21	chr15	18609504	18863527	254023	8	0.582165
CP05	chr15	19774581	20053812	279231	108	0.347959259
CP53	chr15	18571399	18863527	292128	9	0.457204444
CP17	chr15	19774581	20076089	301508	116	0.394820776
CP28	chr15	19774581	20077257	302676	117	0.584919829
CP29	chr15	19774581	20080161	305580	118	0.463534831
CP53	chr15	19774581	20080161	305580	118	0.511916356
CP35	chr15	19774581	20080161	305580	118	0.592008475
CP18	chr15	19774581	20080161	305580	118	0.688115847
CP02	chr15	19774581	20080161	305580	118	0.733365424
CP24	chr15	19774581	20080161	305580	118	0.738707542
CP09	chr15	19774581	20080161	305580	118	0.760169322
Cp16	chr15	19774581	20080161	305580	118	0.976212966
CP04	chr15	19774581	20080161	305580	118	0.979375508
CP21	chr15	19774581	20080161	305580	118	1.123052712
CP13	chr15	19147312	19465053	317741	10	0.432154
CP37	chr15	19080050	19465053	385003	12	0.3106375
CP26	chr15	19080050	19465053	385003	12	0.317015833
CP36	chr15	19080050	19465053	385003	12	0.366929167
CP05	chr15	19080050	19465053	385003	12	0.3676575
CP29	chr15	19080050	19465053	385003	12	0.3697825
CP33	chr15	19080050	19465053	385003	12	0.380080833
CP09	chr15	19080050	19465053	385003	12	0.382439167
CP20	chr15	19080050	19465053	385003	12	0.394354167
CP59	chr15	19080050	19465053	385003	12	0.422100833
CP27	chr15	19080050	19465053	385003	12	0.4275175
CP23	chr15	19080050	19465053	385003	12	0.4533975
CP01	chr15	19080050	19465053	385003	12	0.4729225
Cp22	chr15	19080050	19465053	385003	12	0.480405
CP40	chr15	19080050	19465053	385003	12	0.486094167

Sample	chrom	start	end	size	Probe count	meanSig
CP57	chr15	19080050	19465053	385003	12	0.517815833
CP52	chr15	19080050	19465053	385003	12	0.526089167
CP03	chr15	19080050	19465053	385003	12	0.5288975
CP15	chr15	19080050	19465053	385003	12	0.614275833
CP53	chr15	19080050	19465053	385003	12	0.640141667
CP24	chr15	19080050	19465053	385003	12	0.646348333
CP28	chr15	19080050	19465053	385003	12	0.702321667
CP21	chr15	19080050	19465053	385003	12	0.712635
CP35	chr15	19080050	19465053	385003	12	0.74197
CP02	chr15	19080050	19465053	385003	12	0.76567
CP04	chr15	19080050	19465053	385003	12	0.767229167
CP18	chr15	19080050	19465053	385003	12	0.836205
Cp16	chr15	19080050	19465053	385003	12	0.926906667
CP01	chr16	32529276	32530792	1516	68	-0.450340294
CP59	chr16	13014821	13017052	2231	4	0.635425
CP33	chr16	13014821	13017052	2231	4	0.6776675
CP41	chr16	13014821	13017052	2231	4	0.731365
CP21	chr16	13014821	13017052	2231	4	0.80066
CP53	chr16	33520443	33522880	2437	21	0.305152857
CP11	chr16	32521033	32523734	2701	40	-0.3626685
CP55	chr16	33518773	33521551	2778	30	0.501153667
CP53	chr16	33514032	33516846	2814	12	-0.32191
CP29	chr16	32520808	32523734	2926	42	-0.35403119
CP11	chr16	33500475	33503595	3120	26	-0.388854615
CP53	chr16	32513377	32516522	3145	26	0.310256154
CP30	chr16	32513377	32516887	3510	27	0.320501111
CP28	chr16	33394212	33397968	3756	25	-0.33284
CP32	chr16	32513442	32517294	3852	30	0.323668333
CP11	chr16	33394696	33398557	3861	25	-0.4853292
CP58	chr16	32513377	32517304	3927	33	0.415887273
CP56	chr16	32513377	32517304	3927	33	0.423533333
CP29	chr16	33393926	33397968	4042	26	-0.486992692
