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A case of Feingold type 2 syndrome associated with keratoconus refines keratoconus type 7 locus on chromosome 13q.

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Abstract

We report on a 58-year old woman with microcephaly, mild dysmorphic features, bilateral keratoconus, digital abnormalities, short stature and mild cognitive delay. Except for keratoconus, the phenotype was suggestive for Feingold syndrome type 2 (FGLDS2, MIM 614326), a rare autosomal dominant disorder described in six patients worldwide, due to the haploinsufficiency of *MIR17HG*, a micro RNA encoding gene. Karyotype showed a *de novo* deletion on chromosome 13q, further defined by array-Comparative Genomic Hybridization (a-CGH) to a 17.2-Mb region. The deletion included *MIR17HG*, as expected by the FGLDS2 phenotype, and twelve genes from the keratoconus type 7 locus. Because our patient presented with keratoconus, we propose she further refines disease genes at this locus. Among previously suggested candidates, we exclude *DOCK9* and *STK24*, and propose as best candidates *IPO5*, *DNAJC3*, *MBNL2* and *RAP2A*.

In conclusion, we report a novel phenotypic association of Feingold syndrome type 2 and keratoconus, a likely contiguous gene syndrome due to a large genomic deletion on 13q spanning *MIR17HG* and a still to be identified gene for keratoconus.

1. Introduction

Feingold syndromes are two very rare genetic entities characterized by digital anomalies, microcephaly, short stature and mild intellectual disability. Both are autosomal dominant, and due to the haploinsufficiency of *MYCN* (MIM 164840)(van Bokhoven, et al., 2005), encoding a transcription factor belonging to the MYC family, and Micro RNA 17 Host Gene (*MIR17HG*)(de Pontual, et al., 2011), a miRNA encoding gene.

Feingold type 1 (*FGLDS1*) is also associated with gastrointestinal atresia and short palpebral fissures, whereas type 2 (*FGLDS2*) features include brachymesophalangy, fifth finger clinodactyly and/or other digital anomalies, and dysmorphic features. The patients may also have associated cardiac anomalies (three cases) and hearing loss (two cases). Feingold type 2 is described in six patients worldwide. All have a genomic deletion spanning *MIR17HG* (MIM 609415)(Grote, et al., 2015).

No Feingold type 2 case is reported with keratoconus (MIM 148300, *KTCN*), a typically bilateral, non-inflammatory, progressive corneal disorder, with no gender predominance and incidence of 1 in 2,000 individuals for the isolated form (ranging from 0.0003% in Russia to 2.3% in Central India)(Gokhale, 2013) Keratoconus is associated with stromal thinning and protrusion, which causes an increase in astigmatism and in advanced cases loss of vision. Positive family history is reported in 6-23% of cases (Bechara, et al., 1996, Nowak and Gajecka, 2011). Eight *KTCN* loci have been mapped (see MIM 148300), including a 5.6-Mb region on 13q32 from 95,351,409 to 100,940,866 (*KTCN7*, MIM 614629), containing twenty-five genes.

2. Methods

2.1 Cytogenetics and array-CGH analyses

G-banding karyotype was performed on a peripheral blood sample following standard procedures.

Genomic DNA was extracted from peripheral blood using a standard protocol (Qiagen, Hilden, Germany), and verified using Nanodrop spectrophotometer (Thermo Scientific). Array-CGH was

performed using a 60K whole-genome oligonucleotide microarray following the manufacturer's protocol (Agilent Technologies, Santa Clara, California, USA). Slides were scanned using a G2565BA scanner, and analyzed using Agilent CGH Analytics software ver. 4.0.81 (Agilent Technologies Inc.) with the statistical algorithm ADM-2 and a sensitivity threshold of 6.0. At least three consecutive aberrant probes identified significant copy-number changes. Reference human genomic DNA was GRCh37/hg19. We compared our findings to known CNVs listed in the Database of Genomic Variants (DGV, <http://projects.tcag.ca/variation>) and in the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER, <https://decipher.sanger.ac.uk/application/>). IRB approved the study.

