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Report

A Third Novel Locus for Primary Autosomal Recessive Microcephaly Maps to Chromosome 9q34

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Summary

Primary autosomal recessive microcephaly is a clinical diagnosis of exclusion in an individual with a head circumference ⩾4 SDs below the expected age-and-sex mean. There is associated moderate mental retardation, and neuroimaging shows a small but structurally normal cerebral cortex. The inheritance pattern in the majority of cases is considered to be autosomal recessive. Although genetic heterogeneity for this clinical phenotype had been expected, this has only recently been demonstrated, with the mapping of two loci for autosomal recessive primary microcephaly: MCPH1 at 8p and MCPH2 at 19q. We have studied a large multiaffected consanguineous pedigree, using a whole-genome search, and have identified a third locus, MCPH3 at 9q34. The minimal critical region is ~12 cM, being defined by the markers cen-D9S1872-D9S159-tel, with a maximum two-point LOD score of 3.76 (recombination fraction 0) observed for the marker D9S290.

Autozygosity mapping has been used to identify a third, novel locus for primary autosomal recessive microcephaly, MCPH3, in a consanguineous family of northern Pakistani origins that has four affected individuals. The family was originally reported by Heney et al. (1992), who described the clinical findings in two Pakistani sisters ages 7 and 8 years (VI:7 and VI:8, respectively; see fig. 1). Both were microcephalic and of low-normal intelligence and were, being educated in a mainstream school. Since publication of the original description of the family, a further two individuals with microcephaly have been ascertained (VI:2 and VI:3; see fig. 1). Both have nonprogressive mental retardation; VI:2 has mild mental retardation, whereas VI:3 has moderate mental retardation but also profound congenital sensorineural deafness and infrequent tonic/clonic fits. One of the previously reported individuals, VI:7, assessed as having low-normal intelligence, is now age 17 years and more clearly has mild mental retardation, evidenced by increasing difficulties with further education, at school and at college. The head circumference of all four individuals was noted to be small at birth and continued to be 6–8 SDs below the expected mean. The other previously reported sister, VI:8, developed acute lymphoblastic leukemia (ALL) and has since died, owing to complications arising from this condition. The three remaining individuals with microcephaly are in good health, have normal growth parameters, and have no other congenital anomalies, neurological deficits, or dysmorphic features. Further recent investigations have shown no metabolic or cytogenetic deficiency, no chromosome breakage (spontaneous breaks and rearrangements, ionizing-radiation sensitivity, mitomycin C sensitivity, and sister-chromatid exchange), and no immunoglobulin deficiency. Neuroimaging was not performed, in light of the good health of the three remaining affected individuals. No maternal or environmental causes could be found to explain the finding of microcephaly in the four affected individuals, and a diagnosis of primary autosomal recessive microcephaly (MIM 251200) was made.

