Genotypic and Phenotypic Spectrum of Foveal Hypoplasia: A Multi-centre Study

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Abstract

Purpose: To characterise the genotypic and phenotypic spectrum of foveal hypoplasia (FH)

Design: Multi-centre, observational study

Subjects: 907 patients with a confirmed molecular diagnosis of albinism, *PAX6*, *SLC38A8*, *FRMD7*, *AHR* or achromatopsia *(ACHM)* from twelve centres in nine countries (n=523), or, extracted from publicly available datasets from previously reported literature (n=384).

Methods: Individuals with a confirmed molecular diagnosis and availability of foveal optical coherence tomography (OCT) scans were identified from twelve centres or from the literature, between January 2011 and March 2021. A genetic diagnosis was confirmed by sequence analysis. Grading of FH was derived from OCTs.

Main outcome measures: Grade of FH, presence or absence of photoreceptor specialisation (PRS+ vs PRS−), molecular diagnosis and visual acuity (VA).

Results: The most common genetic etiology for typical FH in our cohort was albinism (67.5%), followed by *PAX6* (21.8%), *SLC38A8* (6.8%) and *FRMD7* (3.5%) variants. *AHR* variants were rare (0.4%). Atypical FH was seen in 67.4% of ACHM cases. Atypical FH in ACHM had significantly worse VA compared to typical FH (p<0.0001). There was a significant difference in the spectrum of FH grades based on the molecular diagnosis (X^2=60.4, p<0.0001). All *SLC38A8* cases were PRS− (p=0.003), while all *FRMD7* cases were PRS+ (p<0.0001). Analysis of albinism sub-types revealed a significant difference in the grade of FH (X^2=31.4, p<0.0001) and VA (p=0.0003) between oculocutaneous albinism (OCA) compared to ocular albinism (OA) and Hermansky-Pudlak syndrome (HPS). OA and HPS demonstrated higher grades of FH and worse VA than OCA. There was a significant difference (p<0.0001) in VA between *FRMD7* variants compared to other diagnoses associated with FH.

Conclusion: We characterised the phenotypic and genotypic spectrum of FH. Atypical FH is associated with much worse prognosis compared to all other forms of FH. In typical FH, our data suggests that arrested retinal development occurs earlier in *SLC38A8, OA, HPS* and *AHR* variants and much later in *FRMD7* variants. The defined time-period of foveal developmental arrest for OCA and *PAX6* variants appears to demonstrate more variability. Our findings provide mechanistic insight into disorders associated with FH and also have significant prognostic and diagnostic value.

Précis

We observe distinctive patterns of arrested foveal development based on genotype and highlight the mechanistic implications with direct clinical relevance to diagnosis and prognosis. This helps prioritise genetic testing, subsequent counselling, and support.

Introduction

The normal foveal anatomy consists of the extrusion of the plexiform layers, a formed foveal pit, cone photoreceptor outer segment (OS) lengthening and outer nuclear layer (ONL) widening relative to the parafoveal OS and ONL.[1] Foveal development begins with the centrifugal displacement of the inner retinal layers, followed by the centripetal migration

of the cone photoreceptors towards the incipient fovea and finally cone photoreceptor specialisation (figure 1A–E).[2–4] Foveal pit formation begins mid-gestation and continues postnatally characterised by pit deepening and widening. Simultaneously, outer retinal specialisation occur at a rapid rate in the first two years of life but ONL widening continues up to the age of 13 years.[4] Lengthening of the OS layer represents cone photoreceptor specialisation and reflects peak cone photoreceptor density.[5] The maturation of the human fovea is a complex process and is critical for visual function.[4] Many genes are involved in the development of the retina. Pathogenic variants in these genes often cause disruption to the foveal developmental process, resulting in foveal hypoplasia (FH), which describes the underdevelopment of the fovea.[1]

FH is characterised by the continuation of inner retinal layers posterior to the foveola, and progressive loss of these foveal elements is represented by increasing grades of FH. Three key foveal development stages form the basis of The Leicester Grading System for FH, developed by Thomas *et al.* (2011) (figure F–H).[1] The Leicester Grading System is divided into four grades of typical FH (grades 1–4) and one grade of atypical FH, which is associated with photoreceptor degeneration.

The Leicester Grading System can be applied to a diverse range of genetic disorders including albinism, achromatopsia and those caused by pathogenic variants in *PAX6*, *SLC38A8*, *FRMD7* and *AHR*. In addition to providing insight into the degree of foveal development, identifying the grade of FH has diagnostic and prognostic implications.[1,6] Recently, Rufai *et al.* (2020) reported that identifying the grade of FH can predict future visual acuity (VA) in preverbal children with nystagmus.[6]

The introduction of optical coherence tomography (OCT) has revolutionised ophthalmic diagnosis, with the ability to promptly visualise retinal morphology in a high-resolution, non-invasive manner.[7,8] Furthermore, the advent of hand-held OCT has facilitated the foveal examination of paediatric patients.[9] OCT is now available in most ophthalmology clinics, and subsequently has rapidly become part of routine ophthalmic assessment.[7] Thus, the necessary technology to identify foveal developmental abnormalities is now accessible in most centres.