CP54	chr16	33518773	33522880	4107	33	0.344211818
CP25	chr16	32513058	32517294	4236	32	0.868513125
CP15	chr16	32546843	32551192	4349	45	-0.331152667
CP54	chr16	32512715	32517304	4589	35	0.433719143
CP55	chr16	32512715	32517304	4589	35	0.625732571
Cp16	chr16	33518206	33522880	4674	35	0.360890571
CP23	chr16	33392441	33397968	5527	28	-0.5166025
CP01	chr16	33511536	33517143	5607	32	-0.473366875
CP11	chr16	33515467	33521208	5741	42	-0.381975238
CP01	chr16	33392441	33398552	6111	30	-0.500523333
CP15	chr16	33391818	33398547	6729	30	-0.478191333
CP15	chr16	32528483	32535900	7417	119	-0.437612941

Sample	chrom	start	end	size	Probe count	meanSig
CP55	chr16	32528512	32536580	8068	117	0.508181026
CP54	chr16	32528495	32536580	8085	118	0.392728136
CP29	chr16	32545376	32553552	8176	58	-0.344820172
CP29	chr16	32528490	32537109	8619	120	-0.444891583
CP42	chr16	32525384	32535373	9989	125	0.32159088
CP07	chr16	32513442	32523937	10495	87	-0.338107011
CP23	chr16	32513058	32523734	10676	88	-0.351157841
CP15	chr16	32513058	32523937	10879	89	-0.356663596
CP04	chr16	33527812	33539025	11213	29	-0.328585862
CP40	chr16	33517143	33529218	12075	54	0.382815926
CP18	chr16	68740176	68753041	12865	6	0.44065
CP21	chr16	33518206	33531207	13001	55	0.445720727
CP28	chr16	32520380	32533552	13172	164	-0.340020366
CP06	chr16	19852126	19866033	13907	4	-0.607885
CP28	chr16	32542979	32557496	14517	68	-0.301907647
CP58	chr16	29911541	29926078	14537	18	0.474557778
CP02	chr16	29912534	29927713	15179	17	-0.532979412
CP21	chr16	33298886	33314218	15332	14	0.416969286
CP27	chr16	33517143	33533681	16538	68	0.320761912
CP31	chr16	26780471	26798187	17716	4	-0.6143175
CP11	chr16	32528483	32547865	19382	195	-0.385293333
CP01	chr16	32532405	32553552	21147	114	-0.38747693
CP33	chr16	19857770	19878979	21209	4	-0.60202
CP57	chr16	33496811	33518211	21400	103	-0.338267282
CP23	chr16	32528946	32551192	22246	196	-0.405006837
CP38	chr16	33496811	33519724	22913	111	-0.328971802
CP21	chr16	32513377	32537396	24019	232	0.331757328
CP07	chr16	32528946	32560267	31321	209	-0.357714211
CP07	chr16	33476960	33517143	40183	113	-0.35383531
CP28	chr16	33498645	33539025	40380	177	-0.336508531
CP42	chr16	33426969	33467950	40981	17	-0.321595294
CP23	chr16	33475178	33516846	41668	122	-0.353177705
CP37	chr16	32518551	32560267	41716	290	-0.310527655
CP15	chr16	33475183	33517143	41960	122	-0.347130492
CP04	chr16	33475178	33517143	41965	123	-0.386817642
CP32	chr16	16069514	16112701	43187	13	0.397683846
CP40	chr16	32494121	32537950	43829	242	0.307107314
CP29	chr16	33476960	33521208	44248	144	-0.3231825
CP04	chr16	32515750	32560267	44517	307	-0.342999674
CP57	chr16	33526526	33571115	44589	35	-0.408229143
CP20	chr16	13796342	13842547	46205	12	-0.758710833
CP52	chr16	68706246	68753041	46795	7	0.578718571
Cp22	chr16	32512715	32560267	47552	328	-0.371943232
CP53	chr16	33253460	33302210	48750	16	0.3301425

Sample	chrom	start	end	size	Probe count	meanSig
CP04	chr16	13014821	13066479	51658	12	0.403758333
CP14	chr16	19507692	19573177	65485	11	0.432036364
CP59	chr16	22538121	22605065	66944	12	-0.305639167
CP27	chr16	22540464	22611974	71510	12	-0.373519167
