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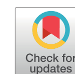
Microcoria due to first duplication of 13q32.1 including the *GPR180* gene and maternal mosaicism



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ABSTRACT

Congenital microcoria (MCOR) is an eye anomaly characterized by a pupil with diameter below 2 mm, and is caused by underdevelopment or absence of the dilator muscle of the pupil. Two types have been described: a recessive, syndromic (Pierson syndrome OMIM 609049) and a dominant, isolated form (MCOR syndrome OMIM 156600). Fares-Taie and colleagues described inherited microdeletions in chromosome band 13q32.1 segregating with dominant microcoria in several families. The *GPR180* gene is located within the smallest commonly deleted region and encodes a G protein-coupled receptor involved in smooth muscle cells growth. We here describe a patient with isolated, non-syndromic MCOR. The patient presented with a blue iris and small pupils, non-reactive to cycloplegic agents. Her mother had a milder ocular phenotype, namely a blue iris with hypoplastic crypts and mild myopia. We present a detailed clinical examination and follow up. DNA from the index patient was analyzed for the presence of chromosomal imbalances using molecular karyotyping. The genetic test revealed a small duplication of chromosome band 13q32.1. The duplication affected a 289 kb region, encompassing 11 genes including *GPR180*. Interestingly, the patient displays only MCOR in contrast to patients with the reciprocal deletion who present with MCOR and iridocorneal angle dysgenesis. This genetic anomaly was inherited from the mother who carries the duplication in mosaic form, which should be considered when offering genetic counselling. In summary, we describe the first 13q32.1 duplication encompassing *GPR180* associated with MCOR.

1. Introduction

Microcoria (MCOR) is a rare congenital eye anomaly characterized by a pupillary opening below 2 mm, Patients with this condition do not dilate after installation of mydriatic drops (Holth and Berner, 1923). Etiologically, MCOR is a heterogeneous condition, as both an autosomal recessive, syndromic and an autosomal dominant, isolated form have been described. The recessive form of MCOR is associated with systemic anomalies and is known as Pierson syndrome (OMIM 609049) (Pierson et al., 1963). In addition to MCOR, ophthalmological aspects include large corneas, lenticonus and retinal abnormalities. These patients also have congenital nephrotic syndrome and neurological abnormalities. Most succumb to their disease within their first years of life. Pierson syndrome is caused by mutations in *LAMB2* (Zenker et al., 2004). Patients with the dominant form of MCOR (OMIM 156600) do not have systemic involvement. The iris is flat with no contraction folds, has a thin periphery and can have transillumination defects. This is due to underdevelopment of the muscle dilator of the pupil. Lack of myofilaments and desmin is described in the anterior pigmented cells of the iris in a patient with MCOR (Ramirez-Miranda et al., 2011; Simpson and Parsons, 1989). Patients with MCOR often develop important myopia or astigmatism. Irido-corneal angle abnormalities can lead to glaucoma at 20 years of age. The average myopia of these patients equals −10 diopters (D), although some have a particularly high myopia (Toulemont et al., 1995). Fares-Taie and colleagues identified small chromosomal deletions on 13q32.1 in six families with dominant MCOR (Fares-Taie et al., 2015). Breakpoint mapping revealed that the *GPR180* gene was invariably deleted. The *GPR180* gene is a G protein-coupled receptor involved in smooth muscle cells growth. Moreover, a *GPR180* variant p.(Gln115*) was found in a family with irido-corneal dysgenesis and a diagnosis of Leber Congenital Amaurosis. The authors showed that the p.(Gln115*) variant segregated with the ocular angle defect and was independent from the congenital retinal dystrophy, showing that these two phenotypes have an independent genetic etiology. Together, these findings indicated that *GPR180* impairment, alone or in combination with the loss of elements that regulate the

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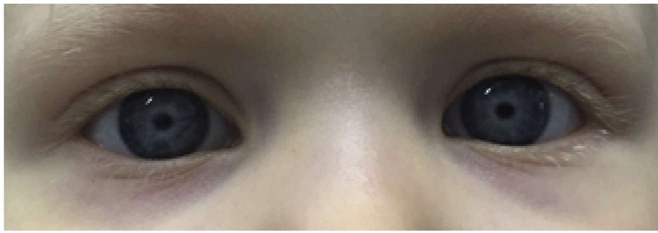

Fig. 1. Irises of the index patient. Small pupils and blue irises with no contraction folds can be seen. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

expression of neighbouring genes by a position effect, underlies MCOR (Fares-Taie et al., 2015).

