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Original Article

Whole exome sequencing of known eye genes reveals genetic causes for high myopia

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Abstract

High myopia [refractive error ≤ −6 diopters (D)] is a heterogeneous condition, and without clear accompanying features, it can be difficult to pinpoint a genetic cause. This observational study aimed to evaluate the utility of whole exome sequencing (WES) using an eye disorder gene panel in European patients with high myopia. Patients with high myopia were recruited by ophthalmologists and clinical geneticists. Clinical features were categorized into isolated high myopia, high myopia with other ocular involvement or with systemic involvement. WES was performed and an eye disorder gene panel of ∼500 genes was evaluated. Hundred and thirteen patients with high myopia [mean (SD) refractive error − 11.8D (5.2)] were included. Of these, 53% were children younger than 12 years of age (53%), 13.3% were aged 12–18 years and 34% were adults (aged > 18 years). Twenty-three out of 113 patients (20%) received a genetic diagnosis of which 11 patients displayed additional ocular or systemic involvement. Pathogenic variants were identified in retinal dystrophy genes (e.g. *GUCY2D* and *CACNA1F*), connective tissue disease genes (e.g. *COL18A1* and *COL2A1*), non-syndromic high myopia genes (*ARR3*), ocular development genes (e.g. *PAX6*) and other genes (*ASPH* and *CNNM4*). In 20% of our high myopic study population, WES using an eye gene panel enabled us to diagnose the genetic cause for this disorder. Eye genes known to cause retinal dystrophy, developmental or syndromic disorders can cause high myopia without apparent clinical features of other pathology.

Introduction

Myopia is a refractive error in which light rays entering the eye focus in front of the retina lead to blurred vision (1). High myopia is the more severe form and is defined as a refractive error ≤ −6 diopters (D) or axial length ≥ 26 mm (1). The risk of high myopia is larger in patients with an early onset (i.e. when refractive error exceeds the age, i.e. when myopia severity is higher than the age) (2). Although myopia can easily be corrected with glasses or contact lenses, it can lead to irreversible vision loss owing to its complications later in life (3,4). Considering the increasing prevalence and incidence, myopia will become one of the leading causes of visual impairment or blindness worldwide, necessitating elucidation of its complex etiology (3,5).

Common myopia caused by a complex interplay between multiple common genetic factors of small effect and environmental influences is often distinguished from Mendelian myopia caused by a single pathogenic variant, which is often accompanied by systemic features (6). Up to now, >500 common variants associated with refractive error have been identified through genome-wide association studies, explaining only 18% of the phenotypic variability of refractive error. Hence, a large part of its heritability is still unresolved (7,8). In contrast, >80 systemic syndromes, such as Marfan and Stickler syndrome, and 27 retinal disorders, such as retinitis pigmentosa or congenital stationary night blindness (CSNB), can co-occur with myopia (9,10). The more pronounced familial aggregation of high myopia compared with low myopia suggests that the genetic contribution to high myopia is higher (11). Several mainly Asian studies have focused on the identification of new genetic variants for high myopia, but results were limited to the identification of a small number of loci (12–18).

In our clinic, whole exome sequencing (WES) with the application of an eye gene panel is commonly used to survey for putative genetic variants in suspected Mendelian

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Table 1. General characteristics of the study population

		N (%)	Children (<12 y)	Adolescents (12–18 y)	Adults (>18 y)	Total
N (%)			60 (53.1)	15 (13.3)	38 (33.6)	113 (100)
Age at presentation (y)		115 (100)	6.3 (2.7)	14.7 (1.9)	45.5 (16.9)	20.5 (20.5)
Age of onset of myopia (y)		86 (75)	2.8 (1.3)	3.4 (1.5)	5.1 (4.0)	3.5 (2.5)
Gender (% male)		115 (100)	39 (65)	8 (53.3)	13 (34.2)	60 (53.1)
Mean RE (D)		111 (98)	−9.9 (4.7)	−13.0 (5.3)	−13.9 (5.2)	−11.8 (5.2)
RE groups	**−0.5D to >−3D**	111 (98)	1 (1.7)	0 (0)	0 (0)	1 (0.9)
	−3D to >−6D		9 (15.3)	0 (0)	0 (0)	9 (8.1)
	−6D to >−10D		21 (35.6)	5 (33.3)	9 (24.3)	35 (31.5)
	−10D to >−15D		22 (37.3)	5 (33.3)	13 (35.1)	40 (36.0)
	≤-15D		6 (10.2)	5 (33.3)	15 (40.5)	26 (23.4)