CP53	chr16	22538121	22611974	73853	14	-0.371190714
CP39	chr16	22538121	22611974	73853	14	-0.340247143
CP04	chr16	33392441	33473683	81242	61	-0.363464098
CP38	chr16	33392441	33473683	81242	61	-0.318139344
CP36	chr16	69236715	69320615	83900	13	0.325915385
CP04	chr16	32727399	32822993	95594	8	-0.30206625
CP35	chr16	69236715	69333114	96399	16	0.325860625
CP35	chr16	22504463	22611974	107511	15	-0.385392667
CP27	chr16	33279393	33395826	116433	53	0.328589811
CP39	chr16	33498969	33615563	116594	180	-0.313431278
CP07	chr16	32727399	32849480	122081	9	-0.346102222
Cp22	chr16	32727399	32849480	122081	9	-0.333051111
CP37	chr16	5366905	5500832	133927	5	0.556194
CP30	chr16	22464337	22611974	147637	16	-0.7457825
CP21	chr16	22464337	22611974	147637	16	-0.36774
CP55	chr16	22464337	22623411	159074	18	-0.408906667
CP07	chr16	33253460	33417330	163870	79	-0.405727975
CP23	chr16	32173377	32364650	191273	18	-0.396225
CP04	chr16	32173377	32364650	191273	18	-0.362315
CP59	chr16	5299353	5500832	201479	7	0.385051429
CP14	chr16	5299353	5500832	201479	7	0.385141429
CP37	chr16	33475178	33678188	203010	209	-0.319365024
CP36	chr16	34333620	34602345	268725	9	-0.381492222
CP20	chr16	34333620	34602345	268725	9	-0.3719
CP21	chr16	34333620	34602345	268725	9	-0.358068889
Cp22	chr16	33253460	33538575	285115	309	0.35636165
CP32	chr16	33392441	33678188	285747	278	-0.33488054
CP52	chr16	34333620	34635771	302151	10	-0.341616
CP05	chr16	5265675	5602110	336435	11	0.41654
CP07	chr16	5198696	5602110	403414	13	0.352326923
CP09	chr16	5265675	5741286	475611	15	0.394664
CP08	chr16	541638	1482914	941276	29	-0.449801034
CP10	chr16	305046	1516494	1211448	37	-0.336998108
CP17	chr17	32213987	32219633	5646	5	-0.689308
CP54	chr17	31889386	31895571	6185	7	0.329082857
CP25	chr17	31889386	31895571	6185	7	0.831522857
Cp16	chr17	32830185	32841713	11528	5	-1.397976
CP18	chr17	32830185	32841713	11528	5	-0.538826
CP11	chr17	34132273	34145880	13607	5	-0.621624
CP33	chr17	32828887	32848639	19752	7	-1.001628571

Sample	chrom	start	end	size	Probe count	meanSig
CP37	chr17	36684803	36707624	22821	4	-0.6407925
CP30	chr17	31470033	31495548	25515	12	0.603909167
CP53	chr17	17943845	17971232	27387	20	0.3724965
CP25	chr17	17943153	17971232	28079	21	0.929214286
CP09	chr17	18076262	18104562	28300	15	-0.52709
CP53	chr17	56819625	56849621	29996	22	0.359757727
CP02	chr17	18075378	18106269	30891	17	-0.587548235
CP08	chr17	18075378	18106269	30891	17	-0.549345882
CP54	chr17	31474852	31505846	30994	13	0.49085
Cp16	chr17	31470033	31503916	33883	14	0.456693571
Cp22	chr17	34139134	34174848	35714	11	-0.442146364
CP07	chr17	31470033	31505846	35813	15	0.39365
CP57	chr17	31470033	31505846	35813	15	0.568242
CP25	chr17	31470033	31505846	35813	15	1.045409333
CP32	chr17	18068366	18108918	40552	20	0.381294
CP06	chr17	32830185	32872197	42012	8	-0.74529625
CP04	chr17	31461568	31503916	42348	16	0.437695625
CP05	chr17	31462054	31505846	43792	16	0.38906375
CP27	chr17	31462054	31505846	43792	16	0.468219375
CP32	chr17	31462054	31505846	43792	16	0.876378125
CP24	chr17	31461568	31505846	44278	17	0.375607647
CP40	chr17	31461568	31505846	44278	17	0.384715294
CP36	chr17	31461568	31505846	44278	17	0.466186471
