Acta Ophthalmologica

ACTA OPHTHALMOLOGICA 2019

The genetic aetiology of retinal degeneration in children in Finland – new founder mutations identified

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ABSTRACT.

Purpose: **To study the genetic aetiology and phenotypes of retinal degeneration (RD) in Finnish children born during 1993–2009.**

Methods: **Children with retinal degeneration ($N = 68$) were investigated during 2012–2014 with a targeted gene analysis or a next-generation sequencing (NGS) based gene panel. Also, a full clinical ophthalmological examination was performed.**

Results: **The cohort covered 44% (68/153) of the Finnish children with inherited RD born 1993–2009. X-linked retinoschisis, retinitis pigmentosa, Leber congenital amaurosis and cone–rod dystrophy were the most common clinical diagnoses in the study group. Pathogenic mutations were found in 17 retinal genes. The molecular genetic aetiology was identified in 77% of the patients (in 77% of the families) analysed by NGS method. Several founder mutations were detected including three novel founder mutations c.148delG in *TULP1*, c.2314C>R (p.Gln772Ter) in *RPGRIP1* and c.533G>A (Trp178Ter) in *TYR*. We also confirmed the previous tentative finding of c.2944 + 1delG in *GYCU2D* being the most frequent cause of Leber congenital amaurosis (LCA) in Finland.**

Conclusions: **Globally, RD is genetically heterogeneous with over 260 disease genes reported so far. This was shown not to be the case in Finland, where the genetic aetiology of RD is caused by a small group of genes, due to several founder mutations that are enriched in the population. We found that X-chromosomal retinoschisis constitutes the major group in Finnish paediatric RD population and is almost exclusively caused by two founder mutations. Several other founder mutations were detected including three novel founder mutations. All in all, the genetic aetiology of 77% of families was identified which is higher than previously reported from other populations, likely due to the specific genomic constitution of the Finns.**

Key words: blindness – founder mutation – molecular genetic aetiology – next-generation sequencing – paediatric retinal degeneration – visual impairment

Acta Ophthalmol.

doi: 10.1111/aos.14128

Introduction

In western countries, the inherited and neuro-ophthalmological disorders are the most prevalent causes for vision impairment (VI) in childhood. VI affects the child's development, education and the care to be provided by families and professionals. The associated lifelong burden of disability is significant and has high economic costs.

The prevalence of VI in children has been studied in different populations, and it has been shown to vary from 10–41/100 000 (Foster & Gilbert 1992) to 181/100 000 (Arnaud et al. 1998). The prevalence of VI in children has also been studied in Finland (Rudanko & Laatikainen 2004), where it was shown to be 49/100 000 in children born during 1972–1989. At the time, the most frequent ophthalmological diagnoses in the children were ocular malformations (34%), retinal diseases (31%) and neuro-ophthalmological disorders (26%). Further, VI was associated with genetic factors in 42% of all children and 53% in the full-terms (Rudanko & Laatikainen 2004).

The recent advances in sequencing technologies, especially next-generation sequencing (NGS) techniques, have dramatically changed our ability to identify causative mutations in genetic diseases. Next-generation

sequencing has been shown to be extremely efficient at identifying causative genes in retinal degeneration (RD), which is known to be a genetically very heterogeneous condition (Farrar et al. 2017). We wanted to find out the genetic aetiologies of RD in children in Finland. We hypothesized that NGS method might reveal new founder mutations in Finnish RD children.

Patients and Methods

The ethical tenets for human scientific research according to the Declaration of Helsinki (2000) (World Medical Association General Assembly, 2001) were adhered, and the research plan was approved by the Ethical Board of the Medical Faculty of the University of Helsinki. A permission for fulfilling the research plan was admitted by the National Institute for Health and Welfare in Finland (THL).

Medical records were received from University and Central hospitals, Visio low vision rehabilitation centre, the Finnish Register of Visual Impairment (FRVI), the Register of Congenital Malformations and the Medical Birth Register. Additional information concerning relatives and their diseases was acquired with a questionnaire.

Prior to investigations, after being well informed on the purpose and implications of the study, every participating patient signed a consent by themselves if they were aged 15 years or over, and by their guardian if they were below 15 years. A separate consent concerning genetic analysis was also signed by the patient or the guardian.

