A Novel Mutation of *KIF11* in a Child with 22q11.2 Deletion Syndrome Associated with MCLMR

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Keywords

Chorioretinal dysplasia · *KIF11* · Lymphedema · MCLMR · Microcephaly · 22q11.2 deletion

Abstract

Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation (MCLMR; OMIM 152950) is a rare autosomal dominantly inherited syndrome. Mutations in the kinesin family member 11 (*KIF11*) gene have been associated with this condition. Here, we report a de novo novel heterozygous missense mutation in exon 12 of the *KIF11* gene [c.1402T>G; p.(Leu468Val)] in a boy with 22q11.2 microdeletion syndrome. His major features were microcephaly, ventricular septal defect, congenital lymphedema of the feet, and distinct facial appearance including upslanting palpebral fissures, a broad nose with rounded tip, anteverted nares, long philtrum with a thin upper lip, pointed chin, and prominent ears. His right eye was enucleated due to subretinal hemorrhage and retinal detachment at age 3 months. Lacunae of chorioretinal atrophy and the pale optic disc were present in the left eye. He also had a de novo 1.6-Mb

microdeletion in the Di George/VCFS region of chromosome 22q11.2 in SNP array, which was confirmed by FISH analysis. In this study, for the first time, we describe the co-occurrence of a *KIF11* mutation and 22q11.2 deletion syndrome in a patient with MCLMR.

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There was an ongoing debate about the phenotypic overlap between microcephaly, primary lymphedema and chorioretinal dysplasia syndrome (MLCRD; OMIM 152950) and chorioretinal dysplasia, microcephaly and mental retardation syndrome (CDMMR; OMIM 156590) [Feingold and Bartoshesky, 1992; Angle et al., 1994; Fryns et al., 1995]. The identification of causative variants in the kinesin family member 11 (*KIF11*) gene in both MLCRD and CDMMR cases, demonstrated that these conditions are allelic with highly variable expression [Ostergaard et al., 2012]. The association of microcephaly with or without chorioretinopathy, lymphedema, or mental retardation is now collectively called MCLMR and merged into one entity (OMIM 152950).

Fig. 1. The clinical appearance of the patient at different ages. **a, b** At 19 months of age. **c** Five years and 6 months of age. **d, e** Six years and 6 months of age. Note prominent ears, upslanting palpebral fissures, a broad nose, anteverted nares, long philtrum, thin upper lip, micro-retrognathia, a pointed chin, and slender and long fingers (**a, c, d**). The bilateral lymphedema remained more pronounced on the right foot (**b, e**).

The 22q11.2 deletion syndrome (OMIM 188400) is a frequent microdeletion syndrome with an estimated incidence ranging from 1:2000 to 1:7000 live births [Shprintzen, 2008]. The clinical features consist of conotruncal heart malformations, palatal abnormalities, learning difficulties, thymus and parathyroid hypoplasia, and characteristic facial features including short palpebral fissures, hypoplastic ala nasi, bulbous nasal tip, small mouth and chin as well as overfolded helices [Forbes et al., 2007; Binenbaum et al., 2008].

Here, we report a case of a boy with MCLMR and 22q11.2 deletion syndrome presenting with overlapping clinical findings.

Patient and Methods

A 1-year-old boy was referred to our department due to an atypical facial appearance. His healthy parents were nonconsanguineous. His birth weight, length, and head circumference (OFC) were 3,230 g (−0.5 SD), 50 cm (−0.06 SD), and 30.1 cm (−2.5 SD), respectively. Postnatal echocardiography revealed a perimembranous ventricular septal defect (VSD). Because of bilateral edema of the dorsum of the feet, lymphoscintigraphy was performed. There was no evidence of tracer uptake in the inguinal lymph nodes or lymphatic tracts confirming the primary lymphedema. The result is characteristic of the functional aplasia typically observed in MCLMR [Ostergaard et al., 2012]. Due to the prediagnosis of reti-

noblastoma, his right eye was enucleated and fitted with a prosthetic eye at age 3 months. Pathological examination of the right eye revealed diffuse retinal detachment and subretinal hemorrhage.