The phenotypic spectrum of albinism has previously been reported, including the variance in degree of arrested retinal development.[10,11] Furthermore, high grades of FH (grades 3 and 4) have recently been consistently associated with *SLC38A8* variants.[12] However, to date, the full spectrum of FH linked with variants of known genes involved in retinal development, has not yet been investigated. Thus, it is unclear whether variants of certain genes known to be related to FH and nystagmus, are associated with a more underdeveloped foveal morphology. We performed a comparative study to investigate and characterise the genotypic and phenotypic spectrum of FH in albinism, achromatopsia, *PAX6*, *SLC38A8*, *FRMD7* and *AHR* variants.

Methods

In this multi-centre study, we collated genotypic and phenotypic data for 907 patients from twelve centres in nine countries, from a cohort of patients with infantile nystagmus and/or FH over a 10-year period (2011–2021). We set up an international consortium with a special interest in foveal developmental disorders, composed of paediatric ophthalmologists, neuro-ophthalmologists, clinical geneticists, data scientists and statistical geneticists. Data was collected by our Foveal Development Investigators Group (FDIG). Inclusion criteria was defined as: (1) a molecular diagnosis and (2) report of foveal morphology on OCT. Genes known to be associated with typical FH (table 1) were selected; *PAX6, SLC38A8, FRMD7, AHR* and genes underlying both syndromic and non-syndromic forms of albinism (*TYR, OCA2, TYRP1, SLC45A2, SLC24A5, LRMDA (C10orf11), GPR143, HPS (1–11), LYST*). An overview of the phenotypical characteristics associated with each of these disorders is included in table 1. We also included the achromatopsia genes (*CNGB3, CNGA3, GNAT2, PDE6C, PDE6H and ATF6*) associated with atypical FH (table 1). Previously reported cases that met the inclusion criteria were additionally identified and collected from the literature between 2011–2021. We included all patients with foveal morphology documented on OCT and pathogenic variants in any one of the genes listed, irrespective of whether they had FH or normal foveal morphology. This study was approved by the local ethics committee and adhered to the tenets of Declaration of Helsinki.

Grading of foveal hypoplasia

FH was graded using the Leicester Grading System for FH (figure 1F,G).[1] Typical FH was diagnosed as Grade 1 if OCT revealed incomplete extrusion of the inner retinal layers posterior to the foveola, the presence of a foveal pit (irrespective of depth), lengthening of the OS layer and widening of the ONL. FH was diagnosed as grade 2 if all features of grade 1 were present, except there was no longer a foveal pit. FH was diagnosed as grade 3 FH if all features of grade 2 FH were present except there was no lengthening of the OS layer. FH was diagnosed as grade 4 FH if all features of grade 3 were present except there was no ONL widening (mimicking the appearance of the peripheral retina).[1] Grades 1 and 2 together can be considered to show evidence of cone photoreceptor specialisation (PRS+), whilst in grades 3 and 4 there is no evidence of cone photoreceptor specialisation (PRS−).[1]

Atypical FH is associated with photoreceptor degeneration and was diagnosed if OCT revealed the disruption of the inner segment ellipsoid associated with continuation of the inner retinal layers posterior to the foveola.[1] Foveal scans were interpreted and graded by experienced clinicians within the FDIG.

Multi-Centre Data Collection

Patients with a confirmed molecular diagnosis and a report of foveal morphology were identified between Jan-2011 and March-2021 from twelve different sites in nine countries. Foveal scans were captured with OCT using site-specific protocols previously described (supplementary table 1).[10,12,13] Patient demographic and basic ophthalmic examination findings were included, where available. Informed consent was obtained from all involved

participants. Ethical approval was received from the research ethics committee (REC references: (1) 20/EM/0040, (2) 31499, (3) 10/H0406/74, (4) 12/EM/0261).

To perform genetic analysis, genomic DNA was extracted from saliva samples or peripheral blood samples, dependent on protocols at each specific site. DNA was extracted as per manufacturer guidelines. Targeted next generation sequencing (NGS) panels,[10,14,15] whole exome sequencing,[16] whole genome sequencing,[16,17] or Sanger sequencing[10,11,18,19] was then performed. Individuals with a genetic diagnosis (including those with missing heritability i.e., just one pathogenic variant) in the appropriate clinical context, were included. Details of genetic analysis protocols have previously been published.[10,14,17] Briefly, our NGS analysis protocol consisted of variant calling using the GATK pipeline (https://gatk.broadinstitute.org/) and further annotation using ANNOVAR (http://www.annovar.openbioinformatics.org/).

Data Extraction from Literature Review

Data was extracted from the literature by five experienced clinicians in order to identify previous reports of cases and cohorts of individuals with a diagnosis and foveal OCT. Literature searches were carried out using the databases PubMed, Medline and Scopus. We applied a filter to search published literature between January-2011 and March-2021. FH grading was introduced in 2011 hence we did not include literature prior to this.