There were 874 children with VI born 1993–2009 at the Finnish Register of Visual Impairment (FRVI) including 153 children with various types of RD as the primary cause of VI. These 153 patients from 2 to 18 years of age were invited to participate in the study. Written information about the study was delivered to patients and families attending Visio, Low Vision Research Centre in Helsinki, and the Ophthalmological Departments of the University and Central hospitals throughout the country. We also advertised this study in a journal published by the retinitis pigmentosa patient organisation.

Families contacted researchers by phone or letter, and 75 families were invited for a first visit into Visio. All of them were of Finnish origin. During the first visit, complete ophthalmological examination was conducted, and the medical records were examined for checking the fulfilment of the recruitment criteria. The children with an obvious genetic RD as the primary cause of visual impairment were selected to participate in the cohort. Some patients with severe motor and cognitive impairment were excluded because of co-operational reasons. Children with persistent fetal vitreous, choroid coloboma, foveal hypoplasia, congenital nystagmus and high myopia without retinal degeneration that is congenital birth defects and malformations without any signs of dystrophic retinal structure were excluded. The final size of the study group was 68 children from 53 families.

The 68 patients were examined ophthalmologically, a detailed family history was taken, medical records were examined and an EDTA-blood sample taken for genetic analysis if the molecular genetic aetiology was unknown. In addition, the DNA samples were taken from parents, siblings and sometimes also from other relatives. When necessary or informative, also parents, grandparents, brothers and sisters were examined ophthalmologically.

The ophthalmological examinations included assessment of visual acuity (ETDRS LogMAR letters or symbols for suitable far and near distances), contrast sensitivity (low contrast Sloan letters or symbols for suitable far and near distances), colour vision (Ishihara Pseudo Isochromatic Plates, Farnsworth's Panel D-15), binocularity, stereo vision (Random Dot (Butterfly) Stereotest), assessment of ocular motor functions, refraction, Haag Streit slit lamp biomicroscopy and ophthalmoscopy. Heidelberg Spectralis^{R} HRA+OCT Multicolor^{TM} with BluePeak (Heidelberg Engineering GmbH, Germany) was used for fundus photography, optical coherence tomography and autofluorescence imaging. Visual fields were tested with Goldmann kinetic perimeter (V/4a, II/4a) and Haag Streit Octopus 101 dynamic perimeter (dM2, central 30 degrees from fixation). Due to vast differences in visual functions of visually impaired children, a large selection of vision tests was used for detecting the best picture of the child's vision during various tasks and lighting conditions.

The targeted gene tests were performed using Sanger sequencing, MLPA analysis or minisequencing for founder mutations in X-linked retinoschisis. Due to unspecific phenotype, samples from 36 families were sent to a NGS-based gene panel analysis (see below). The mutations detected with NGS were confirmed by Sanger sequencing. The samples from affected siblings and parents were analysed with a targeted mutation test to determine the segregation and the phasing of the mutated alleles.

Next-generation sequencing procedure was performed as follows. Enrichment was performed with a custom design (Retinal dystrophy v2) Sure Select Custom Target enrichment kit (Agilent) for the Hiseq 2500 (Illumina) system, following the manufacturer's protocols. The target enrichment design consists of the coding region of transcripts including the immediate splice sites (± 5 bases) for 105 genes associated with retinal dystrophy listed

Table 1. Retinal diagnoses and sex distribution of children with visual impairment born from 1993 through 2009 (N = 68)

Ophthalmic diagnosis	All children[†] (N = 68)	Families (N = 53)	Males (N = 45)	Females (N = 23)
Retinoschisis, juvenile x-linked	20	16	20	0
Retinal pigmentary degeneration	14	12	5	9
Usher syndrome, type 3	2	2	1	1
Leber congenital amaurosis	15	11	8	7
Cone-rod dystrophy	11	6	10	1
Neuronal ceroid lipofuscinosis	2	2	1	1
Achromatopsia	2	2	0	2
Stargardt disease	1	1	0	1
Albinism, oculocutaneous	1	1	0	1

[†]M/F ratio 1.96.