Continuous variables are presented as mean (SD) and categorical variables as N (%). Abbreviations: Y = years; RE = refractive error. The values in bold indicate different refractive error categories.

forms of high myopia (19). Studies investigating the utility of this genetic testing have mainly been performed in high myopia families or small numbers of unrelated high myopia cases (9).

In this study, we investigated the utility of WES with an eye disorder gene panel in high myopia irrespective of accompanying pathology.

Results

This study included 113 participants of whom 53% were male patients and the mean (SD) age of the total population was 20.5 (20.5) years (range: 1–75 years) (Table 1). The majority of our patients were children younger than 12 years of age (53%) followed by adults (aged > 18 years, 34%) and adolescents (aged between 12 and 18 years, 13%). Mean (SD) refractive error in our study population was −11.8D (5.2) and 59% had a refractive error of ≥−10D. Eighty-two (73%) patients presented with isolated high myopia, 17 (15%) presented with systemic involvement and 14 (12%) presented with other ocular features.

With application of WES using an eye disorder gene panel, we identified 48 variants in 40 patients (Fig. 2, Supplementary Material, Table S1). After careful evaluation, 25 variants in 23 patients (20%) were classified as causal pathogenic mutations (final decision). In 17 (15%) patients, the variants were classified as a VUS.

Figure 2 displays the occurrence of causal pathogenic variants in patients with different clinical presentation. The frequency of causal pathogenic variants was 15% in patients with isolated high myopia, 35% in patients with systemic features and 36% in patients with ocular involvement. Considering the refractive error in the total group, the frequency of pathogenic variants was 10% in patients with a refractive error up to −6D, 20% in patients with a refractive error ≤ −6D to −10D and 23% in patients with a refractive error ≤ −10D. Note that the first group only included children aged <12 years, who might have a more myopic refraction when reaching adult age. Patients with a pathogenic variant were significantly younger [the mean (SD) age was 12.3 years (14.3) vs. 22.9 years (20.9), $P = 0.012$ for pathogenic variant vs. no pathogenic variant, respectively] and had a non-significantly more myopic refraction compared with those without a pathogenic variant [mean (SD) refractive error − 12.6D (5.6) vs. −11.3 D (5.1), $P = 0.329$ for pathogenic variant vs. no pathogenic variant, respectively]. About half of the patients with causal pathogenic variants (52%, 12/23) presented with isolated high myopia, 22% had retinal involvement and 26% other systemic features (Fig. 1). In the group of patients without a genetic diagnosis, less patients presented with ocular or systemic involvement (10 and 12% for ocular and systemic involvement, respectively, $P = 0.042$). The chance for a genetic diagnosis was higher in patients with ocular or systemic involvement [OR: 3.21 ($P = 0.017$) for ocular/systemic involvement vs. isolated high myopia].

Onset of myopia was only available for a subgroup of patients [75% (85/113)]. These patients did not differ significantly with respect to refractive error. The median age of onset in our study population was 3 years. Figure 2 displays the difference in frequency of causal pathogenic variants between patients with an age of onset of myopia ≤3 and >3 years. This frequency was non-significantly higher in patients with a lower age of onset (26 vs .13% in ≤3 and >3 years, respectively, $P = 0.123$; Fig. 3). In patients with an onset ≤3 years, most pathogenic variants were located in a connective tissue gene or retinal dystrophy gene (both 36%, Fig. 3).