We here describe a girl with non-syndromic congenital microcoria (MCOR). Molecular karyotyping revealed a small duplication of 289 kb encompassing the critical MCOR region containing the *GPR180* gene. Her mother carried the same duplication in a mosaic state. This report indicates that apart from *GPR180* deletions leading to MCOR, reciprocal duplications can lead to a similar phenotype. This further emphasizes the role of *GPR180* impairment in the molecular pathogenesis of MCOR.

2. Case report

An 18-month-old girl with an otherwise unremarkable medical history was referred for ophthalmological examination because refraction could not be measured at her routine paediatric screening. She has normal visual behaviour. There was no objection at occlusion of neither eye. She had normal ocular motility and was orthophoric. Refraction was impossible. Dilatation could not be obtained using cyclopentolate (0.5% or 1%), tropicamide 0.5% or atropine (0.5%).

Slit lamp examination showed a blue iris with very small pupils (Fig. 1). Iris hypoplasia with absence of iris crypts and no contraction folds was noticed. There was no iris transillumination. The intraocular pressure was 22 mm Hg and 17 mm Hg respectively for the right and left eye measured with i-Care. The fundus was inaccessible because of the very small pupils.

Ocular ultrasound examination showed anterior chamber, lens, vitreous, retina and optic disc with normal appearance. Examination under narcosis was carried out. Gonioscopy revealed normal irido-corneal angle structures, without signs of dysgenesis. The biometry showed axial length of 20 mm and 19.65 mm. Limited examination of the fundus was possible under narcosis and revealed normal optic nerves and retinal vessels.

During the follow up the child was re-examined at 2 years. The clinical examination was stable. The intraocular pressure was 15 mm Hg at both eyes, fundus examination and refraction remained impossible. At 2 years and 6 months, refraction was obtained −1.50 (+1.50 × 105°) and −7.00 (+2.50 × 80°) for the right and left eye, respectively, however the accuracy was uncertain. The visual acuity was 7/10 and 3/10 for the right and left eye, respectively. An optic correction and patching a few hours per day every day for left amblyopia was prescribed. At 3 years, the visual acuity was 9/10 and 4/10 for the right and left eye, respectively. Left eye patching was continued and enhanced.

The mother of the index patient was also examined. She has blue irises, with no iris transillumination. She has a −6.5 D myopia in the both eyes. Gonioscopy showed a normal irido-corneal angle. The intraocular pressure was 14 mm Hg for both eyes. Fundus examination was unremarkable.

3. Materials and methods

Cyclopentolate (0.5% or 1%), tropicamide 0.5% and atropine (0.5%) were administered in an attempt to obtain dilatation. The refraction was performed with Retinomax K-Plus 3 Righton. The vision was obtained by the Key Picture Test. The slit lamp examination was performed with Haag Streit BM 900, fundus examination with Volk Double Asphéric 90D and tonometry with i-Care Pro. The gonioscopy was performed with 3 Mirror Lens 903 Goldmann and echography with Ellex Eyecubed ultrasound.

4. Genetic testing

Molecular karyotyping was performed using 4 × 180 k Agilent arrays with an average genome wide resolution of 100 kb (AMADID#022060, hg19/GRCh37, Feb 2009) according to the manufacturer's instructions with minor modifications (Vergult et al., 2012) on DNA extracted from peripheral blood of the index patient, her mother and her maternal grandparents. This revealed a duplication of chromosomal band 13q32.1, with a minimal size of 289 kb in the index patient (chr13: 95131814–95421095, hg19). The proximal and distal breakpoints are respectively 7.4 kb (chr13:95119866–95127254, hg19) and 5.8 kb (chr13:95432254–95438104, hg19). The duplicated region encodes 7 genes: *DCT*, *TGDS*, *GPR180*, *LOC101927248*, *SOX21*, *SOX21-AS* and *LOC101927284*. In DNA from peripheral blood of the mother, a similar duplication was detected in a mosaic state. No duplication was observed in DNA from the maternal grandparents indicating a *de novo* origin of the duplication in the mother (Fig. 2). The duplication was submitted to DECIPHER (ID 403905).

