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Case Report of a 17q21.31 Microdeletion Associated with *EFTUD2* Mandibulofacial Dysostosis with Microcephaly (MFDM) Identified by Comparative Genomic Hybridization

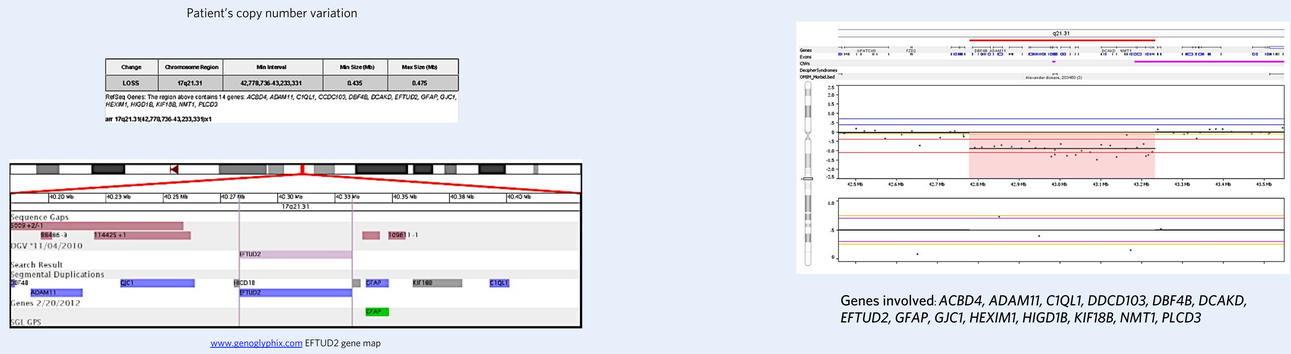
BACKGROUND

- Mandibulofacial dysostosis with microcephaly (MFDM) is a rare congenital craniofacial malformation which is usually sporadic and has been associated with pathogenic variation involving the *EFTUD2* gene (4, 5).
- Although the majority of reported cases of MFD with microcephaly (MFDM) with heterozygous mutations in *EFTUD2* are *de novo*, two deletions involving this gene have been reported in affected probands over the past year (3,4,5).
- Affected individuals have been primarily described as presenting with microcephaly, midface hypoplasia, micrognathia, characteristic external ear abnormalities, and significant global developmental delay. In addition, several affected individuals have also shown abnormal MRI findings, choanal and aural atresia, cleft palate, congenital heart defects, hearing loss, cryptorchidism, proximally placed thumbs, and expressive language delays (3, 4, 5).
- The *EFTUD2* gene is located on chromosome 17q21.31 and encodes a highly conserved spliceosomal GTPase with a central regulatory role in catalytic splicing and post-splicing complex disassembly. In one report, haploinsufficiency in *EFTUD2* has been implicated as a cause for mandibulofacial dysostosis with microcephaly (MFDM) in 14 individuals (3, 4, 5).
- Here we present a female dizygotic twin neonate born at 36 weeks gestation presenting with intrauterine growth retardation (growth discordance of 30%), early feeding difficulties, gastroesophageal reflux, high-pitched cry due to vocal cord paralysis, and reduced suck/swallow/breathe coordination.
- She was immediately admitted into the NICU for ongoing management. The patient also had microcephaly, microcrania, cleft palate, microretrognathia, a small patent ductus ateriosus, oral and pharyngeal dysphagia, bilateral proximal radioulnar synostosis, hearing deficits, and 11 thoracic ribs. She had a flat facial profile, downslanting palpebral fissures, back-sloping forehead, prominent glabella and paranasal region with a "beak" nose, overlapping toes, thin fingers **(Figure 2)**.
- Magnetic resonance imaging studies confirmed microcephaly with simplified gyral pattern, mild dilatation of the posterior bodies of the lateral ventricles, and thinning of the white matter. Bilateral subependymal gray matter heterotopias were also noted posteriorly.
- Her female twin sister, mother, father, and siblings are healthy. Family history was negative for MFDM and consanguinity was denied.

