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A Gene for Autosomal Recessive Symmetrical Spastic Cerebral Palsy Maps to Chromosome 2q24-25

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Summary

Cerebral palsy has an incidence of ~1/500 births, although this varies between different ethnic groups. Genetic forms of the disease account for ~1%–2% of cases in most countries but contribute a larger proportion in populations with extensive inbreeding. We have clinically characterized consanguineous families with multiple children affected by symmetrical spastic cerebral palsy, to locate recessive genes responsible for this condition. The eight families studied were identified from databases of patients in different regions of the United Kingdom. After ascertainment and clinical assessment, we performed a genomewide search for linkage, using 290 polymorphic DNA markers. In three families, a region of homozygosity at chromosome 2q24-q25 was identified between the markers D2S124 and D2S148. The largest family gave a maximum LOD score of 3.0, by multipoint analysis (HOMOZ). The maximum combined multipoint LOD score for the three families was 5.75. The minimum region of homozygosity is ~5 cM between the markers D2S124 and D2S2284. We have shown that a proportion of autosomal recessive symmetrical spastic cerebral palsy maps to chromosome 2q24-25. The identification of genes involved in the etiology of cerebral palsy may lead to improved management of this clinically intractable condition.

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Introduction

Cerebral palsy is a common disorder of childhood, with an incidence of 1/250–1/1,000 births (Pharoah et al. 1987; Bundey and Alam 1993). It is defined as a nonprogressive but not unchanging disorder of posture or movement, caused by an abnormality of the brain and first evident at the stage of rapid brain development (Hughes and Newton 1992). Cerebral palsy can be classified according to the specific type of movement disorder observed (Hagberg et al. 1972). Spastic cerebral palsy accounts for ~60% of cases and is subdivided into hemiplegia, diplegia, quadriplegia, and monoplegia. Other types of movement disorder seen in cerebral palsy are athetoid/dyskinetic, ataxic, and mixed. The athetoid/dyskinetic and ataxic groups each account for 5%–10% of the condition overall, with the mixed group constituting the remainder (Gustavson et al. 1969).

Recent studies have documented that only 10%–15% of cases of cerebral palsy can be attributed to intrapartum problems (Blair and Stanley 1988). The other major risk factors are now recognized to be prematurity, small size for gestational age, and multiple pregnancy (Stanley 1994). The majority of children with cerebral palsy born at >36 wk gestation have no recognized birth asphyxia, and only ~40% have a possible potential cause (Hagberg and Hagberg 1993).

It is among the 60% of idiopathic cases that the rarer genetic forms of cerebral palsy occur. Approximately 2% of all cases of cerebral palsy in Swedish and English children are due to a genetic cause (Gustavson et al. 1969; Bundey and Griffiths 1977). Most of these cases have no recognizable adverse pre- or postpartum events and have marked symmetry of the neurological signs. Thus it follows that hemiplegia and monoplegia are rarely, if ever, genetic. Ataxic cerebral palsy occurring without an obvious predisposing event is likely to be genetic in approximately half the cases, and the same is true for idiopathic athetoid cerebral palsy (Hughes and Newton 1992).

Spastic cerebral palsy is the most common subtype and has a low overall recurrence risk. Spastic cerebral

palsy that is both symmetrical and idiopathic accounts for <4% of all cerebral palsy cases, but its recurrence risk in sibs is ~1/8 (Bundey and Griffiths 1977). Most cases are autosomal recessive, but rare autosomal dominant and X-linked forms have also been described (Bundey and Griffiths 1977; Bundey et al. 1978). There are no previous reports of genetic linkage for autosomal recessive "true" spastic cerebral palsy.

We have used homozygosity mapping to identify genetic loci involved in spastic cerebral palsy in eight consanguineous families originating from the Mirpur region of Pakistan (Mitchell and Bundey 1997). The affected children have a symmetrical, nonprogressive spasticity and no adverse perinatal history or obvious underlying alternative diagnosis. Careful documentation of the clinical phenotype was undertaken. A genomewide search was performed to identify a genetic locus that cosegregated with spastic cerebral palsy in these families.

