Genetics and Genomics of Cerebral Palsy

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Learning Objectives

To understand/gain insight into:

- The type of genetic aberrations underlying cerebral palsy and of the mode(s) of inheritance.
- The reported yield of genetic investigations (including chromosomal micro-array analysis, exome sequencing) in cerebral palsy.
- The difference in yield of genetic testing between typical and atypical cerebral palsy patients.
- The importance of establishing an underlying diagnosis in patients with cerebral palsy.

Highlights

- At least 4% of patients with cerebral palsy have disease-causing copy number variants, and at least 14% have disease-causing single nucleotide variants or indels.
- In patients in whom cerebral palsy-like neuromotor dysfunction occurs with additional malformations or neurodevelopmental abnormalities, the rate of disease-causing genomic lesions is more than twice as high.
- Aberrations of many different genetic loci can produce a cerebral palsy-like phenotype.
- Most, but not all, gene or chromosomal mutations that cause cerebral palsy occur de novo.
- Recognizing the cause of cerebral palsy in an affected patient is essential to providing optimal clinical management, including precision therapy.
- Genome-wide (exome or genome) sequencing is indicated in the initial work-up of patients with cerebral palsy, especially those who have additional neurodevelopmental abnormalities or malformations.

Introduction

Cerebral palsy (CP) is not a homogeneous disease entity but rather an etiologically diverse group of conditions characterized by abnormal movement or posture with onset early in development [1–3]. It has been known for more than 50 years that some patients with clinical features of cerebral palsy have a genetic syndrome or inherited metabolic disorder [4, 5], but for a long time such cases were considered to be highly exceptional. We now know that they are not — it has become apparent in the past decade that many patients with developmental abnormalities of motor function have an underlying genetic disease of major effect, such as a Mendelian disorder or chromosomal abnormality.

The structural and/or functional central nervous system abnormalities that underlie CP may have their origin at conception, during embryonic or foetal development, during the perinatal period, or in early childhood. A major genetic cause is most likely when the condition has obvious prenatal onset, but the clinical features of CP may not become manifest until later in life in other instances. Non-genetic factors, such as teratogenic exposures, hypoxia, hemorrhage or infections, may also cause CP, and in some other patients the cause is a

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combination of non-genetic and genetic factors. 'Genetics' is definitely plural when referring to CP.

Patients with CP are often classified clinically into spastic, hypotonic, dystonic (also called 'dyskinetic'), ataxic, and mixed subgroups and by the limbs involved (diplegia, hemiplegia or quadriplegia, and occasionally other patterns) [1–5]. Each of these clinical subgroups and patterns of involvement is also etiologically and genetically heterogeneous, and while certain major genetic forms of CP characteristically produce only one particular kind of involvement, the clinical presentation of other genetic forms of CP is variable [1, 6].

Clinical definitions of CP require that the condition be non-progressive, and developmental abnormalities of movement or posture that become worse with time are sometimes called 'atypical CP' or 'cerebral palsy mimics' [6, 7]. Distinguishing progressive from non-progressive neuromotor abnormalities is important for clinical management but may present difficulties in genetic analysis of these conditions for several reasons [8]. Firstly, disease progression occurs over time and may not be apparent when a child is initially evaluated. Secondly, the rate of progression may be very slow, and the functional loss may not become apparent until later in life. Thirdly, some patients are very severely involved from birth, and it may not be possible to recognize disease progression clinically. Fourthly, disease progression may not affect motor function but become apparent in other ways, such as intractability of seizures to treatment, loss of vision or speech, or cognitive decline. In addition, genetic diseases that can cause CP are often quite variable in their manifestations and course from patient to patient, so that disease progression may be obvious in some individuals but not in others with the same condition. Finally, specific treatment is available for some diseases that may present as CP [9], and the treatment may prevent progression of the neuromotor symptoms.

In this chapter, we consider the genetics and genomics of both non-progressive and progressive neurodevelopmental movement disorders because almost all reported studies include some patients who have typical CP and others who are atypical or may become so later in their course. The information is organized by study design: twin and other family studies (without genetic testing), association studies, studies of chromosomal abnormalities or genomic copy number variants, studies of Mendelian diseases caused by single nucleotide variants or indels, and epigenetic studies. This organization also generally reflects the time when the studies were done, with genome-wide sequencing and epigenetic studies being most recent, and the others, earlier.

Twin and Other Family Studies

Hundreds of studies have been published that include twins with CP, but such studies are difficult to interpret with respect to genetic causation because being born of a twin pregnancy is itself strongly associated with the occurrence of CP. Luu and Vohr [10] and Pharoah and Dundar [11] summarized data from CP registry studies and found a substantially greater frequency of CP in twins than in birth registries for the same jurisdictions. CP was reported in 6.3–12.6 per 1000 twins who survived infancy in comparison to 1.0–2.3 per 1000 surviving singleton infants. A population-based study performed through the Medical Birth Registry of Norway found 3649 children who developed CP and 22,558 pairs of twins among 2,036,741 infants born between 1967 and 2002. [12] The prevalence of CP was three times greater among the twins (5.1 per 1000) than among singleton births (1.7 per 1000). After reviewing such data, Briana and Malamitsi-Puchner [13] emphasized the importance of low birth weight and premature delivery, which frequently occur in twin pregnancies, in mediating the development of CP.

Twin studies have been used for almost 150 years to infer genetic causation of familial traits based on recognition that monozygotic twins share all of their genes in common, while dizygotic twins resemble ordinary sibs, sharing about half of their genes [14]. The studies discussed in the previous paragraph comparing the rate of CP in twins to that in singleton pregnancies or the general population ignore zygosity and thus cannot be used to assess the importance of genetic factors in the occurrence of CP.

A study of the population-based Western Australia CP Registry identified 74 sets of twins born between 1956 and 1985 in which one or both members of the pair had CP. [15] The rate of concordance for CP in monozygotic twins was significantly higher than that in dizygotic twins ($p = 0.0026$). In contrast, concordance for CP was observed in 4 (20%) of 20 monozygotic twin pairs and 10 (40%) of 25 dizygotic twin pairs in a series collected by a single physician over a 21-year period. [16] The fact that concordance was not complete among monozygotic twins is consistent with the known etiological heterogeneity of CP and formally proves that all cases are *not* caused by major genetic factors.

Very few studies of CP in twins have confirmed zygosity by genetic testing, but monochorionic placentation is strongly associated with the occurrence of CP in twins. [17, 18] Almost all dizygotic pregnancies have dichorionic placentation, and most monozygotic pregnancies are monochorionic, but about 30% of monozygotic pregnancies are dichorionic [19]. Chorionicity is, therefore, an imprecise surrogate for zygosity. The proportion of twin pregnancies that is monozygotic, rather than dizygotic, varies greatly in different populations and has changed in the last few decades as a result of fertility treatments that increase the frequency of pregnancies with two or more genetically distinct foetuses.

The association of monochorionic placentation with CP may largely be attributable to the occurrence of placental vascular anastomoses between the circulatory systems of the twins [20, 21]. Monochorionic placentation is also associated with increased frequencies of intrauterine death of one

or both twins, preterm delivery, severe discordance in birth weight between the twins, foetal growth restriction, and congenital anomalies, all of which are also associated with the occurrence of CP [10, 11, 13].

No twin studies have been reported that assess the effect of genetic factors on the occurrence of CP in proven monozygotic versus dizygotic (or monochorionic vs. dichorionic) twins after removing the effects of placental vascular anastomosis, intrauterine death of one twin, preterm delivery, severe birth weight discordance, foetal growth restriction and other congenital anomalies.

Studies that have compared the frequency of CP in co-twins of unknown zygosity to the frequency of CP in sibs of children with CP born of singleton pregnancies have found higher rates of co-occurrence of CP in the twin sibs.

A Norwegian population-based record linkage study [12] found the prevalence of CP to be 79/1000 in the co-twins of children with CP, 15/1000 in the sibs of singleton children with CP, 8.5/1000 in the children of parents with CP, 2.6/1000 in the second-degree relatives of children with CP, and 2.5/1000 in the third degree relatives of children with CP. The prevalence of CP was 1.5/1000, 1.6/1000 and 1.6/1000, respectively in first-, second- and third-degree relatives of individuals *without* CP in this study. A subsequent publication expanded this investigation by adding four more years of data to include a total of 5707 children with CP and 26,485 twin pairs among 2,297,408 children who survived the neonatal period [22]. In the expanded study, the co-twins of children with CP had a 27-fold greater than expected risk of having CP, and the full sibs of children with CP born of singleton pregnancies had a 6.4-fold greater risk of having CP. The sibs of children with CP born of singleton pregnancies also had higher than expected risks of stillbirth, neonatal death, intellectual disability, autism spectrum disorder, deafness, blindness, epilepsy, attention deficit hyperactivity disorder and schizophrenia. All these risks were even higher among the co-twins of children with CP born of twin pregnancies.

A Swedish population-based record linkage study that included 3997 patients with CP found that the risk of hospitalization for CP was 4.8 times greater than expected among the sibs of individuals with CP born of singleton pregnancies and 29 times greater than expected among the co-twins of individuals with CP born of twin pregnancies. [23]

These data indicate that genetic factors are often important in the aetiology of CP. The studies are compatible with a multifactorial mechanism or with genetic heterogeneity, with some cases resulting from genetic variants of major effect and others having a non-genetic cause. A multifactorial mechanism in some cases, various major genetic factors in other cases, and non-genetic causation in still others seems most likely.

