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Editor's Choice

The genetic basis of cerebral palsy

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ABBREVIATIONS

AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP-4	Activating enhancer binding protein 4
ApoE	Apolipoprotein E
CNV	Copy number variant
OMIM	Online Mendelian Inheritance in Man

Although prematurity and hypoxic–ischaemic injury are well-recognized contributors to the pathogenesis of cerebral palsy (CP), as many as one-third of children with CP may lack traditional risk factors. For many of these children, a genetic basis to their condition is suspected. Recent findings have implicated copy number variants and mutations in single genes in children with CP. Current studies are limited by relatively small patient numbers, the underlying genetic heterogeneity identified, and the paucity of validation studies that have been performed. However, several genes mapping to intersecting pathways controlling neurodevelopment and neuronal connectivity have been identified. Analogous to other neurodevelopmental disorders such as autism and intellectual disability, the genomic architecture of CP is likely to be highly complex. Although we are just beginning to understand genetic contributions to CP, new insights are anticipated to serve as a unique window into the neurobiology of CP and suggest new targets for intervention.

Cerebral palsy (CP) is a major neurodevelopmental disorder, currently estimated to affect approximately 1 in 500 children. As a clinical diagnosis, the aetiology of the syndrome varies and is often unknown.[1] Although prematurity, hypoxia–ischaemia, placental insufficiency, and prenatal infection are well-characterized causes of CP, for other patients, particularly those born at term and/or without a clear aetiology identifiable by magnetic resonance imaging (MRI), the cause of the condition has remained obscure.

Although there is a clear role for hypoxic–ischaemic injury in some cases of CP, estimates suggest that acute intrapartum hypoxia–ischaemia accounts for fewer than 10% of cases.[2–4] Furthermore, despite international consensus criteria for defining perinatal asphyxia,[5–7] many published studies have not applied these rigorous definitions. Despite improved obstetric practice and better antenatal and perinatal care, several studies indicate there has been little reduction in the incidence of CP over the last several decades.[7–9] These data and other findings have led some investigators to suggest that 'unknown pathophysiologic processes' must be at work to account for a significant proportion of CP.[10] We suspect that much of this unknown pathophysiology may be owing to genetic or epigenetic factors. Indeed, current estimates indicate that as many as 30% of CP cases may be genetic in nature.[11–13] Regardless of the final numbers, there is a growing consensus (National Institutes of Health Workshop on Basic and Translational Research in Cerebral Palsy; https://videocast.nih.gov/summary.asp?Live=18384&bhcp=1) that genetic contributions to CP are significant and important for understanding the disorder. There are four main types of DNA variation that contribute to CP pathogenesis (Fig. 1), yet the ultimate effect of most mutations is a loss of the normal cellular function of the protein encoded by that gene.

Few studies to date have been published on the genetics of CP, given the historical focus on other aetiologies. Despite the criticism that CP represents a 'wastebasket diagnosis', we would argue that once one excludes CP mimics (Table I[14]) there is no better term to characterize 'permanent disorders of ... movement and posture' that affect 'the developing ... brain'.[15] As our knowledge continues to advance, it will likely be necessary to refine classifications of individual disorders based on their aetiology, but the concept of CP as a unifying and defining term remains useful. Similar to epilepsy or autism, which have also undergone a similar process of 'lumping and splitting',[16–18] the term CP unifies a cardinal neurodevelopmental disorder in its own right.

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Table II: Hereditary spastic paraplegia genes implicated in inherited forms of cerebral palsy to date

Gene	Function	Inheritance
NIPA1 (*SPG6*)[48]	Bone morphogeneic protein receptor	Autosomal dominant
SPAST (*SPG4*)[49]	Microtubule-severing	Autosomal dominant
SPG34[49]	Thyroid transporter	X-linked
AP4B1 (*SPG47*)[41]	Endolysosomal trafficking	Autosomal recessive

Distinction from hereditary spastic paraplegia

Clinically, it can be difficult to distinguish CP from hereditary spastic paraplegia. In principle, hereditary spastic paraplegia affects the lower limbs and is familial and progressive, while CP may be associated with quadriplegia and is non-progressive. However, these distinctions are readily blurred in clinical practice, as CP may be relatively limited to the lower extremities and, as a neurodevelopmental disorder, its manifestations change over time. Further complicating the issue is the designation of mutations in *AP4M1* as a cause of both CP (OMIM #603513) and hereditary spastic paraplegia (OMIM #614066) (Table II). In the future, we suspect that distinctions between genetic forms of CP and hereditary spastic paraplegia will become increasingly blurred, particularly as one considers 'complicated' forms of hereditary spastic paraplegia. We recommend that clinicians and researchers not be limited by nomenclature, but rather to think broadly and consider cases that highlight 'grey areas' as opportunities to revise and refine diagnostic categories.