Subjects and Methods

Study Families and Diagnosis

The probands from the seven families were ascertained from the database of patients of the West Midlands Paediatric Neurology and Clinical Genetics Service (United Kingdom) and from the records of local special schools and child-development centers within the city of Birmingham (Mitchell and Bundey 1997). One further family was ascertained by the Yorkshire Regional Clinical Genetics Service, Leeds. The following criteria were used: (1) Pakistani ethnic origin, (2) nonprogressive symmetrical spasticity, and (3) no identifiable perinatal cause or known alternative underlying diagnosis.

The numbering of these families corresponds to that used in Mitchell and Bundey's (1997) paper describing the clinical phenotypes. The clinical features of the families are summarized in table 1. The case histories of the linked families are detailed below, to highlight clinical differences between them, as well as the minor phenotypic variability that occurs even between siblings.

Family 3.—The oldest affected boy was born in February 1981 in Pakistan and came to the United Kingdom at the age of 3 years. At that time, he had global developmental delay and was neither walking nor speaking. He attended a day school for children with moderate-to-severe learning difficulties. When seen, he was a friendly but moderately retarded boy with little capacity for speech. His head circumference was at the 10th centile, and there were no abnormalities of either the cranial nerves or the upper limbs. He had a symmetrical spastic diplegia with brisk leg reflexes and equivocal plantar responses, but he was able to walk. There was no evidence of either a neuropathy or an ataxia.

The proband's sister was born in the United Kingdom in July 1986. She also presented with global developmental delay. She could walk with support at age 3 years and without support at age 5–6 years. Her speech was markedly delayed and remains poor. When examined at age 8 years, she was friendly, moderately mentally retarded, and walking. Neurological examination revealed increased tone in her legs, brisk tendon reflexes, and equivocal plantar responses. There was no abnormality of her cranial nerves, and she had neither ataxia nor spasticity in her arms.

Family 4.—All the children were born in the United Kingdom and had no pre- or perinatal problems. The oldest affected girl had bilateral dislocated hips that required surgical correction. She never walked independently and has been in a wheelchair since the age of 4 years. She is severely mentally retarded.

When reviewed at age 14 years, she still had not developed bladder or bowel control. Examination revealed a severely retarded child with no recognizable speech. Her head circumference was at the 25th–50th centile. She had a marked spastic diplegia with increased tone, contractures at the knees, brisk reflexes, and extensor plantar responses. There was no nystagmus, and no cranial nerve abnormality was observed.

Her 12-year-old brother is also severely retarded, with

Table 1

Clinical Features of Linked and Unlinked Families

	Status of				
Family	Homozygosity at 2q24-25?	Mental Retardation	Epilepsy	Microcephaly	Spasticity
3	Yes	Moderate	Absent	Absent	Diplegia
4	Yes	Moderate and severe	Absent	Variable	Diplegia
8	Yes	Severe	Present	Present	Quadriplegia
1	No	Moderate and severe	Present	Variable	Diplegia
2	No	Moderate	Absent	Absent	Diplegia
5	No	Moderate	Absent	Absent	Diplegia
6	No	Severe	Absent	Present	Quadriplegia
7	No	Severe	Present	Variable	Quadriplegia

limited speech, and he too has not attained bladder or bowel control. On examination, his head circumference was <3d centile, and he had a kyphoscoliosis, a spastic diplegia with flexion contractures at the knees and extension contractures at the hips. There was symmetrical wasting of both lower limbs, with brisk tendon reflexes and equivocal plantar responses. Mild hypertonia and ataxia of the upper limbs were noted. No abnormality of the cranial nerves was present.

The next affected boy was one of DZ twins born by cesarean section. He presented with developmental delay, and, when reviewed at the age of 9 years, he was crawling and had symmetrical spasticity of his legs, with brisk reflexes and upgoing plantar responses. There was mild hypertonia of the arms, with some ataxia. He was dysarthric but had no nystagmus, and there was no abnormality of the cranial nerves.