A detailed description of the 23 patients with a pathogenic variant can be found in Supplementary Material, Table S1 and Figure 4. The majority of pathogenic variants was inherited in an X-linked (33%), autosomal recessive (30%) or autosomal dominant (30%) pattern. Only one patient (4%) harbored a *de novo* dominant mutation. In 39% of the patients, a pathogenic variant in a retinal dystrophy gene was detected (*FAM161A, GUCY2D, PDE6H, CACNA1F, NYX, RPGR* and *TRPM1*; Fig. 3). Six (77%) of these patients presented with isolated high myopia (mean age: 17.3 years). In the other patients, nystagmus, nyctalopia, strabismus or amblyopia were clinical ocular features and their mean age was 6.3 years. In three of the patients, a pathogenic variant in *CACNA1F*, involved in X-linked CSNB type 2A, was discovered. In one patient, electroretinography (ERG) was performed, which showed a reduced photopic, scotopic and mixed

WES with eye disorder gene panel
(N=120)

Excluded:
- Segregation analysis N=5

Eligible study population N=115
WES as primary test

Excluded:
- No high myopia (refractive error <-6D (adults) OR axial length <98th percentile (children)) N=2

Included study population
N=113

N=40 patients with at least one variant
- Heterozygote N=24 (2 persons carried two different variants)
- Homozygote N=5
- Compound heterozygote N=6
- Hemizygote N=5

Total number of variants: 48

Clinical presentation:
- Isolated high myopia N=82
- High myopia with ocular involvement N=14
  - Photophobia N=1
  - Nystagmus N=1
  - Color blindness N=1
  - Night blindness N=2
  - Cataract N=1
  - Strabismus N=2
  - Optic hypoplasia N=1
  - Photophobia + color blindness N=1
  - Glaucoma N=1
  - Bulls eye maculopathy N=1
  - High astigmatism N=1
  - CVI N=1
- High myopia with systemic features N=17
  - Dental problems N=1
  - Dysmorphological features N=6
  - Skeletal features N=5
  - Mental retardation N=2
  - Mix N=3

Figure 1. Flow chart of study population selection. Abbreviation: CVI = cerebral visual impairment.

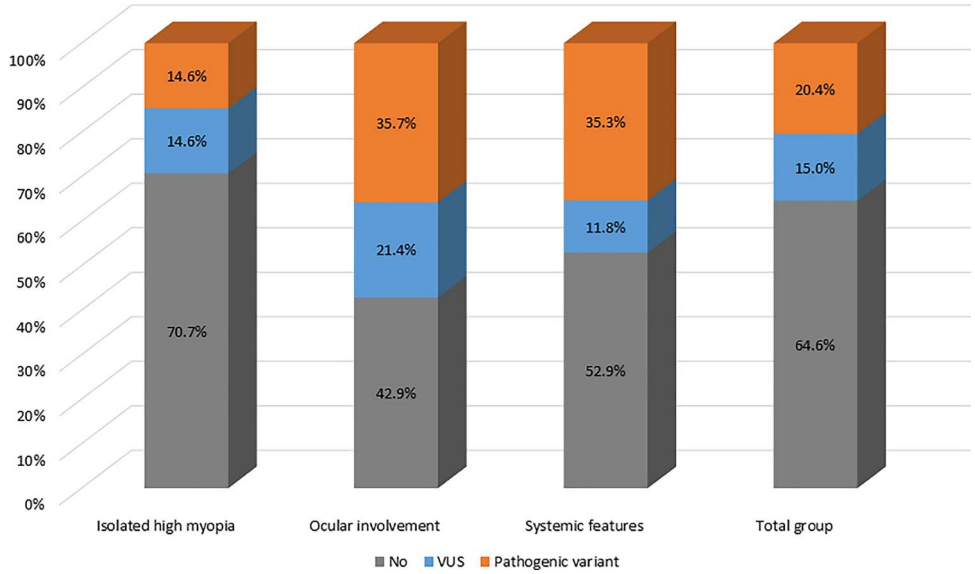

Figure 2. Clinical presentation of the patients undergoing WES and the identification of genetic variants. The presence of genetic variants in four groups are displayed: patients with isolated high myopia, ocualr involvement, systemic features and the total group. Genetic variants were identified using WES and application of an eye disorder gene panel. Values represent percentages.

