

**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 text.

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Gai Lisette McMichael

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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 metalloproteinase 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 (adrenocorticotrophic 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 – Autism

ASD – Autism Spectrum Disorders

Ep – Epilepsy

ID – Intellectual Disability

JS – Joubert 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://projects.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](http://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.