5. Discussion

We here describe a child with inherited MCOR due to a microduplication of 13q32.1. The patient had the typical iris appearance, normal axial length at 18 months while at the age of 2.5 years she gradually developed myopia and reduced vision in the left eye. Interestingly, the mother of the index patient shows some features of MCOR such as blue irises and myopia. The size of her pupils is however normal. Genetic testing suggested mosaicism of the duplication but this could however not be confirmed in additional tissues. Such a parental mosaicism moreover has implications for recurrence risks, and should be considered upon genetic counselling.

Low vision has been shown in patients with MCOR and is related to the high myopia or to the development of glaucoma. Some individuals complain from nyctalopia and the visual fields can be constricted. Abnormal irido-corneal angles with iris processes concealing the trabeculum have been described in most patients with MCOR. The examination under anesthesia of the current patient did not reveal any anterior segment dysgenesis, although it will be repeated later in life to exclude this. Around 30% of MCOR patients develop high pressure glaucoma, with an average age of diagnosis of 20 years (Fares-Taie et al., 2015; Sergouniotis et al., 2017; Tawara et al., 2005). The youngest patient with glaucoma reported by Toulemont and colleagues was 7 years old (Toulemont et al., 1995). Interestingly, there is some intrafamilial variability concerning the development of glaucoma (Sergouniotis et al., 2017; Toulemont et al., 1995). We therefore proposed regular follow up of the intraocular pressure with age appropriate methods.

Fares-Taie and coworkers identified 13q32.1 deletions in six families with autosomal dominant MCOR. The size of the deletions ranged from 35.2 kb to 79.9 kb (Fares-Taie et al., 2015). Another deletion of 69 kb causing MCOR was also recently reported (Sergouniotis et al., 2017) (Fig. 3). The minimal region of deletion overlap covers only *GPR180* and *TGDS*, making it tempting to hypothesize that haploinsufficiency of one or of the two genes of which *GPR180* is the strongest candidate, might contribute to MCOR. The heterozygous nonsense variant of *GPR180* in a patient with irido-corneal angle dysgenesis but no MCOR described by Fares-Taie et al. (2015)challenges this hypothesis however. A possible explanation may be that the effect of the nonsense variant may be less severe than a complete gene