CP08	chr17	31461568	31505846	44278	17	0.531771176
CP21	chr17	31461568	31505846	44278	17	0.550653529
CP29	chr17	31461568	31505846	44278	17	0.634314706
CP59	chr17	31461568	31505846	44278	17	0.675723529
CP02	chr17	18803403	18860424	57021	35	-0.491952857
CP57	chr17	32551654	32612535	60881	48	0.563077708
CP58	chr17	32551654	32619477	67823	52	0.473805769
CP17	chr17	32549625	32619051	69426	51	0.393198431
CP59	chr17	32549625	32619477	69852	53	0.57075
CP54	chr17	32549625	32619477	69852	53	0.596011509
CP32	chr17	32549625	32619477	69852	53	0.625499623
CP27	chr17	32549625	32619477	69852	53	0.644467925
CP13	chr17	32547987	32619350	71363	53	0.638313774
CP41	chr17	32547987	32619477	71490	54	0.518184815
CP40	chr17	32547987	32619477	71490	54	0.55978463
CP06	chr17	32547987	32619477	71490	54	0.573132778
CP39	chr17	32547987	32619477	71490	54	0.574082222
CP52	chr17	32547987	32619477	71490	54	0.575401481
CP42	chr17	32547987	32619477	71490	54	0.57911537
CP28	chr17	32547987	32619477	71490	54	0.604299074
CP56	chr17	32547987	32619477	71490	54	0.624323148

Sample	chrom	start	end	size	Probe count	meanSig
CP53	chr17	32547987	32619477	71490	54	0.629585556
CP23	chr17	32547987	32619477	71490	54	0.629777963
CP55	chr17	32547987	32619477	71490	54	0.629902778
CP36	chr17	32547987	32619477	71490	54	0.634818333
CP35	chr17	32547987	32619477	71490	54	0.637996296
CP31	chr17	32547987	32619477	71490	54	0.642334815
CP18	chr17	32547987	32619477	71490	54	0.644764444
CP20	chr17	32547987	32619477	71490	54	0.666685
CP24	chr17	32547987	32619477	71490	54	0.670056852
CP30	chr17	32547987	32619477	71490	54	0.674478148
CP33	chr17	32547987	32619477	71490	54	0.68397037
CP04	chr17	32547987	32619477	71490	54	0.714708704
CP07	chr17	32547987	32619477	71490	54	0.716383519
CP21	chr17	32547987	32619477	71490	54	0.723974259
Cp16	chr17	32547987	32622705	74718	56	0.618947857
CP12	chr17	32547987	32622705	74718	56	0.688385714
CP38	chr17	32544441	32619477	75036	55	0.535056727
CP37	chr17	32544441	32619477	75036	55	0.601989091
CP03	chr17	32544441	32619477	75036	55	0.611419455
CP01	chr17	32544441	32619477	75036	55	0.677108909
CP05	chr17	32544441	32619477	75036	55	0.701513636
CP10	chr17	32544441	32619477	75036	55	0.717560364
CP09	chr17	32544441	32619477	75036	55	0.762738364
CP14	chr17	32544376	32619477	75101	56	0.604685536
CP26	chr17	32547987	32623105	75118	57	0.61352614
CP19	chr17	32544441	32622175	77734	56	0.587299643
CP02	chr17	32544441	32622705	78264	57	0.642289123
CP56	chr17	17943153	18021621	78468	50	0.4433656
CP58	chr17	17943153	18021621	78468	50	0.4821294
CP55	chr17	17943153	18021621	78468	50	0.5341566
CP54	chr17	17943153	18023282	80129	53	0.327523019
Cp22	chr17	32547987	32628264	80277	59	0.619046949
CP15	chr17	32547987	32628264	80277	59	0.647461017
CP08	chr17	32547987	32628264	80277	59	0.708161525
CP11	chr17	32544441	32628264	83823	60	0.469426333
CP29	chr17	32544441	32628264	83823	60	0.585678167
CP24	chr17	41533854	41648626	114772	8	-0.55137375
CP14	chr17	41533854	41648626	114772	8	-0.4343325
CP36	chr17	41517002	41648626	131624	9	-0.50385
Cp16	chr17	41517002	41648626	131624	9	-0.456983333
CP13	chr17	75245393	75379962	134569	5	0.420842
