Abnormal spindle-like microcephaly gene detection in an autosomal recessive microcephalic Saudi patient with attention deficit hyperactivity disorder and mental retardation

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Human autosomal recessive primary microcephaly (MCPH) is failure of normal fetal brain development, resulting in microcephaly (MIC) and mental retardation. It is caused by an abnormal spindle-like microcephaly (ASPM) gene mutation. It is a heterogeneous disorder, with at least 7 genetic loci. A mutation of the ASPM gene at the MPCH5 locus is the most common cause of MCPH. The ASPM associated protein is known as abnormal spindle protein or ASP Homolog. It is encoded by the ASPM gene, and is located on the long arm of chromosome 1, band 3, sub-band1 (1q31). The expressed protein product of the ASPM gene is essential for normal mitotic spindle function in embryonic neuroblasts.

A 6-year-old Saudi girl presented to the outpatient department with epilepsy, cognitive decline, and hyperactivity. She had a history of 2 febrile seizures at age 9 months and 24 months consecutively followed by afebrile seizures at age 33 months. The seizures were mainly right sided clonic, with secondarily generalization, lasting more than one minute, with up rolling of eyes and oral cyanosis. They used to occur during sleep, and recurred up to 20 times per day. She was born second in order, from a consanguineous marriage after a full term pregnancy through a normal vaginal delivery. Birth weight was 2.75kg, with head circumference (HC) 30 cm, below the 3rd centile. The father's HC is 59 cm, and mother's HC is 55cm. Both at the 50th centiles. There is a strong family history of MIC in the younger brother and 2 paternal second cousins. The Apgar score at birth was within normal range, and neonatal history was uneventful. The child had normal gross motor development, but fine motor delay. Her speech was delayed; as she did not develop more than 2 words. Hearing and vision was intact. She was delayed socially with no symbolic play at 24 months. She was diagnosed with attention deficit hyperactivity disorder (ADHD) at the age of 4.5 years. On examination her HC was 43.1 cm at 33 months, below the 5th centile. Vital signs were stable. There were no neurocutaneous stigmata or dysmorphic features. Neurological examination showed intact cranial nerves, with equally reactive rounded pupils, normal fundi, with no restriction of eye movements, and symmetric face. Mouth opening and closure was normal. She had a normal response to sounds, turning her head to both sides without difficulty. The tongue was normal and in the midline. Bulk, tone, power, and reflexes were normal. The back was normal. All other systems were unremarkable. Renal, liver function, and electrolytes were normal. The serum amino acids and urinary organic acids were unremarkable. Her EEG was reported as left sided frontal spike and wave discharges. The brain MRI was unremarkable (**Figure 1**). On formal neurocognitive testing, her intelligence quotient was 62. Chromosomal karyotyping was unremarkable. A DNA genetic study was sent to assess the microcephalic gene, and the ASPM gene was detected. The cytosine nucleotide was replaced by a thymine nucleotide at position 8017 in a homozygous state (the resultant amino acid changed from glutamine to STOP

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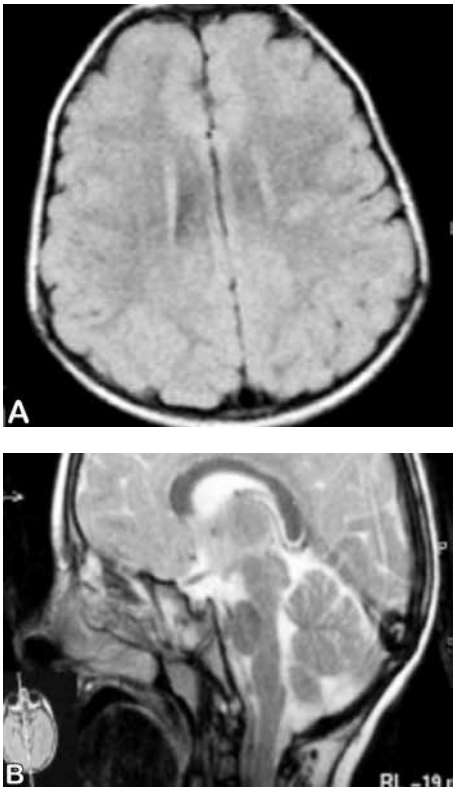

Figure 1 - T2 MRI **A**) axial and **B**) sagittal views of the brain of the child, which was essentially normal.

codon). Seven sequence polymorphs were discovered in the case. The c. 8017 C>T (p. Q2673X) mutation identified in the homozygous state in the ASPM gene. Polymorphism IV87+112G>T, IVS13-83A>G, c.4449A>G, c.5961A<G, C.7674>T, c7684c>a, IV19-42G>A. Valproic acid (VPA) and L-carnitine were started at age 33 months. Seizures were fairly well controlled. After psychiatric evaluation, the child was diagnosed as having ADHD at age 4.5 years. Speech therapy was started.