Search terms included "optical coherence tomography" or "foveal hypoplasia" plus the diagnosis in question (e.g., "albinism", "*PAX6*"). To broaden our search strategy, we also included search terms "aniridia", "infantile nystagmus" and the abbreviations for ocular albinism ("OA") and oculocutaneous albinism ("OCA"). Syndromic forms of albinism; Hermansky-Pudlak syndrome (HPS), Chediak Higashi syndrome (CHS) and Griscelli syndrome (GS) were also searched for. An overview for the literature search approach can be found in supplementary figure 1. Further details of the cohort extracted from the literature is shown in supplementary table 2.

In cases where a foveal tomogram was provided but no formal comment of foveal morphology was made, an experienced clinician would interpret and grade the tomogram, using the Leicester Grading System.[1] Basic demographic and clinical characteristics were also collected, where available. VA scores or fractions were converted to logarithm of minimum angle of resolution (logMAR).

Individuals reported in publications authored by a collaborator site were omitted from the 'literature cases' to avoid repeated data. For the purpose of reporting results, data from study sites and the literature was combined, unless otherwise specified.

Statistical Analysis

Statistical analysis was carried out using SPSS software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). An average of right and left VA measurements was calculated and used for statistical analysis. Normality testing of the VA distribution was carried out with the Shapiro-Wilk test.

The Kruskall-Wallis test was used to test for statistical differences between average VA measurements across different diagnostic groups, grades of FH, and to assess for statistical differences in average VA between the albinism subgroups; OCA, OA and HPS. To test whether cone photoreceptor specialisation was affected (PRS+ versus PRS−) we performed the Pearson chi-squared test. This allowed us to investigate whether there was a difference in the proportion of cases with and without cone photoreceptor specialisation (PRS+ versus PRS−) based on the genotype. We performed a sub-analysis (Pearson chi-squared test) within the albinism group dividing the cohort into OCA, OA and HPS. Post-hoc analysis was performed as previously described,[20] and we report the adjusted residuals (adjusted z-scores) with adjusted *p*-values (Bonferroni correction) to control for a Type 1 error.

All analyses were considered statistically significant when a probability value of $p \leq 0.05$ was identified.

Results

Overview of the cohort

This multi-centre study identified a total of 907 suitable individuals with genotypic and phenotypic data, from study sites (57.7%) and from the literature (42.3%). The mean age of the cohort was 22.7 years (SD=16.7 years), with a higher proportion of males (53.6%) compared to females (46.4%). The age and gender breakdown per diagnostic group is shown in the supplementary figure 2. In the achromatopsia cohort (n=310), atypical FH was observed in 67.4% of cases (figure 2A). Among individuals with variants in genes linked to typical FH (n=597), we observed typical FH in 81.6% (figure 2A).

Comparison of VA between typical foveal hypoplasia, atypical foveal hypoplasia and normal foveal morphology

Atypical FH had a significantly worse VA (*p*<0.0001) compared to the typical FH group and the normal foveal morphology group (figure 3A). Further analysis with typical FH split into each grade of FH revealed significant (*p*<0.05) differences in VA (figure 3B) for all pairwise comparisons except between grade 2 and 3 FH.

Typical FH: genetic etiologies, photoreceptor specialisation and visual acuity

Genetic etiologies—The breakdown of genetic etiologies of typical FH (n=487) included: albinism (67.5%), *PAX6* variants (21.8%), *SLC38A8* variants (6.8%), *FRMD7* variants (3.5%) and *AHR* variants (0.4%) (figure 2B). Grade 4 FH was the most frequently reported grade of FH in this study (43.1%), and grade 2 was the least prevalent (14.0%) (figure 4). An overview of the spectrum of typical FH OCT tomograms associated with each diagnosis is shown in figure 4. The genotypic spectrum for the cases of typical FH is shown in supplementary table 3.

Photoreceptor specialisation—There was a significant difference in the grade of FH (PRS+ versus PRS−) between the genetic etiologies ($X^2 = 60.4$, $p < 0.0001$). All *SLC38A8* variants were PRS− cases (adjusted z-score = 4.0, *p*=0.003). In contrast, all *FRMD7* variants were PRS+ (adjusted z-score = 6.3, $p < 0.0001$). Similarly, sub-analysis for albinism showed

a significant difference in the grade of FH (PRS+ versus PRS−) between the albinism subtypes ($X^2 = 31.4$, $p < 0.0001$). Post-hoc analysis showed that HPS (adjusted z-score = 2.7, p=0.0065) and OA (adjusted z-score = 4.5, $p < 0.0001$) were associated with only PRS− cases. However, OCA had a spectrum of both PRS+ and PRS− cases (adjusted z-score = 5.6, $p < 0.0001$).

Visual acuity—There was a significant difference in VA between genetic etiologies associated with typical FH (H(3)=21.3, $p < 0.0001$) (figure 5). Multiple comparisons revealed that this was due to significant differences in VA between FRMD7 and albinism (median difference = 0.30 logMAR, $p = 0.003$), PAX6 (median difference = 0.40 logMAR, $p = 0.0004$) and SLC38A8 (median difference = 0.31 logMAR, $p = 0.004$) (figure 5A). The FRMD7 group demonstrated the best VA whilst the poorest median VA was associated with *PAX6* variants. Similarly, there was a significant difference in VA between the albinism subtypes (H(2)=21.8, $p < 0.0001$). Multiple comparisons revealed that this was due to significantly better VA in OCA compared to OA (median difference = 0.14 logMAR, $p = 0.008$) and HPS (median difference = 0.28 logMAR, $p = 0.006$) (figure 5B). Sub-analysis of data only from study sites (i.e., excluding cases from the literature) showed similar results for both cone photoreceptor specialisation and VA with genetic etiology (supplementary table 4).

