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Short clinical report

MCPH1 deletion in a newborn with severe microcephaly and premature chromosome condensation

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ABSTRACT

A newborn with severe microcephaly and a history of parental consanguinity was referred for cytogenetic analysis and subsequently for genetic evaluation. While a 46,XY karyotype was eventually obtained, premature chromosome condensation was observed. A head MRI confirmed primary microcephaly. This combination of features focused clinical interest on the *MCPH1* gene and directed genetic testing by sequence analysis and duplication/deletion studies disclosed a homozygous deletion of exons 1–11 of the *MCPH1* gene. This case illustrates a strength of standard cytogenetic evaluation in directing molecular testing to a single target gene in this disorder, allowing much more rapid diagnosis at a substantial cost savings for this family.

1. Introduction

Microcephaly is typically defined as a head circumference of less than three standard deviations below the mean for age and gender-matched peers [1]. The birth prevalence of congenital microcephaly ranges from 1.4 to 150 per 100,000 births [2]. This wide range reflects a higher prevalence of isolated severe microcephaly among communities with a greater frequency of consanguinity, underscoring the contribution of autosomal recessive genetic conditions to this phenotype [3].

The diagnosis of primary microcephaly applies to cases in which a small head size is present at birth, while secondary microcephaly typically becomes evident postnatally. The majority of cases of microcephaly are attributable to genetic or syndromic causes, while the remaining cases are generally linked to environmental insults, such as prenatal exposure to teratogenic agents or postnatal insults including illness or nutritional deficiency. Syndromic causes of microcephaly are often accompanied by dysmorphic features beyond the cranium, while autosomal recessive primary microcephaly (MCPH) often presents as isolated, or non-syndromic microcephaly [4]. The observation of severe congenital microcephaly (MIC) [5] is significant due to a strong association with intellectual disability, as well as a high likelihood for underlying genetic or syndromic etiology.

The first identified patients with an MCPH syndrome also presented with short stature, and demonstrated a cytological phenotype of premature chromosome condensation (PCC). The defective gene was subsequently identified as *MCPH1* (also known as *BRIT1*)

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[6]. In addition to *MCPH1*, nine other loci have been mapped and eight genes have been characterized in families with MCPH [7], but MCPH1 syndrome remains the only genetic primary microcephaly syndrome with PCC as a defining feature.

We report a case involving severe microcephaly, presenting at birth, in a child born to consanguineous parents. The consanguinity raised the question of an autosomal recessive primary microcephaly, for which there may be defects in one of several known genes, and for which multi-gene panel testing is often the approach of choice. The observation of PCC during a routine cytogenetic evaluation and subsequent cooperation between clinical and laboratory personnel turned the focus in this case to the *MCPH1* gene, thus highlighting the value of clinical cytogenetic testing in directing clinical laboratory evaluation and diagnosis of this single gene disorder.

2. Methods

2.1. Cytogenetics

Cytogenetic preparations were obtained from peripheral blood collected with sodium heparin and cultured in RPMI using standard cytogenetic protocols. One culture was harvested at 42.5 h to obtain a STAT chromosome preparation, and two cultures were harvested after 68 h, following standard methotrexate synchronization to produce high resolution chromosome preparations. The mitotic index was estimated by two persons, who counted a total of 300 metaphase spreads, tightly packed or non-spread metaphase cells, and interphase nuclei. A mitotic fraction was calculated as a percentage of mitotic cells (# metaphase cells/[# interphase cells + # metaphase cells]*100%). A contemporaneous and identically processed STAT newborn specimen was evaluated as a control.

2.2. Molecular genetic analysis

Genomic DNA was isolated from blood leukocytes using a Gentra® PureGene DNA Isolation Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. PCR primers were designed for amplification of all coding exons and flanking intronic sequences of *MCPH1* (RefSeq NM_024596) and are available upon request. PCR products were sequenced using the Big Dye terminator version 3.1 chemistry on an ABI 3730 XL Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). Sequences were compared to the *MCPH1* reference sequence using Mutation Surveyor software version 3.01 (SoftGenetics, State College, PA).