Candidate Gene Association Studies

Association studies are used to identify genetic loci that predispose to or protect against the development of a disease. They are usually based on an assumption that the disease is multifactorial, i.e., caused by a complex combination of many different minor genetic and non-genetic factors.

Association studies of at least 160 different genetic variants in at least 60 candidate genes have been reported in patients with CP and corresponding control groups. Table 35.1 lists the genes and variants (mostly SNPs) that have been assessed in these studies. Most of these genetic loci were chosen for study because of their known involvement in blood clotting, vascular regulation or inflammation, processes that are thought to be important in the pathogenesis of, or physiological response to, perinatal intracranial bleeding [24–26]. These studies vary in terms of how the CP was defined, how the patients were ascertained, and what populations the patients represented [26, 27]. Most of the studies are small: the largest candidate gene association study of CP reported to date includes 763 cases [27], but many have fewer than 100 cases.

Although associations with CP have been reported with polymorphic genetic variants near or within a dozen different genes [27–38], none of these associations has been replicated in an independent investigation. Many of the reported associations are inverse or 'protective,' meaning that patients with the more frequent allele in the population are at higher risk, a counterintuitive observation. Even more associations have been observed in *ad hoc* subgroups of CP patients, but none of these has been independently replicated, and such observations are suspect for statistical reasons [24–26].

The inability to replicate candidate gene association studies is a common observation in complex diseases [39, 40]. Independent replication is essential because candidate disease association studies are often confounded by issues related to disease definition, patient ascertainment, population stratification, publication bias and statistical analysis. More recent genetic association studies of many complex diseases address these problems through genome-wide testing of tens of thousands or more SNPs in homogenous groups of thousands to tens of thousands of patients. We are not aware of any published genome-wide association studies of patients with CP [41], and it seems unlikely that this approach would be informative unless the known aetiological and pathogenetic heterogeneity of CP were taken into account in patient selection and data analysis.

Table 35.1 Loci that have been studied in CP candidate gene association studies [24, 27–31, 33–38, 81–88]

Gene	Locus	SNP	Location	Comment
Loci associated with blood clotting				
Annexin A5	*ANXA5*	rs1257049725	5′ UTR	
Factor II	*F2*	rs1799963	3′ UTR	Also known as *F2* (G20210A)
Factor V	*F5*	rs6025	Exon (missense)	Also known as factor V Leiden or *F5* (G1691A)
Factor VII	*F7*	rs6046	Exon (missense)	
		rs5742910	Upstream	
Fibrinogen beta chain	*FGB*	rs4220	Exon (missense)	
		rs1800790	Upstream	
Integrin subunit alpha 2	*ITGA2*	rs1062535	Exon (synonymous)	Also known as *ITGA2* (873G/ A)
Integrin subunit beta 3	*ITGB3*	rs5918	Exon (missense)	Also known as ITGB3 (leu33pro)
Methylenetetrahydrofolate reductase	*MTHFR*	rs1801133	Exon (missense)	Also known as *MTHFR* (C677T)
		rs1801131	Exon (missense)	Also known as *MTHFR* (A1298C)
		rs4846049	3′ UTR	
		rs1476413	Intron	
		rs9651118	Intron	
Plasminogen activator, tissue type	*PLAT*	rs2020918	Upstream	
Protein C receptor	*PROCR*	rs867186	Exon (missense)	Gene also known as *EPCR*
Serpin family B member 2	*SERPINB2*	rs6098	Exon (missense)	Also known as PAI_2–1
		rs6103	Exon (missense)	Also known as PAI_2–2
		rs6104	Exon (missense)	Also known as PAI2
Serpin family E member 1	*SERPINE1*	rs7242	3’ UTR	Also known as PAI1
		rs1799768	Upstream	Also known as PAI1
Thrombomodulin	*THBD*	rs1800576	Exon (missense)	
Tissue factor pathway inhibitor	*TFPI*	rs1189623	Intron	
Loci associated with inflammation				
Arachidonate 5-lipoxygenase activating protein	*ALOX5AP*	rs9551963	Intron	Also known as SG13S32
		rs17222842	Downstream	Also known as SG13S35
		rs4769874	Intron	
C-C motif chemokine ligand 18	*CCL18*	rs1102934	Upstream	
		rs2015086	Upstream	
		rs2015070	Intron	
		rs2735835	Intron	
		rs712044	Intron	
C-reactive protein	*CRP*	rs1205	3′ UTR	
C-X-C motif chemokine ligand 8	*CXCL8*	rs4073	Upstream	Gene also known as *IL-8*
Complement C3d receptor 2	*CR2*	rs3813946	5′ UTR	
		rs1048971	Exon (synonymous)	
		rs17615	Exon (missense)	
Complement factor H	*CFH*	rs1061170	Exon (missense)	
Intercellular adhesion molecule 1	*ICAM1*	rs1799969	Exon (missense)	
Interleukin 1 beta	*IL1B*	rs16944	Upstream	Also known as *IL1B* -511C/T
Interleukin 1 receptor antagonist	*IL1RN*	IL1RN, IVS2, 86-BP DUP	Intron	VNTR
Interleukin 10	*IL10*	rs1554286	Intron	SNP also in *IL19* 5’UTR
		rs1518111		
		rs3024490		
Interleukin 13	*IL13*	rs20541	Exon (missense)	
Interleukin 19	*IL19*	rs1800872	Intron	SNP also in *IL10*
		rs1800896	Intron	SNP also in *IL10*
		rs1800871	Intron	Also known as IL-10 -819

Table 35.1 (continued)

Gene	Locus	SNP	Location	Comment
Interleukin 1 beta	*IL1B*	rs1143623	Upstream	
		rs1143634	Exon (synonymous)	Also known as *IL1B* 3954
		rs4848306	Upstream	
Interleukin 4	*IL4*	rs2243250	Upstream	Also known as *IL4* -589C/T
Interleukin 6	*IL6*	rs1800795	Intron	Also known as IL-6 -174
		rs1554606	Intron	
		rs1800796	Intron	
		rs1800797	Intron	
		rs1880243	Upstream	Also known as IL-6 -7227
		rs2066992	Intron	
		rs10242595	Downstream	
		rs2069837	Intron	
		rs2069840	Intron	
		rs11766273	Downstream	
		rs12700386	Upstream	
Interleukin 6 receptor	*IL6R*	rs952146	Upstream	
		rs4075015	Intron	
		rs4537545	Intron	
		rs4601580	Intron	
		rs4845374	Intron	
		rs4845618	Intron	
		rs4845625	Intron	
		rs6687726	Intron	
		rs7549338	Intron	
Lymphotoxin alpha	*LTA*	rs1041981	Exon (missense)	
Mannose binding lectin 2	*MBL2*	rs5030737	Exon (missense)	Also known as MBL-52
		rs1800450	Exon (missense)	Also known as MBL-54
		rs1800451	Exon (missense)	Also known as MBL-57
		rs7096206	Intron	Also known as MBL −221
		rs7095891	Intron	Also known as MBL +4 C > T (P/Q)
		rs11003123	Intron	
		rs11003125	Intron	Also known as MBL −550
Secreted phosphoprotein 1	*SPP1*	rs2853744	Upstream	Gene also known as osteopontin (*OPN*)
		rs2853749	Intron	Gene also known as osteopontin (*OPN*)
		rs11728697	Exon (missense)	Gene also known as osteopontin (*OPN*)
		rs4754	Exon (missense)	Gene also known as osteopontin (OPN)
		rs1126616	Exon (synonymous)	Gene also known as osteopontin (*OPN*)
Selectin E	*SELE*	rs5361	Exon (missense)	Also known as *SELE* (ser128arg)
		rs5355	Exon (missense)	Also known as *SELE* (leu554phe)
Toll-like receptor 1	*TLR1*	rs5743551	5′ UTR	
Toll-like receptor 2	*TLR2*	rs4696480	Intron	
		rs5743708	Exon (missense)	
Toll-like receptor 4	*TLR4*	rs4986790	Exon (missense)	Also known as *TLR-4* (asp299gly)
		rs4986791	Exon (missense)	
Transforming growth factor beta 1	*TGFB1*	rs1800470	Exon (missense)	
		rs1800469	Upstream	Variant is 2 kb upstream of *TGFB1* and 500 bp downstream of *B9D2*
Tumour necrosis factor	*TNF*	rs1800629	Upstream	Also known as TNF-alpha −308 (G308A)
		rs1800610	Intron	Gene also known as TNF-alpha
		rs361525	Upstream	Also known as TNF-alpha-238
		rs1799964	Upstream	Also known as TNF-alpha-1031 T/C; SNP is also downstream of *LTA*
		rs1799724	Upstream	Also known as TNF-alpha-857 C/T; SNP is also downstream of *LTA*

(continued)

Table 35.1 (continued)

