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Novel Deletion in the *CNNM4* Gene in Siblings with Jalili Syndrome

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Abstract

Different mutations in the *CNNM4* (OMIM 607805) gene are known to cause Jalili syndrome (OMIM 217080) which is characterized by a combination of cone-rod dystrophy and amelogenesis imperfecta. In particular one homozygous missense mutation Leu438Profs*9 in exon 1 of the *CNNM4* gene has been described in patients originating from the Kosovo. This mutation causes a frameshift and generates a new stop codon in the same exon. The two patients described here carry the same Leu438Profs*9 mutation in a heterozygous state. In addition they also carry a novel heterozygous deletion which has not been described in the literature. The deletion includes 29 base pairs and is also located in exon 1 of the *CNNM4* gene. A genetic analysis of the parents revealed both mutations to be compound heterozygous and are therefore the likely cause of Jalili syndrome in the family.

Keywords

Jalili syndrome, *CNNM4*, Leu438Profs*9, 29 basepair deletion, Cone-rod dystrophy, Amelogenesis imperfect

Introduction

Cone-rod dystrophy (CRD) in combination with amelogenesis imperfecta (AI) is a characteristic pattern in a new syndrome first described in 1988 by Jalili & Smith [1] in an extended Arab family from the Gaza Strip with family members affected with photophobia, nystagmus, achromatopsia and abnormal, discolored teeth. Since then additional families affected both with CRD and AI have been described with Jalili syndrome [2-4]. Cone-rod dystrophies are a heterogeneous group of genetically related retinal diseases characterized by progressive loss of visual acuity and achromatopsia [5]. Photophobia and often night-blindness the patients are unable to differentiate colors. Amelogenesis imperfecta includes several different clinical features such as hypoplastic, hypomature or hypocalcified enamel of the teeth [6]. Mutations in the *CNNM4* gene are causative for Jalili syndrome [2,7,8]. The *CNNM4 gene,* located on chromosome 2q11, spans 51kb of genomic DNA in seven exons [9]. It encodes a magnesium transport protein which is necessary for many cellular functions, including a role as cofactor for enzymes of the phototransduction cascade [7,10].

The description of an affected sib pair from Kosovo, affected both with CRD and AI, suggests that the combination of this phenotype may exist as a genetically homogeneous syndrome [2]. The reported sibs were genetically analyzed and a homozygous mutation in exon 1 of the *CNNM4* gene, further in this report termed the Kosovo mutation, has been ascertained. We here report a family with sibs clinically affected with Jalili syndrome carrying the Kosovo mutation in a compound heterozygous state. This mutation occurred with an allelic previously unknown deletion (c.694_722del) located in exon 1 of the *CNNM4* gene.

Patients and Methods

Patients

The family members described here originate from Kosovo. The affected sibs are the only children of non-consanguineous parents. Because of ambiguous retinal findings a genetic diagnostic was taken into consideration. Because of both, the kosovan ancestry and the clinical signs a genetic cause for Jalili syndrome was suspected. At the time of examination and molecular diagnosis the index patient (female) was 20 years old, her brother was 16 years old. The anamnesis resulted in both with loss of visual acuity at the age of about four years. Also at the age of about four years conspicuous dental findings were described. The deciduous teeth of both sibs and the permanent teeth showed yellowish discoloration and carious changes with enamel defect, typical signs of amelogenesis imperfecta (Figure 1). At the age of six years an ophthalmologic examination of the index patient revealed rod-cone dystrophy with juvenile macular degeneration (Figure 2) based on the following findings: fixation nystagmus, range of vision with concentrical constriction of approximately 30 percent, vision defects in the dark. The Panel-D15 test revealed bilateral multiple deviations along the tritan axis. At the age of ten years a further ophthalmolocigal examination confirmed these clinical results and an electroretinography (ERG) revealed a maximal response scotopic with a reduced amplitude. A dysfunction including cones as well as rods was diagnosed. For the index patient the achromatopsia was diagnosed at the age of fifteen by one of the authors (D M) at the Hospital for Pediatrics and Adolescent Medicine in Chemnitz, Germany. At the age of 17 years a control examination revealed a progression of the clinical signs such as retinal pigment degeneration, bilateral effaced ERG-response for scotopic and photopic range, restriction of visual field to 10 degree of the right eye and 12 degree

