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Primary Autosomal Recessive Microcephaly (MCPH1) Maps to Chromosome 8p22-pter

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Summary

Primary (or "true") microcephaly is inherited as an autosomal recessive trait and is thought to be genetically heterogeneous. Using autozygosity mapping, we have identified a genetic locus (MCPH1) for primary microcephaly, at chromosome 8p22-pter, in two consanguineous families of Pakistani origin. Our results indicate that the gene lies within a 13-cM region between the markers D8S1824 and D8S1825 (maximum multipoint LOD score of 8.1 at D8S277). In addition, we have demonstrated the genetic heterogeneity of this condition by analyzing a total of nine consanguineous families with primary microcephaly.

Introduction

Microcephaly is a condition in which the head circumference of an affected individual is >3 SD below the age-related mean and craniosynostosis is not present. The "small head" that is characteristic of microcephaly results from a smaller-than-normal cranial vault relative to the facial skeleton and the rest of the body. The small cranial capacity is apparently due to underlying hypoplasia of the cerebral cortex rather than to abnormal development of the overlying skull (Ross and Frias 1977).

Microcephaly has a heterogeneous etiology (Bundey 1992). A variety of environmental causes have been proposed, including intrauterine infections, drugs taken during pregnancy, prenatal radiation exposure, maternal phenylketonuria, and birth asphyxia (Wood et al. 1967; Ross and Frias 1977). However, with the exception of birth asphyxia, all of these are rare causes of microcephaly (Qazi and Reed 1973). The majority of cases arise as the result of a variety of genetic mechanisms, including cytogenetic abnormalities, single-gene disorders, and syndromes of as-yet-undetermined etiology (Baraitser 1990).

Primary or "true" microcephaly (MIM 251200) appears to be a distinct subtype. It is defined by the absence of associated malformations and of secondary or environmental causes (Ross and Frias 1977; Baraitser 1990). It is inherited as an autosomal recessive trait and has an incidence of 1/30,000 to 1/250,000 (Komai et al. 1955; Van den Bosch 1959). Its clinical manifestations are microcephaly and mental retardation, without weakness, spasticity, or athetosis (Bundey 1992); other features, such as a cheerful, affable personality and a sharply sloping forehead, have been described in some but not all studies (Cowie 1960). Cowie (1960) suggested, on the basis of variation in phenotype between studies, that primary microcephaly is likely to be genetically heterogeneous.

Distinguishing genetic microcephaly from its phenocopies is problematic, particularly in the absence of an environmental factor, a chromosomal abnormality, or syndromal features. However, a number of characteristics strongly indicate a genetic etiology. These include consanguinity, a normal pregnancy and postpartum period, absence of associated neurological features, less-severely delayed developmental milestones, and a symmetrically small cerebrum, as determined by computed tomography (Qazi and Reed 1973; Bundey 1992).

Prenatal diagnosis of microcephaly by serial ultrasonographic measurement of fetal head circumference has not been reliable, since the head circumference measurements do not fall appreciably below normal percentiles until the third trimester of pregnancy (Jaffe et al.

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1987; Tolmie et al. 1987). Elucidation of the genes responsible for microcephaly is therefore important for both genetic counseling and prenatal diagnosis.

To identify loci involved in primary microcephaly, we have used "homozygosity mapping" (Lander and Botstein 1987), also known as "autozygosity mapping," to perform a genomewide search in a large, multiply affected consanguineous family with primary autosomal recessive microcephaly. A further eight pedigrees were then used to test for genetic heterogeneity and to refine the position of the locus.

Subjects and Methods

Subjects

A family containing five individuals affected with primary autosomal recessive microcephaly was ascertained. This family originates from the Mirpur region of Pakistan (fig. 1, family 1). According to the clinical histories, the family confirmed that microcephaly was present from birth in all affected individuals and that there was no history of epilepsy in affected individuals. On examination, head circumferences were 5–9 SD below the population age-related mean. The affected individuals examined were 13–28 years old, and mental retardation ranged from mild to moderate in severity. None were able to read or write, but all could speak and had basic self-care skills. Except for microcephaly, there were no dysmorphic features. No affected individual had a sloping forehead such as that described by Penrose (Cowie 1960). Examinations did not reveal weakness, spasticity, or athetosis. Computed tomography had been performed on one affected individual at 5 years of age, and results were normal. No environmental causes of microcephaly were identified. All parents appeared to be of normal intelligence and had normal head circumferences.

