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Molecular characterization of co-occurring Duchenne muscular dystrophy and X-linked Oculo-Facio-Cardio-Dental syndrome in a girl

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Abstract

Duchenne muscular dystrophy is an X-linked condition at the severe end of the spectrum of dystrophinopathies. Females with dystrophin mutations are at risk for cardiomyopathy, but are usually asymptomatic during childhood. However, some girls can exhibit features of Duchenne muscular dystrophy because of skewed X-inactivation, aneuploidy, or chromosomal rearrangement. Oculo-facio-cardio-dental syndrome is a rare X-linked disorder, lethal in males, that comprises microphthalmia, congenital cataracts, congenital heart defect, canine radiculomegaly, and digital anomalies. We report a 7-year-old girl who was referred for muscular hypotonia, with clinical features of Duchenne muscular dystrophy, including elevated serum creatine phosphokinase, pseudohypertrophy of calf muscles, and muscle weakness, which became evident at 3 years of age. In addition, she had multiple congenital anomalies including atrial septal defect, cataracts, dental and digital anomalies, a constellation that suggested the diagnosis of Oculo-Facio-Cardio-Dental syndrome, a condition caused by mutations in *BCOR*. Immunohistochemistry and western blot analysis of muscle, and mutation analysis of *DMD* showed a maternally inherited deletion of exons 30-43, confirming the diagnosis of Duchenne muscular dystrophy. Studies of lymphocytes showed essentially complete skewing of X-inactivation. Mutation analysis of *BCOR* revealed a *de novo* frameshift mutation (c.1005delC). Thus, we report for the first time an individual with the co-occurrence of Duchenne muscular dystrophy and Oculo-Facio-Cardio-Dental syndrome.

Keywords

Duchenne muscular dystrophy; Oculo-Facio-Cardio-Dental syndrome; X inactivation

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INTRODUCTION

The dystrophinopathies include a spectrum of muscle diseases caused by mutations in the X-linked *DMD* gene, which encodes dystrophin. The mild end of the clinical spectrum includes an asymptomatic increase in the serum creatine phosphokinase (CPK) and muscle cramps with myoglobinuria and isolated quadriceps myopathy. The severe end of the spectrum includes progressive muscle diseases that are classified as the Duchenne and Becker muscular dystrophies when skeletal muscle is primarily affected. Most females heterozygous for *DMD* mutations are asymptomatic or have mild clinical presentations, although they are at an increased risk for cardiomyopathy [Hoogerwaard et al., 1999].

Oculo-facio-cardio-dental (OFCD) syndrome is also an X-linked condition with characteristic ocular, facial, cardiac, and dental findings in females with apparent lethality in males [Obwegeser and Gorlin, 1997; Gorlin, 1998; Opitz et al., 1998]. Ocular findings include congenital cataracts, microphthalmia or microcornea, and secondary glaucoma. Craniofacial features include long narrow face, high nasal bridge, bifid nasal tip, and long philtrum. Cardiac anomalies include atrial and ventricular septal defects and mitral valve prolapse. Dental anomalies include canine radiculomegaly, delayed eruption, oligodontia, and retained primary teeth [Oberoi et al., 2005]. Intelligence is usually normal [Tsukawaki et al., 2005]. Loss of function mutations in the *BCOR* (BCL6 corepressor) gene on chromosome Xp11.4 have been identified in patients with OFCD syndrome [Ng et al., 2004].

We report a 7-year-old girl with Duchenne Muscular Dystrophy and OFCD, and the results of the molecular analyses of the girl and her family, which demonstrate the importance of such analyses to understand the mechanisms underlying the co-occurrence of these phenotypes.

CLINICAL REPORT

The patient is a girl who was first evaluated in the Genetics clinic at the age of 4 years because of elevated CPK (11,660 mU/ml) and congenital anomalies. She was born at full term by spontaneous vaginal delivery without prenatal or perinatal complications. Her birth weight was 6 pounds, 8 ounces, which was normal. She had mild hypotonia and feeding difficulty during infancy. Several anomalies were documented during the first few years of life, including bilateral posterior cataracts that were first noticed at 23 months of age and were extracted at 2 years of age. Her tooth eruption was delayed and the sequence of eruption was abnormal. Her molars were the first to erupt at age 12 months and her incisors appeared at age 2 years. No other dental abnormality was evident from dental X-rays at the age of 7 years. She had an atrial septal defect that required surgical closure at age 4, and surgical repair of left 2-3 cutaneous toe syndactyly. A formal developmental evaluation revealed moderate expressive language delay and apraxia. Her visual and motor skills tested normally, as determined by the Bayley scale; however, her motor development was mildly delayed. She rolled over at 6 to 7 months, crawled at 12 months, and walked independently at 16 months. The family history revealed several members on the paternal side with 2-3 toe syndactyly, including her paternal uncle and grandfather. There was no consanguinity. There were no other individuals affected with DMD, OFCD syndrome, other genetic conditions, major birth defects, multiple miscarriages, or mental retardation. Her growth parameters were normal at 4 years: weight 16 kg (~35th centile), height 96.6 cm (~15th centile), and head circumference 51 cm (~75th centile). She did not have a long face, high nasal bridge, bifid nasal tip, or long philtrum. Facial features that were consistent with OFCD included a wide anterior columella and mildly protuberant, cup-shaped ears with uplifted lobules (Figure 1). She had moderate pseudohypertrophy of the calf muscles but no Gower sign. Her gait was abnormally wide-based. Diagnostic studies included urine organic acid and plasma amino acid analyses, cranial MRI, chromosomal analysis, telomere FISH, chromosomal microarray analysis, muscle biopsy (done at the time she was undergoing