b-wave, which was in line with the electro-retinal findings observed in CSNB. Two of these *CACNA1F* variants were reported in literature before, and these patients showed a CSNB phenotype with ERG changes (Supplementary Material, Table S1). In the patient with CSNB type 1C caused by a pathogenic variant in

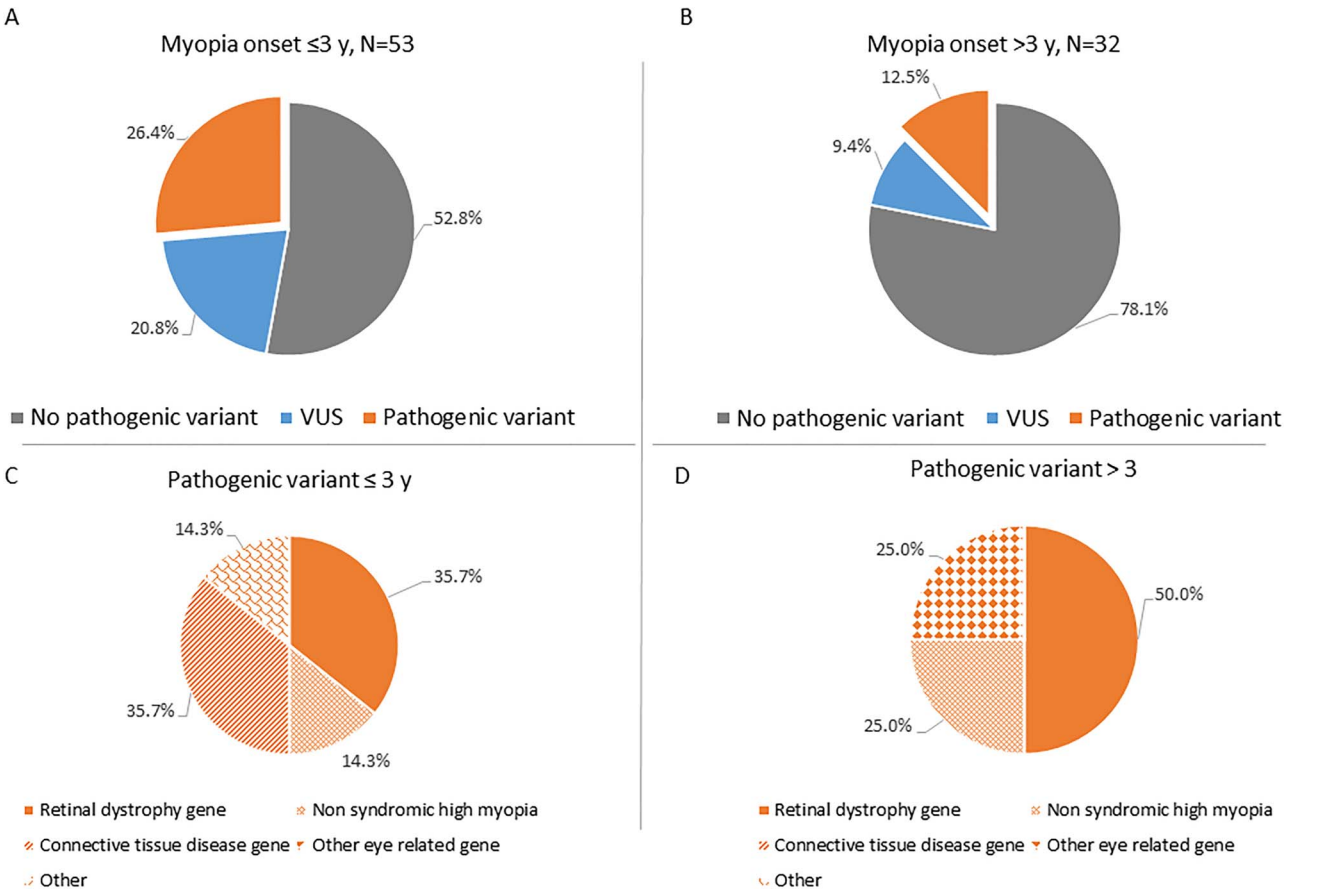

Figure 3. Occurrence of a pathogenic variant, VUS or no pathogenic variant stratified by age of onset of myopia, i.e. ≤3 years (**A**) and >3 years (**B**). The different types of genes in which a pathogenic variant was identified stratified for patients ≤3 years (**C**) and >3 years (**D**) are shown at the bottom. Values represent percentages. Abbreviation: y = years.

TRPM1, ERG showed a negative b-wave corresponding to the CSNB phenotype. The ERG of the patient with a pathogenic variant in *FAM161A* (Cone rod dystrophy) showed reduced amplitudes in both scotopic and photopic conditions. ERG of the young patients with the *GUCY2D* and *RPGR* variant did not show any abnormalities but will be repeated when the patients have reached an older age.

Causal pathogenic variants in connective tissue disease genes [*COL18A1* (Knobloch syndrome, $N = 2$) and *COL2A1* (Stickler syndrome, $N = 5$)] were identified in 30% of patients. In three of the five patients with Stickler syndrome, the clinical presentation was limited to isolated high myopia. One of these variants was identified before with different clinical features, including isolated high myopia (Supplementary Material, Table S1) (20). In one other patient, a pathogenic variant was identified