A further eight multiply affected consanguineous families were ascertained, with a total of 23 affected individuals displaying primary microcephaly. All of these families also originated from the Mirpur region of Pakistan and had pedigrees consistent with autosomal recessive inheritance. The study was approved by Leeds Health Authority/United Leeds Teaching Hospitals NHS Trust Research Ethics Committee.

DNA Extraction and Microsatellite Analysis

DNA was extracted from peripheral blood lymphocytes by means of a standard nonorganic extraction procedure. The ABI Prism linkage mapping primer set was used to perform a genomewide search. This panel contains 358 microsatellite repeat markers spaced at ~10-cM intervals, with an average heterozygosity of 0.81. PCR amplification of all the autosomal markers was performed according to the manufacturer's specifications. Amplified markers were pooled and electrophoresed on the ABI Prism 377 gene sequencer with a 4.2% polyacrylamide gel at 3000 V and 52°C for 2 h. We performed fragment-length analysis using the ABI Prism Genescan and Genotyper 1.1.1 analysis packages.

For fine mapping on 8p22-pter, we used D8S504 and D8S277 from the ABI Prism linkage set, and we selected a further seven polymorphic markers from the Genome Database: tel-D8S1824-D8S1798-D8S1819-D8S1825-D8S552-D8S1731-D8S261-cen. PCR reactions were performed in 10-μl volumes that contained 50 ng genomic DNA; 1 μM primers; 250 μM each dGTP, dCTP, dTTP, and dATP; 5 U *Taq* DNA polymerase; and 1 × reaction buffer (1.5–2.0 mM $MgCl_2$, 10 mM Tris-HCl pH 9.0, 50 mM KCl, and 0.1% Triton X-100). Amplification was performed with a 5-min initial denaturing step at 95°C; 35 cycles of 94°C for 30 s, 54°C–60°C for 30 s, and 72°C for 30 s; and a final incubation step at 72°C for 5 min.

Linkage Analysis

A fully penetrant autosomal recessive mode of inheritance was assumed, and the disease allele frequency was estimated at 1/300. Two-point analysis was performed by the LINKAGE analysis programs (Terwilliger and Ott 1994) and HOMOZ-MAPMAKER was used for multipoint analysis (Kruglyak et al. 1995). An allele frequency of 0.1 was used in the genome screen for all markers. For further analysis of the candidate region, marker allele frequencies were calculated by genotyping 34 unrelated individuals from the same ethnic population, with a lower limit for allele frequencies set at 0.1. Heterogeneity testing was performed with the HOMOG program (Morton 1955; Terwilliger and Ott 1994).

Results

Four of the five affected individuals and four parents from family 1 were analyzed against all 343 autosomal

Table 1

Two-Point LOD Scores for Family 1

	LOD Score at Recombination Fraction of						
Marker	.00	.01	.05	.1	.2	.3	.4
D8S504	−.75	−.32	.09	.21	.23	.15	.06
D8S1824	.65	1.04	1.34	1.30	1.01	.64	.27
D8S1798	2.57	2.51	2.26	1.95	1.35	.81	.34
D8S277	1.53	1.49	1.34	1.16	.80	.44	.13
D8S1819	4.24	4.14	3.76	3.28	2.31	1.40	.60
D8S1825	2.00	1.94	1.71	1.43	.91	.49	.19
D8S552	3.40	3.31	2.99	2.58	1.81	1.09	.46
D8S1731	2.34	2.28	2.04	1.76	1.21	.71	.29
D8S261	−∞	.06	1.12	1.30	1.06	.64	.26