A younger sister, when examined at age 19 mo, was able to neither walk nor stand. Her head circumference was at the 25th centile. There was neither nystagmus nor abnormalities of her cranial nerves or arms. The tendon reflexes in her legs were pathologically brisk, and her plantar responses were extensor.

Family 8.—This family of Pakistani origin has been ascertained recently in a U.K. region geographically separate from that of the other seven families. The second and fourth sons of first-cousin parents were born in the United Kingdom and were found to be microcephalic, with head circumferences >3 SD below the mean. The pregnancies and deliveries for both boys were uneventful. Hypotonia was present during the 1st year of life, but, by age 3 years, quadriplegic spasticity and severe developmental delay were apparent in both boys. Seizures developed in them both at age 6 mo, and grand mal fits have occurred approximately monthly since then. Neither child is dysmorphic or has any features suggestive of a metabolic disorder. Review of the older boy at age 16 years revealed that he has gross contractures, communicates only by grunting, and has a head circumference of 46.5 cm (6 cm below the 3d centile). The affected 4-year-old has severe spasticity, achilles-tendon and knee contractures, a head circumference of 43 cm (6 cm below the 3d centile), and no speech.

Genomewide Search

The markers used for the genomewide search were originally described by Reed et al. (1994) and have an average spacing of one marker every 12–14 cM. Extra markers mapping to regions of possible homozygosity were identified from the CEPH-Généthon Integrated Map. PCR products were labeled either by incorporation of 5′–fluorescently labeled oligonucleotide primers or by the addition of FAM-labeled dUTP to the reaction. Analysis of the PCR products was performed by an automated ABI 377 Gene Scanner.

PCR was performed in a total volume of 20 μl containing 40 ng of DNA, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 50 ng of each primer, 1 unit of *Taq* DNA polymerase, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), and 1% Triton X-100. Annealing temperatures were optimized for each primer pair. PCR cycles were as follows: 95°C for 5 min, followed by 30 cycles of 95°C for 20 s, annealing temperature at 20 s, and 72°C for 20 s, with a final extension at 72°C for 5 min. If the primers were not fluorescently labeled, then 0.02 ml of 100 mM FAM-labeled dUTP was added to each reaction. PCR products were analyzed on a 4% polyacrylamide/6 M urea/1 × Tris-borate EDTA gel for 2 h at 3,000 V and at a fixed temperature of 51°C. The data were captured by the ABI Genescan software package, and genotypes were generated by the Genotyper software package (Applied Biosystems).

Statistical Analysis

Linkage analysis was performed by both LIPED, for two-point analysis (Ott 1976), and HOMOZ/MAPMAKER, for multipoint analysis (Kruglyak et al. 1995). The allele frequencies for each polymorphic marker were estimated on the basis of values in 35 unrelated control individuals originating from the same region of Pakistan as the families involved in the study. The frequency of the allele for each marker associated with the disease is shown, for each family, in table 2. A disease-allele frequency of 1/200 was used. All the families were genotyped with the markers identified within the region of interest. The results for the three families with homozygous-by-descent regions were then combined (fig. 1).

Results

Phenotypes

Table 1 shows the main clinical features seen within each of the families. The families have been classified according to whether they have a region of homozy-

Table 2

Frequency of Disease-Associated Alleles in Each Family

	Disease Allele (Frequency) in		
Marker	Family 3	Family 4	Family 5
D2S142	Heterozygous	Heterozygous	123 (.20)
D2S124	159 (.29)	Heterozygous	159 (.29)
D2S2345	156 (.20)	152 (.34)	156 (.20)
D2S376	269 (.62)	267 (.16)	269 (.62)
D2S2284	Heterozygous	165 (.18)	165 (.18)
D2S333	Heterozygous	203 (.21)	199 (.53)
D2S2302	Heterozygous	189 (.07)	187 (.32)
D2S326	Heterozygous	89 (.06)	89 (.06)
D2S2188	Heterozygous	137 (.18)	139 (.04)
D2S148	Heterozygous	187 (.42)	187 (.42)
D2S300	Heterozygous	Heterozygous	Heterozygous