in the ocular developmental *PAX6* gene and, in another patient, a variant was identified in the *MYOC* gene, causing both high myopia and glaucoma. The remaining pathogenic variants were identified in *ASPH* (Traboulsi syndrome) and *CNNM4* (Jalili syndrome). The patient with a pathogenic variant in *ASPH* presented with astigmatism, plagiocephaly, retrognathia, an elongated face and iridal cysts, which

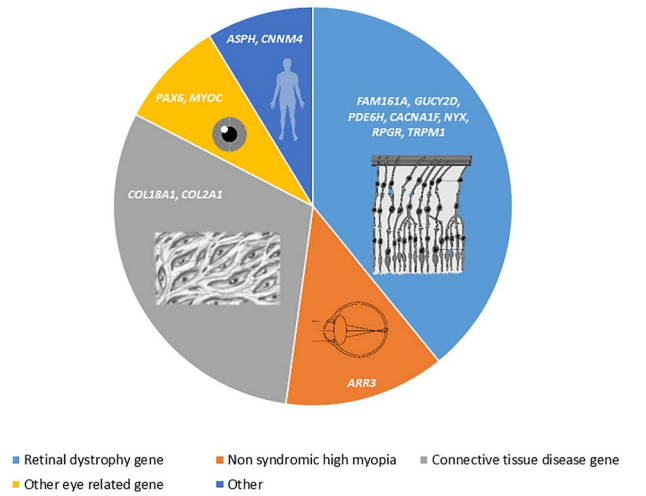

Figure 4. Overview of genes with a pathogenic variant identified using WES with vision related gene panel in our cohort. The total number of patients with a pathogenic variants was $N = 23$ (20.4%). In 39.1% of these patients, a pathogenic variant in a retinal dystrophy gene was identified; in 30.4%, a pathogenic variant in a connective tissue disease gene; in 13.0%, in a non-syndromic high myopia gene; in 8.7%, in another gene and, in 8.7%, in other eye-related genes.

corresponds with the phenotype of Traboulsi syndrome (OMIM 601552). The patient with Jalili syndrome (OMIM 217080) presented with a amelogenesis imperfecta and

high myopia. Follow-up electrophysiological testing (ERG showed low photopic responses) pointed toward a cone dystrophy. The diagnosis of Jalili syndrome was made based on the clinical presentation, biallelic variants in the *CNNM4* gene (a missense variant and a deletion) and the electrophysiological testing.

In three female patients, a pathogenic variant was found in a gene involved in non-syndromic high myopia (*ARR3*). A female-limited inheritance pattern was present in all these three patients' family histories (21).

