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# A new recessively inherited disorder composed of foveal hypoplasia, optic nerve decussation defects and anterior segment dysgenesis maps to chromosome 16q23.3-24.1

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**Purpose:** We have previously described two families with unique phenotypes involving foveal hypoplasia. The first family (F1) presented with foveal hypoplasia and anterior segment dysgenesis, and the second family (F2) presented with foveal hypoplasia and chiasmal misrouting in the absence of albinism. A genome-wide linkage search in family F1 identified a 6.5 Mb locus for this disorder on chromosome 16q23.2–24.1. The aim of this study was to determine if both families have the same disorder and to see if family F2 is also linked to the 16q locus.
**Methods:** Family members underwent routine clinical examination. Linkage was determined by genotyping microsatellite makers and calculating logarithm of the odds (LOD) scores. Locus refinement was undertaken with single nucleotide polymorphism (SNP) microarray analysis.
**Results:** The identification of chiasmal misrouting in family F1 and anterior segment abnormalities in family F2 suggested that the families have the same clinical phenotype. This was confirmed when linkage analysis showed that family F2 also mapped to the 16q locus. The single nucleotide polymorphism microarray analysis excluded a shared founder haplotype between the families and refined the locus to 3.1 Mb.
**Conclusions:** We report a new recessively inherited syndrome consisting of foveal hypoplasia, optic nerve decussation defects and anterior segment dysgenesis, which we have abbreviated to FHONDA syndrome. The gene mutated in this disorder lies within a 3.1 Mb interval containing 33 genes on chromosome 16q23.3–24.1 (chr16:83639061 - 86716445, hg19).

Foveal hypoplasia is a congenital disorder characterized by absent or abnormal foveal or macular reflexes, unclear definition of the foveomacular region, and a poorly defined foveal avascular zone. Foveal hypoplasia is commonly found in association with other eye conditions, including albinism (OMIM 203100 and 300500), achromatopsia (OMIM 216900), aniridia (OMIM 106210), retinopathy of prematurity, and incontinentia pigmenti (OMIM 308300) but has also been reported as an isolated feature [1-3].

We have previously described two families with unique phenotypes involving foveal hypoplasia. The first family (F1) is a consanguineous British family of Pakistani origin who presented with multiple cases of foveal hypoplasia and anterior segment dysgenesis (Figure 1A) [4]. The anterior segment abnormalities observed were posterior embryotoxon (all affected patients) and Axenfeld's anomaly (IV:1 and IV:7 in Figure 1). By performing a whole genome linkage search, we mapped a recessive gene for this disorder to a 6.5 Mb interval on chromosome 16q23.2–24.2. This locus is flanked by markers D16S3098 and D16S2621, and multipoint linkage analysis generated a maximum logarithm of the odds (LOD) score of 5.51 [4]. The second family (F2) is from Afghanistan and is also consanguineous (Figure 2). The two affected females presented with foveal hypoplasia and chiasmal misrouting but showed no other signs of albinism [5].

In this study, we extend the clinical phenotypes observed in each family and show that they have the same disorder,

