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ORIGINAL ARTICLE

Autosomal recessive primary microcephaly: an analysis of locus heterogeneity and phenotypic variation

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Background and objectives: Locus heterogeneity is well established in autosomal recessive primary microcephaly (MCPH) and to date five loci have been mapped. However, the relative contributions of these loci have not been assessed and genotype-phenotype correlations have not been investigated.
Design: A study population of 56 consanguineous families resident in or originating from northern Pakistan was ascertained and assessed by the authors. A panel of microsatellite markers spanning each of the MCPH loci was designed, against which the families were genotyped.
Results: The head circumference of the 131 affected subjects ranged from 4 to 14 SD below the mean, but there was little intrafamilial variation among affecteds (± 1 SD). MCPH5 was the most prevalent, with 24/56 families consistent with linkage; 2/56 families were compatible with linkage to MCPH1, 10/56 to MCPH2, 2/56 to MCPH3, none to MCPH4, and 18/56 did not segregate with any of the loci.
Conclusions: MCPH5 is the most common locus in this population. On clinical grounds alone, the phenotype of families linked to each MCPH locus could not be distinguished. We have also shown that further MCPH loci await discovery with a number of families as yet unlinked.

Autosomal recessive primary microcephaly (MCPH) is a rare human disorder in which the cerebral cortex is reduced in size and, as a consequence, the head circumference is reduced.[1 2] MCPH appears to arise through failure of a normal developmental process and not regression or degeneration of established neuronal tissue. The consequence of the disorder is mild to moderate mental retardation without other neurological deficit.[3] In this study, we defined MCPH as: a congenital disorder in which an affected subject has microcephaly of at least –4 SD (measured as occipitofrontal head circumference and corrected for age and sex), and mild/moderate mental retardation, in the absence of any generalised disorder of growth, abnormal neurological findings, fits, or dysmorphic features and in which other syndromic, neurological, chromosomal, metabolic, and maternal/environmental diagnoses were sought and eliminated. When performed, brain neuroimaging showed that the cerebral cortex is small and symmetrical, other areas of the brain are of normal size, and there are no structural anomalies or evidence of abnormal neuronal migration.[1]

While MCPH apparently has a single clinical phenotype, it exhibits genetic heterogeneity.[2] We had previously used four of the study families to identify MCPH gene loci using autozygosity mapping[4]: MCPH1 (family 18), MCPH2 (family 27), MCPH3 (family 1), and MCPH5 (family 38).[5–9] A further locus, MCPH4, was found by Jamieson *et al*[10] in a consanguineous Turkish family.[10] To date, no MCPH gene has been published (see note added in proof).

The aims of this study were threefold: firstly, to document the phenotype of a large number of subjects affected with MCPH, secondly, to determine the relative frequency of each of the five MCPH loci within one ethnic population, and, thirdly, to use the genotype data to seek interlocus and intralocus phenotype differences.

METHODS

Fifty-six consanguineous families of northern Pakistani origin containing one or more members with MCPH were ascertained. Families were referred to the authors from two sources: from paediatricians in Yorkshire, UK (serving a large northern Pakistani population centred upon Bradford) and from paediatricians and Special Schools in Rawalpindi, Islamabad, and Lahore in Pakistan (which is the geographical origin of the majority of Asians in Yorkshire). One of the authors obtained a medical history and family pedigree and examined all potentially affected subjects. Ethical Committee consent for this study has been given.