2.2 Real-time PCR

TaqMan real-time quantitative PCR analysis was used to measure copy number variants at 5q35.3 in genomic DNA as follows: (a) *ZNF45* (NM_182559.2) exons 2, primers 5'- ggacccttctagcctgagga; 5'- ttagggacaggcaaagtgc; #16 UPL probe (Roche Diagnostics); (b) RNaseP reference gene, VIC-labeled pre-designed TaqMan gene expression assays (P/N 4316844, Applied Biosystems). Reactions were carried out on an ABI 7500 Fast real-time PCR machine using the ABI TaqMan Universal PCR master mix according to the manufacturer's instructions (Applied Biosystems). Efficiencies of the assays were similar and in a range of 90-110%. Samples from affected individuals and unrelated healthy controls were run in triplicate; the mean Ct value was used for calculations using the $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001).

3. Results

3.1 Clinical report

Our proband (II-2, Fig. 1) was born by vaginal delivery at 38 weeks of gestation. No history of prenatal exposure to teratogens or maternal illness was recorded. As a child, she suffered from

psychomotor retardation and growth delay. She underwent growth hormone replacement therapy without any improvement.

At 58-yrs., she presented with short stature (height = 134 cm, <2nd centile), microcephaly (head circumference = 49 cm; <2nd centile). Parents' height was 160 (father) and 150 cm (mother). The proband showed mild dysmorphic features consisting in bulbous nose and wide philtrum. She suffered from bilateral keratoconus: diagnosed at 40 yrs, with curvature of the left cornea Sim K 52.8D (Max), 46.6 (Min) astigmatism 6.2D and right cornea of Sim K 50.8 D (Max), 46.4 (Min) astigmatism of 4.4D. Both corneas showed increased anterior and poster curvature elevation with thin corneas (RE 443, LE 440) and typical asymmetric 'bow-tie' pattern on orbscan topography. Endothelial cell count on specular microscopy was within normal range for her age. She also presented digital abnormalities (brachymesophalangy, clinodactyly of the fifth fingers) and mild thoracic scoliosis (Figure 1 B-D).

She also had mild mental impairment (IQ = 75, Wechsler Adult Intelligence Scale-Revised scale). She had not been suffering from any notable psychiatric illness during her life. Abdominal ultrasound, echocardiography and thyroid profile showed no abnormalities. She had normal female external genitalia. Hearing was normal. She had only one pregnancy that was interrupted because her fetus inherited her 13q deletion. The patient went through menopause at 47 years old.

Her parents (I-1 and I-2), older sister (II-2) and nephews (III-1 and 2) were healthy (Figure 1A).

3.2 Laboratory findings

Because of her psychomotor and growth retardation, she underwent a karyotype analysis, which identified an interstitial deletion on the long arm of chromosome 13. Both parents showed a normal karyotype, proving the deletion was *de novo*. Array-CGH, performed to assess the extension of the deletion, identified two rearrangements: 1) a 400-kb duplication on chromosome 5q35.3, maternally inherited and likely unrelated to the phenotype; 2) a *de novo* 17.2-Mb deletion at 13q31.1q31.2 [arr 5q35.3 (178,078,984-178,457,126)x3,13q31.1q31.2(81,484,523-98,726,969), hg19] (Figure 2A).

This copy number variant (CNV) encompassed *MIR17HG* gene, known to be associated with Feingold syndrome type 2 (Figure 2B). Remarkably, the deletion partially overlapped the keratoconus type 7 locus (*KTCN7*), identified by linkage analysis on Ecuadorian families (MIM 614629) (Figure 2B)(Bechara, et al., 1996, Karolak, et al., 2011).

4. Discussion

We describe a never reported association of Feingold type 2 with keratoconus in a sporadic female patient. We demonstrated the patient carried a *de novo* 17.2-Mb deletion on chromosome 13q, including the *MIR17HG* gene whose haploinsufficiency causes FGLDS2. The deletion extends telomerically into the type 7 keratoconus locus, thus suggesting that the haploinsufficiency of a still to be identified gene at this region is causing keratoconus in our patient. The gene should map within the smallest region of overlap (SRO) between our deletion and *KTCN7* locus (chr13:95,052,254-98,726,969 bp, hg19) (Figure 2B).