Gene	Locus	SNP	Location	Comment
Loci associated with vascular regulation				
Adrenoceptor beta	*ADRB2*	rs1042714	Exon (stop gain)	Also known as *ADRB2* (gln27glu)
		rs1042713	Exon (missense)	Also known as *ADRB2* (arg16gly)
		rs1042717	Exon (synonymous)	
Angiotensin	*AGT*	rs699	Exon (missense)	Also known as *AGT* (met235thr)
Angiotensin II receptor type 1	*AGTR1*	rs5186	3′ UTR	Also known as *AGTR1* (1166A/ C)
Natriuretic peptide A	*NPPA*	rs5063	Exon (missense)	Also known as *NPPA* (664G/A)
		rs5065	Exon (stop loss)	Also known as *NPPA* (2238 T/C)
Neuropeptide Y	*NPY*	rs16135	Intron	Also *LOC10798677* intron variant
		rs16476	Intron	Also *LOC10798677* intron variant
Nitric oxide synthase 1	*NOS1*	rs3782219	Intron	
		rs2293054	Exon (missense)	
		rs10774909	Intron	
		rs3741475	Exon (synonymous)	
		rs2682826	3′ UTR	
Nitric oxide synthase 2	*NOS2*	rs1137933	Exon (synonymous)	Gene also known as iNOS
		(CCTTT)n micro satellite	2.5 kb upstream	
Nitric oxide synthase 3	*NOS3*	rs1800779	Intron	Gene also known as eNOS
		rs3918226	Intron	Gene also known as eNOS
		rs1799983	Exon (missense)	Gene also known as eNOS
Sodium channel epithelial 1 subunit alpha	*SCNN1A*	rs5742912	Exon (missense)	Also known as *SCNN1A* (trp493arg)
		rs2228576	Exon (missense)	Also known as *SCNN1A* (ala663thr)
Other loci				
Adducin 1	*ADD1*	rs4961	Exon (missense)	
Apolipoprotein E	*APOE*	rs429358	Exon (missense)	
		rs7412	Exon (missense)	
		rs769446	Upstream	
		rs405509	Upstream	
		rs121918399	Exon (missense)	
		rs429358	Exon (missense)	
		rs190853081	Exon (missense)	
		ε2	Exon (missense)	Variant includes rs429358(T) and rs7412(t)
		ε3		Variant includes rs429358(T) and rs7412(C) (major alleles at both loci)
		ε4	Exon (missense)	Variant includes rs429358(c) and rs7412(C)
Autophagy related 5	*ATG5*	rs510432	Upstream	
		rs3804338	Intron	
		rs573775	Intron	
		rs2299863	Intron	
		rs6568431	Downstream	SNP also downstream of *PRDM1*
Autophagy related 7	*ATG7*	rs346078	Intron	
		rs1470612	Intron	
		rs11706903	Intron	
		rs2606750	Intron	
		rs2594972	Intron	
		rs4684787	Intron	
Collagen type IV alpha 1 chain	*COL4A1*	rs10492497	Intron	
		rs1961495	Intron	
		rs562992	Intron	
		rs1411040	Intron	
Collagen type IV alpha 2 chain	*COL4A2*	rs4773144	Intron	
		rs3809346	Intron	

Table 35.1 (continued)

Gene	Locus	SNP	Location	Comment
Cystathionine beta-synthase	*CBS*	rs5742905	Exon (missense)	
G protein subunit beta 3	*GNB3*	rs5443	Exon (synonymous)	Also known as *GNB3* (825C / T)
Glutamate decarboxylase 1	*GAD1*	rs379187	Intron	
		rs3791862	Intron	
		rs16858977	Intron	
Matrix metallopeptidase 2	*MMP2*	rs243865	Upstream	
Matrix metallopeptidase 3	*MMP3*	rs602128	Exon (missense)	
		rs3025058	Upstream	Also known as *MMP3* −1171 (5A/6A) (1 bp indel)
Oligodendrocyte transcription factor 2	*OLIG2*	rs6517135	Upstream	
		rs1005573	Intron	
		rs6517137	3′ UTR	
		rs9653711	Downstream	
Phosphodiesterase 4D	*PDE4D*	rs12188950	Intron	

Studies of Chromosomal Abnormalities and Genomic Copy Number Variants

Major genetic factors are those that are both necessary and sufficient to cause a particular disease in a patient. Clinically, major genetic causes of disease include inherited or de novo Mendelian disorders and chromosomal abnormalities. At a molecular level, the changes that cause genetic disease of major effect are alterations of nucleotide sequence, genomic copy number or genomic structure, alone or in combination. Most mutations that cause inherited or de novo Mendelian diseases are alterations of nucleotide sequence, usually single nucleotide variants. Alterations of genomic copy number or structure are conventionally called 'chromosomal abnormalities' because microscopic (cytogenetic) analysis has been used to identify them for more than 60 years. However, most disease-causing genomic alterations are too small to be visualized under the light microscope and require molecular techniques such as chromosomal microarray analysis or genome sequencing for detection.

Anecdotal reports of patients with CP and various chromosomal abnormalities have occasionally appeared in the medical literature [42–45], and a few patients with segmental gain or loss of genomic material large enough to be seen cytogenetically and a 'cerebral palsy' phenotype are reported in the DECIPHER or ClinVar databases (Table 35.2). However, we are not aware of any study describing the results of routine cytogenetic testing in a large series of patients with CP.

In a study of data from eleven European CP registries, 13 (0.3%) of 4584 children with CP born between 1976 and 1996 were reported to have chromosomal abnormalities detected by cytogenetic analysis [46]. This must be a minimal estimate because the techniques available for identifying genomic imbalance were much less sensitive at that time than they are today and because cytogenetic analysis was infrequently done on children with CP, which was usually assumed to have been caused by perinatal anoxia or intracranial bleeding.

Segmental gains or losses of genomic material are usually called 'deletions' or 'duplications' if they can be demonstrated under the microscope and 'copy number variants' (CNVs) if they are smaller (generally <10 Mb) and require the use of molecular techniques, such as chromosomal microarray analysis (CMA) or exome sequencing, to be detected. Much smaller (1–50 bp) genomic gains or losses that can only be identified by sequencing are called 'indels'.

Variability is a normal feature of the human genome. The nucleotides of two unrelated people differ by about 1% of their total nucleotide sequence or content and by more than 20,000 CNVs, on average [47]. Most of these variants occur as polymorphisms in the general population and are inherited from one parent or the other, and most are thought to be unrelated to the occurrence of CP or any other disease. A small fraction of the genomic variants in each of us arise de novo as a result of new mutations.

There are two critical steps in identifying disease-causing CNVs in patients with CP. The first is recognition of the genomic variant, which is usually done by CMA or DNA sequencing, and the second is determining that the variant is, in fact, capable of causing disease. Rare CNVs that are both necessary and sufficient to cause a genetic disease are classified as 'pathogenic' or 'likely pathogenic' according to standard laboratory criteria [48]. Most CNVs that are unrelated to the occurrence of a genetic disease can be classified as 'benign' or 'likely benign'. We are unable to determine whether some other CNVs have an effect on the phenotype – such variants are classified as being of 'uncertain significance'.

A few individual patients with CP and other neurodevelopmental abnormalities who were found to have apparently

Table 35.2 CNVs reported in ClinVar or DECIPHER in patients whose phenotype includes CP

Identifier	Number	Description	Location	Interpretation	Clinical features	Comments
ClinVar variation ID	154737	3.4 Mb copy number gain	1q21.1–21.2	Pathogenic	Autism, delayed speech and language development, pituitary dwarfism, hyperpigmentation of the skin, muscular hypotonia, global developmental delay, failure to thrive, morphological abnormality of the central nervous system, delayed gross motor development, short stature, attention deficit hyperactivity disorder, delayed fine motor development, cerebral palsy, behavioural abnormality	
ClinVar variation ID	144454	4.9 Mb copy number gain	2p25.3–25.2	Pathogenic	Cerebral palsy	
ClinVar variation ID	154597	7.5 Mb copy number loss	2q23.3–24.2	Pathogenic	Failure to thrive, cerebral palsy	
DECIPHER patient	283420	751.6 kb copy number loss	4p13	Pathogenic	Cerebral palsy, congenital hypothyroidism, hemiplegia, intrauterine growth retardation	
DECIPHER patient	283426	446.7 kb copy number gain	5p15.2	Pathogenic	Cerebral palsy, congenital hypothyroidism, hemiplegia, intrauterine growth retardation	
ClinVar variation ID	442344	9.9 Mb copy number gain (4 copy)	5q12.1–13.2	Likely pathogenic	Global developmental delay, hypertonia, abnormal heart morphology, abnormal facial shape, cerebral palsy	
DECIPHER patient	283424*	136 bp copy number loss	5q21.1 (*SLCO6A1* gene)	Pathogenic	Anxiety, autism, cerebral palsy, global developmental delay, intellectual disability, mild, intracranial hemorrhage, periodontitis, tetraplegia	
ClinVar variation ID	443701	5.2 Mb copy number loss	6q14.1–14.3	Likely pathogenic	Triangular face, upslanted palpebral fissure, seizures, absent speech, abnormal facial shape, cerebral palsy	
ClinVar variation ID	154347	1.6 Mb copy number loss	7q11.23	Pathogenic	Cerebral palsy, hearing impairment, microcephaly	Copy number loss does not overlap region associated with NF1 microdeletion syndrome
DECIPHER patient	283422†	567.8 kb copy number gain	7q21.13	Pathogenic	Cerebral palsy, cerebral visual impairment, generalized myoclonic seizure, global developmental delay, spastic diplegia	
DECIPHER patient	388863	**11.8 Mb copy number gain**	**7q32.1-q35**	Likely pathogenic	Abnormal heart morphology, autistic behaviour, cerebral palsy, seizure	
DECIPHER patient	355383‡	273.3 kb copy number gain	7q34	Likely pathogenic	Athetoid cerebral palsy, delayed speech and language development, generalized hypotonia, global developmental delay, growth delay	
DECIPHER patient	355383‡	3.54 Mb copy number gain (4 copy)	7q34-7q35	Likely pathogenic	Athetoid cerebral palsy, delayed speech and language development, generalized hypotonia, global developmental delay, growth delay	