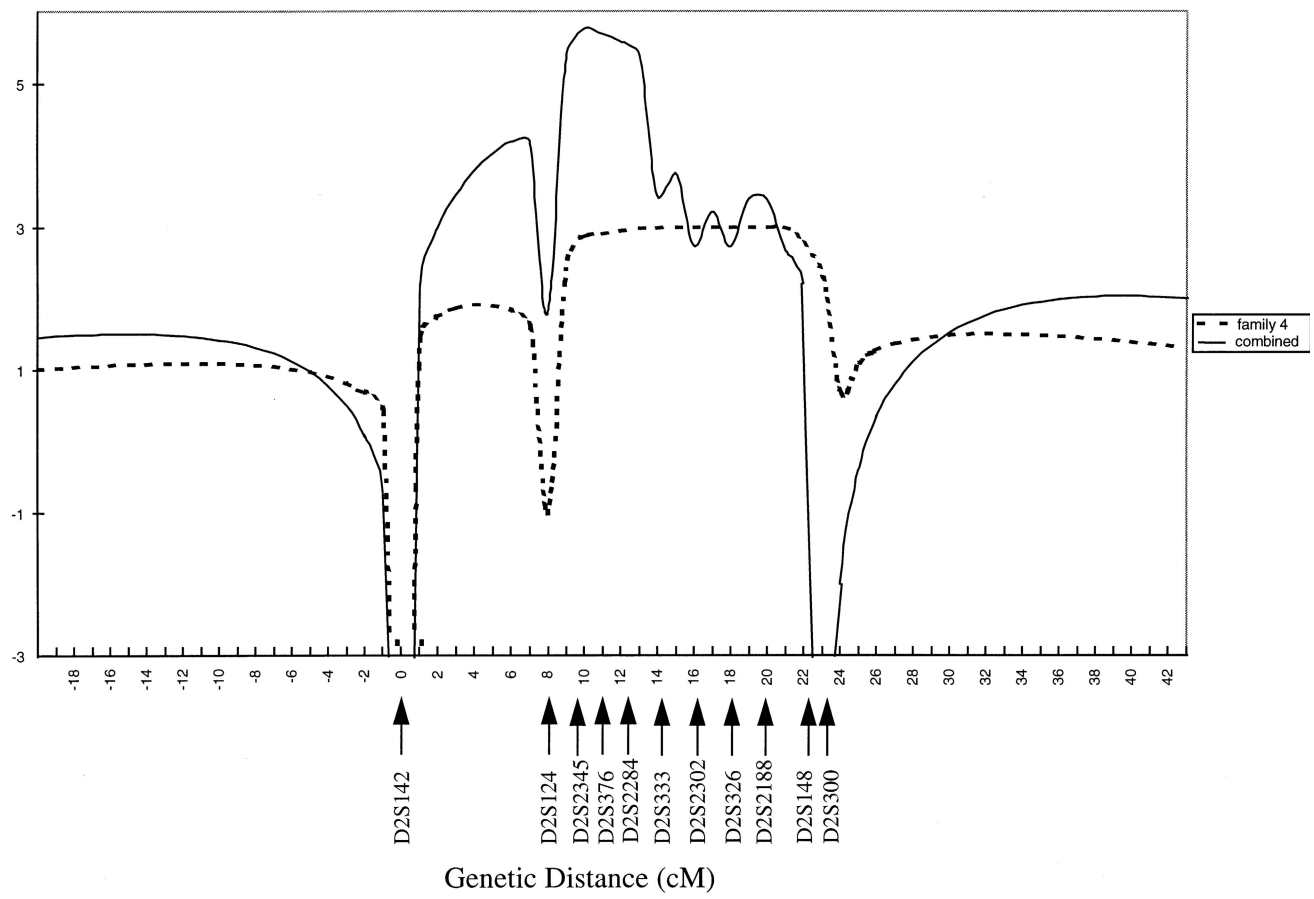

Figure 1 Multipoint analysis of family 4 and combined result for all three linked families. Map distances are from the Genome Database.

gosity ⩾5 cM on chromosome 2q24-25. Although considerable inter- and intrafamilial variability was seen, there were no clinical features that could have been used to distinguish families segregating with this region of chromosome 2 versus those families showing no evidence of linkage.