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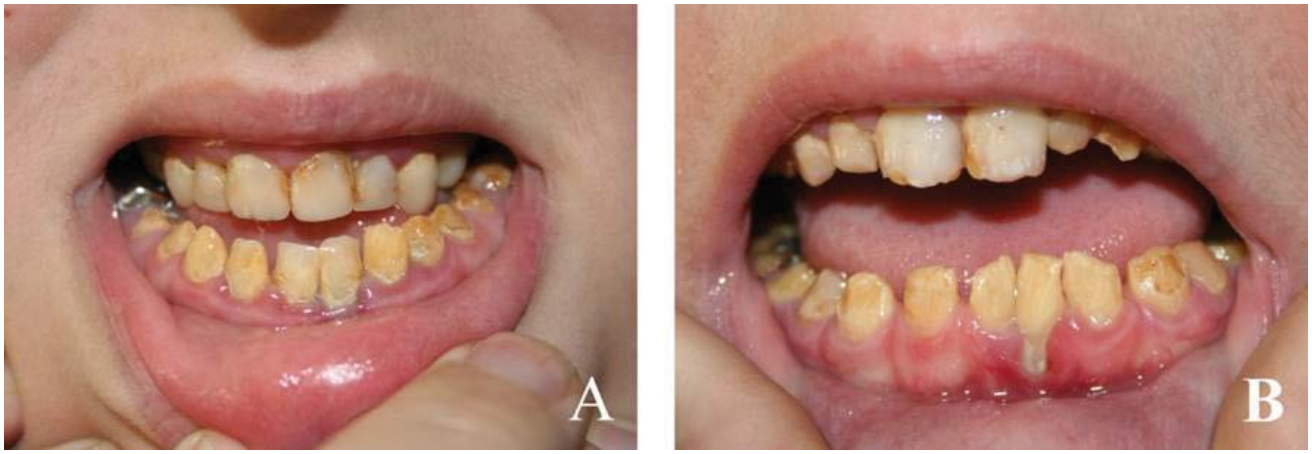

Figure 1: Teeth showing amelogenesis imperfecta and the caries in the sister at the age of fifteen (A) and the brother at the age of eleven (B).

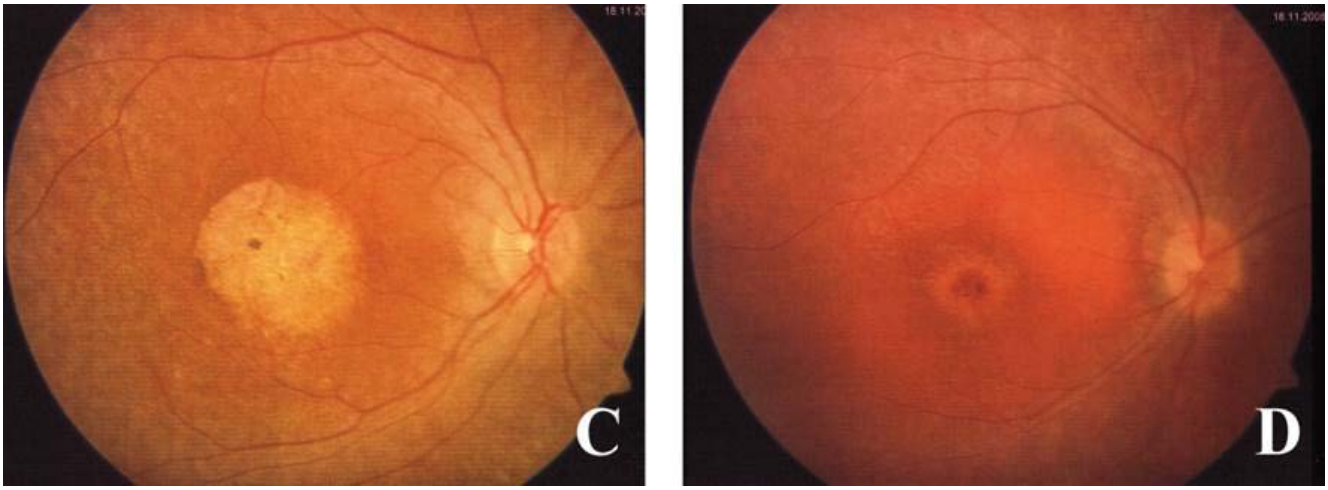

Figure 2: Fundus of the patients: (C) macular dystrophy with pigmentary degeneration at the sisters eye, (D) Bull's eye maculopathy at the brothers eye.

Table 1: Primer Sequences for the fragments of *CNNM4* Exon 1

Exon	Amplicon	Forward primer[1]	Reverse primer[1]	Size (bp)	T_a[2]
1	a	GGCCTCCAATGCAAATGA	GAGCTGCAACAAGTCGT	353	56
	b	CAGAGCCAGAGCAACATGG	TCTGGCTGCACATTCTCCTA	579	56
	c	GTGGACGGACAAGGACTCAC	CCAACACCATCCTTCTCACC	433	58
	d	TTGGGGAGATCCTACCTCAG	ATACTCGCATCCCGGTGTT	388	55
	e	CTTCAACACCATGTCGGAGA	TCTTGACGCCTCTTTTCCC	278	55

[1]All Primer sequences are given in 5' to 3' directi on, [2]optimal annealing temperatures given in °C

diameter (vertical) and 18 degree diameter horizontal of the left eye. Panel- D15 test again showed bilateral multiple deviations along the tritan- and the deutan axis. Altogether several signs, which are typical for Jalili syndrome, were present at the index patient and her brother. The parents do not have any problems with vision and have no dental changes. All participating persons gave their informed consent prior to the genetic examination.