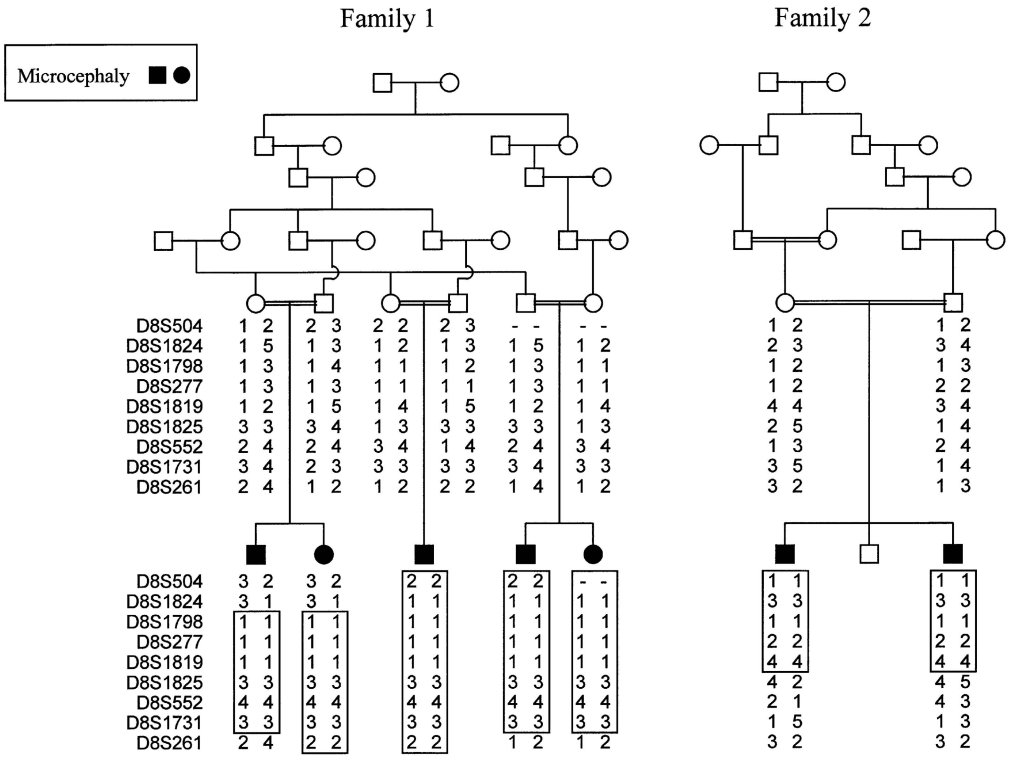

Figure 1 Haplotypes for nine markers from 8p22-pter, for families 1 and 2. Unaffected siblings from family 1 have been omitted, for clarity. Marker order and relative distances are presented here as deduced from the Généthon map: D8S504-3 cM-D8S1824-3 cM-D8S1798-3 cM-D8S277-2 cM-D8S1819-5 cM-D8S1825-13 cM-D8S552-5 cM-D8S1731-5 cM-D8S261.

markers from the ABI Prism Linkage mapping set. A single autozygous region at 8p22-pter was identified in all four affected individuals. No other region was identified in which a marker was fully informative and homozygous in all affected individuals. Multipoint analysis of the entire genome search data set confirmed that this was the only genomic region with a LOD score >3 (fig. 2).

An extended map of the autozygous region was constructed (tel-D8S504-D8S1824-D8S1798-D8S277-D8S1819-D8S1825-D8S552-D8S1731-D8S261-cen) and used to analyze all 11 members of family 1. This confirmed the presence of a large region of autozygosity in all five affected individuals (fig. 1, family 1). Two-point linkage analysis of these markers gave a maximum LOD score of 4.24 with D8S1819, at a recombination fraction of zero (table 1). Multipoint analysis gave a maximum LOD score of 5.70 at D8S1819.

To determine whether other families with primary microcephaly also displayed genetic linkage to the chromosome 8p22-pter region, we analyzed eight more families, using the markers described above. Multipoint LOD scores were generated and used to test for locus heterogeneity, with the HOMOG program. Linkage to this locus at 8p22-pter, as well as significant evidence for the presence of other loci, was demonstrated (table 2). A maximum log likelihood of 14.07 was obtained for $\alpha = .25$, at D8S277, where α is the proportion of families with linkage. In addition, HOMOG analysis supports linkage to this locus in family 2. For the other families, linkage is unlikely (table 3).

In autozygosity mapping, the minimal critical region is defined as the smallest region of homozygosity common to all affected individuals. Since family 2 had a maximum multipoint LOD score of 2.5 (at D8S1824), we combined families 1 and 2 to define a critical region of 13 cM between the markers D8S1824 and D8S1825 (fig. 1). A combined multipoint analysis of these two families gave a total multipoint LOD score of 8.1 at

Table 2

HOMOG Analysis

Hypothesis[a]	df	χ^2	Likelihood Ratio
H2 vs. H1	1	22.535	7.823×10^4
H1 vs. H0	1	5.611	16.5348
H2 vs. H0	2	28.146	1.293×10^6

[a] H0 = no linkage; H1 = linkage, homogeneity; and H2 = linkage, heterogeneity.