CP42	chr17	41533854	41692616	158762	9	-0.441648889
CP39	chr17	41533854	41692616	158762	9	-0.363063333
CP04	chr17	41533854	41692616	158762	9	-0.358903333

Sample	chrom	start	end	size	Probe count	meanSig
CP59	chr17	41533854	41692616	158762	9	-0.353092222
CP32	chr17	41533854	41692616	158762	9	-0.352003333
CP33	chr17	41533854	41692616	158762	9	-0.35037
CP36	chr17	21255581	21456756	201175	45	0.393720667
CP36	chr17	1658656	1860085	201429	7	-0.488907143
CP13	chr17	74718121	74953266	235145	8	0.47456875
CP36	chr17	50802718	51175608	372890	12	-0.346041667
CP29	chr18	11998792	12004613	5821	8	-0.46824875
CP55	chr18	12018360	12026473	8113	9	0.561552222
CP02	chr18	11998792	12022943	24151	13	-0.502597692
CP26	chr18	11998792	12026197	27405	16	-0.39056875
CP24	chr18	22490922	22591419	100497	4	-1.0097575
CP07	chr18	45849713	45950607	100894	4	1.0682175
CP11	chr19	53856151	53856655	504	6	-0.938613333
CP14	chr19	53856151	53856655	504	6	-0.659596667
CP17	chr19	53856151	53856655	504	6	-0.613333333
CP13	chr19	53856151	53856655	504	6	-0.56065
CP09	chr19	53856151	53858542	2391	7	-0.993495714
CP10	chr19	53856151	53858542	2391	7	-0.985531429
Cp22	chr19	53856151	53858542	2391	7	-0.838137143
CP04	chr19	53856151	53858542	2391	7	-0.784645714
CP03	chr19	53856151	53858542	2391	7	-0.769025714
CP21	chr19	53856151	53858542	2391	7	-0.731552857
CP15	chr19	53856151	53858542	2391	7	-0.726898571
Cp16	chr19	53856151	53858542	2391	7	-0.717384286
CP18	chr19	53856151	53858542	2391	7	-0.699957143
CP26	chr19	53856151	53858542	2391	7	-0.6891
CP59	chr19	53224546	53231067	6521	7	-0.35905
CP24	chr19	53856151	53870208	14057	8	-0.73861625
CP19	chr19	53856151	53870298	14147	9	-0.676757778
CP23	chr19	53856151	53876392	20241	10	-0.647741
CP05	chr19	53830829	53858542	27713	11	-0.678133636
CP08	chr19	55061838	55094225	32387	27	-0.516043704
CP12	chr19	53856151	53889686	33535	11	-0.777710909
CP02	chr19	55053686	55094066	40380	30	-0.572524
CP09	chr19	55061863	55105250	43387	35	-0.467779714
CP07	chr19	53829384	53889686	60302	16	-0.4194475
CP02	chr19	53798876	53889686	90810	37	-0.488126216
CP12	chr19	24279378	24386625	107247	4	0.3476875
CP02	chr19	24279378	24386625	107247	4	0.3868525
CP05	chr19	35799733	36001250	201517	7	0.575097143
CP01	chr19	32495113	33041987	546874	17	0.349518824
CP02	chr19	32423668	32971847	548179	17	0.318821765
CP12	chr19	32423668	33041987	618319	19	0.349622632

Sample	chrom	start	end	size	Probe count	meanSig
Cp22	chr20	62280170	62418379	138209	5	-0.582428
CP01	chr20	62213395	62418379	204984	7	-0.624497143
CP28	chr20	62213395	62418379	204984	7	0.640028571
CP24	chr20	62045208	62418379	373171	12	-0.432534167
CP10	chr20	61675582	62418379	742797	23	-0.362784783
CP09	chr20	61171503	62418379	1246876	38	-0.364624474
CP08	chr20	61137963	62418379	1280416	39	-0.370177949
CP29	chr22	22676398	22714114	37716	4	-1.718255
CP32	chr22	22676398	22714114	37716	4	-1.56422
CP52	chr22	22676398	22714114	37716	4	-1.4907625
CP36	chr22	18087632	18135835	48203	15	0.339803333
CP52	chr22	18087632	18135835	48203	15	0.377514667
CP55	chr22	18087632	18135835	48203	15	0.690384
CP54	chr22	21741145	21792636	51491	15	0.438767333