Methods

Blood samples were taken after informed written consent. Genomic DNA was purified using the MasterPure™ DNA Purification Kit for Blood Version II (Epicentre Technologies Corp., Chicago). In view of the large size of exon 1 (1500 bp), it was divided into five overlapping fragments (a-e) (Table 1). The Primers were designed using the 'Primer3' software (http://frodo.wi.mit.edu/). For the GC-rich sequence in parts a and b of exon 1 the Advantage®-GC Genomic LA Polymerase Mix (Clontech Laboratories Inc., USA) was used for amplification according to the manufacturer's protocol. The PCR reaction was performed with the following modifications: initial denaturation at 95°C for 10 minutes, followed by 35 cycles for denaturation at 95°C for 1.5 minutes, 1-minute hold at the indicated annealing temperature (Table 1), and extension at 72°C for 3 minutes, with a final extension step at 72°C for 10 minutes. For amplification of the fragments c-e of exon 1 a standard PCR (Maxima Hot Start Taq DNA Polymerase, Thermo Fisher Scientific Inc. Fermentas, PA/USA) was used according to the manufacturer's protocol. The PCR conditions were as follows: initial denaturation at 95°C for 7 minutes, followed by 35 thermo cycles for denaturation at 95°C for 30 seconds, 1-minute hold at the indicated annealing temperature (Table 1), and extension at 72°C for 1 minute, with a final extension step at 72°C for 10 minutes. Samples were sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Reaction kit (Applied Biosystems, Foster City, CA/USA) according to the manufacturer's protocol. For analysis the 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA) was used. Bidirectional sequencing using primers specific for *CNNM4* exon 1 were used to screen for the expected mutations (Table 1). Sequence variants were analyzed using CodonCode Aligner Vs. 3.7.1, mutation data were indicated using the reference-sequence: NM_020184.3.

Table 2: Families with compound-heterozygote mutations in the *CNNM4* gene

Origin	Consanguinity	Mutation 1	Mutation 2
Kosovo	no	c.1312dupC; p.Leu438Profs*9	c.694_722del; p.Ile232Profs*80
Guatemala	no	c.2149C > T; p.Gln717*	c.62_145del; p.Leu21Hisfs*185
Scotland	no	c.971T > C; p.Leu324Pro	c.1690C > T; p.Gln564*

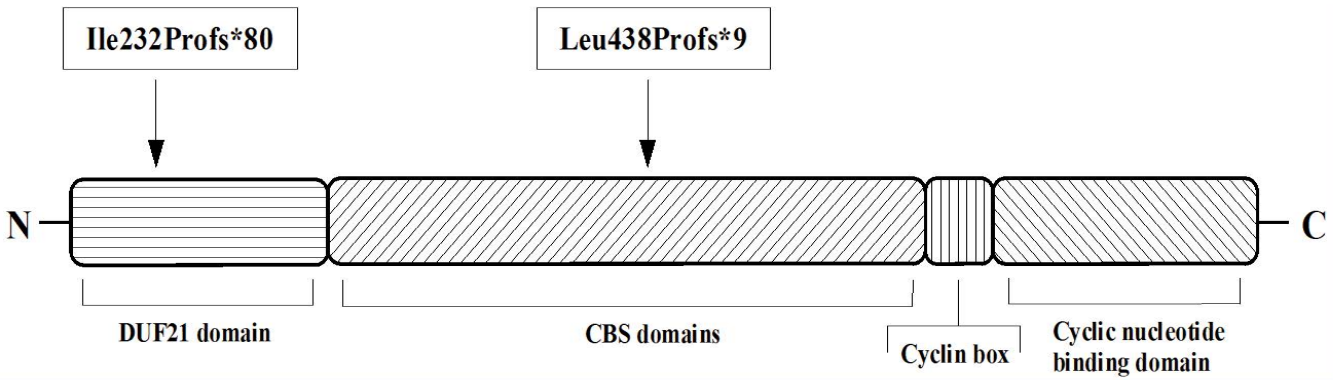

Figure 3: Structure of *CNNM4* including protein domains (according to Parry et al. [7]) and mutations discovered in the members of the family described in this study. Abbreviations: CBS domain: cystathionine beta-synthase core domain; DUF21 domain: domain of unknown function DUF21 [1].