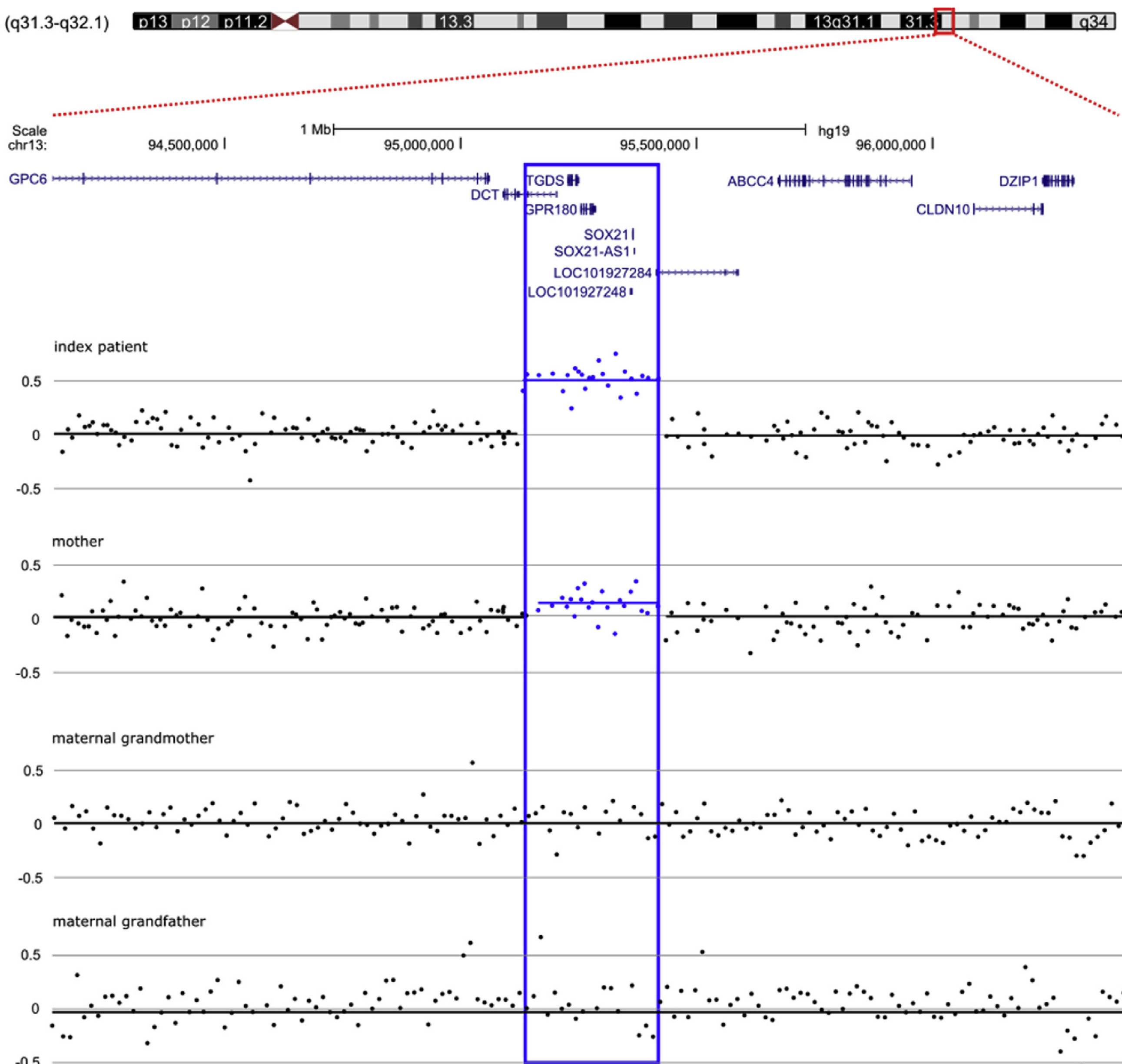

Fig. 2. The figure shows a small duplication on the long arm of chromosome 13. The minimal size of the duplication is ~290 kb (chr13: 95131814–95421095, hg19) and contains 7 genes, including *GPR180*. Upper panel: detail of the 13q31.3-q31.2 region with the duplicated genes indicated by the blue box. Bottom panel: molecular karyotyping result of the index patient, her mother and maternal grandparents. The profile of the index patient indicates a duplication with a ratio of 0.5 between duplicated and non-duplicated probes. The profile of the mother indicates mosaicism with a ratio of ~0.2 between duplicated and non-duplicated probes. No duplication is observed in DNA from the maternal grandparents. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

deletion, consequently not resulting in a full MCOR phenotype. The observed irido-corneal angle dysgenesis in these patients is therefore regarded as an MCOR endophenotype. Based on these observations Fares-Taie and colleagues could not exclude that haploinsufficiency of *GPR180* is necessary, but not sufficient, for MCOR to manifest. Consequently, they hypothesized that the full range of MCOR symptoms is due to deletions of this gene, with or without the loss of elements regulating the expression of neighbouring genes (Fares-Taie et al., 2015). Here we present a 289 kb duplication overlapping the previously described deletions, in a patient with MCOR. The duplication-associated phenotype is similar to that of the deletion-associated phenotype, except for the irido-corneal angle dysgenesis. Based on this phenotypic difference, we can support the hypothesis that disruption of regulatory elements of *GPR180* or of a neighbouring gene is necessary to cause MCOR while a genetic defect leading to a (partially) nonfunctional GPR180 protein leads to irido-corneal angle dysgenesis. As the deletions and duplication do not overlap with the boundary of the topologically associating domain (TAD) in which *GPR180* is located (Fig. 3), the overall regulatory landscape seems not to be affected by these copy number variations. Instead these intra-TAD deletions could change enhancer dosage resulting in loss of function while the intra-TAD duplication may result in either dose-dependent upregulation or tissue-specific misexpression (Spielmann et al., 2018). In conclusion, this report further emphasizes the role of *GPR180*, likely in combination with regulatory elements, in MCOR.