Table 35.2 (continued)

Identifier	Number	Description	Location	Interpretation	Clinical features	Comments
DECIPHER patient	283421[§]	219.4 kb copy number gain	8p23.1	Pathogenic	Cerebral palsy, hemiplegia, hypoplasia of the corpus callosum, periventricular leukomalacia, porencephalic cyst	
ClinVar variation ID	441537	4.7 Mb copy number loss	10p15.3–15.1	Pathogenic	Cerebral palsy	
DECIPHER patient	283429	234.3 kb copy number gain	10q26.13	Pathogenic	Cerebral palsy, global developmental delay, hemiplegia	
ClinVar variation ID	155548	6.8 Mb copy number loss	10q26.2–26.3	Pathogenic	Cerebral palsy	
ClinVar variation ID	443505	6.4 Mb copy number gain	10q26.2–26.3	Pathogenic	Cerebral palsy	
ClinVar variation ID	154432	**14.4 Mb copy number loss**	**11p15.1–13**	Pathogenic	Cerebral palsy, cataract, global developmental delay, hydrocephalus, aniridia, microcephaly	WAGR 13p13 deletion occupies 651 kb within this much larger deletion
DECIPHER patient	283422[†]	386.6 kb copy number loss	12p12.2	Pathogenic	Cerebral palsy, cerebral visual impairment, generalized myoclonic seizure, global developmental delay, spastic diplegia	
DECIPHER patient	283425	211.2 kb copy number gain	14q23.1	Pathogenic	Cerebral palsy, spastic diplegia	
DECIPHER patient	283421[§]	534.6 kb copy number gain	15q11.2	Pathogenic	Cerebral palsy, hemiplegia, hypoplasia of the corpus callosum, periventricular leukomalacia, porencephalic cyst	
DECIPHER patient	341043	467.9 kb copy number loss	15q11.2	Likely pathogenic	Bicuspid aortic valve, cerebral palsy, intellectual disability, moderate	
ClinVar variation ID	58,073	5.0 Mb copy number gain	15q11.2–13.1	Pathogenic	Seizure, cerebral palsy	
ClinVar variation ID	154724	7.2 Mb copy number gain (4 copy)	15q11.2–13.2	Pathogenic	Seizures, global developmental delay, abnormal heart morphology, cerebral palsy	
ClinVar variation ID	154725	1.9 Mb copy number gain	15q13.2–13.3	Pathogenic	Seizures, global developmental delay, abnormal heart morphology, cerebral palsy	
ClinVar variation ID	144213	3.4 Mb copy number gain	17p11.2	Pathogenic	Cerebral palsy	Duplication compatible with Potocki-Lupski syndrome. The usual features of this syndrome are mild developmental delay/ intellectual disability, autistic features, attention-deficit hyperactivity disorder, failure to thrive in early childhood, dysmorphic facial features and sometimes structural cardiovascular abnormalities
DECIPHER patient	283423	4.5 kb copy number loss	17p11.2 (*COPS3* gene)	Pathogenic	Cerebral palsy, delayed gross motor development, generalized myoclonic seizure, hemiplegia, periventricular leukomalacia, porencephalic cyst	
ClinVar variation ID	153240	1.4 Mb copy number gain	17p12	Pathogenic	Areflexia, autism, gastroesophageal reflux, aortic aneurysm, peroneal muscle atrophy, cerebral palsy	
ClinVar variation ID	59561	382.8 kb copy number loss	17p13.3	Pathogenic	Seizure, cerebral palsy	

(continued)

Table 35.2 (continued)

Identifier	Number	Description	Location	Interpretation	Clinical features	Comments
DECIPHER patient	283428	**68.7 Mb copy number loss**	**17p13.3-q25.1**	Pathogenic	Cerebral palsy, generalized myoclonic seizure, intellectual disability, moderate, polymicrogyria, tetraplegia	
ClinVar variation ID	443555	1.4 Mb copy number gain	17q12	Likely pathogenic	Muscular hypotonia, failure to thrive, respiratory failure, short stature, hypoxemia, cerebral palsy	
ClinVar variation ID	155320	483.7 kb copy number loss	17q21.31	Pathogenic	Autistic behaviour, intellectual disability, seizures, abnormality of the corpus callosum, cortical dysplasia, cerebral palsy	Deletion compatible with Koolen de Vries syndrome. The usual features of this syndrome include intellectual disability, hypotonia, seizures, structural brain abnormalities, and autistic behaviour. Other findings include dysmorphic facial features, cardiovascular malformations, renal anomalies and abnormalities of the skin and hair. Cerebral palsy not a recognized feature of this syndrome
DECIPHER patient	283427	612.3 kb copy number gain	17q25.3	Pathogenic	Autism, cerebral palsy, generalized myoclonic seizure, global developmental delay, hemiplegia, intellectual disability, mild	
DECIPHER patient	283424*	64.5 kb copy number gain	18p11.21	Pathogenic	Anxiety, autism, cerebral palsy, global developmental delay, intellectual disability, mild, intracranial haemorrhage, periodontitis, tetraplegia	
ClinVar variation ID	58724	145.0 kb copy number gain	18p11.32	Pathogenic	Autism, cerebral palsy, gait disturbance	
ClinVar variation ID	812928	9.5 kb copy number loss	19p13.12	Likely pathogenic	Cerebral palsy; global developmental delay; visual impairment	
ClinVar variation ID	442027	228.6 kb copy number loss	20q13.33	Pathogenic	Abnormality of vision, intellectual disability, seizures, abnormalfacial shape, scoliosis, short stature, cerebral palsy	
DECIPHER patient	303619	2.1 Mb copy number loss	22q11.21	Pathogenic	Abnormal facial shape, broad forehead, cerebral palsy, clinodactyly of the fourth toe, global developmental delay, microcephaly, rheumatoid arthritis, ventricular septal defect	
ClinVar variation ID	57671	6.7 Mb copy number loss	22q13.31–13.33	Pathogenic	Cerebral palsy, gait disturbance, autism	The 142 kb critical region of the Phelan-Mcdermid syndrome is included at one end of this much larger deletion. The usual features of this syndrome are moderate to severe intellectual disability with particular difficulty in speech, autistic behaviour, seizures, tall stature and dysmorphic facial features. Some affected children have been diagnosed with cerebral palsy because they have neonatal hypotonia, delayed walking and unsteady gait

Table 35.2 (continued)

Identifier	Number	Description	Location	Interpretation	Clinical features	Comments
DECIPHER patient	283424*	169.2 kb copy number gain	22q13.33 (*MC2R* gene)	Pathogenic	Anxiety, autism, cerebral palsy, global developmental delay, intellectual disability, mild, intracranial haemorrhage, periodontitis, tetraplegia	
DECIPHER patient	284245	29.0 kb (male)	Xp11.4 (*OTC* gene)	Likely pathogenic	Cerebral palsy, episodic ammonia intoxication	
ClinVar variation ID	154959	1.6 Mb copy number loss (presumed male)	Xp22.31	Pathogenic	Autism, dystonia, cerebral palsy, cortical visual impairment	
ClinVar variation ID	443632	2.0 Mb copy number loss (presumed male)	Xq26.2–26.3	Pathogenic	Intellectual disability, seizures, cerebral palsy	
DECIPHER patient	388870	**13.3 Mb (male)**	**Xq27.1-q28**	Pathogenic	Cerebral palsy, intellectual disability, moderate, short stature	
ClinVar variation ID	154936	470.7 kb copy number loss (presumed male)	Xq28	Pathogenic	Seizure, cerebral palsy, global developmental delay	
ClinVar variation ID	154935	6.5 Mb copy number gain (presumed male)	Xq28	Pathogenic	Cerebral palsy, global developmental delay, seizure	

Data are from https://www.ncbi.nlm.nih.gov/clinvar/ or https://www.deciphergenomics.org/. Copy number changes highlighted in **bold font** are large enough to be detected by routine cytogenetic analysis. DECIPHER patients with the same superscript symbol (*, †, ‡, §) are individuals who are reported to have two or more pathogenic/likely pathogenic copy number changes

disease-causing CNVs have been described in the medical literature [49–51], but it is impossible to determine if the co-occurrence of CP and the CNV in these anecdotal cases reflects a causal or coincidental relationship. Dozens of patients with various pathogenic or likely pathogenic CNVs and CP are listed in the ClinVar [52] or DECIPHER [53] databases (Table 35.2). Almost all of these patients have other neurodevelopmental conditions in addition to CP, and a few are reported also to have malformations of other organ systems or dysmorphic features. Most of the CNVs seen in these patients are unique; very few are recurrent copy number changes that are recognized causes of specific genetic syndromes (Table 35.2).