Table 2. The results of the genetic analyses from 37 non-XLRS families with 48 patients and from 16 X-linked juvenile retinoschisis (XLRS) families with 20 patients

Causative mutation found (families 1–25)

Case	Phenotype	Causative gene	Mutations	Reference	Allele frequency	SIFT	Mutation taster	Polyphen
1a	LCA	GUCY2D	c.2944 + 1deIG homoz.	Hanein et al. (2002)				
1b	LCA	GUCY2D	c.2944 + 1deIG homoz.	Hanein et al. (2002)				
2	LCA	GUCY2D	c.2944 + 1deIG homoz.	Hanein et al. (2002)				
3a	LCA	GUCY2D	c.2944 + 1deIG homoz.	Hanein et al. (2002)				
3b	LCA	GUCY2D	c.2944 + 1deIG homoz.	Hanein et al. 2002;				
4	*LCA*	*GUCY2D*	*c.2994 + 1delG homoz.*	Hanein et al. 2002;				
5	*LCA*	*GUCY2D*	*c.2994 + 1delG homoz.*	Hanein et al. (2002)				
6a	LCA	RPGRIPI	c.1445T>A (p.Leu482Ter) heteroz., c.2314C>T (p.Gln772Ter) heteroz.	Ellingford et al. (2016)				
6b	LCA	RPGRIPI	c.1445T>A (p.Leu482Ter) heteroz., c.2314C>T (p.Gln772Ter) heteroz.	Ellingford et al. 2016				
7	LCA/RCD	CEP290	c.1666delA heteroz., c.2991 + 1655A>G heteroz.	Brancati et al. (2007), den Hollander et al. (2006)				
8	RP	EYS	c.1155T>A (p.Cys385Ter) homoz., c.8648_8655del8 homoz	Avela et al. (2018)				
9	RP	EYS	c.1155T>A heteroz. (p.Cys385Ter), c.8229delA heteroz.	Avela et al. (2018)				
10	RP	RPGR (ORF15)	c.2452delG hemiz., also present in affected mother		Not in gnomAD, not in HGMD			
11a	RP	RPGR (ORF15)	c.2569delinsGG hemiz.		Not in gnomAD, not in HGMD			
11b	RP	RPGR (ORF15)	c.2569delinsGG hemiz.		Not in gnomAD, not in HGMD			
12	RP	RDH12	c.883C>T (p.Arg295Ter) homozygous	Thompson et al. (2005)				
13	RP	RHO	c.888G>C (p.Lys296Asn) heteroz., *de novo*, not present in unaffected parents	Sohocki et al. (2001)				
14	RP	PRPF8	c.6942C>G (p.Phe2314Leu) heteroz., also present in affected father	Mckie et al. (2001)				
15	CRD	RPGR	c.334G>T (p.Gly112Cys) hemizygous, also present in affected mother		Not in gnomAD, not in HGMD	Deleterious	Disease causing	
16a	CRD	TULP1	c.148delG homoz.	Avela et al. (2018)				
16b	CRD	TULP1	c.148delG homoz.	Avela et al. (2018)				
17a	CRD	CRX	c.238G>A (p.Glu80Lys)	Sankila et al. (2000)				
17b	CRD	CRX	c.238G>A (p.Glu80Lys)	Sankila et al. (2000)				
17c	CRD	CRX	c.238G>A (p.Glu80Lys)	Sankila et al. (2000)				
17d	CRD	CRX	c.238G>A (p.Glu80Lys)	Sankila et al. (2000)				
18	Achromatopsia	CNGB3	c.1148delC homoz.	Sundin et al. (2000)				
19	Achromatopsia	CNGB3	c.1148delC homoz.	Sundin et al. (2000)				
20	Usher syndrome type 3	CLRN1	c.98G>A (p.Trp33Ter) heteroz., c.567T>G (p. Tyr189Ter) heteroz.	Joensuu et al. (2001)				
21	Usher syndrome type 3	CLRN1	c.359T>A (p.Met120Lys) heteroz., c.567T>G (p. Tyr189Ter) heteroz.	Joensuu et al. (2001)				
22	JNCL	CLN3	2.8 kb deletion homoz.	The Batten Disease Consortium (1994)				

Table 2. (Continued)

Causative mutation found (families 1–25)