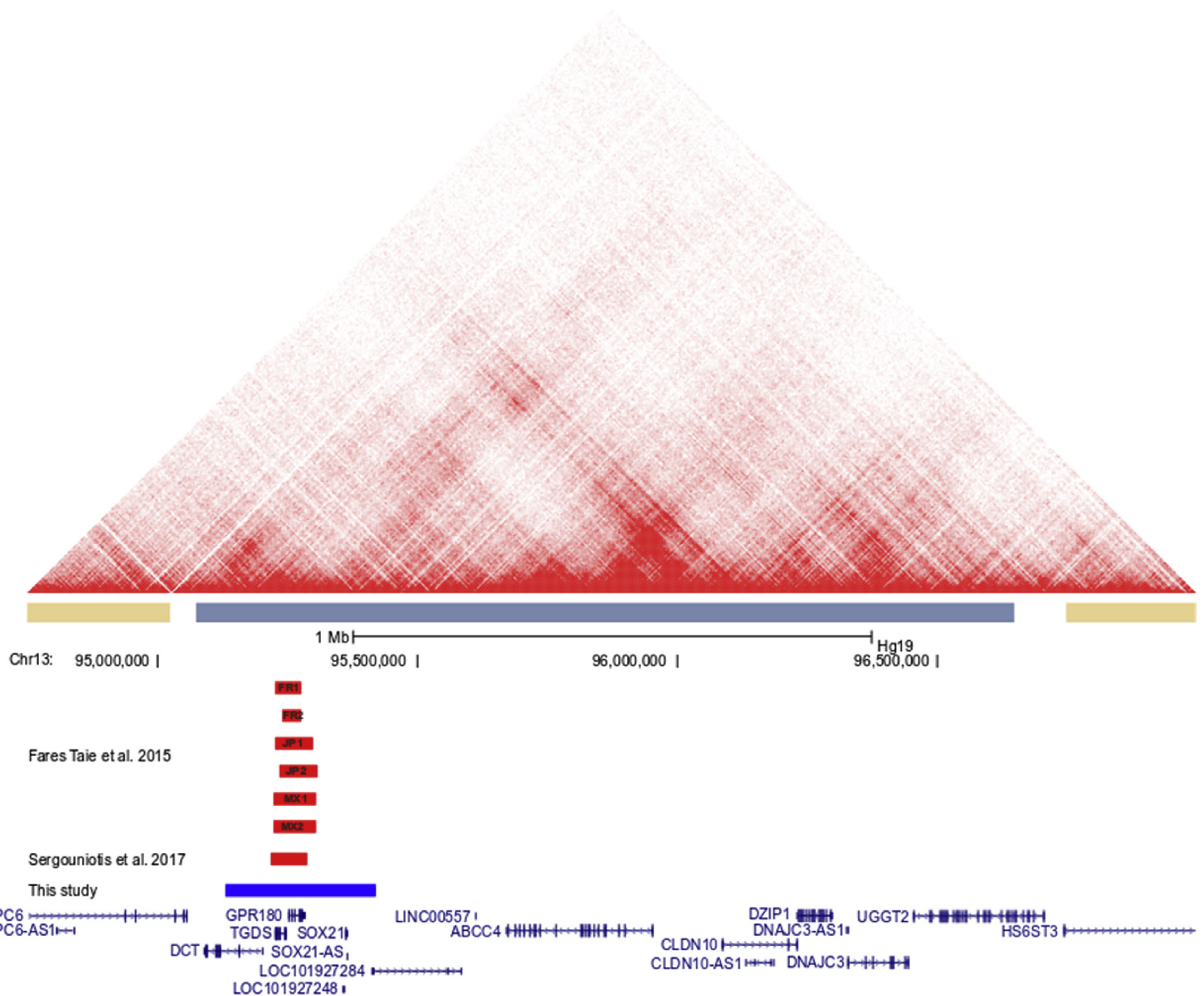

Fig. 3. Overview of the 13q32.1 region. The two-dimensional heat map representing normalized Hi-C interaction frequencies in GM12878 was visualized with the 3D Genome Browser (Wang et al., 2018). The topologically associating domain (TAD) in which *GPR180* is located, is indicated with a light blue bar (chr13:95075000–96650000, Hg19). Neighbouring TADs are shown as yellow bars. The deletions previously reported by Fares-Taie et al. (2015) and Sergeouniotis et al. (2017) are displayed as red bars. The duplication identified in this study is represented by a blue bar. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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