A novel mutation of *KIF11* in a child with 22q11.2 deletion syndrome associated with MCLMR

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Running title: 22q11.2 deletion syndrome and *KIF11* mutation

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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. We report here 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 (VSD), congenital lymphedema of the feet, and distinct facial appearance including upslanting palpebral fissures, a broad nose with rounded tip, anteverted nares, long philtrum with 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 fluorescence *in situ* hybridization analysis. Here, we report, for the first time the co-occurrence of a *KIF11* mutation and 22q11.2 deletion syndrome in a patient with MCLMR.

Keywords: MCLMR, microcephaly, lymphedema, chorioretinal dysplasia, *KIF11*, 22q11.2 deletion.

Introduction

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) [Angle et al., 1994; Feingold and Bartoshesky, 1992; Fryns et al., 1995]. With the identification of causative variants in the kinesin family member 11 (*KIF11*) gene in both MLCRD and CDMMR cases, it was 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).

The 22q11.2 deletion syndrome (OMIM 188400) is a frequent microdeletion syndrome with an estimated incidence ranging between 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 and overfolded helices [Binenbaum et al., 2008; Forbes et al., 2007].

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

Subjects and Methods

A 1-year-old boy was referred due to the atypical facies. His healthy parents were non-consanguineous. His birth weight, length, and head circumference (OFC) were 3230 gr (-0.5 SD), 50 cm (-0.06 SD), and 30.1 cm (-2.5 SD), respectively. Postnatal echocardiography revealed a perimembranous VSD. Because of bilateral edema of the dorsum of the feet, lymphoscintigraphy was performed. There was no evidence of uptake of tracer in the inguinal

lymph nodes or lymphatic tracts confirming the primary lymphedema. The result is typical of the functional aplasia typically observed in MCLMR [Ostergaard et al., 2012]. Due to the prediagnosis of retinoblastoma, 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-old, he had microcephaly (OFC: 38 cm, -6.7 SD), prominent ears, upslanting palpebral fissures, a broad nose with rounded tip, anteverted nares, long philtrum with thin upper lip, 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 revealed spontaneous closure of the VSD. No venous insufficiency in lower extremities was seen via venous Doppler. Cranial MRI revealed 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. He had hyperactive behavior and attention problems but could speak in long sentences fluently by age 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 could count fingers at 2 meters. His OCT imaging revealed severe retinal thinning. The bilateral lymphedema remained more pronounced on the right foot (Fig. 1E).

For detection of copy number aberrations, Human CytoSNP-12 bead chip 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. Fluorescence *in situ* hybridization analysis (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 Mutation Taster and SIFT prediction tools [Schwarz et al., 2010; Kumar et al., 2009].

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). The SNP-array analysis 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 Mutation Taster (probability>0.9999) and SIFT (SIFT score: 0.03) and has not been observed in 1000 Genome 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, that drives microtubule sliding and contributes to the assembly of the mitotic spindle [Jones et al., 2014]. EG5 also has non-mitotic functions such as involvement in endothelial cell lineage proliferation, secretory protein transportation and protein translation [Bartoli 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 were ranging 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 seven, optic disc pallor in three patients with *KIF11* mutations. Their long-term observation of three of the *KIF11* mutation positive patients with chorioretinal atrophy over 2, 6 and 9 years revealed no progression of fundus examinations. Consistently 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, eye 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 lower limbs in four 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 three 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 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 as upslanting palpebral fissures, broad nose with rounded tip, long philtrum with 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, hearing loss were not present in the patient.

Recent observational WES studies have found that approximately 5% of patients were carriers of two 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 the microcephaly, eye problems and congenital lymphedema and a novel

de novo variant in *KIF11* was identified. We propose that these two genetic syndromes are the cause of the mixed phenotype of the patient.