Genotypes

Seven of the eight families were typed with 290 markers evenly spaced across the genome. All the affected members of family 4 were homozygous for the same allele at the marker D2S326. This generated a two-point LOD score of 2.6, by LIPED. Further markers (cen–D2S142, D2S124, D2S2345, D2S376, D2S2284, D2S333, D2S2302, D2S2188, D2S148, D2S300–tel) from this region were identified from the CEPH-Généthon Integrated Map map. Multipoint analysis was performed by the HOMOZ/MAPMAKER computer program; the results are shown in figure 1.

The marker D2S326 was the only marker in the genomewide search that was fully informative and homozygous in all four affected children from family 4. Haplotype analysis ruled out other potential homozygosity-by-descent regions from the initial screen.

All the families were genotyped with the marker D2S326 and with the additional markers from the region of interest. The LOD scores from the multipoint analyses of the families with regions of homozygosity >5 cM were combined and are shown in figure 1. The genotypes are shown in figure 2.

Discussion

The results of the genomewide search revealed that all four affected individuals from family 4 were homozygous for the marker D2S326. Additional markers from this region of chromosome 2q24-25 (D2S142, D2S124, D2S2345, D2S376, D2S2284, D2S333, D2S2302, D2S2188, D2S148, and D2S300) were selected at spacings of ~2 cM. These identified a 12-cM interval of homozygosity, flanking D2S326 between the markers D2S124 and D2S300. Multipoint analysis was performed on the results for this family, by HOMOZ/MAPMAKER (Kruglyak et al. 1995), and a maximum LOD