Family members gave blood samples from which DNA was obtained by standard methods. For each MCPH locus, a panel of microsatellite markers was devised (table 1). Each of the 56 families was genotyped against the five known MCPH loci using these panels. As all of the families are consanguineous, identity by descent could be sought and used to assess evidence of linkage to a particular locus.[11]

RESULTS

In the 56 study families, there were 131 MCPH affected members with a male to female ratio of 70:61. Of these families, 20/56 contained affected subjects in more than one sibship, 22/56 were a single sibship with multiple affected subjects, and in 14/56 a single offspring was affected. All affected subjects were microcephalic at birth with a head circumference between –4 and –14 SD (fig 1). In fifteen of them, the degree of microcephaly at birth could be compared to that at 5-10 years of age and no difference was seen, implying that this is not a progressive condition. There was no sex difference in the degree of microcephaly or the gross cognitive ability. Within multiply affected families, head circumferences usually varied only by ± 1 SD. Where there was a greater variation in head circumference, this was confined to older affected members (>30 years of age) of the family, who had a reduced relative head circumference compared to their younger affected family relatives. All study subjects enjoyed good health, comparable to their unaffected family members. All had normal early motor milestones but some had delay in speech (in acquisition of vocabulary and grammar but without dysarthria), none had

Table 1 MCPH locus information

Primary microcephaly locus	Chromosomal location	Size of locus (genetic)	Markers used (pter flanking - within locus - qter flanking)
MCPH1[5]	8p23.1	13 cM	D8S1824-D8S1798/D8S1819/D8S1825/D8S552/D89S1731-D8S261
MCPH2[6]	19q13	5 cM	D19S569-D19S896/D19S897/D19S200-D19S223
MCPH3[7]	9q34	25 cM	D9S195-D9S1682/D9S1821/D9S290-D9S159
MCPH4[10]	15q14	19 cM	D15S118-D15S1012/D15S994/D15S990-D15S117
MCPH5[8 9]	1q31.3	5 cM	GATA135F02-D1S1726/D1S1723-D1S1647

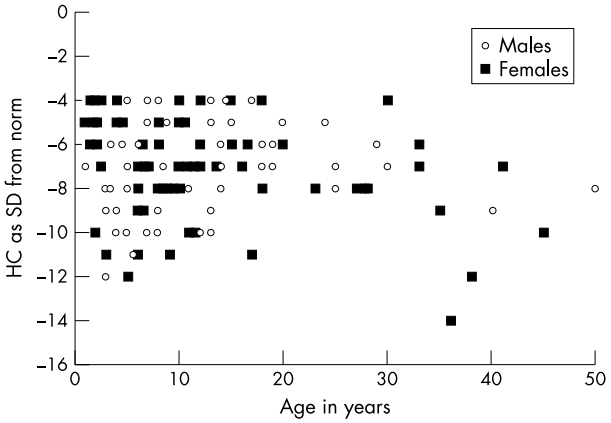

Figure 1 Head circumference measurements of all affected subjects shown as standard deviation from norms by age and sex. Each point represents one or more persons with a diagnosis of autosomal recessive primary microcephaly. The head circumference as a standard deviation from the age and sex norm is shown on the y axis.[12–14] The age of the subjects is shown in years on the x axis. The sex is shown as a circle for a male and a square for a female.

severe or profound mental retardation, and none had epilepsy. Most were physically able and the majority were personable, biddable, and trainable in life skills. Some had "sloping foreheads", but within a family this was an inconsistent feature.[1] All parents were of normal physical and mental ability and none had microcephaly.

The genotype results are reported in table 2: 2/56 families were consistent with linkage to MCPH1 (4%), 10/56 to MCPH2 (18%), 2/56 to MCPH3 (4%), none to MCPH4, and 24/56 to MCPH5 (43%). In two multi-affected families likely to be linked to MCPH5, a subject was homozygous for a smaller region while carrying the larger family haplotype. We have used these results to refine this locus further. One singleton was homozygous for all markers for both the MCPH2 and MCPH3 loci. In 18/56 families (32%), there was no definite evidence of linkage to one of the five known loci. However, three of these families had affected subjects with a concordant haplotype for MCPH3 markers (that is, the same heterozygous allele results for all polymorphic microsatellite markers assessed) suggesting the possibility of linkage to a smaller homozygous region contained within this haplotype.