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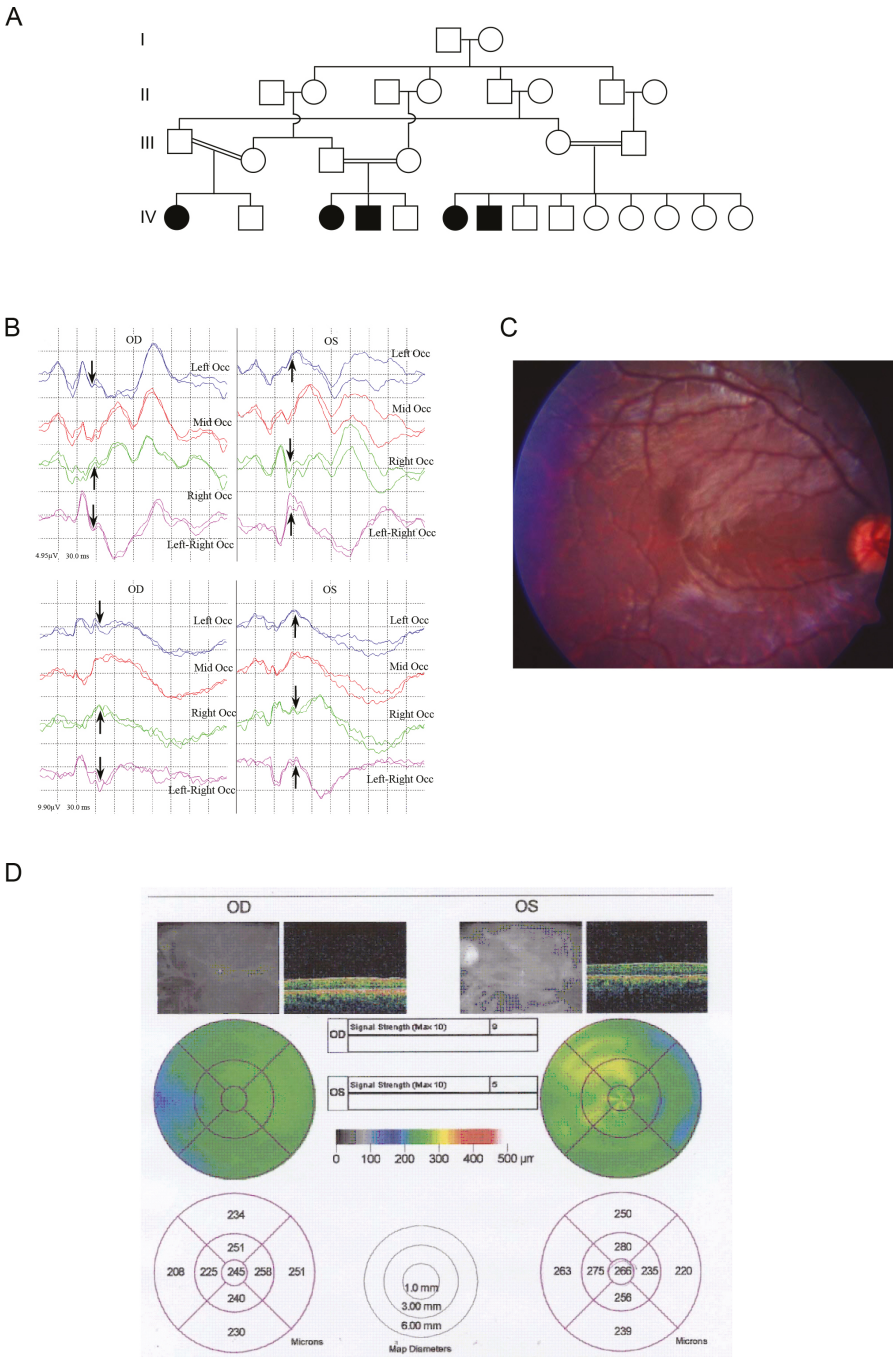


Figure 1. Clinical results of family F1. **A**: Pedigree of family F1. Individuals represented by solid symbols have been confirmed as affected by clinical examination. **B**: Ocular coherence tomography (OCT) macular thickness data for family member IV:1 showing bilateral foveal hypoplasia in an otherwise normal retina. **C**: Fundus image showing foveal hypoplasia in family member IV:1. **D**: Flash visual evoked potential (VEP) results of IV:1 (upper panel) and IV:3 (lower panel) showing contralateral asymmetry of VEP. The arrow shows the N2 peak, which is similar to that seen in albinos. OD, right eye; OS, left eye. Note the time on the x-axis begins at time-point -15 ms.

which we have termed foveal hypoplasia, optic nerve decussation defects and anterior segment dysgenesis (FHONDA syndrome). Through genetic studies, we present data suggesting that the same gene is mutated in the two families and refine the previously published 16q locus to a 3.1 Mb interval containing 33 genes.