Discussion

This multi-centre observational study represents the largest cohort of patients with FH providing significant insight into retinal development and visual prognosis based on a molecular diagnosis. We identified that albinism and *PAX6* variants are associated with a wide spectrum of arrested retinal development (grade 1–4 FH). Interestingly within the albinism group, OA and HPS had higher grades of FH (grade 3 and 4 FH), whilst a spectrum of FH (grade 1–4 FH) was observed in OCA. A narrow spectrum of FH was identified in *SLC38A8* variants (grade 3–4 FH) and *FRMD7* variants (grade 1 FH). Only two patients with *AHR* variants were reported in the literature, both with grade 3 FH, thus more data on *AHR* variants must be analysed to determine any association with degree of arrested retinal development. Consistent with the grade of FH, the *FRMD7* cohort had the best VA. Taken together, this highlights the central role of determining foveal morphology in patients due to its strong correlation to genetics and visual prognosis. Moreover, in scenarios where phenotypic data is lacking (for example an uncooperative patient), but genotype is available, our data can guide clinicians in providing visual prognosis based on the correlations we describe in this study.[14,16] There is a paucity of studies systematically comparing the different genetic etiologies associated with FH.[1,4] Previously this has been limited to comparatively smaller cohorts or without a molecular diagnosis.[1,4] Consistent with previous studies characterising foveal development in albinism or *PAX6* variants,[1,10,22] we highlight a spectrum of FH seen in both of these genetic conditions. This spectrum of FH is indicative of a variable timeframe of foveal developmental arrest in albinism and *PAX6* variants. On the contrary, *SLC38A8* and *AHR* variants were consistently associated with high grades of FH (grade 3 and 4),[12,21] thus we hypothesize that arrested retinal development occurs at a more defined time period, earlier in development.[12] *FRMD7*

variants were associated with grade 1 FH or normal foveal morphology,[23] therefore likely indicative of retinal developmental arrest at a much later, defined time-point.

High grades of FH (grade 3 and 4) were consistently associated with OA and HPS. In contrast, OCA demonstrated a spectrum of FH (grade 1–4 FH). Genes involved in non-syndromic OCA are generally enzymes or ion channels/exchangers.[24] Previous *in vitro* studies have shown that hypomorphic variants in *TYR* can exhibit reduced enzymatic activity of tyrosine hydroxylase and DOPA oxidase.[25] Thus, the FH spectrum seen in OCA could be attributed to the variable enzymatic function. In the presence of residual enzyme activity partial pigmentation maybe present as previously described in OCA1B patients.[26] Conversely, *HPS* genes encode proteins that regulate intracellular vesicle trafficking, whilst OA is caused by variants in *GPR143*, which is an intracellular G-protein coupled receptor.[24] Both *GPR143* and *HPS* genes are considered to be crucial for melanosome biogenesis. Previous *in vitro* studies show that deletions and nonsense *GPR143* variants produce either no protein or rapidly degraded truncated proteins. Similarly, the majority of *GPR143* missense variants cause significant protein misfolding with inability to exit the endoplasmic reticulum, thus these variants are thought to have a similar pathogenesis to the large deletion and splice pathogenic variants.[27,28] We hypothesize that this cellular phenotype and impact on melanosome biogenesis translates to a more severe clinical phenotype with earlier arrested retinal development and worse visual prognosis.

The proportion of atypical FH in the achromatopsia group is similar to previous studies, with Thomas *et al* (2011) previously identifying 69% of achromatopsia individuals with atypical FH.[29] Interestingly earlier work has shown that the foveal avascular zone is intact in achromatopsia[30] This implies that normal cone photoreceptor development and function is an important process for structural development of the fovea. The outer retinal changes in achromatopsia can be progressive based on longitudinal studies.[31] However, further studies are needed to understand the relationship between cone photoreceptor dysfunction and foveal development.

We explored whether there was a difference in VA based on a molecular diagnosis. Albinism, *SLC38A8* and *PAX6* variants had similar median VA's (0.60–0.70 logMAR), whereas *FRMD7* had significantly better median VA of 0.30 logMAR. *FRMD7* variants were consistently associated with grade 1 FH or normal foveal morphology, thus demonstrating OS lengthening. OS lengthening is a surrogate marker for peak foveal cone density.[5] Thus, the OS lengthening in *FRMD7* indicates a more tightly packed cone photoreceptor mosaic with foveal specialisation compared to other genetic etiologies, thus resulting in good visual prognosis. This is consistent with previous literature describing better median VA in *FRMD7* compared to albinism.[32] Albinism, *PAX6* and *SLC38A8* had worse VA's which could be due to the predominantly higher grades of FH (grade 3 and 4 FH) observed, this represents a lack of OS lengthening (or PRS−).[12] Although the median VA for albinism and *PAX6* group were 0.60 logMAR and 0.70 logMAR respectively, a large distribution of VA was observed, thus reflecting the spectrum of FH grade (grade 1–4 FH) associated with both conditions. Furthermore, *PAX6* variants are associated with pan-ocular phenotypes and significant phenotypic heterogeneity, thus likely providing a further explanation for the variance in VA.[4,33] In the achromatopsia group, individuals