METHODS

- A blood sample was collected from the proband and sent to Ambry Genetics (Aliso Viejo, CA) for 400K CGH+ SNP analysis (Agilent Technologies, Santa Clara, CA). Genomic deoxyribonucleic acid (gDNA) was isolated from the patient's specimen using QIAsymphony DNA kit (Qiagen, Valenica, CA) and quantified using a Nanodrop (Thermoscientific, Pittsburg, PA).
- The aCGH method is based on the hybridization of fluorescently labeled patient gDNA (Cy-5) with fluorescently labeled reference DNA (Cy-3) to a 400K oligonucleotide array. Genomic patient DNA relative to the reference DNA are represented as fluorescent ratios (Cy5/Cy3) that are further quantified by image analysis software (Agilent Technologies, Santa Clara, CA and BioDiscovery, Hawthorne, CA). Quantified results indicate each targeted-DNA sequence as loss of copy number (deletion), gain of copy number (duplication), or normal copy number.
- The Ambry CMA 400K CGH+ SNP array contains 400,000 probes (~ 300,000 CGH probes and ~100,000 SNP probes) covering >400 genetic disorders.
- The array includes probes for pericentromeric and subtelomeric regions with dense probe coverage spanning 10 Mb at each subtelomere. The backbone spacing of the probes is set at an average of 13 Kb throughout the entire human genome and at 5 Kb on the X chromosome. There is no probe coverage of the pseudoautosomal regions 1 and 2 (PAR1 and PAR2) on the X and Y chromosomes.

