Primary microcephaly: *microcephalin* and *ASPM* determine the size of the human brain

In microcephaly (small head), the size of the head as measured by the occipito-frontal circumference of an affected individual is greater than three standard deviations below the population age-related mean. The cranial vault in a microcephaly patient is smaller than normal relative to the facial skeleton and the rest of the body. The small cranial capacity results from underlying hypoplasia of the cerebral cortex rather than abnormal development of the overlying skull and there is no major abnormality in cortical architecture (Jackson *et al* 1998; Mochida and Walsh 2001). Microcephaly is known to have a heterogeneous etiology with environmental and genetic causes. Among the environmental causes are intrauterine infections, drugs (alcohol) taken during pregnancy, prenatal radiation exposure, maternal phenylketonuria and birth asphyxia. All of these except birth asphyxia are known to be rare causes of microcephaly. The majority of microcephalic cases are caused by a variety of genetic mechanisms including cytogenetic abnormalities and single-gene disorders (Jackson *et al* 1998).

Primary or true microcephaly or microcephaly vera (MCPH; OMIM 251200) is a distinct subtype that is defined by the absence of associated malformations and of secondary or environmental causes. It is inherited as an autosomal recessive trait and has an incidence of 1/30,000 to 1/250,000 live births in western populations. The actual incidence of microcephaly is not known in India. Mental retardation in primary microcephaly ranges from mild to severe, but other neurological deficits are absent. Brain weight in a primary microcephaly patient is typically 430 g compared with 1,450 g in a normal male, and the cerebral cortex is disproportionally small, although the gyral pattern is relatively well preserved with no abnormality in cortical architecture (Jackson *et al* 2002; Mochida and Walsh 2001). Microcephaly is diagnosed after exclusion of (1) craniosynostosis (premature fusion of skull sutures), (2) microcephaly occurring as a part of a malformation syndrome (e.g. Cri-Du-Chat syndrome), and (3) known causes of secondary microcephaly (e.g. birth asphyxia). Prenatal diagnosis of microcephaly by serial ultrasonographic measurement of foetal head circumference has not been reliable until the third trimester. Therefore, the identification and characterization of the gene(s) responsible for microcephaly is important for both genetic counseling and prenatal diagnosis (Jackson *et al* 1998).

Mapping of primary microcephaly has been problematic due to unavailability of families with multiple affected individuals. Using homozygosity mapping, Jackson *et al* (1998) were first to map a locus (MCPH1) for primary microcephaly on chromosome 8p22-pter in two families from the Mirpur region of Pakistan with five and two affected individuals respectively. Homozygosity mapping efficiently maps recessive conditions in consanguineous families (here 'homozygosity' is a term used to mean homozygosity of markers identical by descent). Individuals with recessive diseases in consanguineous families are likely to be homozygous (autozygous) for markers linked to the disease locus. By combining the haplotype data from both families, Jackson *et al* (1998) mapped the candidate region for the MCPH1 locus to a 13 cM region between D8S1824 and D8S1825. Subsequently four more loci for primary microcephaly were identified: MCPH2 on chromosome 19q13·1–13·2 mapped in two families from the northern region of Pakistan; MCPH3 on chromosome 9q34 mapped in a single family also from the northern region of Pakistan; MCPH4 on chromosome 15q in a single family from Morocco; and MCPH5 on chromosome 1q25–q32 mapped in two families from Turkey and Multan, Pakistan (Jamieson *et al* 1999; Roberts *et al* 1999; Jamieson *et al* 2000; Moynihan *et al* 2000; Pattison *et al* 2000).

We have collected seventeen families with primary microcephaly from the state of Karnataka in India and 11 families have been genotyped so far using markers from the candidate regions of each of

the five known loci (unpublished data). None of the cases in our families appear to be linked to the MCPH1 locus. In three families the trait showed linkage to MCPH5. Linkage could not be established in other families because of small sizes of the families. There is a possibility of there being a sixth locus for microcephaly as one of our families with three affected individuals did not show linkage to any of the known five loci (unpublished data). Similar observations have been made by others (Roberts *et al* 2002).