cardiac surgery), and DNA mutation studies for *DMD* and *BCOR* genes. *DMD* mutation analysis was performed after muscle immunohistochemistry showed a deficiency of dystrophin protein.

METHODS

Genomic Southern blot analysis for *DMD*

Genomic Southern blot analysis for the *DMD* gene was performed as previously described [Koenig et al., 1987]. Genomic DNA samples from the patient and both parents were digested overnight with Hind III restriction enzyme, along with male and female control samples. Heterozygous deletions were detected by visualization of the films and were quantified by densitometry.

X inactivation studies

An X chromosome inactivation study was done by the Greenwood Genetic Center Molecular Diagnostic Laboratory (Greenwood, SC 29646). Peripheral blood was collected for isolation of DNA. X-inactivation analysis was done at the Androgen Receptor (AR) locus as previously described [Allen et al., 1992]. Inactivation ratios of less than 80:20 are considered random patterns. Ratios between 80:20 and 90:10 are reported as moderately skewed and ratios greater than 90:10 are considered highly skewed and possibly clinically significant.

Immunostaining and western blot analysis

Cryosections of skeletal muscle were processed for immunostaining using antibodies against dystrophin (rod and C-terminal domains), dysferlin, laminin alpha 2, alpha, beta and gamma sarcoglycans, emerin, and utrophin. Immunostaining for beta spectrin served as a tissue integrity control, while beta dystroglycan served as a control for the sarcoglycans. Laminin gamma 1 served as a control for laminin alpha 2. Immunoblots were run in duplicate and stained for dystrophin with antibodies against the rod and C-terminal domains, dysferlin, laminin alpha 2, alpha and gamma sarcoglycans with beta dystroglycan as a control, and calpain 3 with antibodies to exons 1 and 8. All studies were carried out using commercially available antibodies and the manufacturers' conditions (laminin alpha 2 antibody, Chemicon International, Inc., Bellerica, MA; all other antibodies, Novocastra Laboratories Ltd., Newcastle upon Tyne, UK).

BCOR mutation analysis

BCOR analysis was performed under a research protocol approved by the NHGRI IRB. DNA isolation, PCR amplification, and sequencing was performed as described [Ng et al., 2004]. The position of the mutation was deduced from the sequence of GenBank accession number, NM_017745.

RESULTS

Genomic Southern blot analysis of the dystrophin gene

Southern blot analysis using probes 30-1 and 47-4a showed reduced intensities of the Hind III fragments corresponding to exons 30 through 43 with an abnormal ~9 kb Hind III fragment that may have contained exons 28 and 29 (supplemental Figure S1A for probe 30-1 & S1B for probe 47-4a). The results were consistent with a heterozygous deletion involving at least exons 30 through 43. The 5′ and 3′ breakpoints of this deletion were not further characterized. Genomic Southern blot analysis also showed that the proband's mother, who was tested after the *DMD* mutation was identified in the patient, carried the same deletion in the *DMD* gene (supplemental Figures S1A and S1B).

X inactivation analysis

An X-inactivation study was done using peripheral blood, analyzing the Androgen Receptor (AR) locus as previously described [Allen et al., 1992]. An X-inactivation ratio of 100:0 was observed, indicating a highly skewed pattern in peripheral blood lymphocytes.

Histology and immunostaining of muscle samples

Histological study of a rectus abdominis muscle specimen showed moderate to marked fiber size variation, and atrophic and hypertrophic fibers for both fiber types. Multiple or single necrotic and regenerating myofibers and mild endomysial fibrosis were also present, consistent with a dystrophic process (supplemental Figure S2A). Immunostaining for dystrophin with the C-terminal antibody showed a mosaic pattern and positivity in less than 5% of fibers (supplemental Figure S2C). In addition, antibody to the dystrophin rod domain showed a mosaic pattern with positive staining in approximately 15% of fibers (supplemental Figure S2D). The immunostaining for dystrophin contrasted with the prominent, more widespread staining for beta spectrin (supplemental Figure S2B). In contrast, immunostaining for beta dystroglycan was slightly reduced (data not shown). Immunostaining for alpha and gamma sarcoglycans corresponded to the pattern of staining for beta dystroglycan. Laminin alpha 2 immunostaining corresponded to the pattern of staining for laminin gamma 1; up-regulation of utrophin was evident in nearly 100% of the fibers; and emerin immunostaining was normal (data not shown). Taken together, the muscular dystrophy protein analysis was consistent with a dystrophinopathy, and the severity of protein depletion was consistent with a Duchenne-type muscular dystrophy.