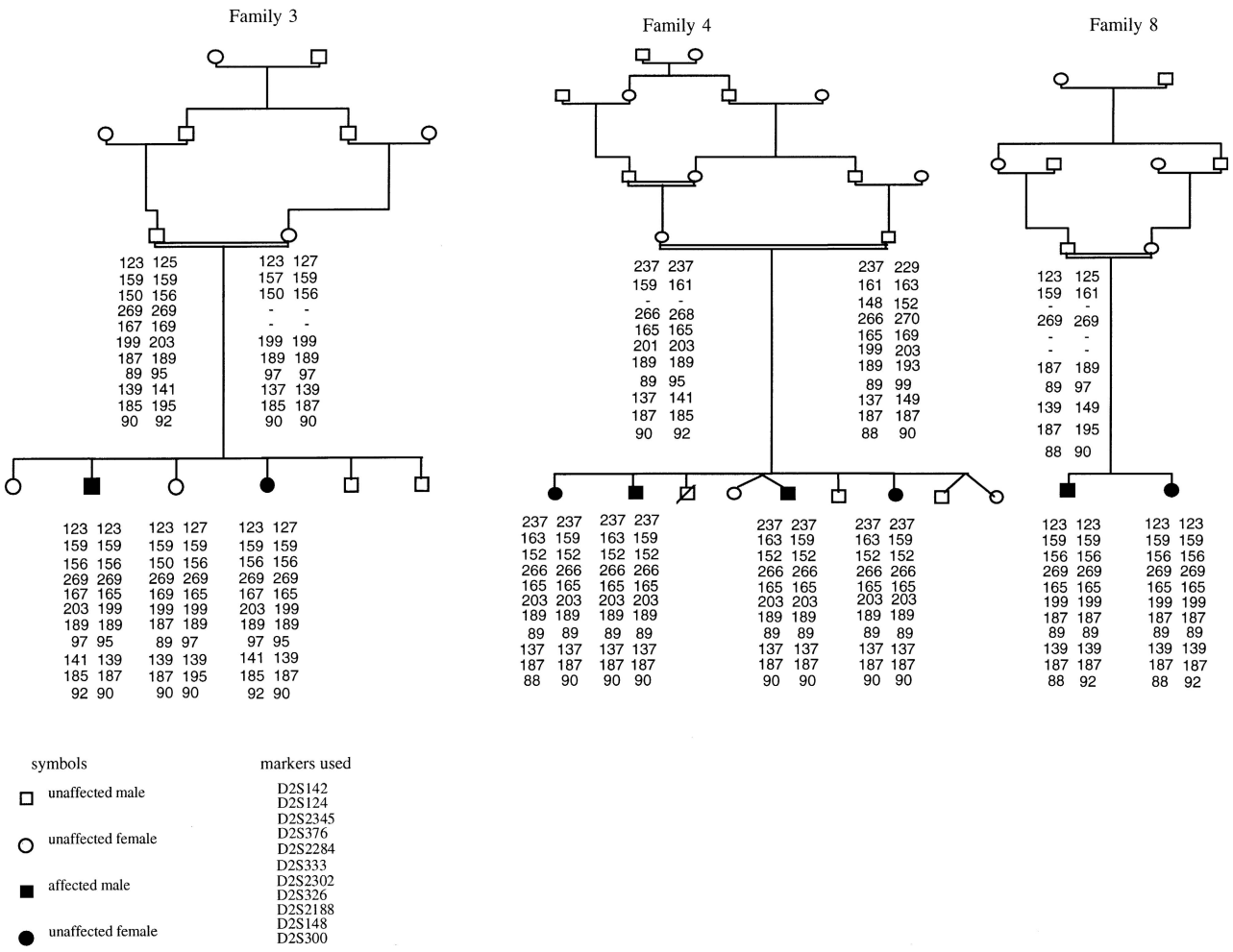

Figure 2 Genotype data for families with linkage to chromosome 2q24-25. The allele sizes of the respective microsatellite markers used are indicated, in chromosomal order, beneath genotyped individuals

score of 3.0, between the markers D2S2345 and D2S300, was generated (fig. 1).

The remaining seven families that we had characterized were then genotyped with the marker set from the critical region. A region of homozygosity ⩾5 cM was also present in two of these families (families 3 and 8). The combined, multipoint-analysis result from the initial family and these two other families gave a maximum LOD score of 5.75, between the markers D2S124 and D2S2284 (fig. 1). All affected individuals from these three linked pedigrees have a region of homozygosity of ~5 cM, between the markers D2S124 and D2S2284 (fig. 2). In the remaining five families, the LOD scores ranged from negative infinity to .5, but in no one family was a LOD score of −2 observed across the whole region. This was due to both the small size of the families, with all except one having only two affected members, and the fact that some of the markers were only partially informative, resulting in homozygous markers but no definite region of homozygosity. Formal analysis with HOMOG led to equivocal results for each of these five families.

No haplotype common to the three pedigrees was observed. The families used in the study all originate from the Mirpur region of Pakistan. It is possible that these families are distantly related, potentially allowing the use of linkage-disequilibrium mapping to define a smaller candidate region, by use of a higher density of polymorphic markers. Ascertainment of additional linked families may also allow the narrowing of the common region of homozygosity, prior to both physical mapping of the region and candidate-gene analysis.

There are 59 cDNA transcripts reported to map to the region, according to the Genome Maps 96 database. Of these, there are two known genes that would make plausible candidates. The first is a brain-specific voltage-gated sodium channel. The *Drosophila* homologue of this gene is called "*paralytic,*" and mutations result in abnormal—or, in some cases, no—conduction within

parts of the CNS. A second candidate gene is *GAD1,* which codes for a 67-kD isoform of glutamate decarboxylase. This enzyme is involved in the production of gamma-aminobutyric acid (GABA), which is a potent inhibitory neurotransmitter. Many of the remaining cDNA species are expressed in neural tissue and would therefore be positional candidates for the gene that causes symmetrical spastic cerebral palsy.

Genetic forms of spastic cerebral palsy usually present with symmetrical neurological signs and no other obvious potential cause (Mitchell and Bundey 1997). Several other variable features are seen, including mental retardation, microcephaly, and epilepsy. Despite careful clinical assessment, there was no consistent overall disease phenotype segregating in the families with linkage to chromosome 2q24-25, compared with the unlinked families. In addition, considerable inter- and intrafamilial variation was seen, consistent with complex gene × environment and gene × gene interactions. Thus, clinical assessment alone is not sufficient to distinguish between the group of children suffering from inherited spastic cerebral palsy caused by mutations in a gene mapping to chromosome 2q24-25 versus those children in whom the mutations are at another locus.