Variants were classified as a VUS in 17 (15%) of patients. In a significant proportion (35%), this VUS was located in a non-syndromic high myopia gene (*ARR3, SLC39A5, LRPAP1, PRIMPOL* and *SCO2*); in 24%, this VUS was located in a retinal dystrophy gene (*TUB, OPN1LW, IFT140* and *CNGB1*) and, in 24%, this VUS was located in a connective tissue disease gene (*COL2A1* and *COL11A1*) (Supplementary Material, Table S1).

Discussion

Using WES with application of a panel of known eye disorder genes in patients with high myopia in a tertiary care setting, we were able to make a genetic diagnosis in one out of five patients with isolated high myopia and high myopia and comorbidity (total population) and in one out of seven patients with isolated high myopia. The variants identified were located in genes involved in retinal dystrophies, non-syndromic high myopia, connective tissue diseases, several other syndromes and disorders of ocular development. This study demonstrates that in patients who do not appear to have a Mendelian inheritance pattern of myopia, single gene mutations as well as sub-clinical retinal and systemic symptoms might be overlooked.

The majority (39%) of pathogenic mutations in our study was identified in retinal dystrophy genes. Many retinal dystrophies are characterized by the presence of myopia (10). However, without specific clinical symptoms, i.e. defects in color vision or night blindness, these disorders can be easily overlooked. This observation has been described before in two community-based studies in children with high myopia, warranting the awareness of its co-occurrence with myopia (22,23). In our patients, findings of the ERG which was done after WES often confirmed the pathogenicity of detected variants. However, in some patients, it did not reveal any abnormalities, and myopia may be the first or only symptom of a retinal dystrophy. These findings suggest that ERG should be performed to guide the interpretation of genetic variants (especially, variants of unknown clinical significance) in retinal dystrophy genes in patients with isolated high myopia even in the absence of decreased visual acuity of color blindness.

Some diagnoses in our study were made more frequently than expected. Three patients had a mutation in *ARR3*, encoding a cone specific protein. An extensive description of the families is described elsewhere (24). Up to now, there were only very few reports on patients with mutations in this gene (21,25–27). Pathogenic variants in *ARR3* are associated with a specific female-limited inheritance pattern of high myopia, caused by the cellular inference between the active and inactivated X-chromosome present in females (21). The exact role of the cone Arrestin 3 protein in the visual signaling cascade is not known, but an effect on circadian rhythm, dopamine release, blue light and melanopsin has been proposed (25). In a bovine animal model, the location of this protein varied depending on the light conditions, i.e. it was more present in the outer segments of cones after light adaptation and increased in the inner segments in dark-adapted eyes, suggesting a role in light-adaption (28). Given the high frequency in our cohort, X-linked female-limited *ARR3* mutations should be considered in families with high myopia with this specific female-only inheritance pattern.

Another group of the causal genes that we have identified is related to connective tissue disorders. In five patients, we identified a pathogenic variant in the *COL2A1* gene causing Stickler syndrome, which is a disorder known to be characterized by high myopia but is also often reported to be accompanied by specific vitreous changes, midfacial underdevelopment, cleft palate and joint abnormalities (29). Nonetheless, in three of the five patients with Stickler syndrome, the clinical presentation was limited to isolated high myopia, suggesting that Stickler syndrome should also be suspected in case of a clinical presentation limited to high myopia. Surprisingly, two patients with Knobloch syndrome were identified in this study. In contrast to Stickler syndrome, the prevalence of Knobloch syndrome is thought to be very low and its clinical presentation more severe (29–31). The clinical spectrum of Knobloch syndrome reported in literature is heterogeneous but is mainly characterized by high myopia, vitreoretinal degeneration, often leading to retinal detachment, and occipital encephalocele (30,32–34). The Knobloch patients in this study presented with only minor occipital defects and high myopia. These findings suggest that the occurrence of this condition is probably higher than reported in literature and that the diagnosis might be missed in case of mild symptomatology.