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

Statement of Ethics

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

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgements

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Figure Titles and Legends

Fig. 1. The clinical appearance of the patient at different ages: At 19 months of age **(A, B)**, 5 years and 6 months of age **(C),** 6 years and 6 months of age **(D, E)**. Note prominent ears, upslanting palpebral fissures, broad nose, anteverted nares, long philtrum, thin upper lip, micro-retrognathia, a pointed chin and slender and long fingers **(A, C, D)**. Lymphedema of the feet has remained bilaterally, dominantly on the right side **(B, E)**.

Fig. 2. A SNP-array identified 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 white arrow) confirms the deletion. Green band (indicated by red arrows) demonstrates the 22qter control probe. **C** A heterozygous variant was identified in exon 22 of *KIF11* by Sanger sequencing in the patient (upper panel), but not in either of the parents (mother, middle panel; father, lower panel). Schematic representation of the genomic organisation of *KIF11* with the c.1402T>G mutation and the representation of the EG5 protein with the p.Leu468Val variant **(D, E)**.

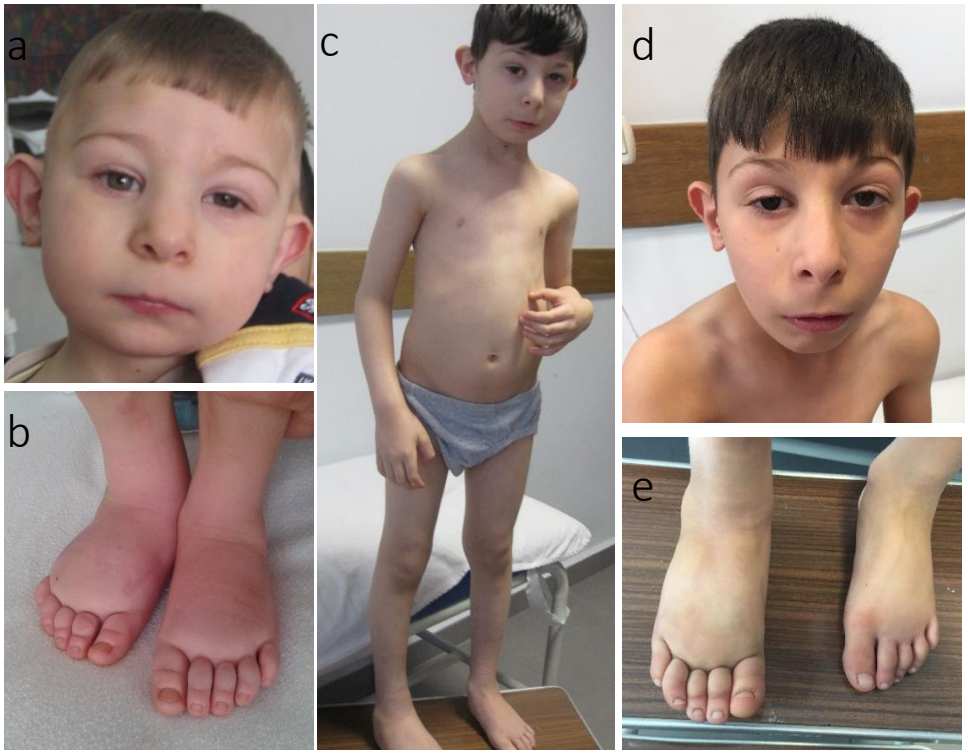

Figure 1

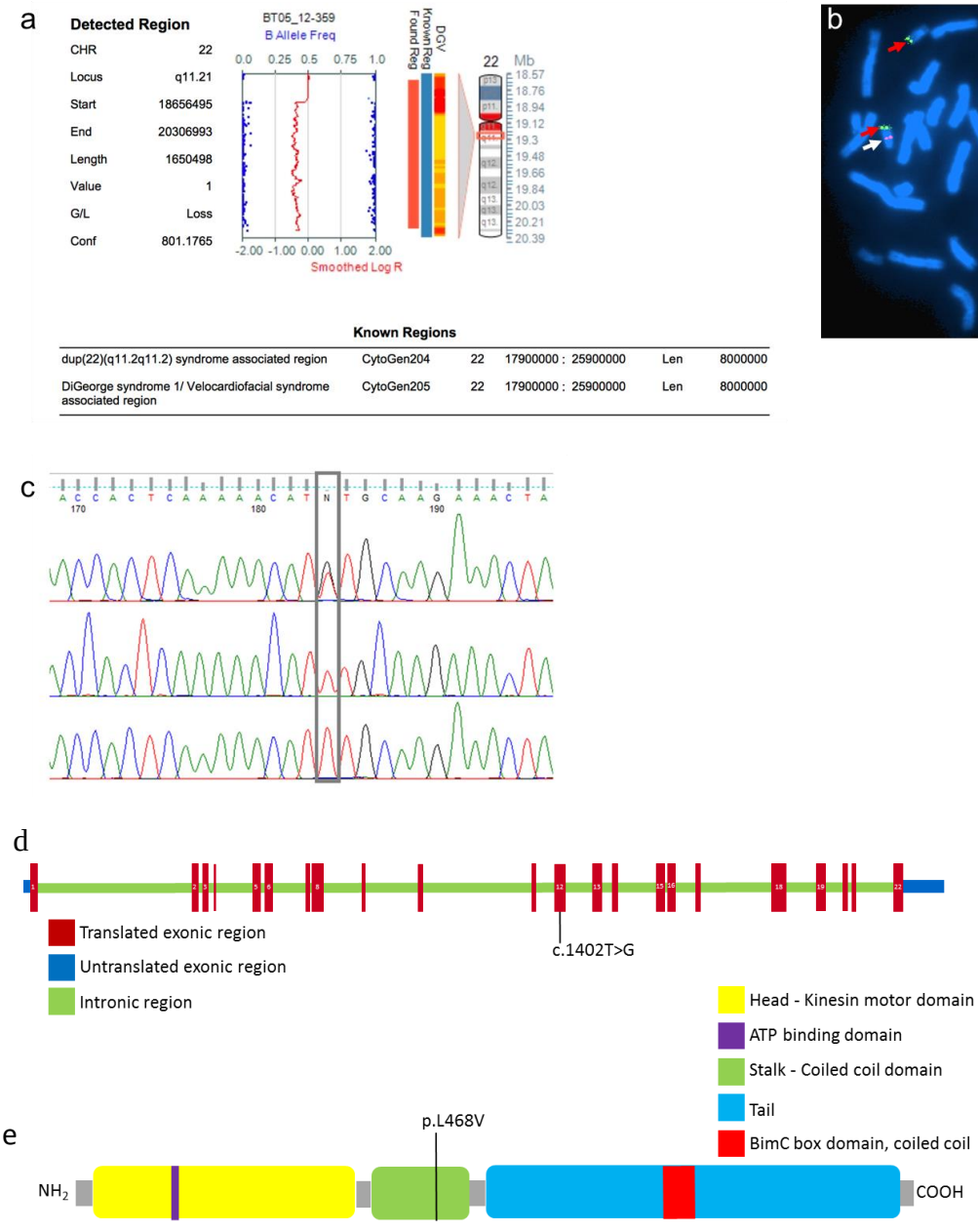
a
Detected Region
CHR 22
Locus q11.21
Start 18656495
End 20306993
Length 1650498
Value 1
G/L Loss
Conf 801.1765
BT05_12-359
B Allele Freq
Smoothed Log R
Known Regions
dup(22)(q11.2q11.2) syndrome associated region CytoGen204 22 17900000 : 25900000 Len 8000000
DiGeorge syndrome 1/ Velocardiofacial syndrome associated region CytoGen205 22 17900000 : 25900000 Len 8000000
b
c
d
Translated exonic region
Untranslated exonic region
Intronic region
c.1402T>G
Head - Kinesin motor domain
ATP binding domain
Stalk - Coiled coil domain
Tail
BimC box domain, coiled coil
e
p.L468V
NH2
COOH

Figure 2