Bearing in mind the possibility of a child of first cousin parents carrying a homozygous region by descent rather than as the disease allele is 1 in 16, whereas in multi-affected pedigrees this chance decreases, and that clinical misdiagnosis must always be considered in a "diagnosis of exclusion" such as MCPH, and such phenocopies are more likely in single affected families,[3] then, excluding singletons from the analysis, 2/42 families are compatible with linkage to MCPH1 (5%), 6/42 to MCPH2 (14%), 1/42 to MCPH3 (2%), none to MCPH4, 19/42 to MCPH5 (45%), and 14/42 families (33%) did not segregate with any of the loci. This suggests that inclusion of singletons in our analysis was not a major cause of error. All individual head circumferences and the mean for each family were analysed against the MCPH loci genotype data and there was no discernable difference (table 3).

DISCUSSION

Given the large number of disorders that cause "mental retardation with microcephaly", it is important from a clinical perspective to attempt to split such a broad phenotypic category into more discrete entities. To this end we report a large cohort of subjects in whom we have made a clinical diagnosis of autosomal recessive primary microcephaly (MCPH). Supporting the definition of MCPH as a clinical neurodevelopmental disorder is both the numbers of cases we have amassed and the fact that genetic linkages for MCPH loci have been identified. We suggest that autosomal recessive primary microcephaly may be a more exact and useful term than others in use, for example, "true microcephaly", "recessive microcephaly", or "microcephaly vera".[3] Certainly, in our study population the "true microcephaly" phenotype with the sloping forehead was not a consistent finding in subjects affected with MCPH. It has also been suggested that carriers of MCPH have diminished intelligence as a heterozygote effect,[15] though we found no evidence of this.

In conclusion, we suggest that MCPH can be defined as a clinical entity. It was considered that it would exhibit genetic heterogeneity[16 17] and this has now been confirmed, with five MCPH loci reported to date.[2] We have established the relative incidence of each locus in the northern Pakistani population and that MCPH5 is the most common in this ethnic group. Interestingly, none of our northern Pakistani families link to the MCPH4 locus,[10] which was identified in a Turkish population. Following genotyping of the study families, we were unable to differentiate clinically between families linked to

Table 2 Study family data summary

Pedigree No*	Mean head circum-ference†	No of affecteds in each sibship‡	Loci that were homozygous or concordant for each pedigree§
1	–7	2+2	MCPH3
2	–5	1+1	MCPH5
3	–4	4	None
4	–4	2	MCPH5
5	–11	1	MCPH5
6	–6	2	MCPH2
7	–4	1	MCPH3
8	–7	1	MCPH2
9	–6	1	MCPH5
10	–4	2+1	MCPH2
11	–12	1	MCPH2
12	–11	3	MCPH5
13	–6	2	None
14	–4	2+1	None
15	–6	2	MCPH5
16	–7	2+1	MCPH5
17	–4.5	2	None
18	–7	2+2+1	MCPH1
19	–7.5	2	MCPH5
20	–8.5	2	MCPH5
21	–8	3	MCPH5
22	–8	1+1	MCPH5
23	–6	3	Concordant for MCPH3
24	–10	2	MCPH1
25	–6	2+1	MCPH2
26	–5	1	MCPH5
27	–6.5	2+2+1+1	MCPH2
28	–7.5	1+1	None
29	–10	1	MCPH5
30	–9	1+1+2+3+1	MCPH2
31	–4	3+1+1	None
32	–5	2	Concordant for MCPH4
33	–10	2+1	MCPH5
34	–6	2	Concordant for MCPH3
35	–7.5	2	MCPH2
36	–9	2+2	None
37	–5	3+1+1	None
38	–9	1+1+1	MCPH5
39	–8	2	None
40	–4.5	1+1	None
41	–9.5	2	MCPH5
42	–10	1	MCPH5
43	–6	2	Concordant for MCPH3
44	–6	3+1	MCPH5
45	–5	1	None
46	–9	2	MCPH5
47	–6	3	MCPH5
48	–6	2	MCPH5
49	–5	1	MCPH2 and MCPH3
50	–6	1	MCPH2
51	–4	1	None
52	–5	1	None
53	–4	1	MCPH2
54	–6	2	MCPH5
55	–9	2+1	MCPH5
56	–9	1+5+4+2	MCPH5