FIGURE 1: Patient's Copy Number Variation on Microarray Analysis

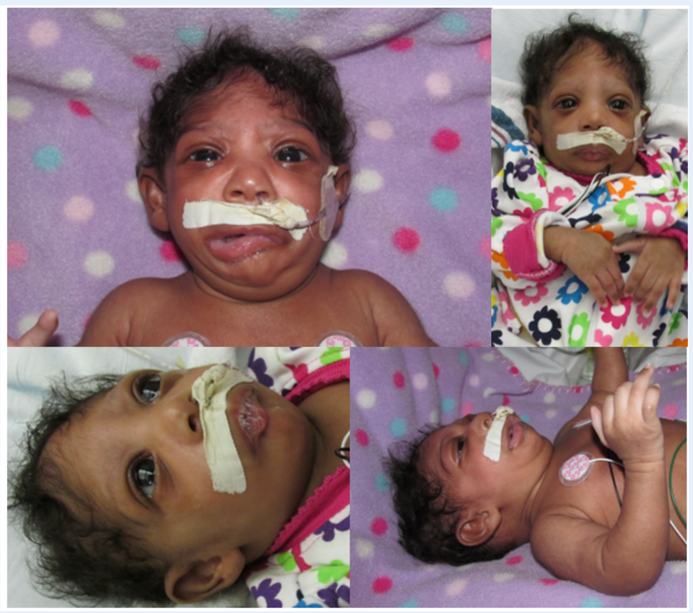


www.genoglyphix.com EFTUD2 gene map

RESULTS/ DISCUSSION

- Results of the patient's 400K microarray analysis revealed a deletion in copy number in chromosome 17q21.31 which contained 14 genes and spanned a minimum size of 0.435 Mb and a maximum size of 0.475 Mb. These genes encompassed in this interval were *ACBD4, ADAM11, C1QL1, CCDC103, DBF4B, DCAKD, EFTUD2, GFAP, GJC1, HEXIM1, HIGD1B, KIF18B, NMT1, and PLCD3* **(Figure 1)**.
- No other copy number variations or regions of homozygosity were identified.
- Upon interpretive analysis, it was concluded that only 3 genes in this deletion interval were potentially clinically relevant. The first gene investigated was *CCDC103*, which is known to be associated with primary ciliary dyskineria-17 (PCD) (7). The second gene, *GFAP*, is known to be associated with Alexander disease (1, 2, 9). It was determined that neither of these genes were conclusively implicated in the proband's phenotype.
- Based on our patient's significant clinical overlap to recently reported cases of *EFTUD2*-associated MFDM, it was determined that *EFTUD2* haploinsufficiency was responsible for her condition. Parental samples and a sample from the proband's twin were unable to be obtained for further analysis; however, based on the known sporadic nature of MFDM and negative family history,
- it was also concluded that this proband's genetic alteration was likely *de novo*.
- Diagnostic testing has changed dramatically over the last decade, especially with the introduction of DNA-based techniques such as cytogenetic microarrays (6). Researchers have found that in cases of individuals with cognitive impairment, standard molecular karyotyping would miss approximately 0.6% of cases with pathogenic balanced *de novo* aberrations (8). In addition, much discussion has surfaced over the years regarding the use of cytogenetic microarray analysis for the investigation of developmental delay/intellectual disability, multiple congenital anomalies, and/or autism spectrum disorders (6).
- Our patient's 17q21.3 microdeletion fell below the traditional detection size for standard karyotype analysis (0.5 Mb), thus G-band chromosome studies would not have provided a diagnosis. Haploinsufficiency in *EFTUD2* has been implicated as the primary known cause of MFDM in several reported cases, as was reiterated here (3, 4, 5). In addition, this case supports the claim that more cases involving *EFTUD2* haploinsufficiency likely exist and are becoming more frequently identified with the use of advanced genetic testing technologies like chromosomal microarray analysis.
- The American College of Medical Genetics (ACMG) practice guidelines for array-based technology for the detection of chromosomal abnormalities clearly states that a chromosomal microarray analysis should not be ordered in cases when a rapid turnaround time is needed (<48 hours).
- Moreover, limitations of microarray analysis include the inability to detect balanced chromosome rearrangements such as translocations and inversions, potential sex chromosome abnormalities (if the wrong gender control is used), marker chromosomes, and low-level mosaicism (6).
- However, as illustrated in this case, G-band karyotype analysis would have failed to identify this patient's diagnosis.

FIGURE 2: Proband's Clinical Presentation



Clinical features include prenatal IUGR, early feeding difficulties, gastroesophageal reflux, high-pitched cry due to vocal cord paralysis, reduced suck/swallow/breathe coordination, microcephaly, microcrania, cleft palate, microretrognathia, small PDA, oral and pharyngeal dysphagia, bilateral proximal radioulnar synostosis, hearing deficits, 11 thoracic ribs, flat facial profile, downslanting palpebral fissures, back-sloping forehead, prominent glabella and paranasal region with "beak " nose, overlapping toes, thin fingers, and abnormal MRI findings.

CONCLUSIONS

- Here we show that chromosomal microarray analysis undoubtedly plays a significant role in the "first-tier" diagnostic process in many cases, especially in the identification of *de novo* copy number variations and single-gene disruptions (including deletions and duplications).
- Moreover, clinical judgment is pivotal in making these testing decisions, as proper time management is significant.
- Based on the reported cases in the literature to date, it may also be argued that *EFTUD2* sequencing is a superior analysis technique for this condition; however, single-gene sequencing is a lengthy process with no current clinical access on a commercial basis.
- Therefore, diagnostic exome sequencing may be considered an excellent alternative strategy when pathogenic copy number variations and other abnormal chromosomal rearrangements have been ruled-out.
- In conclusion, traditional karyotype analysis should be ordered as a first-tier diagnostic test in neonatal cases presenting with a suspected congenital craniofacial disorder resembling MFDM when rapid turn-around time is a priority.
- Alternatively, in cases of patients whose presentations are highly suspicious for MFDM, and time is not as critical, it is suggested that chromosomal microarray analysis be performed first, followed by step-wise sequential reflex to single-gene sequencing for *EFTUD2* mutations or diagnostic exome sequencing if possible.

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