CP39	chr22	21741961	21797711	55750	16	0.35935625
CP07	chr22	21496024	21554231	58207	9	-0.570406667
CP53	chr22	18074502	18135835	61333	17	0.448166471
CP54	chr22	18074502	18135835	61333	17	0.465461765
CP33	chr22	22644038	22714114	70076	5	-1.428294
CP03	chr22	21469032	21562882	93850	18	-0.470926667
CP41	chr22	21469032	21562882	93850	18	-0.353298333
CP20	chr22	21442180	21562882	120702	20	-0.355935
CP54	chr22	21435919	21562882	126963	21	-0.317107143
CP05	chr22	17258338	17387467	129129	15	-0.706246
CP13	chr22	21392127	21562882	170755	26	-0.301096154
CP11	chr22	21387369	21559293	171924	25	-0.3970712
CP53	chr22	21382020	21562882	180862	29	-0.385193103
CP02	chr22	21361539	21562882	201343	32	-0.664810313
CP17	chr22	49338574	49542684	204110	7	-0.429032857
CP04	chr22	21356425	21562882	206457	34	-0.343902647
CP29	chr22	21290391	21562882	272491	40	-0.38133875
CP26	chr22	21273457	21562882	289425	44	-0.439543636
CP09	chr22	21199673	21562882	363209	60	-0.516364333
CP15	chr22	21132033	21559293	427260	66	-0.305976818
Cp16	chr22	21090152	21562882	472730	75	-0.315506133
CP19	chr22	21081688	21562882	481194	77	-0.409044286
CP54	chrX	148736125	148738705	2580	13	0.415263077
CP25	chrX	48443941	48453189	9248	9	0.640544444
CP08	chrX	48438558	48453189	14631	16	-0.303114375
CP25	chrX	48453954	48469130	15176	16	-0.5607325
CP09	chrX	48700018	48717368	17350	13	-0.354741538
CP12	chrX	48453954	48473718	19764	20	1.184961
CP02	chrX	48453954	48475457	21503	21	1.092990952
CP24	chrX	48453954	48475457	21503	21	1.096327143

Sample	chrom	start	end	size	Probe count	meanSig
CP55	chrX	48230540	48258814	28274	16	1.165255625
CP56	chrX	48230540	48263781	33241	17	0.399337059
CP35	chrX	48230859	48284433	53574	22	0.941313636
CP13	chrX	48230859	48284433	53574	22	0.972416818
CP57	chrX	48859196	48957261	98065	24	0.36348375
CP39	chrX	38378381	38512481	134100	5	0.658906
CP06	chrX	7611459	7780154	168695	6	-0.488873333
CP12	chrX	7174799	8048471	873672	27	1.185137778
CP04	chrX	88358618	89731225	1372607	38	0.334424211
CP07	chrX	88358618	92293681	3935063	109	0.323086422
CP09	chrY	22203134	22207101	3967	7	0.668847143
CP32	chrY	8368376	8468047	99671	61	-1.121885246
CP14	chrY	12077282	12179540	102258	4	-0.49793
CP28	chrY	10523737	10694396	170659	6	-0.620043333
CP26	chrY	10523737	10694396	170659	6	-0.459761667
CP02	chrY	10523737	10694396	170659	6	-0.366875
CP20	chrY	10523737	10694396	170659	6	-0.3597
CP07	chrY	10523737	10694396	170659	6	-0.332605
CP01	chrY	10523737	10694396	170659	6	-0.326228333
CP24	chrY	10523737	10694396	170659	6	-0.304043333
CP28	chrY	12308679	12479342	170663	6	-0.329543333
CP02	chrY	26867996	27208624	340628	11	-0.386198182
CP12	chrY	11654367	12179540	525173	16	-0.401074375
CP04	chrY	11654367	12179540	525173	16	-0.34246625
CP07	chrY	11654367	12179540	525173	16	-0.317310625
CP35	chrY	2982792	4836353	1853561	51	-0.429156078
CP23	chrY	2982792	4836353	1853561	51	-0.333571373
CP04	chrY	2844077	4836353	1992276	55	-0.318033455
CP28	chrY	2709824	4836353	2126529	59	-0.320657288
CP01	chrY	2709824	4836353	2126529	59	-0.310696271
CP12	chrY	2709824	4836353	2126529	59	-0.305310169
CP36	chrY	2709824	4836353	2126529	59	-0.304298644