In one remarkable family, nine individuals with spastic quadriplegia and intellectual disability where found by molecular techniques to carry a 225 kb copy number loss of chromosome 9p24.3 that includes the *KANK1* (*ANKRD15*) gene [54]. This CNV, which was transmitted through at least four generations, is incompletely penetrant but appears to have caused the CP in affected family members. Individuals with various *KANK1* copy number losses from other families do not usually have CP [55].

DECIPHER provides a list of 66 genetic syndromes that are caused by CNVs [56]. None of these conditions includes CP as a cardinal feature. However, some children with the Phalen-McDermid (22q13 deletion) syndrome are diagnosed with cerebral palsy because they have neonatal hypotonia, delayed walking and unsteady gait [57].

Seven patient series have determined the frequency of CNVs among individuals with CP (Table 35.3). Most of these studies found that relatively few (0–6%) of the CP patients had disease-causing CNVs. One exception was a series of 52 patients with disabling non-progressive pyramidal and/or extra pyramidal signs beginning before 3 years of age and no periventricular leukomalacia or spinal cord lesions and no history of hypoxic ischemic encephalopathy, brain infarction, encephalitis or head trauma [58]. Sixteen pathogenic or likely pathogenic CNVs were found in 16 (31%) of these atypical CP patients. Patients in the other series who had disease-causing CNVs often had other neurodevelopmental disorders such as intellectual disability, autism or epilepsy, and some had structural malformations of the brain or other organ systems. Unfortunately, however, the clinical descriptions, apart from their CP, reported for patients in these series are limited.

Most disease-causing CNVs in CP patients occur de novo, rather than being inherited from one of the parents. This is true of disease-causing CNVs in other neurodevelopmental disorders as well [59, 60].

The pathogenic/likely pathogenic CNVs reported in these CP patient series involved many different chromosomal regions. This observation is consistent with the het-

Table 35.3 Studies of disease-causing CNVs in CP patient series

Study	CP patients studied	Patient group studied	Method of CNV testing	Diagnostic rate	Comments	Pathogenic/likely pathogenic CNVs observed
McMichael (2014) [75]	50	Children with CP diagnosed by specialist physicians using standard criteria (non-progressive)	CMA	No pathogenic or likely pathogenic CNVs found	14 rare CNVs found in 10 cases; no proven de novo CNVs; all classified as VUS by FRANKLIN.	None
Segel (2015) [58]	52	CP with undetermined aetiology and disabling non-progressive pyramidal and/or extra pyramidal signs; periventricular leukomalacia, perinatal anoxia excluded	CMA	16 pathogenic or likely pathogenic CNVs found in 16 patients (31%)	9 de novo pathogenic/likely pathogenic CNVs, 7 pathogenic/likely pathogenic CNVs inherited from a parent; 6/16 pathogenic or likely pathogenic CNVs explained the CP phenotype. Most individuals with pathogenic or likely pathogenic CNVs also had ID and/or epilepsy	154 kb del(1)(p21.3) 5.21 Mb del(2)(p23.1p22.2) 862 kb dup(2)(q13) 3.46 Mb del(5)(q14.3) 152 kb del(7)(q31.1) 226 kb del(9)(p24.3) 147 kb del(9)(q34.13q34.2) 11.15 Mb del(14)(q12q21.2) 3.23 Mb del(14)(q32.31q32.33) 387 kb dup(17)(p11.2) 445 kb dup(18)(p11.21) 1.96 Mb del(19)(q13.12) 679 kb dup(20)(p12.3p12.2) 2.82 Mb del(22)(q11.21) 4.29 Mb del(X)(p11.23p11.22)(male patient) 298 kb trp(X)(q28)(male patient)
Oskoui (2015) [76]	147	Children with CP diagnosed by specialist physicians at paediatric rehabilitation centres	CMA	8 pathogenic or likely pathogenic CNVs found in 6 patients (4.1%)	All but one pathogenic/likely pathogenic CNVs thought to be de novo but in two cases involving 4 CNVs, one of the parents may have carried a balanced reciprocal translocation	2.08 Mb del(1)(q21.1q21.2) 73.97 Mb dup(2)(p25.3p13.1) and 30.97 Mb del(X)(p22.33p21.2) (female patient) 12.11 Mb dup(2)(p25.3p24.3) 25.49 Mb del(4)(p16.3p15.2) and 8.10 Mb del(9)(p24.3p24.1) 5.79 MB del(15)(q11.2q13.1) 2.76 Mb dup(22)(q13.31)
Zarrei, (2018) [89]	97	Patients with hemiplegic CP	CMA	5 pathogenic or likely pathogenic CNVs found in 4 patients (4.1%)	4 de novo pathogenic/likely pathogenic CNVs, 1 likely pathogenic CNV inherited from a parent;	1.40 Mb del(17)(p12) 2.55 Mb dup(22)(q11.21) 155.27 Mb dup(X)(p22.33q28) (male patient, Klinefelter syndrome) 84.89 Mb del(X)(q13.1q28) and 70.38 Mb dup(X)(p22.33q13.1) (female patient)
Takezawa [65]	17	CP patients born at term with no apparent acquired cause of CP and no typical findings on brain MRI	CMA	Pathogenic CNV found in 1 patient (6%)	One patient with CP, ID, epilepsy, and microcephaly found to have 47, XXY	155.27 Mb dup(X)(p22.33q28) (male patient, Klinefelter syndrome)

Table 35.3 (continued)

Study	CP patients studied	Patient group studied	Method of CNV testing	Diagnostic rate	Comments	Pathogenic/likely pathogenic CNVs observed
Corbett (2018) [77]	136 cases not previously studied	Children with CP diagnosed by specialist physicians using standard criteria (non-progressive)	Trio exome sequencing with bioinformatic analysis for CNVs	9 pathogenic or likely pathogenic CNVs found in 7 patients (5.1%)	8 de novo pathogenic/likely pathogenic CNVs, 1 pathogenic CNV for which both parents were not tested. In 4/9 patients with pathogenic or likely pathogenic CNVs the copy number change was thought to explain the CP phenotype	4.09 Mb dup(1)(q21.1) 7.51 Mb dup(1)(q43q44) and 50.35 Mb del(X)(p22.33p11.22) (unbalanced reciprocal translocation in female patient) 2.55 Mb del(2)(p25.3) and 8.01 Mb dup(20)q13.2q13.33) (unbalanced reciprocal translocation) 519 kb del(3)(p22.3) 726 kb del(16)(p11.2-p12.2) 2.82 Mb del(22)(q11.21) 2.81 Mb dup(22q11)
Rosello, (2020) [67]	20	Children with CP diagnosed by standard criteria who do not have a multiple congenital anomaly syndrome, ataxic CP, progressive encephalopathy, or neuroradiological findings of hypoxic-ischemic encephalopathy, periventricular leukomalacia, cerebral malformation, or leukoencephalopathy	CMA	No pathogenic or likely pathogenic CNVs found		

CNV copy number variant, *CMA* chromosomal microarray analysis. FRANKLIN <https://franklin.genoox.com/clinical-db/home> is a website that provides on-line assessment of genomic variants using the ACMG criteria

erogeneous genetic aetiology of CP discussed above. However, it is interesting that some specific CNVs were reported in patients in two different series: del(22)(p11.21), dup(22)(p11.21), and duplication of the entire X chromosome in males (Table 35.3). The clinical syndromes associated with these CNVs (velocardiofacial/Di George syndrome, 22q11 duplication syndrome and Klinefelter syndrome, respectively) are well characterized, but cerebral palsy is not a usual feature of any of them.

Studies of Single Nucleotide Variants and Indels

Each of us has 4,000,000 to 5,000,000 single nucleotide variants (SNVs) and 700,000 to 800,000 indels (insertions or deletions of 1 to 50 nucleotides) in comparison to the reference human genome sequence [47]. Such 'small' alterations of nucleotide sequence are more frequent major causes of genetic disease than larger changes such as chromosomal abnormalities or genomic CNVs. Although small sequence variants can cause Mendelian diseases, most SNVs and indels are simply genomic differences that are transmitted from generation to generation without any apparent effect on the phenotype. SNVs and indels also arise by new mutation in every person. Most of these de novo changes, like the majority of inherited variants, occur outside of the genes and have no effect on the phenotype. However, if a mutation affects a gene, the change may abrogate or alter the gene's normal function.

Although many different technologies were used to identify disease-causing SNVs and indels in the past, the advent of accurate, rapid, and increasingly cost-effective 'next-generation' or 'second-generation' DNA sequencing has made it routinely possible to test panels of hundreds or thousands of genes, all protein-coding segments of every gene (the 'whole exome'), or all of a person's DNA (the 'whole genome') at once. Rare SNVs or indels that are both necessary and sufficient to cause a genetic disease are conventionally classified as 'pathogenic' or 'likely pathogenic' variants according to standard laboratory criteria [61]. Most SNVs or indels have no influence on the phenotype and can be classified as 'benign' or 'likely benign', but some variants cannot easily be interpreted and must be classified as variants of uncertain significance. Recognizing the one or two genomic variants that cause a Mendelian disease in an affected person's exome or genome sequence data requires sophisticated bioinformatics and clinical analysis of the results.

OMIM [62], an online catalogue of human genes and genetic phenotypes, lists 58 genetic diseases that may pres-

ent as cerebral palsy (Table 35.4). These Mendelian disorders are caused by alterations of 54 different genes. It is important to note that other neurodevelopmental abnormalities occur in all of these diseases and some have multisystem manifestations. Some are progressive and can be recognized as being different from typical CP once this becomes apparent clinically.