Eight genes were screened in an original Ecuadorian family where *KTCN7* locus was mapped (*MBNL2*, *IPO5*, *FARP1*, *RNF113B*, *STK24*, *DOCK9*, *ZIC5* *ZIC2* and *RNF113B*) without finding the causative mutation. Three possible candidates were suspected because of the variants found: *DOCK9*, *STK24*, and *IPO5*. In particular, the c.2262A>C substitution (p. Gln753His; rs191047852) in *DOCK9* segregated with the disease, and it was further shown to alter *DOCK9* splicing pattern using an *in vitro* assay (Karolak, et al., 2015). This variant is reported three times in the ExAC database (~60,000 cases), and was not found in a group of 42 Polish patients with keratoconus (Karolak, et al., 2016). Furthermore, *DOCK9* and *STK24* lay outside the SRO, and are excluded by our patient.

Using a combination of bioinformatics analyses evaluating haploinsufficiency constraint [(ExAC) database (<http://exac.broadinstitute.org/>)(<http://dx.doi.org/10.1101/030338>)], expression analysis of genes in the cornea and literature data (Chng, et al., 2013), we tried to prioritize the best candidates for *KTCN* among the 16 genes spanning our SRO (Table 1). We suggest four genes, namely importin 5 (*IPO5*), Muscleblind-Like Splicing Regulator 2 (*MBNL2*), DnaJ heat shock protein family (Hsp40)

member *C* (*DNAJC3*) and Ras-related protein 2A (*RAP2A*), can be considered as candidates and should be extensively screened in the original Ecuadorian family, because they are expressed in the target tissue and with a high haploinsufficiency constraint (Czugala, et al., 2012). Among them, *IPO5* –a nuclear protein import as nuclear transport receptor - and *MBNL2* - a member of the muscle blind protein family that regulates alternative splicing is required for terminal differentiation of muscle and photoreceptor tissues- have already been screened for point mutations in the coding region (Czugala, et al., 2012), but small deletions/duplications, mutations in regulatory regions, deep intronic splice-site mutations or even position effects may have been missed.

Seven cases reported in Decipher database and six described in the literature have a deletion encompassing *MIR17HG*. None of them was reported with keratoconus (see figure 2 for a scheme). Only three of them have a rearrangement spanning *KTCN7* locus; however, because these patients are infants, a genotype-phenotype correlation is not possible, considering keratoconus generally occurs during puberty (cases A, J and K, fig. 2B). One deletion (Sharaidin, et al., 2013) overlaps ~500 kb with the *KTCN7* locus without including *MBNL2* and *IPO5* (Tassano, et al., 2013), and two extend ~4-5 Mb with the *KTCN7* locus (cases A and J, fig.2B).

Among the deleted genes in our patient, we also noted *SLITRK1*, which has been previously associated with psychiatric diseases such as trichotillomania, Gilles de la Tourette syndrome and obsessive-compulsive disorder (Abelson, et al., 2005). No such psychiatric illnesses were present in our proband, likely due to the incomplete penetrance and variable expressivity associated to *SLITRK1* haploinsufficiency (Proenca, et al., 2011).

In conclusion, we describe a possible contiguous gene syndrome phenotypically characterized by Feingold syndrome type 2 and keratoconus, due to a large genomic deletion on 13q overlapping *MIR17HG* and a still to be identified gene for keratoconus.

Figure legends

Figure 1. Pedigree and clinical phenotype

A) Family pedigree: filled circles - affected subject; empty symbols - unaffected subjects; arrow indicates the proband; asterisk on the symbol indicates available genomic DNA; dup: duplication; del: deletion; wt: wild type. B) Mild facial dysmorphisms consisting in bulbous nose and wide philtrum. C, D) Brachydactyly of hands and feet.

Figure 2. Array-CGH analysis and overview of the deleted genomic region

A) Array-CGH results using the 60K Agilent platform showing the 17.2-Mb deletion on chromosome 13q. B) Genes located in the 13q31.1q31.2 genomic region between 81-101 Mb (hg19). In black Keratoconus type 7 locus as described in (Czugala, et al., 2012) and boxed in light grey the SRO defined by our patient's deletion. In red, our patient deletion. Below in grey, cases with a *MIR17HG* gene deletion, reported in the Decipher database and literature. Boxed in light purple the SRO of Feingold 2 disease. The figure 2B was modified from UCSC genome browser, using custom-generated tracks (Raney, et al., 2014).