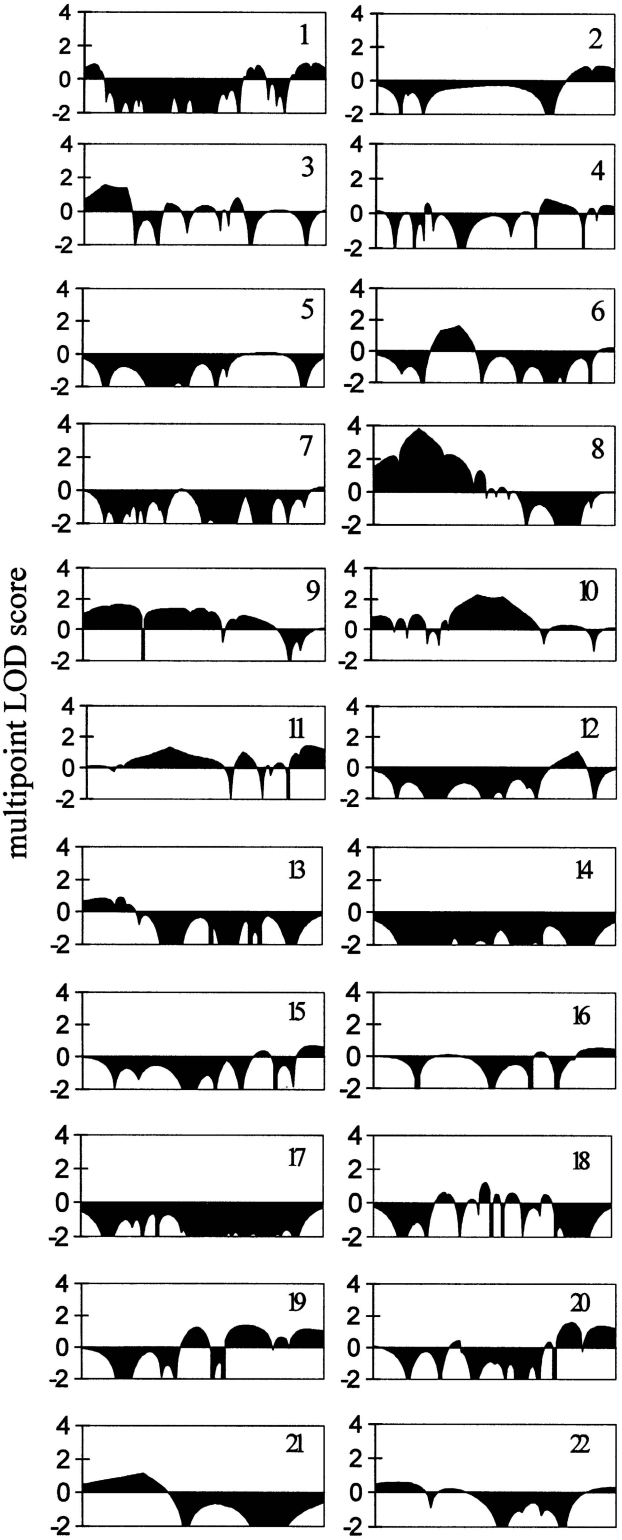

Figure 2 Results of HOMOZ multipoint analysis of Genome search data from family 1. HOMOZ multipoint LOD scores plotted against distance along chromosome, for chromosomes 1–22.

D8S277 (fig. 3). Interestingly, at the marker D8S1798, allele 1 (which has an allele frequency of 0.33) is shared in the two pedigrees. This could indicate the presence of an allelic association, possibly as the result of a founder effect in this population.

Discussion

We report the mapping of a locus for primary autosomal recessive microcephaly, MCPH1 (microcephaly 1). Two families with primary microcephaly map to 8p22-pter, with a multipoint LOD score of 8.1 at D8S277. We have also shown that recessive microcephaly displays locus heterogeneity. Identifying additional families in this and other populations will refine the critical region and determine whether this locus is a common cause of primary microcephaly. Furthermore, such families will be of value in defining phenotype:genotype correlations.

Genetic counseling regarding recurrence risks for sporadic nonsyndromic microcephaly (microcephaly without associated malformations) is difficult because of the attendant etiological uncertainties. The recurrence risk for microcephaly attributable to environmental causes is minimal, whereas the risk for autosomal recessive microcephaly is 1 in 4. At present, empiric recurrence risks ranging from 1 in 5 to 1 in 8 are advised for primary microcephaly (Baraitser 1990; Bundey 1992; Tolmie 1996). Identification of this locus, MCPH1, and subsequent identification of additional loci will facilitate the determination of Mendelian recurrence risks in a proportion of families and will allow reliable prenatal diagnosis.