2.3. Targeted array-comparative genomic hybridization

Deletion and duplication of the *MCPH1* gene was performed using a high resolution, custom-designed, exon targeted 8 × 60 K array-CGH platform (Agilent Technologies, Santa Clara, CA). A total of 2333 probes spanning the *MCPH1* gene and flanking regions were present on this design. Genomic DNA samples of the patient and a gender-matched control were processed and co-hybridized onto microarray slides according to the manufacturer recommended procedures (Agilent Technologies). Microarray images were scanned at 2 micron resolution, the data was extracted using ImaGene (9.0) and analyzed using the Nexus software (6.0) (BioDiscovery, Hawthorne, CA). The genomic copy number was defined by analysis of the normalized log2 (Cy5/Cy3) ratio average of the CGH signal. Regions that reached a threshold of at least −0.32 for four or more consecutive probes were considered to be consistent with deletion.

2.4. Whole genome oligonucleotide microarray analysis

Microarray analysis was performed using a custom-designed whole genome oligonucleotide array consisting of 135,000 oligonucleotide probes (NimbleGen CGX-3, Roche NimbleGen, Madison, WI). Arrays were scanned using a GenePix 4000B microarray scanner (Molecular Devices, Sunnyvale, CA) and analyzed using Agilent Feature Extraction paired with Agilent CGH Analytics software by Agilent Technologies. Array results were displayed using Genoglyphix v2.8 software (Signature Genomics Laboratories/Perkin Elmer). Criteria for the detection of copy number gain or loss consisted of five consecutive probes demonstrating a log2 ratio of 0.5 or −0.5 respectively, in a single hybridization experiment. Data analysis was based on information from the UCSC Genome Browser (hg 18, March 2006 NCBI build 36.1).

3. Results

3.1. Clinical findings

The patient presented as a one-day-old Asian Indian male, born at full term to a married, 22-year-old, G1P0->1 female. The parents are first cousins, and the mother's parents are also first cousins. The mother reportedly received standard prenatal care, including an ultrasound evaluation that was non-concerning. The pregnancy was complicated by two urinary tract infections, one requiring hospitalization for intravenous antibiotic treatment. Exposure to known teratogenic agents, vaginal bleeding, and preterm labor were all denied. Delivery was by cesarean section due to failure to progress, and Apgar scores were reportedly normal. Birth weight and length were average, at 3.63 kg and 50.3 cm respectively. Severe microcephaly was noted at birth, with a head circumference of 30.4 cm (−3 SD). A head ultrasound performed on day of life 2 was interpreted as normal. Cytogenetic evaluation was requested by the neonatologist, and the patient was discharged to home on day of life three.

Neurological evaluation at day of life 10 confirmed severe microcephaly with symmetrical features, a small anterior fontanel that was difficult to palpate, a closed posterior fontanel and non-overlapping sutures. An MRI of the head without contrast was subsequently performed at day of life 19 (Fig. 1), and showed a smaller than expected cranium relative to the face, a normal cortical thickness without a focal migrational abnormality but with a primitive, coarsened gyral pattern with a reduced number of sulci. The myelination pattern was normal for age. The cerebellum and brain stem appeared normal and no extra-axial fluid collections were present. Fluid opacification of the mastoid air cells was present bilaterally. This scan supported the diagnosis of primary congenital microcephaly (MIC).

At two months of age, the patient was evaluated in the genetics clinic. Interval health was good, and the patient was primarily breast fed with supplemental formula. His weight was 4.7 kg (−1 SD), length was 54.3 cm (−2 SD), and head circumference was 34.1 cm (<−3 SD). His growth was calculated at 16.8 gm/day, consistent with failure to thrive. A physical exam identified symmetric microcephaly with essentially closed anterior fontanel, upslanting palpebral fissures, and prominent beaked nose with splayed nasal root.

At a return visit for neurology clinic follow-up at 10 months of age, the patient weighed 6.8 kg (<−2 SD), height was 66.5 cm (<−2 SD) and head circumference was 38 cm (<−5 SD), consistent with continued slowing of growth velocity. Developmentally, he had a social smile, stranger recognition, verbalized with coos and babbling, clapped when asked, and was able to progress from lying recumbent to rolling over, sitting and crawling without assistance.