Results

The sequencing analysis of the index patient revealed the presence of the known c.1312dupC mutation in exon 1 located in the CBS domain of the *CNNM4* gene, the so called Kosovo mutation. The duplication of cytosine leads to a substitution of the amino acid residue at amino acid position 438 and the resulting frameshift generates a stop codon nine amino acid residues downstream (p.Leu438Profs*9). Presumably this generates a truncated non-functional protein. Surprisingly this mutation in the index patient was present in a heterozygous state. A second heterozygous mutation was identified in fragment 1c (c.694_722del), which was upstream of the known mutation. The heterozygous deletion of 29 base pairs is leading to a substitution of isoleucine to proline at amino acid position 232. Since this frameshift generates a stop codon 80 amino acid residues downstream (p.Ile232Profs*80) it will lose its function as in the typical Kosovo mutation. The analysis of the remaining exons of the *CNNM4* gene did not reveal any abnormalities. Based on the clinical phenotype of the patients, we assume both mutations to be compound heterozygous and thus to be causative for the Jalili-syndrome. The analysis by direct sequencing revealed that the affected brother also carry both mutations in a compound heterozygous state. At father's DNA sample the so-called Kosovo mutation was detectable in a heterozygous state. The mother carries the mutation in exon 1, fragment 1c of the *CNNM4* gene in a heterozygous state. The remaining exons of the *CNNM4* gene did not contain any mutations in the index patient. The brother as well as the parents was tested for exon 1 of the *CNNM4* gene only.

Discussion

CNNM4 encodes a protein which is suggested to have functions in magnesium transport. [11] Studies revealed that *CNNM4* mediates transcellular Mg^{2+} transport and posseses characteristics of a Na^{+}/Mg^{2+} exchanger [10]. Magnesium is an essential element that is required for the catalytic activity of numerous metalloenzymes [12]. Deficiency of Mg^{2+} among others can cause several cardiovascular, neurological, and metabolic diseases [13]. The loss of functional *CNNM4* protein observed here may have pathological effects such as hypoplastic and hypomineralized enamel resulting in amelogenesis imperfecta present in families clinically diagnosed with Jalili syndrome [2].

The *CNNM4* protein consists of four functional domains (Figure 3), whereas the DUF21 domain contains four transmembrane helices (TM1-4) [12]. The novel deletion of 29 bp found in our study involves the amino acid residue 232, located between TM1 and TM2. Furthermore, the Kosovo mutation c.1312dupC affects the CBS-domain of the protein. Both are located in exon 1, the promoter region of the *CNNM4* gene. Thus an alteration of the highly conserved sequences very likely leads to a loss of protein. Additional investigations using RNA- and protein analyses could reveal further details. The typical phenotype of patients affected with Jalili syndrome has been classified into two types, A and B, by Jalili [14]. In type A ocular features occur in early infancy and can be present at birth, whereas the type B phenotype is milder and of later onset [14]. The macular degeneration together with the loss of visual acuity at early infancy of both sibs indicate the presence of type A.

Based on the Kosovan ancestry of our patients, we expected to find the Kosovo mutation Leu438Profs*9 which has been described previously in different patients in a homozygous state [2,7,15-17]. Our findings reveal that the Kosovo mutation may be associated with a different previously unknown mutation in the *CNNM4* gene as genetic cause for the Jalili-syndrome. Thus our findings extend the spectrum of the mutations in the *CNNM4* gene causative for Jalili syndrome. Genetic tests when suspecting Jalili syndrome should include the entire coding regions of *CNNM4* including the promoter region because causative mutations are potentially located here.

Now the phenotype described includes amelogenesis imperfecta and cone-rod dystrophy as described in five affected members originating from Kosovo [2,4,7,10]. Different types of amelogenesis imperfecta exist which differ in pattern of inheritance, autosomal dominant (OMIM 104510), autosomal recessive (OMIM 204650) and X-linked (OMIM 301200) types. Autosomal recessive amelogenesis imperfecta types are more common in the Middle East [18,19]. The pathogenic mutation Leu438Profs*9 in exon 1 of the *CNNM4* gene is known to be associated with Jalili-syndrome in a homozygous state. It is well known that recessive mutations can occur in religious or geographical isolates as a result of a founder effect. Patients with Jalili syndrome originate from different territories but only patients originating from the Kosovo have been found to carry the mutation Leu438Profs*9 [4,7,17,20-22]. Referring to the two cases described by Parry [2009], the clinical Jalili syndrome of the patients in this study is also caused by two compound-heterozygote mutations in the *CNNM4* gene (Table 2) [7]. Considering that the family originates for at least three generations from Kosovo and thus we supported the existence of a founder effect of this mutation.

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