On his examination at 1 year of age, he presented with microcephaly (OFC: 38 cm, −6.7 SD), prominent ears, upslanting palpebral fissures, a broad nose with a rounded tip, anteverted nares, long philtrum with a thin upper lip, a high-arched palate, micro-retrognathia, and congenital lymphedema of the feet (Fig. 1a, b). He achieved head control, sitting, and walking at 2, 8, and 12 months, respectively. Complete blood count, calcium metabolism, thyroid functions, immunoglobulins and T-lymphocyte subsets were in normal range. Echocardiography detected spontaneous closure of the VSD. No venous insufficiency in the lower extremities was seen via venous Doppler. Cranial MRI exhibited microcephaly without any structural abnormalities. Fundoscopic examination of the left eye revealed the pale optic disc and lacunar chorioretinal atrophy. Electroretinography showed generalized rod-cone dysfunction. Hearing test was normal. Developmental quotient of Denver II Developmental Test at age 6 was 60. Hyperactive behavior and attention problems were observed, but he could speak fluently in long sentences by the age of 6. He has been followed up regularly and was 8 years old at the last examination. During the follow-up period, his growth parameters (height and weight) were normal. At 8 years of age, his weight and length were 20 kg (−1.89 SD) and 122 cm (−1.04 SD), respectively. However, his OFC was 45 cm (−5.3 SD) (Fig. 1c, d). The findings of the left fundus remained the same. He had low visual acuity and was able to count fingers from 2 meters. His optical coherence tomography imaging revealed severe retinal thinning. The bilateral lymphedema remained more pronounced on the right foot (Fig. 1e).

Fig. 2. **a** SNP array showing a 1.6-Mb deletion of the 22q11.2 region. **b** The standard probe (Di George/VCFS TUPLE 1) for 22q11.2 deletion syndrome showing a single 22 homolog signal (red band indicated by a white arrow) confirms the deletion. The green band (indicated by red arrows) denotes the 22qter control

probe. **c** A heterozygous variant was identified in exon 22 of *KIF11* by Sanger sequencing in the patient, but not in either of the parents. **d, e** Schematic representation of the genomic organization of *KIF11* with the c.1402T>G mutation and the representation of the EG5 protein with the p.Leu468Val variant.

For detection of copy number aberrations, HumanCytoSNP-12 BeadChip array (Illumina), which contains approximately 300,000 SNPs per sample, was used with a targeted and overall resolution of 62 kb and 72 kb, respectively. FISH was carried out using the standard probe (Di George/VCFS TUPLE 1) for 22q11.2 deletion. MCLMR was suspected based on the retinal detachment history, microcephaly, pale optic disc, chorioretinal atrophy, and congenital lymphedema. Direct sequencing of all 22 exons and flanking introns of *KIF11* was performed using methods described previously [Ostergaard et al., 2012]. Pathogenicity and potential functional effects of detected variants in *KIF11* were assessed using MutationTaster and SIFT prediction tools [Kumar et al., 2009; Schwarz et al., 2010].

Results

The karyotype analysis was normal (46,XY). SNP-array analysis detected a de novo 1.6-Mb microdeletion in the Di George/VCFS region of the 22q11.2 locus (Fig. 2a), which was confirmed by FISH (Fig. 2b) and showed no additional pathological CNVs. A de novo heterozygous

missense mutation in exon 12 of the *KIF11* gene (NM_004523:c.1402T>G; p.Leu468Val) was detected (Fig. 2c–e). The variant is predicted pathogenic according to MutationTaster (probability >0.9999) and SIFT (SIFT score: 0.03) and has not been observed in 1000 Genomes Project (<http://www.internationalgenome.org/>) or Genome Aggregation (<http://gnomad.broadinstitute.org/>) databases. The variant was submitted to the LOVD database (variant number 72895; <https://databases.lovd.nl/shared/genes/KIF11>).

Discussion

KIF11 encodes a homotetrameric protein, EG5, which drives microtubule sliding and contributes to the assembly of the mitotic spindle [Valentine et al., 2006; Jones et al., 2014]. EG5 also has non-mitotic functions such as involvement in endothelial cell lineage proliferation, secretory protein transportation, and protein translation [Bar-

toli et al., 2011; Exertier et al., 2013; Wakana et al., 2013]. EG5 possibly also has a role in the cilia [Birtel et al., 2017].