We compared our diagnostic yield of 20% with other studies which performed WES in high myopia (Supplementary Material, Fig. S1) (20,27,35,36). Sun *et al.* (20) used a gene panel of 234 (mainly retinal dystrophy) genes and found a yield of 24% (71/298) in patients with early onset high myopia (<−6D). Zhou *et al.* (36) performed a replication study using the same gene panel and identified a yield of 13% (42/325) and 1.5% (3/195) in the early onset and late onset high myopia, respectively, when considering pathogenic variants (ACMG class 5) only. Wan *et al.* (35) applied a phenotype-driven filtering strategy on a similar early onset high myopia group and identified 20 pathogenic variants in 16 out of 20 (80%) patients. Recently, Liu *et al.* (27) identified a yield

of 10% in 67 patients using a gene panel consisting of 17 ocular genes. Unfortunately, these studies did not all perform a formal classification according to ACMG and not all data were available to perform this by ourselves, which could partly explain the wide range in yield. Furthermore, the selection procedure of study participants differed between these studies. Interestingly, however, the genes with pathogenic variants could be categorized into the same categories as in our study (retinal dystrophy genes, connective tissue disease genes and other).

This is the first European study evaluating the diagnostic yield of WES in a highly myopic population. Among the strengths of our study are the relatively large number of patients included and the detailed clinical information available. Unfortunately, we were not able to perform segregation analysis in all family members of patients with a variant of unknown significance (VUS). Furthermore, we were not able to provide new functional evidence for the variants of unknown significance or pathogenic variants but relied on previous functional studies reported in literature. Lastly, selection bias might have occurred owing to the tertiary care hospital based setting of this study, inclusion of mainly young patients with a potential more extreme phenotype (worse treatment response and high myopia progression) and the retrospective design. However, given the relevant diagnostic yield, which is comparable to other studies (20,27,35,36), and the potential consequences of finding a genetic cause, our study reflects the utility of genetic testing in individuals with high myopia.

Since our analyses were limited to the genes included in the gene panel, future research should focus on the evaluation of WES data without the application of a gene panel. GWAS results have suggested that myopia might be explained by genetic variants located in regulatory elements of the DNA, leading to different expression of genes. Furthermore, assessment of intergenic regions of DNA by using whole genome sequencing will have the potential to unravel some of the yet unsolved cases (9). Although the yield identified in our study encourages to use diagnostic WES in a clinical setting, the availability and costs of WES, as well as genetic counseling, should be born into mind, especially in countries with limited health care budget. Nevertheless, when genetic testing is considered, WES offers a good alternative for targeted sequencing in case of high myopic patients without specific clinical clues for a diagnosis.

A clinical diagnostic guideline for patients with high myopia is currently lacking. Therefore, we have created an easy-to-use clinical management guideline for myopia patients presenting in the clinic, implementing the findings of this study (Supplementary Material, Table S2). First, and extensive medical history should be taken from each patient presenting with high myopia, including hearing and vision problems, as well as taking the family history to pinpoint a certain inheritance pattern. This should be followed by an ophthalmological examination and clinical examination to search for specific features, which could highlight a certain Mendelian form of myopia or specific syndrome. This could be followed by targeted DNA examination in case of a specific phenotype or WES when no specific Mendelian form of myopia is suspected. We recommend to perform WES in children <6 years of age when their refractive error exceeds their age, in children with therapy-resistant progressive myopia (axial length above the 98th percentile line) and in adults with a refractive error ≤ -10D (19). It is important to mention that not all mutations can be picked up using WES (e.g. copy number variants, structural chromosomal rearrangements, intronic variants and mitochondrial DNA mutations). Also, differences in the coverage of genes on the gene panel or other technical sequencing issues (this is specifically the case for high myopia genes *RPGR* and the opsin genes involved in blue cone monochromatism) might cause mutations not being identified. In those cases, additional genetic testing, such as targeted gene sequencing, an array-based approach or whole genome sequencing, is required.