Developing new models for CP

Current animal models for CP are largely limited to hypoxia–ischaemia or inflammation-based rodent or large-animal models with neuronal development analogous to humans. Such animal models have led to many advances, particularly in perinatal and neonatal care.[53–55] However, moving forward, as we define what are likely to be many additional CP-related genes, vertebrate and invertebrate models such as zebrafish, Drosophila, or *C. elegans* may be valuable in understanding the effects of mutations on the developing nervous system. An advantage of these lower eukaryotes is that they are highly genetically tractable, and models can thus be generated much faster and for less cost than mouse or large-animal models. Such systems can thus be used as supportive 'proof of pathogenesis' for a given gene, and many genes can be tested in parallel in a higher throughput than is currently possible for models such as mice.

Worms, flies, and zebrafish are widely used to study other human neurodevelopmental and neurodegenerative diseases, and a large tool kit of methods has been developed to exploit the unique advantages of these models.[56–58] About 70% of all human genes have orthologues in zebrafish, while 65% of human proteins linked to diseases have similar counterparts in *Drosophila*.[57,59] Genetic conservation is especially true for proteins required for essential cellular functions like the regulation of actin dynamics and maintaining cytoskeleton integrity[60,61]; several such proteins have already been linked to CP. In particular, an intact cytoskeleton is crucial to integrate sensory input with motor output, and mutations that disrupt the actin cytoskeleton interfere with learning and memory, locomotion, and neuronal integrity in zebrafish and flies.[62,63] We have previously shown that disruption of *Drosophila Hts*, the orthologue of human adducin, leads to altered brain development and locomotor abnormalities.[23]

These comparatively simple model organisms can thus provide a powerful strategy for investigating how disease-associated changes in human proteins interfere with normal function and cause pathology. For example, knocking down *Kank* expression in flies leads to motor dysfunction (unpublished results). Identifying how mutations in CP candidate genes interfere with normal function *in vivo* can thus increase our understanding of CP pathology and may pinpoint targets for the development of new therapeutics which can then be tested in higher animals.

CONCLUSIONS

Available evidence indicates that genetic mutations may be responsible for a substantial proportion of CP cases. Clinically, microarray analysis in individuals with unexplained forms of CP may yield a unifying diagnosis, and, in select cases, whole-exome sequencing may be informative. Nevertheless, our understanding of genes that lead to CP is in its early stages, and the existing nomenclature is inadequate. As progress continues, partnerships between physicians, basic scientists, and families and advocacy networks will become increasingly important. Clinicians will need to be meticulous in phenotyping patients, while researchers will need to be similarly thorough in characterizing genotype–phenotype relationships as novel genes are discovered in order to inform clinical care.

Eventually, specific molecular subtypes of CP may be found to respond better or worse to specific treatments, facilitating a personalized medicine approach to CP. Similarly, it may be worthwhile targeting those with a genetic susceptibility to CP who suffer an environmental 'second hit'; these children might be appropriately targeted for intensive early intervention to prevent poor outcomes. Finally, clinical studies of CP interventions could benefit from incorporating genetic analysis into their study design, allowing clinical investigators to compare, potentially, gene variants in 'responders' with 'nonresponders'.

The discovery of monogenic contributors to CP may have substantial near- and long-term benefits. The identification of rare Mendelian forms of CP may provide a window into CP neurobiology, as has occurred for other neurological diseases such as Alzheimer and Parkinson diseases.[64,65] Of potential immediate impact would be the diagnosis of a genetic form of CP in a given patient. Such insight has the potential to provide a sense of closure for families. A genetic diagnosis may also be invaluable for guiding preventative health care, treating disease-specific

clinical manifestations, and for accurate counselling regarding recurrence risk.

Confirming rare genomic variants as contributors to CP will require diligent validation studies. Most immediately, validations may be possible by sequencing large cohorts of individuals with CP; if recurrent mutations in a given gene are found in a cohort of patients with CP but not seen in healthy individuals, this provides an important genomic validation. Subsequent studies may utilize a combination of transcriptomic, proteomic, and/or metabolomic analysis of patient samples to characterize evidence for disruption of important pathways at multiple levels. Both established cell lines and patient-derived cell lines, including induced pluripotent stem cell-derived neurons, will be useful to demonstrate that a given genetic variant is deleterious. Developing and employing small-animal models, such as Drosophila, zebrafish, and mice will be crucial as the first steps to translate findings to and from humans. Large-animal models will be a crucial part of therapeutic development. In sum, we believe that genomic discoveries will set the stage for follow-up *in vitro* and *in vivo* studies needed to characterize important molecular and cellular mechanisms that lead to CP when they fail. When such mechanisms are understood, this knowledge can be used to develop effective new therapies seeking not to merely control symptoms, but also to treat the fundamental pathophysiology of CP.

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