Microcephaly may result from aberrations in neuronal proliferation, neuronal migration, or programmed cell death during cellular differentiation in the neocortex. It is difficult to discern which of these processes are perturbed in primary microcephaly, because of the confounding variables introduced by the presence of genetic heterogeneity and, indeed, uncertainty as to whether genetic mechanisms are responsible in apparently "sporadic" cases. An understanding of the pathophysiology of genetic microcephaly is currently hindered by the paucity of associated neuroimaging and neuropathology studies. Given the range of possible pathophysiological mechanisms, it is likely that genes with diverse functions could cause microcephaly.

At present, 43 expressed sequence tags (ESTs) are known to map into the 13-cM MCPH1 critical region, 23 of which are known to be expressed in brain (National Center for Biotechnology Information, Human Gene Map) (Schuler et al. 1996). Although there are no obvious candidate genes for primary microcephaly, two of the brain-specific ESTs (A005X23 and stSG3758) have homologies to known genes. The first encodes a zinc finger protein, MSN4, which is homologous to a

Table 3

HOMOG Analysis: Conditional Probability of Linkage, by Family

Family Number	Conditional Probability of Linked Type	Lower Support Limit	Upper Support Limit
1	1.0000	.9999	1.0000
2	.9904	.0000	.9983
3	.0580	.0045	.4289
4	.0000	.0000	.0002
5	.0010	.0001	.1039
6	.0481	.0000	.9626
7	.0000	.0000	.0001
8	.0003	.0000	.0191
9	.0000	.0000	.0455

transcription factor in the stress-response system in yeast (Martinez-Pastor et al. 1996). The second is homologous to an ecdysone-induced protein in *Drosophila*, EIP 28/29 (Cherbas et al. 1986). Ecdysone is the steroid-molting hormone in *Drosophila*, and, in embryonic cell lines, it causes differentiation and proliferative arrest. Only one characterized human gene maps to this region, that for multifunctional protein ADE2 (Minet and Lacroute 1990). This gene codes for an enzyme that catalyzes two consecutive steps in the de novo purine biosynthesis pathway, which has been shown to be essential for mouse embryonic development (Alexiou and Leese 1992).

The only potential animal genetic model of microcephaly currently available is the *Drosophila minibrain* mutant (Tejedor et al. 1995). *Minibrain* is characterized by markedly smaller central brain hemispheres and optic lobes due to smaller-than-normal numbers of neuronal progeny from outer proliferation center neuroblasts. The causative gene mutation for this phenotype is in a serine/threonine protein kinase, which has a human homologue

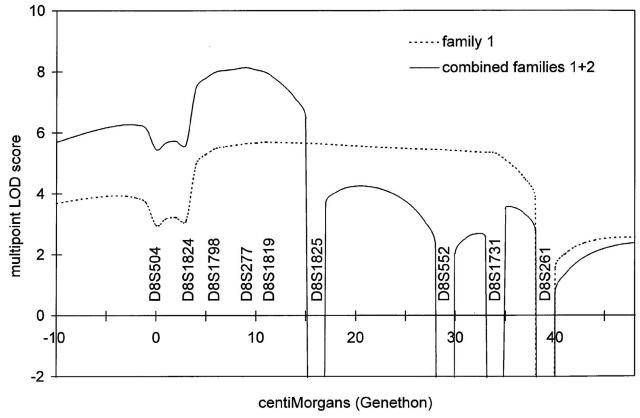

Figure 3 Results of HOMOZ multipoint analysis for family 1 and for combined analysis of families 1 and 2, for markers on 8p22-pter.

on chromosome 21. The scarcity of microcephalic models in other species may result from the genes being species-specific, as one of the most marked differences between humans and other mammals is the relative size of their cerebral cortexes.

The mapping of MCPH1, the first locus for autosomal recessive microcephaly, will aid clinical diagnosis and management of this condition in affected individuals. The eventual cloning of this gene will provide insights into the complex processes of neurodevelopment, learning, and evolution of the neocortex.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, http://www.genethon.fr/genethon_en.html (for marker order and relative distances of markers from 8p22-pter)

Genome Database, http://www.gdb.org (for polymorphic markers used for fine mapping on 8p22-pter)

National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/cgi-bin/SCIENCE96/msrch2 (for Human Gene Map)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for primary recessive microcephaly [MIM 251200])

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