Microcephaly denotes a cranium that is significantly smaller than the standard for the individual's age and gender. It should be considered as a neurologic sign instead of a disorder. Microcephaly is usually defined as occipitofrontal circumference (OFC) –that is more than 2 standard deviations (SD) below the mean for age and gender. However, because this criterion includes many developmentally normal individuals, researchers usually define severe MIC as OFC more than 3 or 4 SD below the mean. Microcephaly can be caused by many different underlying etiologies. Generally, the etiology is divided into genetic causes of MIC, and nongenetic causes. In "genetic" MIC, failure of brain growth is determined by intrinsic genetic information. This category includes chromosomal disorders, and many syndromes. In "nongenetic" MIC, the brain fails to grow after a certain point because of external insults; for example, infections, intrauterine drug or toxin exposure, and hypoxic-ischemic injury. Seven genetic loci and 6 genes for autosomal recessive MIC have been identified using linkage analysis. These loci, termed MCPH1 through MCPH7, map to chromosomes 8p22-pter, 19q13.1-13.2, 9q34, 15q15-q21, 1q25-32, 13q12.2, and 1p32.[1-3] The genes that have been discovered all play a role in cell division, and cell cycle regulation. Hereditary MIC can be seen in autosomal recessive, autosomal dominant, and X-linked recessive inheritance. Also, it can occur as a sporadic case. When a genetic form of MIC is suspected in a first affected child, it is often difficult to determine the pattern of inheritance if the parents are unrelated. There is no reliable way to distinguish different modes of inheritance based on clinical phenotype, unless there are features of a certain syndrome associated with MIC.

It was not until the late 19th century that MIC started to attract scientists' attention. Many theories concerning the pathogenesis of MIC have been proposed since that time. Some authors viewed MIC as a form of atavism, and others thought that it was due to mechanical compression of the fetal brain by contraction of the uterus. Many different classifications and terminologies of MIC have emerged. Genetic and nongenetic MIC is an etiological classification. Primary MIC is often used synonymously as congenital MIC, in which MIC is present at the time of birth. In contrast, secondary MIC or acquired MIC is used when the HC is normal at birth, and subsequently falls into the microcephalic range. Approximately 1% of referrals to child neurologists are specifically for evaluation of MIC, and approximately 15% of children referred to child neurologists for evaluation of developmental disabilities have MIC.

Primary MIC is a term that describes a group of disorders, many with etiologies not yet known. The clinical manifestations associated with MIC are remarkably heterogeneous including obvious small head size, sloping forehead, and a flat occiput. Cognitive impairment varies between moderate to severe and profound in others. Recognition of the relation between MIC and mental retardation has been reported since the late 1800s. There is a 50% increased risk for development delay in children with MIC compared with children without MIC (for example, 15.3 versus 7 percent), and a strong correlation between the severity of MIC and developmental outcome.

In one study,[4] 49.9% of 66 children with MIC (≤2 SD) had epilepsy. Epilepsy is more common in postnatal-onset than in congenital MIC. Severe primary MIC is usually due to autosomal recessive inheritance, while the autosomal dominant form is associated with mild MIC. Abnormal spindle-like MIC is intimately linked to both evolutionary increases and decreases in brain size in anthropoids, and is a key target for natural selection acting on brain size.[5] Mutations in ASPM are the most common cause of autosomal recessive primary microcephaly (MCPH).[6] Reduced HC at birth with variable degrees of mental retardation is encountered in MCPH, a rare disorder of neurogenic mitosis. Seven genetic loci and 6 genes for autosomal recessive MIC have been identified using linkage analysis (see above). Primary MIC patients are phenotypically indistinguishable. The mechanisms by which mutations in MCPH genes cause the disease phenotype are possibly: 1) disturbing the orientation of mitotic spindles, 2) alteration of chromosome condensation mechanism during embryonic neurogenesis, 3) DNA damage-response signaling, 4) alteration of transcriptional regulations and microtubule dynamics, and 5) disturbing unknown centrosomal mechanisms controlling the number of neurons generated by neural precursor cells.[3] Our patient had MCPH, mild mental retardation, ADHD, epileptic seizures fairly responsive to VPA, and confirmed ASPM gene mutation.

In conclusion, in our patient autosomal recessive inheritance is most likely due to the presence of parental consanguinity, as well as MIC in the younger brother

and 2 paternal second cousins. The genetic analysis confirmed the mutation of the ASPM gene.

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