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Table 1. Constraint metrics (ExAC database) and expression in cornea for genes within the SRO.

<i>Gene</i>	<i>Z</i>	<i>pLI</i>	<i>Cornea expression^a</i>
<i>ABCC4</i>	0.79	0	No expression
<i>CLDN10</i>	0.92	0.05	Young CECs
<i>DNAJC3</i>	1.14	0.95	Relatively low in all
<i>DZIP1</i>	0.35	0	Cultured Cells
<i>FARP1</i>	0.77	0.05	Cultured and Young
<i>HS6ST3</i>	2.16	0.75	No expression
<i>IPO5</i>	2.64	1	Cultured and Young
<i>MBNL2</i>	3.03	0.9	Relatively low in all
<i>OXGR1</i>	1.07	0	No expression
<i>RAP2A</i>	3.63	0.72	Relatively low in all
<i>SOX21</i>	n.d.	n.d.	No expression
<i>UGGT2</i>	1.77	0	Old CECs

Note:

CEC – Corneal Endothelial Cells, Low expression <15 Read per million

^a) see supplementary table for details

Figure 1

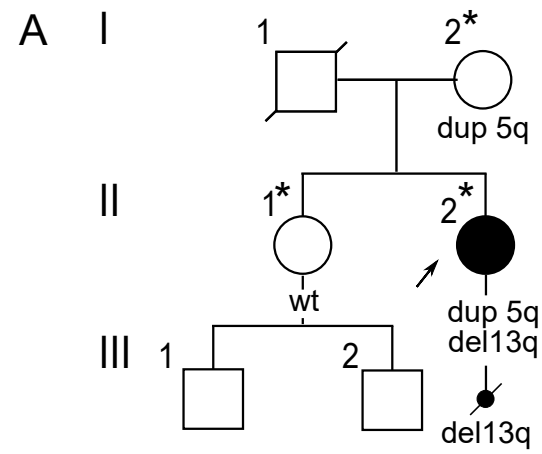
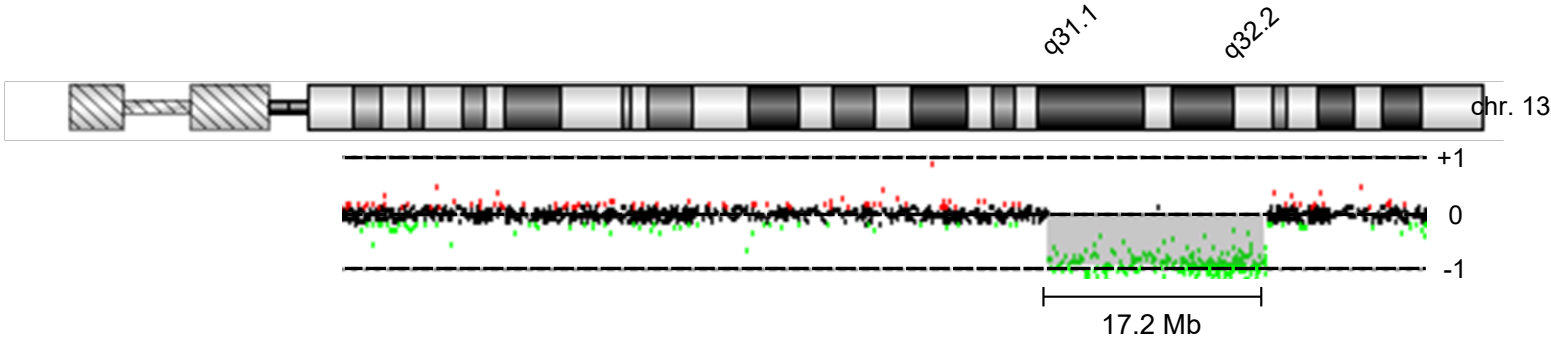


Figure 2

A



B