The results of exome sequencing have been reported in more than 350 CP or atypical CP patients (Table 35.5). The largest published series, which was recently reported by Jin

Table 35.4 Mendelian conditions that may present with cerebral palsy. Data are from Online Mendelian Inheritance in Man <https://www.omim.org/>

MIM number	Disease	Associated gene	Inheritance	CP phenotypes
201450	Acyl-CoA dehydrogenase, medium-chain, deficiency of	ACADM	Autosomal recessive	Cerebral palsy; hypotonia
617008	Cerebral palsy, spastic quadriplegic, 3	ADD3	Autosomal recessive	Spastic quadriplegia; spastic diplegia
614066	Spastic paraplegia 47, autosomal recessive	AP4B1	Autosomal recessive	Spasticity; inability to walk unaided
613744	Spastic paraplegia 51, autosomal recessive	AP4E1	Autosomal recessive	Spastic quadriplegia
612936	Spastic paraplegia 50, autosomal recessive	AP4M1	Autosomal recessive	Spastic quadriplegia
614067	Spastic paraplegia 52, autosomal recessive	AP4S1	Autosomal recessive	Spasticity; loss of ability to walk
207800	Argininemia	ARG1	Autosomal recessive	Spastic quadriplegia
615926	Webb-Dattani syndrome	ARNT2	Autosomal recessive	Spasticity; cerebral palsy
271900	Canavan disease	ASPA	Autosomal recessive	Initial hypotonia, followed by spasticity
182600	Spastic paraplegia 3, autosomal dominant	ATL1	Autosomal dominant	Lower limb spasticity; lower limb weakness; spastic gait
208900	Ataxia-telangiectasia	ATM	Autosomal recessive	Cerebellar ataxia; choreoathetosis; dystonia
615474	Primary aldosteronism, seizures and neurologic abnormalities	CACNA1D	Autosomal dominant	Cerebral palsy; movement disorder
618522	Mental retardation, autosomal dominant 59	CAMK2G	Autosomal dominant	Hyptonia; cerebral palsy
175780	Brain small vessel disease 1 with or without ocular anomalies	COL4A1	Autosomal dominant	Infantile hemiparesis; hemiplegia; tetraparesis; spasticity; limb dystonia
617976	Developmental and epileptic encephalopathy 63	CPLX1	Autosomal recessive	Hyptotonia; inability to walk
250800	Methemoglobinemia, type II	CYB5R3	Autosomal recessive	Hypertonia; spasticity
300958	Intellectual developmental disorder, X-linked, syndromic, snijders blok type	DDX3X	X-linked dominant or recessive	Dystonia; dyskinesia; spasticity; wide-based gait
310200	Muscular dystrophy, duchenne type	DMD	X-linked recessive	Hypotonia; waddling gait
614219	Adams-Oliver syndrome 2	DOCK6	Autosomal recessive	Hypotonia; spasticity; cerebral palsy
158600	Spinal muscular atrophy, lower extremity-predominant, 1, autosomal dominant	DYNC1H1	Autosomal dominant	Difficulty running and climbing stairs; waddling gait
617046	Spastic paraplegia 77, autosomal recessive	FARS2	Autosomal recessive	Spastic paraplegia
618557	Developmental and epileptic encephalopathy 78	GABRA2	Autosomal dominant	Hypotonia, axial; hypertonia, limb; choreiform movements; spasticity
603513	Cerebral palsy, spastic quadriplegic, 1	GAD1	Autosomal recessive	Spastic diplegia, symmetric; spastic quadriplegia
619124	Developmental and epileptic encephalopathy 89	GAD1	Autosomal recessive	Axial hypotonia; peripheral hypertonia; peripheral spasticity; spastic quadriplegia; dystonia; inability to walk
231670	Glutaric acidemia I	GCDH	Autosomal recessive	Dystonia; hypotonia; choreoathetosis
128230	Dystonia, dopa-responsive	GCH1	Autosomal dominant	Postural dystonia; action dystonia; gait abnormalities; gait ataxia

Table 35.4 (continued)

MIM number	Disease	Associated gene	Inheritance	CP phenotypes
603903	Sickle cell Anaemia	HBB	Autosomal recessive	Stroke; cerebral palsy
300322	Lesch-Nyhan syndrome	HPRT1	X-linked recessive	Hypotonia; spasticity; dystonia; choreoathetosis
117360	Spinocerebellar ataxia 29	ITPR1	Autosomal dominant	Broad-based gait; limb ataxia
206700	Gillespie syndrome	ITPR1	Autosomal recessive	General hypotonia; ataxia
160120	Episodic ataxia, type 1	KCNA1	Autosomal dominant	Ataxia, episodic; leg stiffness; spastic gait
605259	Spinocerebellar ataxia 13	KCNC3	Autosomal dominant	Cerebellar ataxia; hypotonia; inability to run
615834	Mental retardation, autosomal dominant 26	KIAA0442	Autosomal dominant	Hypertonia; stiff movements
210200	3-Methylcrotonyl-CoA carboxylase 1 deficiency	MCCC1	Autosomal recessive	Cerebral palsy; hypotonia
251280	Diencephalic-mesencephalic junction dysplasia syndrome 1	PCDH12	Autosomal recessive	Spastic quadriplegia; axial hypotonia; inability to stand or walk; dystonia
312170	Pyruvate dehydrogenase E1-alpha deficiency	PDHA1	X-linked recessive	Hypotonia; ataxia, episodic; choreoathetosis; dystonia
245349	Pyruvate dehydrogenase E3-binding protein deficiency	PDHX	Autosomal recessive	Hypotonia, neonatal; spastic paraplegia; spastic quadriplegia; ataxia; dystonia
312080	Pelizaeus-Merzbacher disease	PLP1	X-linked recessive	Hypotonia; ataxia; spasticity; dystonia; choreoathetosis
312920	Spastic paraplegia 2, X-linked	PLP1	X-linked recessive	Lower limb weakness; lower limb spasticity; spastic gait; ataxia
612304	Thrombophilia due to protein C deficiency, autosomal recessive	PROC	Autosomal recessive	Spastic cerebral palsy
128200	Episodic kinesigenic dyskinesia 1	PRRT2	Autosomal dominant	Dyskinesia, episodic; choreoathetosis, episodic; dystonia, episodic
600118	Warburg micro syndrome 1	RAB3GAP1	Autosomal recessive	Hypotonia; spastic diplegia
610181	Aicardi-Goutieres syndrome 2	RNASEH2B	Autosomal recessive	Spastic paraplegia; dystonia
616260	Tenorio syndrome	RNF125	Autosomal dominant	Hypotonia; abnormal gait; cerebral palsy
300523	Allan-Herndon-Dudley syndrome	SLC16A2	X-linked recessive	Hypotonia, proximal; spastic paraplegia; spastic quadriplegia; ataxia; inability to stand or walk
618973	Neurodegeneration, infantile-onset, biotin-responsive	SLC5A6	Autosomal recessive	Hypertonia; inability to walk; ataxia; dyskinetic movements; spasticity
613135	Parkinsonism-dystonia, infantile, 1	SLC6A3	Autosomal recessive	Truncal hypotonia; limb dystonia; dyskinesia; hypertonicity
609136	Peripheral demyelinating neuropathy, central dysmyelination, Waardenburg syndrome and Hirschsprung disease	SOX10	Autosomal dominant	Spastic paraparesis, spastic quadriplegia, ataxia
612716	Dystonia, dopa-responsive, due to sepiapterin reductase deficiency	SPR	Autosomal recessive	Dystonia; spasticity; axial hypotonia; choreoathetosis; ataxia
600224	Spinocerebellar ataxia 5	SPTBN2	Autosomal dominant	Cerebellar ataxia
615386	Spinocerebellar ataxia, autosomal recessive 14	SPTBN2	Autosomal recessive	Gait ataxia; spasticity
605407	Segawa syndrome, autosomal recessive	TH	Autosomal recessive	Truncal hypotonia; limb dystonia; hypokinesia
618730	Neurodevelopmental disorder with microcephaly, cortical malformations and spasticity	TMX2	Autosomal recessive	Inability to walk; spasticity; spastic tetraplegia
618201	Developmental and epileptic encephalopathy 68	TRAK1	Autosomal recessive	Hypotonia; spasticity
225750	Aicardi-Goutieres syndrome 1	TREX1	Autosomal recessive or dominant	Tetraplegic spasticity; truncal hypotonia; dystonia
105830	Angelman syndrome	UBE3A	Autosomal dominant	Ataxia with jerky arm movements; wide-based gait; clumsiness, unsteadiness
224050	Cerebellar ataxia, mental retardation and Dysequilibrium syndrome 1	VLDLR	Autosomal recessive	Cerebellar ataxia; broad-based gait; quadrupedal gait
314580	Wieacker-Wolff syndrome	ZC4H2	X-linked recessive	Hypotonia; dystonia; spasticity