BCOR mutation studies

Because of congenital cataracts, heart defect, and dental and digital anomalies, we suspected that the patient had OFCD syndrome. *BCOR* sequencing showed a heterozygous c.1005delC mutation in exon 5. This result was consistent with the clinical diagnosis of OFCD syndrome. No *BCOR* mutation was found in the mother, and mutation analysis of the father was not done, as mutations in the *BCOR* gene are lethal in males.

DISCUSSION

We report a 7-year-old girl with both DMD and OFCD syndrome. Both diagnoses were confirmed by molecular evidence with a deletion of exons 30 through 43 of the *DMD* gene and a frameshift mutation of the *BCOR* gene. To our knowledge, this is the first reported case with this combination. Both conditions are X-linked with *DMD* mapping to Xp21.2 and *BCOR* to Xp11.4. The patient's muscular dystrophy seems to be at the severe end of the spectrum even when compared to males with DMD. Although she has many of the features commonly seen in other patients with OFCD, the typical facial features were very mild. Her specific dental anomaly of both delayed and abnormal order of tooth eruption has not been reported before. Also, her language development is significantly delayed. Speech articulation problems have been reported in 1–3% of males with DMD [Cotton et al., 2005; Hinton et al., 2007]], and recent reports suggest that males with DMD may have delayed language development [Giliberto et al, 2004; Cyrulnik et al, 2007]. Hendriksen and Vles [2008] surveyed 351 male patients with DMD and found that 3.1% had autism spectrum disorder, which is significantly higher than would be predicted for random co-occurrence [Hendriksen and Vles, 2008]. In another survey, Young and colleagues [2008] reported that 8.3% of males with Becker muscular dystrophy have autism [Young et al., 2008]; however, no information was provided regarding developmental outcomes in females affected with DMD. Language development is usually normal in girls with OFCD syndrome [Gorlin, 1998], but others have reported developmental delay in one individual with OFCD [Hedera and Gorski, 2003]. Our patient is severely affected with DMD, however, and so it seems reasonable to conclude that her language

delay would be comparable to that of a male with DMD, or that the combination of DMD and OFCD syndrome may have more severe effects on language and development than either condition alone. An assessment of her neurodevelopmental status in the future may provide additional insight in this regard.

Interestingly, 2-3 toe syndactyly, a common trait that is also a part of the OFCD syndrome spectrum [Ng et al., 2004], was present in several of the patient's family members on the paternal side including her uncle and grandfather, but not her father. The male-to-male transmission from her paternal grandfather to her uncle, however, suggests that the syndactyly is coincidental, representing variable expressivity of an autosomal dominant familial trait, and unrelated to OFCD syndrome.

The manifestation of DMD in the patient is most likely the result of unequal Lyonization in favor of the maternally inherited X-chromosome, a contention supported by the nearly 100% skewing of X-inactivation in lymphocytes, the presence of the *DMD* mutation in her mother, and the paucity of dystrophin immunostaining. Skewed X-inactivation has been documented in individuals with OFCD syndrome, with >90% skewing in all cases [Hedera and Gorski, 2003; Ng et al., 2004]. Two familial cases with mother to daughter transmission showed preferential inactivation of the maternal chromosomes with the *BCOR* mutations [Hedera and Gorski, 2003; McGovern et al, 2006]. These observations indicate that the mutations in *BCOR* that cause OFCD syndrome are likely to cause a selective disadvantage for cells that express the mutated allele. In the patient reported here, ~100% skewing of X-inactivation occurred in lymphocytes. In contrast to lymphocytes, however, X-inactivation is unlikely to be 100% in all tissues since the patient has many of the characteristic features of OFCD syndrome. Since no *BCOR* mutation was identified in the mother, and because observations from published studies indicate that the X chromosome with the *BCOR* mutation is strongly inactivated, we reason that the mutation in our patient likely occurred on the paternal X chromosome, and did not occur on the maternal chromosome with the *DMD* mutation. As null *BCOR* mutations are lethal in hemizygous males, we argue that the mutation occurred during spermatogenesis and that somatic mosaicism in the father is conceivable, but unlikely. Finally, since the hemizygous *BCOR* mutation is embryonic lethal while the *DMD* mutation is not, and because X inactivation is presumably skewed in favor of the chromosome with the *DMD* mutation in our patient, one can reasonably speculate that the skewed *DMD* expression is a direct consequence of the *BCOR* mutation instead of a chance event as seen in other manifesting DMD females.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Facial features of the patient at age 7 years of age. No dysmorphic facial features were evident except a wide anterior nasal columella and mildly protuberant ears with uplifted lobules.