Jones et al. [2014] assessed 37 individuals with *KIF11* mutations and found microcephaly, ocular abnormality, lymphedema, epilepsy, and cardiac anomaly as major clinical features. The OFCs of the patients ranged from −9.5 to −1.1 SDs. Similarly, Robitaille et al. [2014] reported a *KIF11* mutation-positive patient with microcephaly (OFC: −4 SD), bilateral chorioretinal atrophy, and retinal detachment. Her mother, with the same mutation, had borderline microcephaly (OFC: −2 SD). However, her eye examination remained normal, indicating variable expression of the condition.

In the study of Jones et al. [2014], major ocular abnormalities were chorioretinopathy, hypermetropia, myopia, bilateral retinal folds, and microphtalmia. Lacunar chorioretinopathy was found in approximately 60% of the patients. Balikova et al. [2016] showed generalized rod-cone and severe macular dysfunction in 7 and optic disc pallor in 3 patients with *KIF11* mutations. The long-term observation of 3 of the *KIF11* mutation-positive patients with chorioretinal atrophy over 2, 6, and 9 years revealed no progression of fundus examinations. In our patient, focal areas of lacunar chorioretinal atrophy and optic atrophy were observed. Electroretinography showed generalized rod-cone dysfunction. He had unilateral retinal detachment and a history of enucleation because of the prediagnosis of retinoblastoma. During his follow-up, the findings of the left fundus remained the same. Therefore, we highlight that the ocular findings of the condition may resemble retinoblastoma.

The lymphedema seen in *KIF11* mutations is described as congenital, bilateral, and confined to the dorsa of the feet. However, adult onset and intermittent lymphedema were also reported [Jones et al., 2014]. Interestingly, a very recent study has reported primary lymphedema of the lower limbs in 4 patients with 22q11.2 deletion syndrome [Unolt et al., 2018]. Unlike the patients with MCLMR, the lymphedema in these patients was not confined to the feet and extended to the calves in one and to the thighs in 3 patients. The patient presented here had a bilateral and pedal congenital lymphedema typical of that seen in other MCLMR cases. Thus, we have considered that the congenital lymphedema in our patient is related to the *KIF11* mutation rather than the 22q11.2 deletion syndrome.

The characteristic facial phenotype of *KIF11* mutations was described including upslanting palpebral fissures, a broad nose with rounded tip, long philtrum with a thin upper lip, and prominent ears [Jones et al., 2014].

Since bulbous nasal tip, small mouth and chin are common features of the 22q11.2 deletion syndrome [Binenbaum et al., 2008], we have postulated that the facial features of our patient are in association with both conditions.

In the MCLMR review, 8% of the patients had cardiac anomalies including patent foramen ovale, thickened pulmonary valve, hypertrophic cardiomyopathy, and atrial septal defect [Jones et al., 2014]. We have considered that VSD in our patient is related to the 22q11.2 deletion rather than the *KIF11* mutation. The common findings of 22q11.2 deletion syndrome, including thymus and parathyroid hypoplasia as well as hearing loss were not present in the patient.

Recent observational whole-exome sequencing studies have found that approximately 5% of the patients were carriers of 2 gene defects. Diagnosing the cases with dual molecular findings can be difficult, especially if the phenotypes overlap [Yang et al., 2014; Posey et al., 2017]. As the 22q11.2 deletion did not explain the whole clinical picture of our proband, MCLMR was suspected based on microcephaly, eye problems, and congenital lymphedema; a novel de novo variant in *KIF11* was also identified. We propose that these 2 genetic syndromes are the cause of the mixed phenotype of the patient.

In conclusion, we identified the association of 22q11.2 deletion syndrome and a *KIF11* mutation in a patient for the first time and discussed the overlapping clinical findings such as distinct facial appearance, VSD as well as microcephaly, congenital lymphedema, and chorioretinopathy.

Acknowledgment

This work was supported by the Turkish Pediatric Association (14.08.14/08).

Statement of Ethics

Signed informed consent and permission for the publication of photos of the patient were obtained from the parents. The data presented in this study were retrieved from the routine clinical care facilities of Cerrahpasa School of Medicine, Istanbul, Turkey.

Disclosure Statement

The authors declare no conflicts of interest.

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