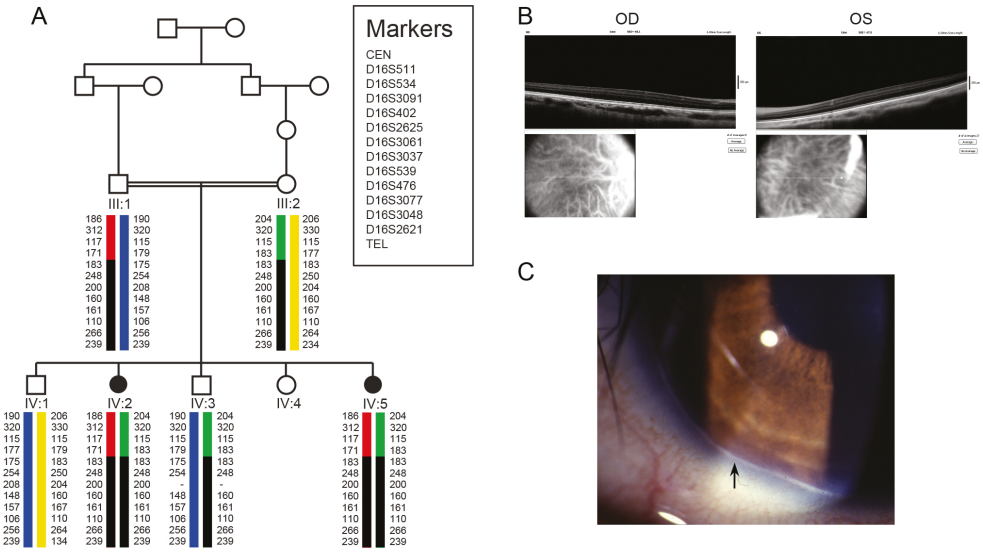


Figure 2. Clinical and genotyping results for family F2. **A**: Pedigree of family F2. Haplotypes spanning 16q23–24 are shown below each symbol. Each different haplotype block is represented by a different color with the disease haplotype colored black. **B**: Ocular coherence tomography (OCT) macular thickness data for family member IV:5 showing bilateral foveal hypoplasia in an otherwise normal retina. **C**: Slit-lamp result for IV:5 showing posterior embryotoxon (arrow).

## METHODS

*Patients:* Ethical approval was obtained from the Leeds East Teaching Hospitals Trust Research Ethics Committee, and informed consent was obtained for all subjects. Family F1 is a UK family of Pakistani origin. Nineteen members were recruited for the study, eleven females and eight males. The ages of the affected individuals at recruitment ranged from 4-14. Family F2 is a Dutch family of Afghan origin. Six members were recruited for the study, three males and three females. The ages of the affected individuals at recruitment were 16 and 10. All subjects were in good general health at the time of the recruitment. The research conducted in this study adhered to the tenets of the Declaration of Helsinki. Family F1 has been described in detail by Pal et al. [4]. Two members of family F2 were described by van Genderen et al. [5]. Detailed clinical examination of family members was undertaken. Blood samples for DNA extraction (2-6 ml depending on age) were collected by venepunture from the antecubital fossa and stored in BD vacutainer® EDTA blood collection tubes (BD Biosciences, Oxford UK). Skin color of the affected individuals was compared to that of the parents and unaffected siblings, and history was taken regarding the individuals' skin color at birth.

*Ophthalmic examination:* The eye examinations of all patients included best-corrected visual acuity, slit-lamp biomicroscopy, and indirect ophthalmoscopy. Color vision was tested using Ishihara color plates. Ocular coherence tomography (OCT) scans of the macular area were obtained using a Stratus 3000 (Carl Zeiss Meditec, Jena, Germany). Visual evoked potential (VEP) tests were performed using International Society for Clinical Electrophysiology of Vision (ISCEV) standard protocols but with a brighter flash luminance. The VEP was recorded with a common reference electrode at Fpz (10:20 system), from active electrodes at O3, Oz, and O4. The stimulus used was a Grass PS33+ stroboscope on setting 4 under scotopic conditions. The unstimulated eye was double-patched to exclude all light.

*Linkage analysis:* Genotyping was performed using fluorescently tagged microsatellite markers as described previously [6]. Briefly, standard PCR reactions were performed in a 25 µl volume containing 50 ng genomic DNA using Invitrogen Taq DNA polymerase and buffers (Invitrogen, Life Technologies Ltd, Paisley, UK). Twelve microsatellite markers (Table 1) surrounding the previously published 16q locus were selected from the UCSC Genome Browser. Following amplification, PCR products were resolved using an ABI 3730 xl DNA sequencer and analyzed using GeneMapper v 4.0 software from the same manufacturer (Applied Biosystems, Carlsbad, CA). Linkage analysis was performed under the assumption of a recessive model with 100% penetrance and 0.0001 frequency of the disease allele and equal marker allele frequencies. Multipoint linkage analyses were performed using Superlink [7]. The UCSC Genome Browser was used to determine the genetic distances.

*Single nucleotide polymorphism microarray analysis:* DNA from one affected member of each family was analyzed using Affymetrix 6.0 SNPchip. This analysis was performed with AROS Applied Biotechnology (Århus, Denmark). The results were analyzed using IBDfinder software [8].