with atypical FH demonstrated worse VA compared to those with typical FH or normal foveal morphology. This is consistent with our previous reports and is likely due to the cone photoreceptor dysfunction.[1] The grade of FH has been recognised to facilitate the prediction of future VA in preverbal children.[1,6] Identifying the grade of FH therefore provides significant prognostic value.[6]

A limitation of our study was the exclusion of data from individuals without a molecular diagnosis, but with a report of foveal morphology on OCT. Individuals with deep intronic variants or novel variants therefore may have been missed from our analysis. Similarly, data extracted from the literature represents a subset of patients which met the inclusion criteria therefore may not be representative of the entire cohorts reported within each study. Whilst every effort was made to record BCVA, it is possible that in PAX6 the presence of panocular phenotypes (e.g., cataracts and anterior segment dysgenesis) could also be contributory factors to the reduced VA, in addition to the presence of FH. Similarly, nystagmus can be variable between cases, and this could also be contributary to reduced VA. Furthermore, it is possible that our cohort has some selection bias as we included patients that had FH as determined by an OCT, thus potentially excluding patients with media opacities, such as cataracts and associated keratopathy. The inclusion of data obtained prior to the introduction of the Leicester Grading System in 2011 posed a challenge, due to the inconsistency with reported degree of arrested retinal development, attributed to the lack of consensus on a suitable grading system for foveal underdevelopment.[1,34–37]. Moreover, most reports prior to 2011 utilised time-domain OCT with limited spatial resolution for sufficient FH grading. We therefore only included data from 2011 onwards, to ensure the standardised grading of FH, by using the same classification system across all disorders.[1] Wilk *et al.* (2014) proposed the subdivision of grade 1 FH into grade 1a (extrusion of plexiform layers but with a nearly normal pit) and 1b (extrusion of plexiform layers with a shallow pit),[38] which has since been included as part of the Leicester Grading System.[6] However, due to its recent introduction, many centres and subsequent publications are yet to adopt the new subclassification, therefore we categorised grade of FH only as grade 1–4 to maintain consistency with grading. Often in clinical environments, patients may be identified for analysis due to an obvious phenotype, for example, those demonstrating nystagmus. This may give rise to selection bias, where more severe grades of FH have been overrepresented in our cohort. Our study design included only qualitative reports of foveal morphology and genotype. Further studies to perform quantitative analysis across the retinal developmental disorders are required, to investigate the potentially more subtle relationships with foveal morphology, function and genotype. Furthermore, our study looked at genotype on a gene level only, therefore future work investigating the genotype-phenotype relationships on a variant level is required.

In summary, this multi-centre collaborative study has utilised the largest dataset so far, to reveal genotypic correlations with foveal development and its consequence to vision. This provides mechanistic insight into how genes involved in foveal development interact at different temporal points resulting in varying degrees of arrested retinal development. Variants of *SLC38A8, GPR143, AHR* and genes involved in HPS are associated with high grades of FH and poor cone photoreceptor specialisation which translates to earlier foveal developmental arrest and worse visual prognosis. This has significant diagnostic and

prognostic value and can help with prioritisation of genetic testing, subsequent counselling, and support.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

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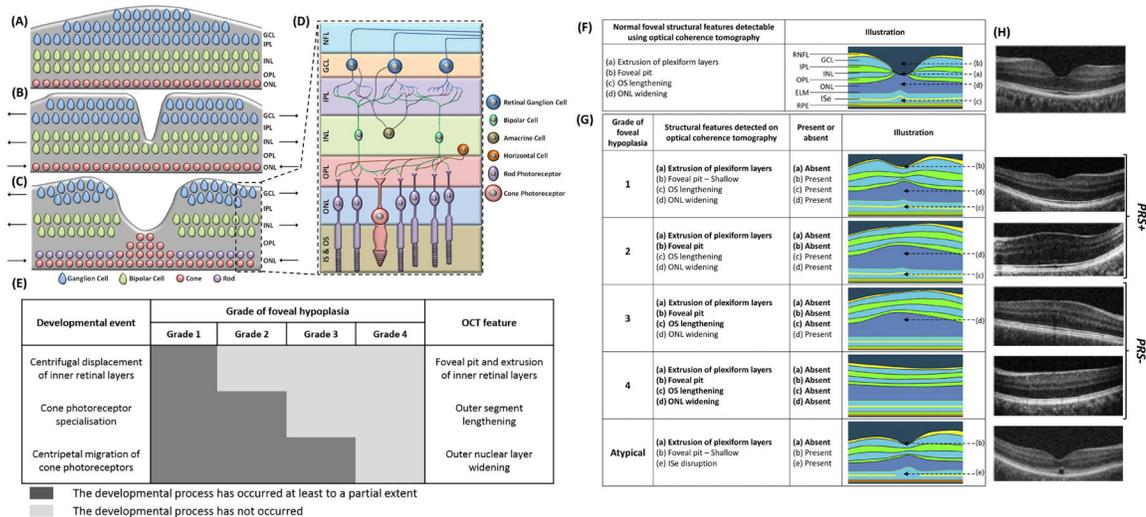
Figure 1:

