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

Musallam Al-Araimi,¹ Bishwanath Pal,¹ James A. Poulter,¹ Maria M. van Genderen,² Ian Carr,³ Tomas Cudrnak,⁴ Lawrence Brown,⁵ Eamonn Sheridan,⁶ Moin D. Mohamed,¹ John Bradbury,¹ Manir Ali,¹ Chris F. Inglehearn,¹ Carmel Toomes¹

(The first two authors contributed equally to this work.)

¹Section of Ophthalmology and Neuroscience, Leeds Institutes of Molecular Medicine, University of Leeds, Leeds, UK; ²Bartiméus Institute for the Visually Impaired, Zeist, Netherlands; ³Section of Translational Medicine, Leeds Institutes of Molecular Medicine, University of Leeds, Leeds, UK; ⁴Department of Ophthalmology, Bradford Royal Infirmary, Bradford, UK; ⁵Department of Ophthalmology, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK; ⁶Section of Genetics, Leeds Institutes of Molecular Medicine, University of Leeds, Leeds, UK; ⁷Department of Clinical Genetics, St James’s University Hospital, Leeds, UK

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,
which we have termed foveal hypoplasia, optic nerve decus-
sation 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.
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 venepuncture 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 x1 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].
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 are as follows: 16cen – D16S511 (110.4-cM) – D16S334 (111.12-cM) – D16S3091 (111.12-cM) – D16S402 (113.52-cM) – D16S2625 (120.59-cM) – D16S3061 (121.45-cM) – D16S3077 (121.45-cM) – D16S539 (124.73-cM) – D16S476 (128.53-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 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).

| Microsatellite | Forward primer (5′-3′) | Reverse primer (5′-3′) | Size (bp) |
|---------------|-----------------------|------------------------|-----------|
| D16S511       | CCCGGAGCAAGTTCA       | CAGCCCAAAGCCAGATTA     | 182–222   |
| D16S334       | CAACAAGCAAGACCCTGTC   | CATCTGGGTCTTTTCTCTC    | 294–364   |
| D16S3091      | GGGAGATAGCCTTTAATCTTTTAC | TGTGTCAATAACACTAGGCCA | 115–129   |
| D16S402       | TTTGTAACCATGACCCCC    | ATTTATAGGGCCATGACCAG   | 161–187   |
| D16S2625      | TACGAAGTCAGACAGCCTC   | GGACATGAGACCCTGCTC     | 183       |
| D16S3061      | CTACTGGGTACGTAGGTTG   | ATATCTCGGGATTGTGTCTTTAC | 241–253   |
| D16S3037      | GAGCCAGATGACACCACT    | GCACCGGGAACCTAAGGA     | 201–217   |
| D16S539       | GATCCAAGCTCTTCTCTTCTCT | ACCTTGTGTGTCATCCTGT   | 157       |
| D16S476       | TTGCATCCACTCTGGGCA    | TTGCCTTGCTTTCTGTTGG    | 144–181   |
| D16S3077      | AGCAAGCCGTGACTGGGT    | CATGAGTAGTGTCCTGGGGG   | 253–267   |
| D16S3048      | AGCAAGCCGTGACTGGGT    | CATGAGTAGTGTCCTGGGGG   | 253–267   |
| D16S2621      | GTCATATGGGCAATTCCTCC  | TACCGCGTAGTGAAGCTTTG   | 239–263   |
| 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
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]. 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 modify this aspect of the phenotype [11,17].
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.

ACKNOWLEDGMENTS

We would like to thank the families for their participation in this study. The financial support of the Oman Ministry of Higher Education and the Oman Ministry of Health (MA-A) and the Royal Society (CT is a Royal Society University Research Fellow) is gratefully acknowledged. Part of this work has previously been presented at the ARVO conference in 2009 (Investigative Ophthalmology and Visual Science 50: 41,200).