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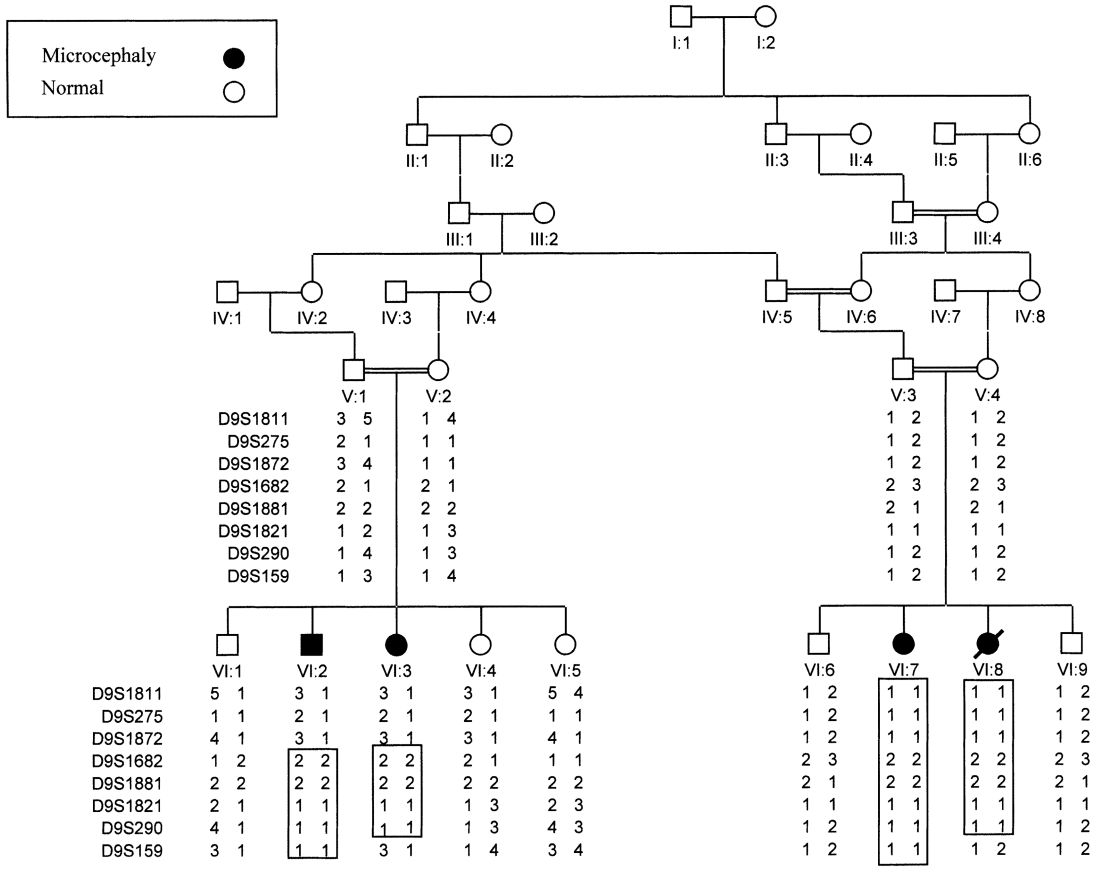

Figure 1 Revised and extended pedigree (see Heney et al. 1992) with primary autosomal recessive microcephaly, showing genotypes for eight microsatellite markers at 9q34. The marker order is cen-D9S1811-D9S159-tel. The disease phenotype for affected individuals is microcephaly. The boxed region indicates the homozygous regions in each affected individual.

After linkage to the MCPH1 locus (Jackson et al. 1998) and the MCPH2 locus (Roberts et al. 1999) was ruled out, a low-resolution whole-genome trawl was performed, with 258 microsatellite DNA markers (Reed et al. 1994). No homozygous regions were identified that were common to the affected individuals with microcephaly. Therefore, a higher-resolution screen was performed, with the ABI Linkage Mapping Panel, version 1 (PE Biosystems), decreasing the average marker spacing to 5–10 cM. The only region of homozygosity identified in all four microcephalic individuals was on chromosome 9, within band q34, with the minimal critical region, of ~12 cM, being defined by the markers cen-D9S1872-D9S159-tel (fig. 1). The MLINK program was used for two-point linkage analysis (Cottingham et al. 1993; Schäffer et al. 1994). A fully penetrant autosomal recessive mode of inheritance and a disease-gene frequency of .003 were assumed for primary microcephaly. Additional polymorphic markers and information regarding marker order and relative distances were obtained from the Center for Medical Genetics, Marshfield Medical research Foundation. Allele frequencies were calculated by genotyping a panel of 30 unrelated individuals from the same ethnic group as that of the research family, with a lower limit, .1, set for allele frequencies. A maximum two-point LOD score of 3.76 at recombination fraction (θ) 0 was observed for marker D9S290 (table 1).