Foveal pit formation and movement of retinal cells during formation of the area of high acuity. The laminar retinal structure prior to foveal pit formation is shown in (A). The inner retinal layers were displaced centrifugally (away from the future fovea) during foveal pit formation (B). The cone photoreceptors migrate centripetally (towards the fovea) and form the pure cone area (C). Arrows point in the direction of movement of the cellular layers. The magnified laminar structure (D) with the different retinal cell types and the inner segment & outer segment (IS & OS) of the photoreceptors are shown in (D). NFL = Nerve Fibre Layer; GCL = ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer. (A, B, C are based on developmental theory proposed by Springer and Hendrickson).[39] Chart showing the 3 developmental processes involved in formation of a structural and functional fovea. In grade 1 foveal hypoplasia, all processes occur to a certain extent. However, in grade 4 foveal hypoplasia, none of these processes occur; thus, the retina resembles that of the parafovea. In grade 2 and 3 foveal hypoplasia, there is outer nuclear layer widening, but no foveal pit. The difference between grade 2 and 3 foveal hypoplasia is occurrence of cone photoreceptor specialization. Identifying these specific features on optical coherence tomography (OCT) enables us to understand whether the respective developmental process has occurred. F, Illustration showing the unique features of a normal fovea detectable on optical coherence tomography. G, Illustration of typical and atypical grades of foveal hypoplasia. All grades of foveal hypoplasia had incursion of inner retinal layers. Atypical foveal hypoplasia also had incursion of the inner retinal layers. Grade 1 foveal hypoplasia is associated with a shallow foveal pit, outer nuclear layer (ONL) widening, and outer segment (OS) lengthening relative to the parafoveal ONL and OS length, respectively. In Grade 2 foveal hypoplasia, all features of grade 1 are present except the presence of a foveal pit. Grade 3 foveal hypoplasia consists of all features of grade 2 foveal hypoplasia except the widening of the cone outer segment. Grade 4 foveal hypoplasia represents all the features seen in grade 3 except there is no widening of the ONL at the fovea. Finally, an atypical form of foveal hypoplasia also is described in which there is a shallower pit with disruption of the inner segment ellipsoid (ISe). (Adapted with permission from Thomas et al. 2011).[1] H, original OCTs