Case	Phenotype	Causative gene	Mutations	Reference	Allele frequency	SIFT	Mutation taster	Polyphen
23	JNCL	CLN3	2.8 kb deletion homoz.	The International Batten Disease Consortium (1995)				
24	OCA1	TYR	c.533G>A (p.Trp178Ter)heteroz., c.650G>A (p.Arg217Gln) heteroz.	Giebel et al. (1991), Oetting & King (1993)				
25	Stargardt disease	ABCA4	c.3364G>A (p.Glu1122Lys) heteroz, c.4773 + 3A>G heteroz.	Lewis et al. (1999), Duno et al. (2012)				

Carriership or a variant of unknown significance was found

	Phenotype	Gene	Variants	Reference	Allele frequency	SIFT	Mutation Taster	Polyphen-2
26	LCA/RP	RDH12 (ad, ar)	c.731T>A (p.Leu244His) homoz. (VUS)		Not in gnomAD or HGMD	Tolerated	Polymorphism	
27	LCA/early CRD	PRPH2 (ad, ar)	c.586A>T (p.Ile196Phe) heteroz. (VUS), present in unaffected mother		Not in gnomAD or HGMD	Deleterious	Disease causing	
28a	EO-RCD	EYS (ar)	c.1155T>A (p. Cys385Ter) heteroz., at least a carrier of mutation	Avela et al. (2018)				
28b	EO-RCD	EYS (ar)	c.1155T>A (p. Cys385Ter) heteroz., at least a carrier of mutation	Avela et al. (2018)				
29	RP	RP1L1 (ad)	c.326_327insT heteroz., present in unaffected mother		gnomAD 0.89% in Finnish, not in HGMD			
30	CRD	CERKL (ar)	c.375C>G (p.Cys125Trp) heteroz., at least a carrier of mutation	Avela et al. (2018)				
31	Atypical RP	TEAD1 (ad)	c.1063C>T p.(Arg355Cys) heteroz. (VUS), present in unaffected mother		gnomAD 0% in Finnish, all 0.00071%; not in HGMD	Deleterious		Probably damaging
32	LCA/ChReD	TTC8 (ar)	c.284A>G (p. Lys95Arg) heteroz.,		gnomAD 0% in Finnish, all 0.0032%; not in HGMD	Tolerated		
32	LCA/ChReD	TTC8 (ar)	c.997G>A (p. Gly333Arg) heteroz.		gnomAD 0% in Finnish, all 0.0016%; not in HGMD			

No findings

	Phenotype	Gene	Variants
33a	LCA	NM	None reported
33b	LCA	NM	None reported
34	RP	NM	None reported
35	RP	NM	None reported
36a	RP	NM	None reported
36b	RP	NM	None reported
37	CRD	NM	None reported

X-linked retinoschisis

Families	Phenotype	Gene	Mutations	Reference
38–46 (12 patients in 9 families)	XLRS	RS1	c.214G>A (p.Glu72Lys)	Huopaniemi et al. (1999)
47–51 (5 patients in 5 families)	XLRS	RS1	c.325G>C (p.Gly109Arg)	Huopaniemi et al. (1999)

Table 2. (Continued)

X-linked retinoschisis Families	Phenotype	Gene	Mutations	Reference
52 (2 patients in 1 family)	XLRS	RS1	c.533G>C (p.Gly178Asp)	The Retinoschisis Consortium (1998)
53 (1 patient)	XLRS	RS1	exon1 deletion	Bowles et al. (2011)

If a variant in next-generation sequencing analysis was found in the index case (case a) the analysis of the siblings (case b, c, d) was performed with a targeted mutation analysis.
In family 17 (patients a,b,c,d) genetic analysis was performed as described previously Sankila et al. (2000).
ChReD = chorioretinal dysplasia, CRD = cone-rod dystrophy, GnomAD = the genome aggregation database (gnomAD), HGMD = The human gene mutation database, JNCL = juvenile neuronal ceroid lipofuscinosis, LCA = leber congenital amaurosis, Mutation Taster = evaluates DNA sequence variants for their disease-causing potential, OCA1 = oculo-cutaneous albinism, Polyphen = predicts possible impact of an amino acid substitution on the structure and function of a human protein, RCD = rod-cone dystrophy, RP = retinitis pigmentosa, SIFT = predicts effects in protein function based on sequence homology and the physical properties of amino acids, XLRS = X-linked retinoschisis.