Table 1. Microsatellite primer details

| Microsatellite | Forward primer (5′-3′) | Reverse primer (5′-3′) | Size (bp) |
|---|---|---|---|
| D16S511 | CCCCGGAGCAAGTTCA | CAGCCCAAAGCCAGATTA | 182–222 |
| D16S534 | CAACAAAGCAAGACCCTGTC | CATCTGCGGTTCTTTCCTC | 294–364 |
| D16S3091 | GGGAGATAGCCTTAAACTTTCTTAC | TGTTGCTAATAACACTAGGCCA | 115–129 |
| D16S402 | TTTTGTAACCATGTACCCCC | ATTTATAGGGCCATGACCAG | 161–187 |
| D16S2625 | TACGCAAGTCAAAGAGCCTC | GGACACATGAGACCCTGTCT | 183 |
| D16S3061 | CTACTGGTGAGGCTGAGGTG | ATATCTCGGGATTTGTTGCTTTAC | 241–253 |
| D16S3037 | GAGCCAAGATGACACCACT | GCACTGGGAACCTAAGGA | 201–217 |
| D16S539 | GATCCCAAGCTCTTCCTCTT | ACGTTTGTGTGTGCATCTGT | 157 |
| D16S476 | TTGCACTCCACTCTGGGCA | TTGCCTTGGCTTTCTGTTGG | 144–181 |
| D16S3077 | AGCAAGCCGTGACTGGGT | CATGAGTAGTGTCCTGGGGG | 253–267 |
| D16S3048 | AGCAAGCCGTGACTGGGT | CATGAGTAGTGTCCTGGGGG | 253–267 |
| D16S2621 | GTCATATGGGCCAATTCCC | TACCGCGTAGTGAGACTGTG | 239–263 |

## RESULTS

The presence of foveal hypoplasia in the absence of well-recognized syndromes such as albinism and aniridia is rare. This prompted us to investigate whether families F1 and F2 could have the same clinical disorder. To address this question, both families underwent additional clinical examination. Electrophysiological testing to measure flash VEPs was performed in two members of family F1 (IV:1 and IV:3) to determine if they had any optic nerve misrouting defects. The results in Figure 1 show that both patients have a contralateral predominance indicating increased crossover at the level of the optic chiasm. Ocular coherence tomography analysis in these patients confirmed that they lacked a foveal pit but showed that the thickness and overall structure of the retina were normal (Figure 1). All affected members of this family have absent iris transillumination and have dark pigmented skin and hair, which has been present since birth, and is similar to that of their parents and siblings. Affected members of family F2 were reexamined for signs of anterior segment dysgenesis. Both members had posterior embryotoxon (Figure 2). Thus, the two families have the same clinical phenotype of foveal hypoplasia, optic nerve decussation defects and anterior segment dysgenesis in the absence of albinism. We termed this new phenotype FHONDA syndrome. The clinical features found in patients with FHONDA syndrome are summarized in Table 2.

We previously mapped the recessive gene mutated in family F1 to a new locus on chromosome 16q [4]. To determine if the gene mutated in family F2 also mapped to this locus, we genotyped 12 microsatellites spanning this locus in family members. The haplotype data for these markers is shown in Figure 2. The order and Marshfield genetic location of these markers are as follows: 16cen – D16S511 (110.4-cM) – D16S534 (111.12-cM) – D16S3091 (111.12-cM) – D16S402 (113.52-cM) – D16S2625 (120.59-cM) – D16S3061 (121.45-cM) – D16S3037 (121.45-cM) – D16S539 (124.73-cM) – D16S476 (128.53-cM) – D16S3077 (127.99-cM) – D16S3048 (127.99-cM) – D16S2621 (130.41-cM) −16qtel (genetic distances are taken from the chromosome 16p telomere). As the genetic position of marker D16S476 did not correlate with its physical position (determined from the genome sequence), we corrected the marker order and used the genetic position of 127.53-cM in our linkage analysis.

Affected individuals IV:2 and IV:5 from family F2 share a region of homozygosity from marker D16S402 to the end of the 16q telomere. To determine the significance of this finding, multipoint LOD scores were generated using all markers. The results are shown in Figure 3. A maximum LOD score of 2 was generated with the telomeric 7 markers, which is the cutoff point for determining significant linkage when testing a known locus.