REFERENCES

1. Curran RE, Robb RM. Isolated foveal hypoplasia. Arch Ophthalmol 1976; 94:48-50. [PMID: 1247409].
2. Oliver MD, Dotan SA, Chemke J, Abraham FA. Isolated foveal hypoplasia. Br J Ophthalmol 1987; 71:926-30. [PMID: 3427001].
3. Azuma N, Nishina S, Yanagisawa H, Okuyama T, Yamada M. PAX6 missense mutation in isolated foveal hypoplasia. Nat Genet 1996; 13:141-2. [PMID: 8640214].
4. Pal B, Mohamed MD, Keen TJ, Williams GA, Bradbury JA, Sheridan E, Inglehearn CF. A new phenotype of recessively inherited foveal hypoplasia and anterior segment dysgenesis maps to a locus on chromosome 16q23.2–24.2. J Med Genet 2004; 41:772-7. [PMID: 15466012].
5. van Genderen MM, Riemslag FC, Schuil J, Hoeben FP, Stilma JS, Meire FM. Chiasmal misrouting and foveal hypoplasia without albinism. Br J Ophthalmol 2006; 90:1098-102. [PMID: 16707527].
6. Toomes C, Downey LM, Bottomley HM, Mintz-Hittner HA, Inglehearn CF. Further evidence of genetic heterogeneity in familial exudative vitreoretinopathy; exclusion of EVR1, EVR3 and EVR4 in a large autosomal dominant pedigree. Br J Ophthalmol 2005; 89:194-7. [PMID: 15665352].
7. Silberstein M, Tzemach A, Dovgolevsky N, Fishelson M, Schuster A, Geiger D. Online system for faster multipoint linkage analysis via parallel execution on thousands of personal computers. Am J Hum Genet 2006; 78:922-35. [PMID: 16685644].
8. Carr IM, Sheridan E, Hayward BE, Markham AF, Bonthron DT. IBDFinder and SNPssetter: tools for pedigree-independent identification of autozygous regions in individuals with recessive inherited disease. Hum Mutat 2009; 30:960-7. [PMID: 19405095].
9. Charles SJ, Green JS, Grant JW, Yates JR, Moore AT. Clinical features of affected males with X linked ocular albinism. Br J Ophthalmol 1993; 77:222-7. [PMID: 8494858].
10. Shiono T, Tsunoda M, Chida Y, Nakazawa M, Tamai M. X linked ocular albinism in Japanese patients. Br J Ophthalmol 1995; 79:139-43. [PMID: 7696233].
11. Hutton SM, Spritz RA. A comprehensive genetic study of autosomal recessive ocular albinism in caucasian patients. Invest Ophthalmol Vis Sci 2008; 49:868-72. [PMID: 18326704].
12. Preising M, Op de Laak JP, Lorenz B. Deletion in the OA1 gene in a family with congenital X linked nystagmus. Br J Ophthalmol 2001; 85:1098-103. [PMID: 11520764].
13. Liu JY, Ren X, Yang X, Guo T, Yao Q, Li L, Dai X, Zhang M, Wang L, Liu M, Wang OK. Identification of a novel GPR143 mutation in a large Chinese family with congenital nystagmus as the most prominent and consistent manifestation. J Hum Genet 2007; 52:565-70. [PMID: 17516023].
14. Zhou P, Wang Z, Zhang J, Hu L, Kong X. Identification of a novel GPR143 deletion in a Chinese family with X-linked congenital nystagmus. Mol Vis 2008; 14:1015-9. [PMID: 18523664].
15. Fang S, Guo X, Jia X, Xiao X, Li S, Zhang Q. Novel GPR143 mutations and clinical characteristics in six Chinese families with X-linked ocular albinism. Mol Vis 2008; 14:1974-82. [PMID: 18978956].
16. Grønskov K, Ek J, Brondum-Nielsen K. Oculocutaneous albinism. Orphanet J Rare Dis 2007; 2:43. [PMID: 17980020].
17. King RA, Willaert RK, Schmidt RM, Pietsch J, Savage S, Brott MJ, Fryer JP, Summers CG, Oetting WS. MC1R mutations modify the classic phenotype of oculocutaneous albinism type 2 (OCA2). Am J Hum Genet 2003; 73:638-45. [PMID: 12876664].
18. Grønskov K, Ek J, Sand A, Scheller R, Bygum A, Brixen K, Brondum-Nielsen K, Rosenberg T. Birth prevalence and mutation spectrum in Danish patients with autosomal recessive albinism. Invest Ophthalmol Vis Sci 2009; 50:1058-64. [PMID: 19060277].
19. Petros TJ, Rebsam A, Mason CA. Retinal axon growth at the chiasm: to cross or not to cross. Annu Rev Neurosci 2008; 31:295-315. [PMID: 18558857].
20. Provis JM, Dubis AM. Maddess, Carroll J. Adaptation of the central retina for high acuity vision: Cones, the fovea and the avascular zone. Prog Retin Eye Res 2013; 35:63-81. [PMID: 23500668].