The gene(s) involved in the etiology of inherited spastic cerebral palsy will presumably encode an important group of human CNS developmental proteins that, although necessary both for early neural development and to establish cerebral function, do not necessarily cause worsening problems if absent during childhood and later life. This suggests that these genes may prove to have temporal- or site-specific expression patterns within the developing brain and may only be essential during the relatively early stages of growth of the immature CNS. It also seems likely that a proportion of sporadic cases of spastic cerebral palsy may be attributable to environmental factors that have an impact on the expression of these genes in utero. Cloning of these genes may provide unique insights into the mechanisms involved in the differentiation of neuronal pathways and, in particular, those factors involved in motor function. Greater understanding of the early developmental mechanisms in these neural networks during fetal life may lead to the possibility of coordinated regeneration of damaged tissue, in an attempt to improve function in a variety of conditions involving neuronal damage.

Cerebral palsy is a common, poorly understood, and intractable group of conditions seen by pediatricians, family practitioners, neurologists, and general physicians, since many of these children survive into adulthood. Although genetic forms of this condition account for only 1%–2% of all cases, they offer a unique insight into both the causes of the disease and the development of specific neuronal pathways. Elucidation of these pathways will undoubtedly improve our understanding of the pathophysiology of cerebral palsy.

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Electronic-Database Information

URLs for data in this article are as follows:

CEPH-Généthon Integrated Map, http://www.cephb.fr/bio/ceph-genethon-map.html (for markers)
Genome Maps 96, http://www.ncbi.nlm.nih.gov/science96/ (for cDNA transcripts)
Genome Database, http://www.hgmp.mrc.ac.uk/gdb/ (for map distances)

References

Blair E, Stanley FJ (1988) Intrapartum asphyxia: a rare cause of cerebral palsy. J Pediatr 112:515–519
Bundey S, Alam H (1993) A five year prospective study of the health of children in different ethnic groups with particular reference to the effect of inbreeding. Eur J Hum Genet 1: 206–219
Bundey S, Griffiths MI (1977) Recurrence risks in families of children with symmetrical spasticity. Dev Med Child Neurol 19:179–191
Bundey S, Griffiths MI, Young JA (1978) Recurrence risk of symmetrical spastic cerebral palsy. Dev Med Child Neurol 20:390–391
Gustavson KH, Hagberg B, Sanner G (1969) Identical syndromes of cerebral palsy in the same family. Acta Paediatr Scand 58:330–340
Hagberg B, Hagberg G (1993) The origins of cerebral palsy. In: David TJ (ed) Recent advances in paediatrics. Vol 11. Churchill-Livingstone, New York, pp 67–83
Hagberg B, Sanner G, Steen M (1972) The dysequilibrium syndrome in cerebral palsy: clinical aspects and treatment. Acta Paediatr Scand Suppl 226:1–63
Hughes I, Newton R (1992) Genetic aspects of cerebral palsy. Dev Med Child Neurol 34:80–86
Kruglyak L, Daly MJ, Lander ES (1995) Rapid multipoint linkage analysis of recessive traits in nuclear families, including homozygosity mapping. Am J Hum Genet 56: 519–527
Mitchell S, Bundey S (1997) Symmetry of neurological signs in Pakistani patients with probable inherited spastic cerebral palsy. Clin Genet 51:7–14
Ott J (1976) A computer program for linkage analysis of general human pedigrees. Am J Hum Genet 28:528–529
Pharoah POD, Cooke T, Rosenbloom I, Cooke RWI (1987)

Trends in birth prevalence of cerebral palsy. Arch Dis Child 62:379–384

Reed PW Davies JL, Copeman JB, Bennett ST, Palmer SM, Pritchard LE, Gough SC, et al (1994) Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. Nat Genet 7:390–395

Stanley FJ (1994) The aetiology of cerebral palsy. Early Hum Dev 36:81–88