MIM Mendelian inheritance in man

Table 35.5 Studies of disease-causing SNVs and indels in CP patient series

Study	Number of CP patients studied	CP group studied	Sequencing performed	Diagnostic rate	Comments
Schnekenburg et al. (2015) [64]	10	Ten patients with congenital ataxia	Trio exome sequencing or 118 gene panel sequencing	3 pathogenic or likely pathogenic SNVs found in 10 patients (30%)	All three patients had de novo autosomal dominant conditions
Takezawa et al. (2018) [65]	17	CP patients who were born at term and do not have an apparent acquired cause of CP or findings characteristic of CP on brain MRI	Trio exome sequencing	10 pathogenic or likely pathogenic SNVs or indels found in 9 patients (53%)	Two patients had an autosomal recessive disease—one was homozygous and the other compound heterozygous. The other pathogenic or likely pathogenic variants were all heterozygous and de novo
Zhu et al. (2018) [90]	9	Children with CP diagnosed by specialist physicians using standard criteria (non-progressive)	Singleton exome sequencing	0	No variants classified as pathogenic or likely pathogenic using current standards
Matthews et al. (2019) [66]	50 individuals in 49 families	Children with impaired motor function of unknown cause within the first year of life and one or more of the following: Severe intellectual disability, progressive neurological deterioration, other neurological abnormalities, multiorgan disease, congenital anomalies outside of the CNS, abnormal neurotransmitter profile, positive family history or brain imaging findings not typical for CP	Trio exome sequencing	Pathogenic or likely pathogenic variants found in 21 (43%) of 49 probands	Eleven patients had de novo autosomal dominant variants. Five patients had autosomal recessive diseases. One was a homozygote, and the others were compound heterzygotes. Five patients, two of them females, had X-linked diseases. The authors suggest that VUSs or likely pathogenic variants in genes that do not have an established association with CP found in 11 other patients may also be disease-causing
Van Eyk et al. (2019) [91]	271	Children with CP diagnosed by specialist physicians using standard criteria (non-progressive)	112 gene panel	Pathogenic or likely pathogenic variants found in 5 (1.8%) of 271 patients	Three patients had an autosomal dominant disease; one case was de novo and the parents were not both studied in the other two cases. One homozygous variant was found in a patient with an autosomal recessive disease, and one male had a variant for an X-linked recessive disease
Jin et al. (2020) [63]	250	Patients with CP defined as a non-progressive developmental disorder of movement and/or posture with onset before age 2 years. Cases with chromosomal anomalies, pathogenic CNVs, other clinically or molecularly diagnosed syndromes, mitochondrial disorders or traumatic brain injuries were excluded	Trio exome sequencing	The authors estimate that at least 14% of CP cases studied can be attributed to a disease-causing SNV or indel	The authors estimate that 11.9% of the CP cases studied can be attributed to a damaging de novo mutation and that 2.1% can be attributed to damaging recessive genotypes. These estimates are based on case-control analyses rather than on classification of individual variants with respect to pathogenicity, as was done in all other studies included in this table. Data in this study include 91 patients previously reported by McMichael et al., 2015 [92].
Rosello et al. (2020) [67]	20	Children with CP diagnosed by standard criteria who do not have a multiple congenital anomaly syndrome, ataxic CP, progressive encephalopathy, or neuroradiological findings of hypoxic-ischemic encephalopathy, periventricular leukomalacia, cerebral malformation or leukoencephalopathy	Trio exome sequencing	13 pathogenic or likely pathogenic SNVs found in 11 patients (55%)	Three patients had an autosomal recessive disease -- one was a homozygote and two were compound heterozygotes. One male patient had X-linked disease variant. The other disease-causing variants were all de novo autosomal dominants

and associates [63], includes 250 patients with CP defined by standard clinical criteria. This study was performed to explore genetically mediated disease mechanisms in CP, and SNVs and indels were assessed using case-control analyses of patient groups rather than by classification of variants for pathogenicity in each individual patient, as is done when exome sequencing is used clinically. On the basis of their analysis, Jin and associates [63] estimated that CP can be attributed to disease-causing SNVs or indels in at least 14% of patients. This clearly is a minimal estimate of the rate of disease-causing small nucleotide sequence changes among patients with conventionally defined CP [63]. Substantially higher proportions of patients with disease-causing SNVs or indels were observed in the patient series reported by Schnekenberg et al. [64], Takezawa et al. [65], Matthews et al. [66] or Rosello et al. [67], but all of these studies are much smaller and many of the patients included have an atypical form of CP (Table 35.5).

Disease-causing SNVs or indels reported in patients with CP or atypical CP involve 54 different genes (Table 35.6). The diseases caused by genetic alterations at some of these genetic loci are recognized as being associated with clinical features of CP, but 42 (78%) of the genes are *not* included in the list of

Table 35.6 Mendelian causes of CP reported in series studied by exome sequencing (Table 35.5). Phenotypes that are not known to include features of CP are marked with an asterisk

Gene	Gene listed in Table 35.4	Mendelian	OMIM number	Phenotype	Patients
AKT3	***N***	AD	615937	Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 2*	Matthews 16
ALS2	***N***	AR	607225	Spastic paralysis, infantile onset ascending	Srivastava 2
AMPD2	***N***	AR	615809	Pontocerebellar hypoplasia, type 9	Takezawa 11, Jin F033–003
AP4B1	***Y***	AR	614066	Spastic paraplegia 47, autosomal recessive	Rosello 1
AP4M1	***Y***	AR	612936	Spastic paraplegia 50, autosomal recessive	Jin F623–003
AP5Z1	***N***	AR	613647	Spastic paraplegia 48, autosomal recessive	Jin F342–003
ASXL1	***N***	AD	605039	Bohring-Opitz syndrome*	Matthews 10
ATL1	***Y***	AD	182600	Spastic paraplegia 3A, autosomal dominant	Rosello 5, Rosello 11, Rosello 18, Jin F050–003
ATP1A3	***N***	AD	614820	Alternating hemiplegia of childhood 2	Matthews 21
CACNA1A	***N***	AD	617106	Developmental and epileptic encephalopathy 42	Takezawa 9
COL4A1	***Y***	AD	175780	Brain small vessel disease 1 with or without ocular anomalies	Van Eyk 204
CSTB	***N***	AR	254800	Epilepsy, progressive myoclonic 1A (Unverricht and Lundborg)	Matthews 2
CTNNB1	***N***	AD	615075	Neurodevelopmental disorder with spastic diplegia and visual defects	Takezawa 3, Jin F066–003
CYP2U1	***N***	AR	615030	Spastic paraplegia 56, autosomal recessive	Takezawa 5
DGUOK	***N***	AR	251880	Mitochondrial DNA depletion syndrome 3	Srivastava 3
EHMT1	***N***	AD	610253	Kleefstra syndrome 1*	Matthews 5
ELP2	***N***	AR	617270	Mental retardation, autosomal recessive 58	Srivastava 1
ERLIN2	***N***	AR	611225	Spastic paraplegia 18, autosomal recessive	Srivastava 64
FARS2	***Y***	AR	617046	Spastic paraplegia 77, autosomal recessive	Jin F629–003
GCDH	***Y***	AR	231670	Glutaricaciduria, type I	Matthews 4
GNAO1	***N***	AD	615473	Developmental and epileptic encephalopathy 17	Rosello 15, Takezawa 7, Matthews 1
GNB1	***N***	AD	616973	Mental retardation, autosomal dominant 42	Rosello 17
IFIH1	***N***	AD	615846	Aicardi-Goutieres syndrome 7	Rosello 2
ITPA	***N***	AR	616647	Developmental and epileptic encephalopathy 35	Matthews 18
ITPR1	***Y***	AD	117360	Spinocerebellar ataxia 29, congenital non-progressive	Schnekenburg 2
KCNC3	***Y***	AD	605259	Spinocerebellar ataxia 13	Schnekenburg 1
KCNJ6	***N***	AD	614098	Keppen-Lubinsky syndrome	Matthews 13
KCNQ2	***N***	AD	613720	Developmental and epileptic encephalopathy 7	Srivastava 50
KIDINS220	***N***	AD	617296	Spastic paraplegia, intellectual disability, nystagmus, and obesity	Matthews 22

(continued)

Table 35.6 (continued)