Primary autosomal recessive microcephaly is a clinical diagnosis of exclusion in an individual with a head circumference 3 SDs below the expected age-and-sex mean. There is usually associated mild-to-moderate mental retardation, but no other neurological or significant dysmorphic features, with all discernible maternal, gestational, postnatal, chromosomal, metabolic, or syndromic etiologies being sought and eliminated (Bundey 1997). In the few individuals with primary microcephaly who have undergone neuroimaging, the cerebral cortex is small and structurally normal (Bundey 1997). The inheritance pattern in the majority of cases of primary microcephaly is considered to be autosomal recessive (Sujatha and Kumari 1989) with empirical recurrence

Table 1

Two-Point LOD Scores Calculated on the Basis of Markers Located at 9q34

	LOD Score at θ =						
Marker	.00	.01	.05	.10	.20	.30	.40
D9S1811	$-\infty$	−.07	.82	.95	.67	.31	.08
D9S275	−.06	.45	.79	.75	.47	.18	.01
D9S1872	.68	1.07	1.32	1.20	.72	.27	.03
D9S1682	3.30	3.21	2.83	2.36	1.46	.71	.22
D9S1881	1.63	1.57	1.33	1.05	.53	.16	.00
D9S1821	2.18	2.13	1.93	1.67	1.15	.68	.29
D9S290	3.76	3.66	3.26	2.75	1.76	.89	.30
D9S159	$-\infty$	−.43	.61	.79	.60	.33	.14

[a] Order is cen-D9S1811-D9S159-9qter.

risks of 1/5 for a white family and 1/4 for a consanguineous couple or in the case of a child with symmetrical mirencephaly demonstrated by neuroimaging (Tolmie et al. 1987; Bundey 1997).

In the family that we are reporting, one of four microcephalic individuals (i.e., VI:8) had ALL, whereas the remaining three all are age >18 years and have not developed any tumors. We therefore suggest that ALL is most likely to have occurred by chance, rather than to be due to an underlying predisposition. At present we have not identified any additional families with primary autosomal recessive microcephaly linked to this locus. It would be interesting to know whether the family described by Teebi et al. (1987) has linkage to MCPH3, since it is a consanguineous Arab kindred with autosomal recessive nonsyndromal microcephaly, apparently normal intelligence, and a "peculiar facies" and in whom one child has also developed a leukemia.

It had been expected that more than one gene could cause primary microcephaly, because of both clinical variation between families (Cowie 1960) and the complexity of human brain development. This has recently been confirmed, with the previous identification of two loci—MCPH1 at 8p23 (Jackson et al. 1998) and MCPH2 at 19q13 (Roberts et al. 1999)—and, now, of a third locus, MCPH3.

Of the genes and expressed sequence tags mapped to this region of chromosome 9q34 (GeneMap'99; also see Deloukas et al. 1998), there are two of interest—the PBX3 gene and the C3G gene. The PBX genes (pre-B cell–leukemia transcription factors) are a family of homeodomain proteins that have been shown to modulate the biological activities of the Hox proteins (van Dijk et al. 1995), including transcriptional control during neurogenesis (Knoepfler and Kamps 1997). C3G (also known as "guanine nucleotide–releasing factor 2") has been suggested to be involved with signal transduction from tyrosine kinase receptors to the RAS protein in a number of different tissues (Takai et al. 1994; Tanaka et al. 1994). As such, it may participate in the signaling mechanism used by the neurotrophin/TRK pathway to promote neurone growth and survival. Both the PBX3 gene and the C3G gene are potential candidates for MCPH3 primary microcephaly, although their role in brain formation is not fully defined.

The identification of candidate genes for primary microcephaly is currently hampered by both the lack of animal models of microcephaly and an incomplete understanding of the embryological events occurring during the development of the mammalian cerebral cortex. The six-layered structure of the cerebral cortex is mammalian specific, and in humans the cerebral cortex has reached its largest size, thus far, in comparison with body mass (this process of an increase in the size of the brain is what anthropologists call "encephalization"; the brain volume of *Australopithecus afarenis* was ~500 cc, that of *Homo habilis* was ~800 cc, and that of *H. neanderthalensis* and *H. sapiens* was/is ~1,500 cc). It is therefore likely that the genes in which mutations can cause primary microcephaly have also been involved in the evolutionary development of the current size and cognitive abilities of the human brain. Therefore, it is to be hoped that the future identification of the MCPH3 gene will provide insights into the evolutionary biology of the cerebral cortex, as well as being of clinical use for families with primary microcephaly.

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Electronic-Database Information

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

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics (for genetic linkage maps)

GeneMap'99, http://www.ncbi.nlm.nih.gov/genemap99 (for expressed sequence tags and gene assignments within the critical interval D9S1872-D9S159)

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

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