As both of the MCPH1 locus linked families originated from the same geographical region of Pakistan and shared a common allele at one (D8S1798) of the nine markers genotyped, Jackson *et al* (2002) hypothesized that both families might have a common ancestral origin of the disease. Jackson *et al* (2002) genotyped both families again with additional markers from the homozygous region and identified an ancestral haplotype of nine markers shared by all affected individuals from both families. This refined the critical region for MCPH1 to a 2·1 mb interval between the markers AC019176CA17 and D8S277. Two genes were identified in this interval, angiopoetin-2 (*ANGPT2*) and an uncharacterized gene, *AX087870*. Sequence analysis of *ANGPT2* did not reveal any mutation in patients, where as it revealed a C → G change at nucleotide position 74 in exon 2 of the *AX087870*, creating a premature stop codon at amino acid position 25 (S25X). As expected the S25X mutation was found in all the affected individuals from both families; parents were heterozygous for this mutation. The mutation was not detected in 202 Pakistani control alleles. The *AX087870* gene was named appropriately as *microcephalin*. This mutation truncated the 835 amino acid long microcephalin protein to a 25 amino acid-long protein, suggesting that microcephaly could be caused by the loss of function of microcephalin. The absence of MCPH1-linked families in our data set suggests that microcephaly families with mutation(s) in *microcephalin* may not be all that common (our unpublished data; Roberts *et al* 2002).

Microcephalin has 14 exons and shows 57% sequence identity with a mouse protein of 822 amino acids. *Microcephalin* is expressed in several organs including kidneys, heart, lungs, spleen, thymus and skeletal muscles in addition to the brain. *In situ* hybridization showed that *microcephalin* is expressed in the developing forebrain and in particular in the walls of the lateral ventricles (Jackson *et al* 2002). This is the area where progenitor cells divide to produce neurons that migrate to form the cerebral cortex, suggesting that the gene has a role in neurogenesis and in regulating the size of the cerebral cortex (Jackson *et al* 2002). In other words, the expression pattern of *microcephalin* strongly suggests its role in the disease process. However, it is not clear what could be the role of microcephalin in organs other than the brain where it is also expressed? Or why is the phenotype restricted only to the brain although it is expressed in other organs also?

Microcephalin has three BRCT (BRCA1 C-terminal) domains. BRCT domains of human microcephalin show 80% sequence identity with the BRCT domains in mouse protein. BRCT domains are known to be present in several key proteins controlling the cell division cycle in bacteria to higher organisms (examples are the breast cancer gene *BRCA1*, terminal deoxynucleotidyl transferase (TdT), *Escherichia coli* NAD + dependent DNA ligase, DNA ligase III, DNA topoisomerase II binding protein and several others (Huyton *et al* 2000). Based on this observation, Jackson *et al* (2002) have suggested that the mutation in *microcephalin* may cause primary microcephaly by perturbing normal cell cycle regulation in neural progenitors.

Recently the same group which isolated the MCPH1 gene has also identified the gene for the MCPH5 locus located on chromosome 1q31 by noticing a region of 600 kb in which one haplotype was shared between three different families and a second haplotype was shared by three other families, suggesting common ancestral origins (Bond *et al* 2002). This region contained four potential candidate genes and one gene, *ASPM (*abnormal spindle-like microcephaly associated) was found to be mutated in four families. The four homozygous mutations detected in this gene were: 719–720delCT in exon 3, 1258–1264delTCTCAAG in exon 3, 7761T → G (nonsense mutation) in exon 18, and 9159delA in exon 21 (Bond *et al* 2002). All four mutations are predicted to produce truncated ASPM proteins. The *ASPM* gene codes for a 10,434 bp long transcript with 28 exons and spans 62 kb of genomic DNA. ASPM contains four regions: a putative N-terminal microtubule-binding domain, a putative calponin-homology domain, multiple calmodulin-binding IQ domains and a terminal region (Bond *et al* 2002). Interspecies comparisons of ASPM proteins have shown an overall conservation, but also a consistent correlation of greater protein size with larger brain size (Bond *et al* 2002). The

increase in protein sizes across the species is due mainly to the number of IQ repeats. For example, the asp protein of *Caenorhabditis elegans* has 2 IQ repeats, *Drosophila melanogaster* has 24 IQ repeats, mouse has 61 IQ repeats and in humans the IQ repeat size is 74 (Bond *et al* 2002).

Aspm is expressed at embryonic day 11–17 and shows preferential expression during cerebral cortical neurogenesis, especially in the cerebral cortical ventricular zone at embryonic day 14·5 and embryonic day 16·5 (Bond *et al* 2002). Fetal expression was found to be greatest in the ventricular zones, which contain the progenitor cells for cerebral cortical pyramidal neurons (Bond *et al* 2002). *Aspm* expression was greatly reduced by postnatal day 0 (day of birth), when neurogenesis in the cortical ventricular zone is completed and gangliogenesis is increasing, suggesting that *Aspm* was preferentially expressed in progenitor cells which give rise to neurons rather than glia (Bond *et al* 2002). These expression data suggest a preferential role for *Aspm* in regulating neurogenesis. The *D. melanogaster* ortholog for human *ASPM*, abnormal spindle gene (*asp*), is known to be essential for both the organization and binding together of microtubules at the spindle poles and the formation of the central mitotic spindle during mitosis and meiosis. *Asp* mutations cause dividing neuroblasts to arrest in metaphase, resulting in reduced central nervous system development (see for references in Bond *et al* 2002). This suggests that mutations in *ASPM* might be operating in a similar fashion for the reduced nervous system development of the human brain leading to microcephaly.