demonstrating the different grades of foveal hypoplasia. Grades 1 and 2 can be considered to show signs of photoreceptor specialisation (PRS+), however grades 3 and 4 do not show signs of photoreceptor specialisation (PRS−). ELM = external limiting membrane; GCL = ganglion cell layer; INL = inner nuclear layer; IPL = inner plexiform layer; OPL = outer plexiform layer; RNFL = retinal nerve fibre layer; RPE = retinal pigment epithelium.
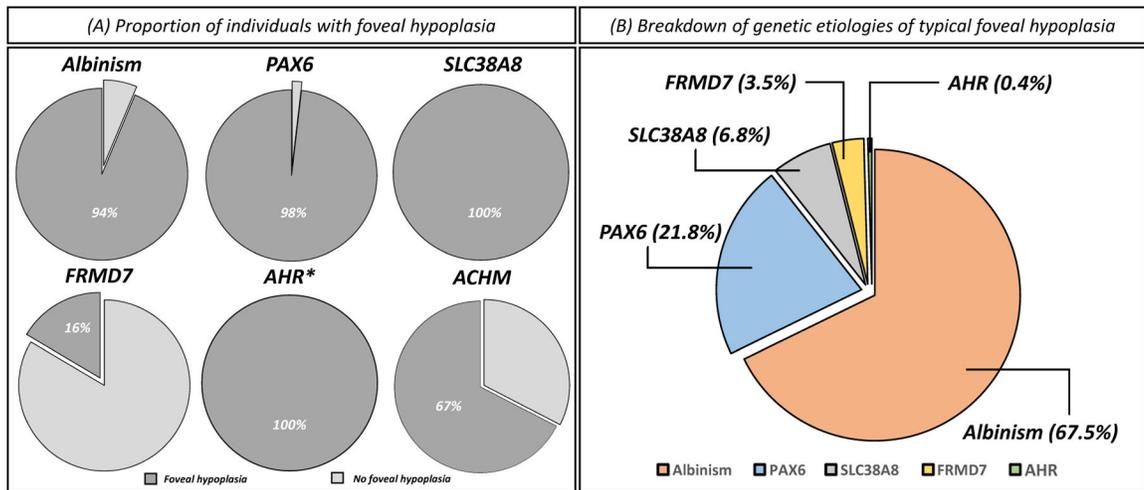
Figure 2:
(A) Proportion of individuals with foveal hypoplasia within each genetic diagnosis. (B) Breakdown of genetic etiologies causative of typical foveal hypoplasia. *Only two cases of AHR variants with foveal hypoplasia were identified.
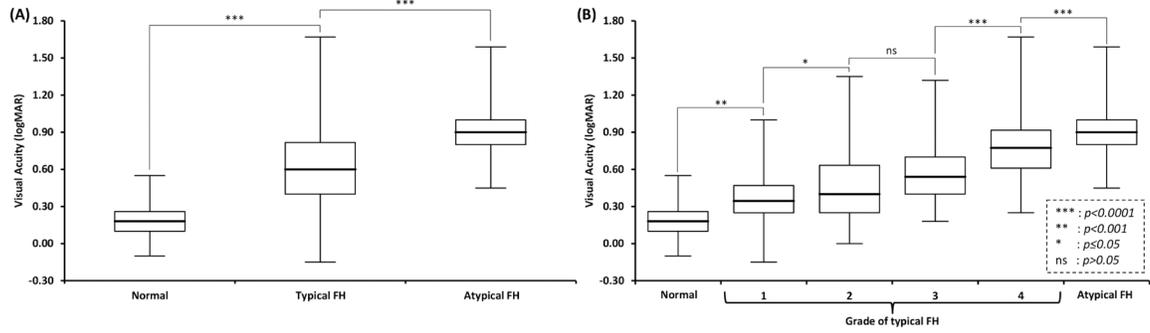
Figure 3:
(A) Box and whisker plots of visual acuity in individuals with normal foveal morphology compared to typical foveal hypoplasia (FH) and atypical FH. Pairwise comparisons show significant differences between the three groups. (B) Pairwise comparisons with typical FH split into individual grades (1–4) shows significant differences across all groups except between grade 2 and 3 FH.
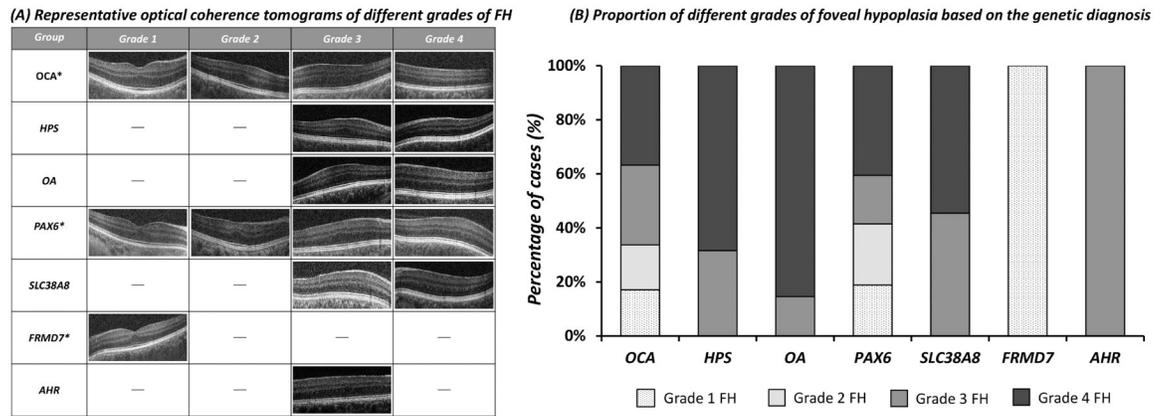
(A) Representative optical coherence tomograms of different grades of FH

(B) Proportion of different grades of foveal hypoplasia based on the genetic diagnosis

Figure 4:

(A) Representative tomograms of typical foveal hypoplasia in different genetic etiologies. (B) The relative proportions of each grade of foveal hypoplasia within each genetic diagnosis is shown in this bar chart. Non-syndromic oculocutaneous albinism (OCA) and PAX6 variants had the full spectrum of foveal hypoplasia. However, SLC38A8 variants, GPR143 variants associated with ocular albinism (OA) and variants associated with Hermansky Pudlak Syndrome (HPS) only had grade 3 and grade 4 foveal hypoplasia. FRMD7 only had grade 1 foveal hypoplasia. AHR only had grade 3 foveal hypoplasia. *Albinism, PAX6 and FRMD7 can also have normal foveal morphology.
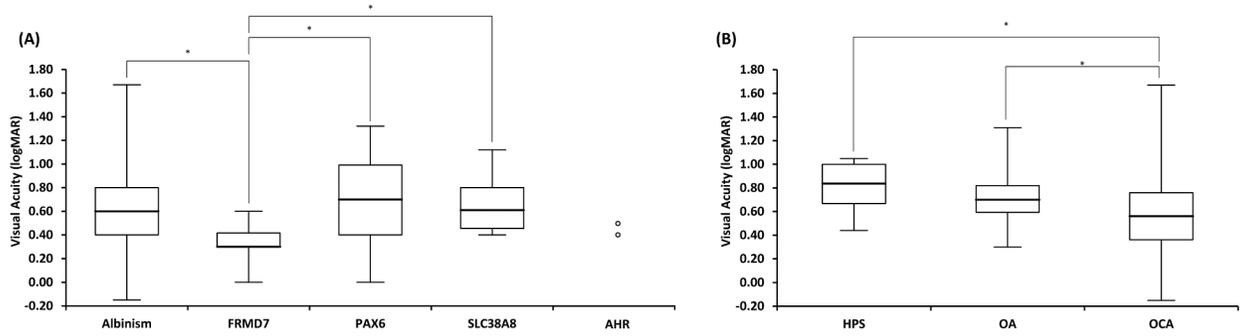
Figure 5:
(A) Box and whisker plots of visual acuity in each genetic etiology associated with foveal hypoplasia. Only individuals with foveal hypoplasia from each diagnostic group were included. (B) Box and whisker plots of visual acuity for albinism subtypes: Non-syndromic Oculocutaneous Albinism (OCA), Hermansky Pudlak Syndrome (HPS) and Ocular Albinism (OA) due to GPR143 variants. Box represents the interquartile range, line through the box represents the median and extent of whiskers represents the range. Significant comparisons (p 0.05) are shown with *.