*The families are numbered sequentially based upon their chronological ascertainment into the study.
†The mean head circumference for the affected subjects in each family is given as a standard deviation from the age and sex norm.
‡The number of affected subjects is given by sibships, for example, two sibs and a cousin would be represented as "2+1", and only includes those assessed as affected by the authors and in whom genotyping was performed.
§The MCPH locus to which each family is most likely to be linked was derived from the genotyping results as follows: if all affected member(s) of a family had the same homozygous haplotype for a MCPH locus the family was designated linked; if the affected members had different heterozygous marker results or different homozygous haplotypes the family was regarded as not linked; and if all affected subjects had identical concordant, but heterozygous, haplotypes the family were not regarded as proven linked to the locus but noted as concordant.

Table 3 Mean head circumference measurement for families linked to each MCPH locus. The mean head circumference is corrected for age and sex. Only families with >1 affected member are included to reduce the effect of incorrect clinical diagnosis. MCPH4 is not shown as none of the study families were linked. "Unknown MCPH" includes all families not linked to a known MCPH locus

MCPH locus	No of multi-affected families	Mean head circumference (SD (range))
MCPH1	2	–8.5 (–7 to –10)
MCPH2	6	–6.5 (–4 to –9)
MCPH3	1	–7
MCPH5	19	–7.5 (–4 to –11)
Unknown MCPH	14	–5.5 (–4 to –9)

the five MCPH loci. We have also shown that further MCPH loci await discovery with a number of families as yet unlinked. It seems likely that determination of the MCPH genes will give valuable insights into normal brain development (and possibly the evolutionary process of encephalisation, where the surface area of the cerebral cortex has tripled in size from primates to man[18]), as well as providing new therapeutic options for affected families.

NOTE ADDED IN PROOF

The MCPH1 gene has now been identified.[19]

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ECHO

Variant of endothelial nitric oxide synthase gene determines severity of atherosclerosis

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A common variant of an enzyme which produces nitric oxide in endothelial cells may in future become a genetic marker for susceptibility to atherosclerosis.

Researchers in Italy have shown for the first time that the polymorphic Glu→Asp variant of endothelial nitric oxide synthase (eNOS) is linked to the extent and severity of coronary artery disease (CAD).

Of the three possible eNOS genotypes, the Asp/Asp homozygous genotype had a higher frequency in patients with CAD than controls (15.9% *v* 6.1%) whereas the Glu/Glu and Glu/Asp genotypes did not. The likelihood of having the Asp/Asp genotype and CAD was 2.9 (95% confidence interval 1.2 to 6.8) when compared with the other genotypes combined. Multiple regression showed that Asp/Asp was an independent risk factor for CAD. This genotype had no relation with other known risk factors but was significantly associated with number of narrowed vessels (mean (SEM) 2.3 (0.1) for Asp/Asp *v* 1.9 (0.1) for Glu/Glu, and 1.8 (0.1) for Glu/Asp, respectively) and higher Duke score, denoting severity of CAD (mean (SEM) 56.1(3.1), 46.7(2.0), and 46.1(1.9), respectively).

The study population included 201 patients with CAD, who had more than 50% narrowing of more than one vessel on angiography, and 114 controls with no CAD awaiting valve replacements. Amplification of DNA by polymerase chain reaction and restriction fragment length polymorphism (RFLP) analysis were used to ascertain eNOS genotype.

Nitric oxide in endothelium inhibits many pathogenetic mechanisms in atherosclerosis, and the Glu^{298}→Asp polymorphism has previously been reported in CAD and myocardial infarction.

▲ *Heart* 2002;**87**:525–528.