To conclude, this study showed the utility of WES with an eye disorder gene panel in patients with high myopia. We identified a genetic cause in one out of five of all high myopia patients and in one out of seven of patients with isolated high myopia. Information on the genetic cause is important since it facilitates concise clinical management of these patients and their family members. First, it might have prognostic implications since progression of visual decline is dependent on the genetic defect underlying the disease. Given the risk of related systemic features which could impact health, referral to another medical specialist, e.g. a cardiologist in case of Stickler syndrome, may be needed. Second, it might predict the course of refractive error development and response to treatment. Third, it might have implications for future therapy, i.e. inclusion in gene therapy trials in case of retinal dystrophies, and last but not least, it provides the opportunity to provide reproductive options (e.g. pre-implantation genetic testing) for future pregnancies and options for predictive or carrier testing in family members.

Materials and Methods

Study population

We included all European patients who underwent WES with indication high myopia at the department of Clinical Genetics at Erasmus Medical Center in Rotterdam, The Netherlands (Fig. 1). These patients were referred to this tertiary care hospital for genetic advice, (treatment of) progressive myopia, other visual complaints or systemic involvement. High myopia was defined as a refractive error ≤ -6 D in adults and as an axial length above the 98th percentile in children (37). Patients who underwent WES in the context of segregation analysis, i.e. they were not the proband, were excluded from analysis. Patients

were stratified into three groups based on their clinical presentation: isolated high myopia, high myopia with ocular involvement and high myopia with systemic features. Patients with both ocular involvement and systemic features were classified in the group with systemic features. Written informed consent was obtained from all participants.

Whole exome sequencing

Genomic DNA was extracted from EDTA anticoagulated blood according to standard protocols. WES was performed as follows [outsourced at GenomseScan (Leiden, The Netherlands)] (38). In short, exome-coding DNA was captured with the Agilent Sure Select Clinical Research Exome SureSelect kit [V4 (March 2015), CRE V1 (March 2015–2017), CRE V2 (March 2017–December 2019) and V7 (December 2019 till now)] and paired-end sequenced on the Illumina platform [average coverage 50×, coverage per gene on (39)]. Reads were aligned to Hg19 and variants were called using the GATK haplotype caller (v2.7-2). Detected variants were annotated, filtered and prioritized using Alissa Interpret (Santa Clara, USA, formerly Cartagenia Bench Lab NGS, Leuven, Belgium). Filter steps included a gene panel restricted to a set of ∼500 genes associated with eye disorders, which included retinal dystrophy genes, cataract genes, corneal dystrophies, connective tissues disease genes, ocular developmental genes and high myopia genes (505 genes, versions available on https://www.erasmusmc.nl/nl-nl/patientenzorg/laboratoriumspecialismen/klinische-genetica (39), Supplementary Material, Table S3). Targeted sequencing of the familial variant was performed in relatives when available (parents or other relatives). Variants were initially classified according the ACMG guidelines using Alamut Visual, the CADD scoring website (40) and the online Franklin tool (https://franklin.genoox.com/clinical-db/home, 17 August 2021); ACMG class 5 was classified as pathogenic, class 4 was classified as likely pathogenic and class 3 was classified as a VUS (41). All class 3–5 variants were determined to be causative after follow-up examinations. First, segregation analysis was performed if possible. Subsequently, further clinical phenotyping was performed, e.g. an ERG was performed in the case of a retinal dystrophy gene defect. The final decision about causality was reached during a multi-disciplinary expert consensus meeting.

Statistical analysis

Descriptive statistics of the patients were presented as frequencies and means (SD). Refractive error was calculated by adding half the cylindrical value to the spherical value. Mean refractive error was calculated by dividing the sum of the refractive error of both eyes by two. Differences in refractive error, age and age of onset between groups were compared using Kruskal Wallis test or Mann-Whitney test since these data were not normally distributed. Frequencies between groups were compared using Chi-square test or Fisher's exact test. For all analyses, a *P*-value of <0.05 was considered to be statistically significant. The IBM SPSS Statistics version 25 (IBM Corp. Armonk, NY) was used for the statistical analyses.

Supplementary Material

Supplementary Material are available at *HMGJ* online.

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Conflict of Interest statement. None declared.

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