No clinical difference exists among patients linked to either of the known five MCPH loci. This may mean that the five MCPH genes work in the same pathway involved in neuronal cell-cycle regulation. However, the genes for other three loci have not been isolated and characterized yet. Moreover, no functional overlap can be discerned at present between microcephalin and ASPM proteins as microcephalin is predicted to be involved in cell-cycle regulation and ASPM in modulation of mitotic spindle activity in neuronal progenitor cells. The identification of other three MCPH genes (and possibly some more) and their roles, and an understanding of the precise functional roles of microcephalin and ASPM proteins are awaited. When that is done, it may be possible to determine the pathway(s) in which these genes work and learn about the complex process of cortex development in humans and possibly other organisms. Further, this work will also be facilitated by the production of knock-out mice models for the genes involved in the microcephaly phenotype.

Acknowledgements

Financial support from the Sir Dorabji Tata Center for Tropical Diseases (PC11014), Indian Institute of Science, Bangalore is gratefully acknowledged.

References

Bond J, Roberts E, Mochida G H, Hampshire D J, Scott S, Askham J M, Springell K, Mahadevan M, Crow Y J, Markham A F, Walsh C A and Woods C G 2002 ASPM is a major determinant of cerebral cortical size; *Nat. Genet.* **32** 316–320

Huyton T, Bates P A, Zhang X, Stenberg M J and Freemont P S 2000 The BRCA1 C-terminal domain: structure and function; *Mutat. Res.* **460** 319–332

Jackson A P, Eastwood H, Bell S M, Adu J, Toomes C, Carr I M, Roberts E, Hampshire D J, Crow Y J, Mighell A J, Karbani G, Jafri H, Rashid Y, Mueller R F, Markham A F and Woods C G 2002 Identification of microcephalin, a protein implicated in determining the size of the human brain; *Am. J. Hum. Genet.* **71** 136–142

Jackson A P, McHale D P, Campbell D A, Jafri H, Rashid Y, Mannan J, Karbani G, Corry P, Levene M L, Mueller R F, Markham A F, Lench N J and Woods C G 1998 Primary autosomal microcephaly (MCPH1) maps to chromosome 8p22-pter; *Am. J. Hum. Genet.* **63** 541–546

Jamieson C R, Fryns J P, Jacobs J, Matthijs G and Abramowicz M J 2000 Primary autosomal recessive microcephaly: MCPH5 maps to 1q25–1q32; *Am. J. Hum. Genet.* **67** 1575–1577

Jamieson C R, Govaerts C and Abramowicz M J 1999 Primary autosomal recessive microcephaly: homozygosity mapping of MCPH4 to chromosome 15; *Am. J. Hum Genet.* **65** 1465–1469

Mochida G H and Walsh C A 2001 Molecular genetics of human microcephaly; *Curr. Opin. Neurol.* **14** 151–156

Moynihan L, Jackson A P, Roberts E, Karbani G, Lewis I, Corry P, Turner G, Mueller R F, Lench N J and Woods C G 2000 A third locus for primary autosomal recessive microcephaly maps to chromosome 9q34; *Am. J. Hum. Genet.* **66** 724–777

Pattison L, Crow Y J, Deeble V J, Jackson A P, Jafri H, Rashid Y, Roberts E and Woods C G 2000 A fifth locus for primary autosomal recessive microcephaly maps to chromosome 1q31; *Am. J. Hum. Genet.* **67** 1578–1580

Roberts E, Hampshire D J, Pattison L, Springell K, Jafri H, Corry P, Mannon J, Rashid Y, Crow Y, Bond J and Woods C G 2002 Autosomal recessive primary microcephaly: an analysis of locus heterogeneity and phenotypic variation; *J. Med. Genet.* **39** 718–721

Roberts E, Jackson A P, Carradice A C, Deeble V J, Mannan J, Rashid Y, McHae D P, Markham A F, Lench N J and Woods C G 1999 The second locus for autosomal recessive primary microcephaly (MCPH2) maps to chromosome 19q13·1–13·2; *Eur. J. Hum. Genet.* **7** 815–820

ARUN KUMAR*
M MARKANDAYA
S C GIRIMAJI**
Department of Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore 560 012, India

***Department of Psychiatry, National Institute of Mental Health and Neurosciences, Bangalore 560 012, India*

**(Email, karun@mrdg.iisc.ernet.in)*