To define the smallest region of homozygosity in each family, DNA from a single affected individual from each family was processed using whole-genome single nucleotide polymorphism (SNP) microarrays (Affymetrix 6.0). These results refined the region of homozygosity in family F1 to an area between the markers D16S505 and rs11863161, and the region in family F2 to that between SNP rs8059833 and the end of 16q. No shared haplotype between the families was evident, suggesting that each family has a different mutation. By combining the SNP data of families F1 and F2, we refined the location of the FHONDA syndrome gene to a 3.1 Mb interval containing 33 genes (chr16:83639061 - 86716445, hg19).

Table 2. Clinical findings in FHONDA patients.

| Patient | Visual acuity (Snellen) OD, OS | Refraction OD, OS | Color Vision | Anterior Segment | Posterior Segment | Chiasm | Iris Transillumination | Other findings |
|---|---|---|---|---|---|---|---|---|
| F1 IV:1 | 20/120, 20/120 | −0.25D/-3.25Dx175 −0.50D/-3.0D x10 | Normal | Axenfeld's anomaly | Foveal hypoplasia | Misrouting | Absent | Esotropia |
| F1 IV:3 | 20/120, 20/120 | +6.50D/-2.0Dx172 +6.0D/-2.0Dx168 | Normal | Posterior embryotoxon | Foveal hypoplasia | Misrouting | Absent | Congenital esotropia |
| F1 IV:4 | 20/200, 20/200 | +1.5D/+3.0Dx95 +0.75D/+3.5Dx95 | Normal | Posterior embryotoxon | Foveal hypoplasia | NT | Absent | Esotropia |
| F1 IV:6 | 20/200, 20/200 | −1.75D/+3.5Dx90 −0.5D/+3.0Dx95 | Normal | Posterior embryotoxon | Foveal hypoplasia | NT | Absent | |
| F1 IV:7 | 20/400, 20/400 | +0.25D/+2.25Dx90 +0.25D/+3.25 Dx90 | Normal | Axenfeld's anomaly | Foveal hypoplasia | NT | Absent | Microcephaly and epilepsy |
| F2 IV:2 | 20/120, 20/100 | +2.0D/-2.0Dx180 +2.5D/-2.0Dx180 | Normal | Posterior embryotoxon | Foveal hypoplasia | Misrouting | Absent | |
| F2 IV:5 | 20/200, 20/120 | +2.0D/-5.0Dx10 +2.5D/-5.5Dx180 | Normal | Posterior embryotoxon | Foveal hypoplasia | Misrouting | Absent | |