Gene	Gene listed in Table 35.4	Mendelian	OMIM number	Phenotype	Patients
KIF1A	***N***	AD	614255	NESCAV syndrome	Van Eyk 174, Van Eyk 781
L1CAM	***N***	XL	303350	MASA syndrome	Van Eyk 724
MECP2	***N***	XL	312750	Rett syndrome	Matthews 8
MECP2	***N***	XL	300055	Mental retardation, X-linked, syndromic 13	Matthews 14
NAA10	***N***	XL	300855	Ogden syndrome	Matthews 9
NT5C2	***N***	AR	613162	Spastic paraplegia 45, autosomal recessive	Van Eyk 718, Jin F444–003
PANK2	***N***	AR	234200	Neurodegeneration with brain iron accumulation 1	Srivastava 15
PGK1	***N***	XL	300653	Phosphoglycerate kinase 1 deficiency	Rosello 8
PLP1	***Y***	XL	312080	Pelizaeus-Merzbacher disease	Matthews 3
RNASEH2B	***Y***	AR	610181	Aicardi-Goutieres syndrome 2	Rosello 20
SCN2A	***N***	AD	613721	Developmental and epileptic encephalopathy 11	Takezawa 17
SCN3A	***N***	AD	617938	Developmental and epileptic encephalopathy 62	Matthews 28
SPAST	***N***	AD	182601	Spastic paraplegia 4, autosomal dominant	Rosello 4, Takezawa 6, Takezawa 10, Srivastava 44, Matthews 20, Jin F082–003
SPATA5	***N***	AR	616577	Epilepsy, hearing loss, and mental retardation syndrome	Rosello 14
SPG11	***N***	AR	604360	Spastic paraplegia 11, autosomal recessive	Jin 84084P
SPTBN2	***Y***	AD	600224	Spinocerebellar ataxia 5	Schnekenburg 4
ST3GAL5	***N***	AR	609056	Salt and pepper developmental regression syndrome	Srivastava 54
STXBP1	***N***	AD	612164	Developmental and epileptic encephalopathy 4	Takezawa 12, Srivastava 71
TBCK	***N***	AR	616900	Hypotonia, infantile, with psychomotor retardation and characteristic facies 3	Matthews 25
TCF4	***N***	AD	610954	Pitt-Hopkins syndrome	Matthews 11
TMEM67	***N***	AR	216360	COACH syndrome 1	Matthews 6
TUBA1A	***N***	AD	611603	Lissencephaly 3	Jin (Table 35.2)
TUBB4A	***N***	AD	612438	Leukodystrophy, hypomyelinating, 6	Matthews 15
UBE3A	***Y***	AD	105830	Angelman syndrome	Srivastava 51
WDR45	***N***	XL	300894	Neurodegeneration with brain iron accumulation 5	Matthews 7
ZBTB18	***N***	AD	612337	Mental retardation, autosomal dominant 22*	Srivastava 8

genetic conditions that may present as CP (Table 35.4). The clinical features of most of these conditions in Table 35.6 are known to overlap with those of CP, but this is not true for a few of them (marked with an asterisk in Table 35.6). Whether the observation of apparently disease-causing variants of these genetic loci among patients with CP or atypical CP represents an expansion of our knowledge about the phenotypic spectrum of these rare genetic diseases or is simply coincidental is currently uncertain. It is noteworthy, however, that all of the genetic conditions in which a CP-like phenotype occurs also include other neurological abnormalities, and often non-neurological anomalies as well (Tables 35.4 and 35.6). Jin et al. [63] also demonstrated substantial overlap among the genes associated with CP and those associated with intellectual disability, autism or epilepsy.

Epigenetic Studies

Epigenetic mechanisms regulate the transfer of information from the genome, allowing different cell types, organs, body systems and the individual to develop from an undifferentiated zygote and to function throughout life. Although this concept is easy to understand, defining epigenetics in a precise scientific fashion has been surprisingly controversial [68]. Key aspects of epigenetic mechanisms are their dependence on features of the chromatin outside of the DNA sequence itself and the stable, but not invariably fixed, transmission of the epigenetic state of a cell through mitosis and over time. Epigenetic mechanisms also provide a means by which the environment can influence genomic function [69].

Laboratory animal studies have clearly established the importance of epigenetic mechanisms in neurodevelopment and adult neurological function, and many observational investigations are consistent with similar roles in humans [70]. The best-studied epigenetic systems are methylation of DNA and acetylation of histone proteins, but other covalent DNA or histone modifications, non-coding RNAs, and four-dimensional alterations of chromatin structure and its relationship to the nuclear membrane may also act in epigenetic regulation. Moreover, epigenetic changes of one kind can affect other kinds of epigenetic alterations in a multidimensional regulatory network [70].

Crowgey and her associates [71] performed genome sequencing of white blood cell DNA from 16 adolescents with spastic CP and 16 control subjects. Sequencing reads from 1.5 million CpG methylation sites throughout the genome were selected bioinformatically, and the degree of methylation at each site was quantified. Comparison of the CP and control groups found significantly increased or decreased methylation at 0.4% of the CpG sites assessed. Because the study was performed in adolescents, it was not possible to determine whether the methylation differences found reflected the presence of spastic CP (or its treatment) or were markers of the processes that caused the CP in these patients.

This issue was not a concern in a study performed on DNA obtained from archived newborn blood spots of 23 children with various forms of CP and 21 unaffected controls [72]. Using a standard microarray assay of 450,000 variably methylated genomic loci, this study found significantly different methylation of 0.05% of the loci tested. The authors suggest that differential methylation at these loci might predict the development of CP in a child, but, given the probable aetiological heterogeneity of the patients studied, it is unlikely that these differences provide any insight into underlying genetic factors.

Monozygotic twins, who are identical genetically but are discordant with respect to CP, provide an opportunity to assess the effect of non-genetic factors on methylation patterns. Mohandas and her colleagues [73] used a standard 450,000 locus methylation microarray to test archived newborn blood spots from 15 monozygotic pairs in which one twin developed CP and the other did not. No probes were found that exhibited statistically significant differential methylation between the twins with CP and the unaffected co-twins after adjusting for multiple testing, but top-ranked differentially methylated probes below the statistical cutoff involved genes that were associated with immunity and inflammation or with epileptic encephalopathy.

The findings were different in a study of four pairs of monozygotic twins who were discordant for CP and in whom genome-wide methylation was assayed by reduced representation bisulphite genome sequencing [74]. One hundred ninety differentially methylated genes were identified among the discordant twins. Enrichment analysis showed associations with genes involved in cerebral atrophy, and pathway analysis suggested involvement in the biosynthesis of antibiotics, glycolysis/gluconeogenesis and propanoate metabolism.

The Genetics and Genomics of Cerebral Palsy

Building on earlier family and twin studies, recent genomic investigations have clearly demonstrated that genetic factors of major effect cause CP in many patients. Most CNVs and small alterations of nucleotide sequence that have been found to cause CP or atypical CP arise as a result of de novo mutations, so studies that depend on the recurrence frequency within families substantially underestimate genetic contributions to the aetiology of CP.

Studies of series of patients with typical CP suggest that at least 4% have disease-causing CNVs [75–77] (Table 35.3) and at least 14% have disease-causing SNVs or indels [63] (Table 35.5). The rates of disease-causing genomic lesions are substantially higher among patients with atypical CP (Tables 35.3 and 35.5). Mutations of many different genetic loci can produce a CP-like phenotype (Tables 35.2, 35.3, 35.4, and 35.6). It seems likely that additional major genetic causes of CP will be recognized as more patients are tested, more sensitive tests (e.g. sequencing of the entire genome) are used, and bioinformatics and clinical interpretation of genomic data improve.

The importance of genetic variants of minor effect and of epigenetic modifications in producing a multifactorial predisposition to CP is less clear. These factors are likely to exist on theoretical grounds, but their involvement has been difficult to demonstrate convincingly. This is probably because of the variety and complexity of such multifactorial predispositions and of the interactions among them in different combinations.

Recognizing the specific cause of CP in a patient is essential to providing optimal clinical management for each affected individual. The financial, emotional and social costs for patients and families affected with CP are great, and obtaining a precise diagnosis provides families an ‘enhanced compass’ that improves overall well-being [78, 79]. Recognizing a specific genetic cause may also facilitate access to educational and social services beyond those that are related to the patient’s physical disability. In addition, treatment targeting pathophysiology is available for a subset of atypical CPs, namely those caused by inherited metabolic diseases [80]. Examples include congenital neuro-transmitter defects and inherited disorders of amino acid metabolism. Early recognition and initiation of therapy (e.g. medical diet, vitamin supplementation, liver transplantation or medica-

tion) is essential before irreversible damage is done in patients suffering a treatable Mendelian inherited metabolic disease. Time is brain!

Patients who receive genetic diagnoses and their families benefit by obtaining knowledge of the cause and projected natural history of their condition, and a precise genetic diagnosis is essential for accurate genetic counselling about recurrence in a family. Finally, obtaining a genetic diagnosis ends an expensive, time-consuming and emotionally draining 'diagnostic odyssey' for many families.

In a substantial fraction of patients with CP, and especially in those whose CP is atypical, an underlying genetic disease is responsible for the neuro-developmental abnormalities. Trio exome sequencing and chromosomal microarray analysis or trio genome sequencing with bioinformatics analysis for CNVs as well as SNVs and indels are clinically indicated in the initial workup of CP patients.

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Multiple Choice Questions

1. The mode of inheritance in the majority of cerebral palsy patients is:
   (a) X-linked dominant (de novo)
   (b) Autosomal recessive
   (c) **Autosomal dominant (de novo)**
   (d) None of the above
2. Establishing a diagnosis in cerebral palsy has implications for
   (a) Supportive care
   (b) Prognosis and counselling
   (c) Prevention and treatment
   (d) **All of the above**
3. In patients with cerebral palsy, genetic aberrations occur with the following frequencies
   (a) **disease-causing copy number variants: 4%, and single nucleotide variants or indels: 14%**
   (b) disease-causing copy number variants: 4% and epigenetic signatures: 21%
   (c) single nucleotide variants or indels: 14% and epigenetic signatures: 21%
   (d) structural and numeric chromosomal abnormalities: 13% and single nucleotide variants or indels: 14%
4. The yield of genetic/genomic testing increases if the following features are present:
   (a) positive family history for cerebral palsy, periventricular leukomalacia on neuro-imaging, progressive disease course
   (b) **progressive disease course, multi-organ involvement, affected siblings**
   (c) unexplained death in the family, progressive disease course, normal neuro-imaging
   (d) abnormalities on prenatal sonogram, normal newborn screening, behavioural problems

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