Table 1:

List of genes associated with typical and atypical foveal hypoplasia with genes reported in this study highlighted in bold

Condition	Clinical Phenotype	Gene	Location	MIM Gene ID	Phenotype title	MIM phenotype ID	Inheritance
Oculocutaneous Albinism (OCA)	A clinically and genetically heterogenous disorder. Characterised by pigmentation defects of the hair, skin, and eyes. Foveal hypoplasia, chiasmal misrouting, infantile nystagmus, iris transillumination defects, fundus hypopigmentation.	**TYR**	11q14.3	606933	OCA1	203100, 606952	AR
		OCA2	15q12-q13	611409	OCA2	203200	AR
		TYRP1	9p23	115501	OCA3	203290	AR
		SLC45A2	5p13.2	606202	OCA4	606574	AR
		SLC24A5	15q21.1	609802	OCA6	113750	AR
		LRMDA (C10orf11)	10q22.2-q22.3	614737	OCA7	615179	AR
Ocular Albinism (OA)	Shares the same clinical characteristics as OCA, however with pigmentation defects generally limited to the eyes	**GPR143**	Xp22.2	300808	OA1	300500	XL
Hermansky-Pudlak Syndrome (HPS)	A syndromic form of albinism demonstrating the same clinical characteristics as OCA, in addition to blood platelet dysfunction with prolonged bleeding	**HPS1**	10q24.2	604982	HPS1	203300	AR
		AP3B1	5q14.1	603401	HPS2	608233	AR
		HPS3	3q24	606118	HPS3	614072	AR
		HPS4	22q12.1	606682	HPS4	614073	AR
		HPS5	11p15.1	607521	HPS5	614074	AR
		HPS6	10q24.32	607522	HPS6	614075	AR
		DTNBP1	6p22.3	607145	HPS7	614076	AR
		BLOC1S3	19q13.32	609762	HPS8	614077	AR
		BLOC1S6	15q21.1	604310	HPS9	614171	AR
		AP3D1	19p13.3	607246	HPS10	617050	AR
		BLOC1S5	6p24.3	607289	HPS11	619172	AR
Chediak-Higashi Syndrome (CHS)	A syndromic form of albinism demonstrating the same clinical characteristics of OCA, in addition to immune deficiency and ability to bruise and bleed easily	**LYST**	1q42.3	606897	CHS1	214500	AR
FHONDA	Foveal hypoplasia, chiasmal misrouting, infantile nystagmus, and anterior segment dysgenesis in some cases (minor association)	**SLC38A8**	16q23.3	615585	FHONDA, FVH2	609218	AR
Aniridia	A pan-ocular condition which can cause corneal and lens abnormalities, iris abnormalities (aniridia), raised intraocular pressure, foveal hypoplasia, infantile nystagmus and optic nerve abnormalities	**PAX6**	11p13	607108	AN1, FVH1	106210, 136520	AD

Condition	Clinical Phenotype	Gene	Location	MIM Gene ID	Phenotype title	MIM phenotype ID	Inheritance
FRMD7-related infantile nystagmus	*Associated with idiopathic infantile nystagmus Most commonly associated with normal foveal morphology. Rare association with foveal hypoplasia.*	**FRMD7**	Xq26.2	300628	NYS1	310700	XL
AHR-related foveal hypoplasia and infantile nystagmus	*A recently reported condition characterised by foveal hypoplasia and infantile nystagmus (only two cases reported in the literature, from the same family)*	**AHR**	7p21.1	600253	*	-	AR
Achromatopsia	*Characterised by cone photoreceptor dysfunction, reduced vision and infantile nystagmus. Known to be associated with atypical foveal hypoplasia and inner segment ellipsoid disruption*	**CNGB3**	8q21.3	605080	ACHM3	262300	AR
		CNGA3	2q11.2	600053	ACHM2	216900	AR
		GNAT2	1p13.3	139340	ACHM4	613856	AR
		PDE6C	10q23.33	600827	ACHM5	613093	AR
		PDE6H	12p12.3	601190	ACHM6	610024	AR
		ATF6	1q23.3	616517	ACHM7	605537	AR