OD, right eye; OS, left eye; NT, not tested

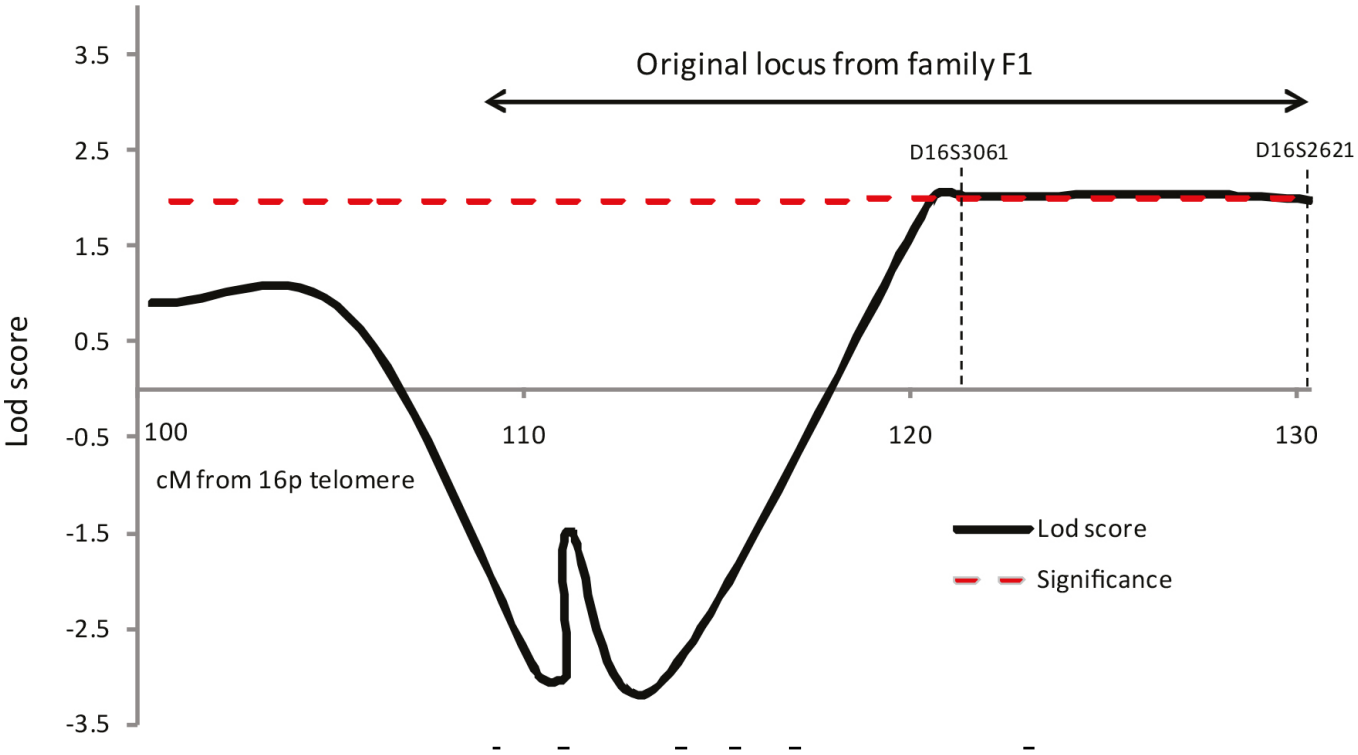


Figure 3. Multipoint graph showing linkage to the foveal hypoplasia, optic nerve decussation defects and anterior segment dysgenesis locus in family 2. The approximate position of the locus published by Pal et al. is shown above the graph [4]. Logarithm of the odds (LOD) scores above 2 are statistically significant for linkage when testing is limited to one known locus.

## DISCUSSION

In this study, we defined a new disorder called FHONDA syndrome comprising foveal hypoplasia, optic nerve decussation defects and anterior segment abnormalities. Combined foveal hypoplasia and optic nerve decussation defects have, up to now, been exclusively found in albinism. Although the anterior segment abnormality posterior embryotoxon is not a well-reported feature, studies have shown that 30% of ocular albinism cases have this feature [9,10]. However, none of the patients reported in this study have skin or ocular pigmentation defects and have absent iris transillumination. This suggests that FHONDA syndrome is not a variant of albinism or caused by a defect in the melanin biosynthesis pathway. Nevertheless, pigmentation defects in albinism can be difficult to detect. Mutation screening in a cohort of patients with autosomal recessive ocular albinism showed that at least 69% (26/36) had mutations within the oculocutaneous albinism genes, particularly *TYR*, but the mild cutaneous pigmentation defects in these patients had been missed at diagnosis [11]. Similarly, patients with mutations in the ocular albinism gene, *OA1,* but without the classic ocular pigmentation defects have been reported [12-16]. In both instances, the lack of hypopigmentation has been attributed to factors such as hypomorphic alleles, genetic background, and/or environmental factors that modify this aspect of the phenotype [11,17]. Thus, we cannot categorically rule out that FHONDA syndrome is allelic with albinism and that similar modifier factors influence the pigmentation, especially as we report only two families of Asian origin. Therefore, once the mutated gene has been identified, it should be screened in a cohort of patients with mutation-negative albinism. A recent mutation screen of the known albinism genes in a cohort of Danish patients with albinism showed that they accounted for only 48% (10/21) of the patients with autosomal recessive ocular albinism, indicating that new genes remain to be identified [17].

Through a combination of microsatellite and SNP genotyping, the gene mutated in both families with FHONDA has been mapped to the same telomeric region of chromosome 16q. This region contains only 33 genes, but none are obvious candidates apart from the forkhead box C1 gene, which has previously been excluded as the FHONDA gene [4]. The mechanism underlying optic nerve decussation at the chiasm has been an intensively studied model for neuronal patterning and guidance [18,19]. Similarly, foveal development has been studied as this highly specialized area of the eye underlies the majority of human visual function [20]. Over the years, these studies have implicated several molecules that individually

influence the development of these structures, but only those involved in the melanogenesis pathway have been shown to impact the development of both. Presumably, the presence of melanin in the retinal pigment epithelium influences the transcription of several retinal genes, but the exact mechanism remains unknown. The identification of the gene mutated in FHONDA syndrome will hopefully shed light on these processes and provide a new melanin-independent aspect to this research.

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