in the supplement. The samples were sequenced using a HiSeq 2500 (Illumina), according to manufacturer's protocols. Sequence data were mapped with GenomeAnalysisToolKitLite-v2.0.39 (GATK) and with hg19 human genome as a reference. Known polymorphisms were subsequently filtered out of the data obtained using bioinformatic analysis. 99.4% of the target coding region of the transcripts was covered to a minimum depth of 50X. The 105 genes analysed in the NGS panel are listed in Appendix S1. Alamut Visual software was used to analyse the meaning of detected variants. Alamut uses information from different public databases such as NCBI, EBI and UCSC, as well as other sources including gnomAD, ESP, Cosmic or ClinVar. Moreover, Alamut Visual integrates several missense variant pathogenicity prediction tools and algorithms such as SIFT, PolyPhen, AlignGVGD or MutationTaster.

Results

After a thorough ophthalmological examination, a clinical diagnosis was established for each patient. These clinical diagnoses are presented in Table 1 as well as the sex distribution of the patients. There were altogether 68 patients (44 males and 24 females) from 53 families in the cohort. The three biggest groups of patients were X-linked juvenile retinoschisis (XLRS) with 29% (20/68) of the patients and 30% (16/53) of the families, retinitis pigmentosa with 21% (14/68) of the patients and 22% (12/53) of the families, and Leber congenital amaurosis with 22% (15/68) of the patients and 20% (11/53) of the families. 16% (11/63) of the patients had cone–rod dystrophy, and 6% (6/53) of the families had a member with cone–rod dystrophy. In addition, some other diagnoses were found in the cohort (see Table 1).

The genetic aetiology was identified in 77% (41/53) of all the families and 77% (52/68) of all the patients. 47% (25/53) of the families had a causative mutation in NGS analysis, and 30% (16/53) of the families had a *RS1* mutation. Findings of unknown significance were received in 13% (7/53) of all families and in 10% (8/68) of all children. In 9% of the families and children no genetic findings were identified.

The NGS gene panel with 105 RP genes was used in cases of 41 children from 33 families, which revealed the genetic aetiology of RDs in 63% of the children (26/41) and in 64% of the families (21/33).

The XLRS comprised the largest group of patients and the molecular aetiology was found in all cases. 60% (12/20) patients had a c.214G>A (p.Glu72Lys) mutation in *RS1 gene*, 25% (5/20) had a c.325G>C (p.Gly109Arg) mutation, two had a c.533G>A (p.Gly178Asp) mutation and one had a deletion of the exon 1 in *RS1* gene.

The genetic results of this study are presented in Table 2. Interestingly, in LCA patients, a homozygous c.2944 + 1deIG mutation in *GUCY2D* gene was found to occur in five families with seven patients (cases 1–5). c.2944 + 1deIG mutation in *GUCY2D* gene accounted for 70% (14/20) of the Finnish LCA-associated alleles in this study.

The allele frequencies of all the missense and nonsense mutations detected in this study were compared in the gnomAD database (Lek et al. 2016) between the Finnish population and European, non-Finnish population. Interestingly, seven of the disease alleles were more prevalent in the Finnish population than elsewhere in the Europe. They are listed in Table 3. The list includes previously reported founder mutations c.567T>G (p.Tyr189Ter) in *CLRN1* (Joensuu et al. 2001), c.214G>A (p.Glu72Lys) and c.325(p.Gly109Arg) in *RS1* (Huopaniemi et al. 1999), and 2.8 kb deletion in *CLN3* (1998), and most recently, c.1155T>A (p.Cys385Ter) in *EYS* (Avela et al. 2018), whereas c.148delG in *TULP1*, c.2314C>T (p.Gln772Ter) in *RPGRIP1*, and c.533G>A (p.Trp178Ter) in *TYR* were novel findings.

The interesting phenotypes and the genotypes of the two Finnish *EYS* patients are described in more detail in Table 4, and their spectral fundus images are presented in Fig. 1. In addition, two RP siblings were found to be carriers of c.1155T>A, but MLPA analysis of their *EYS* gene did not reveal any large deletion or duplications. As the ophthalmological presentations in RD are overlapping, it is impossible to know whether a missing other mutation is more likely than a

Table 3. Disease alleles enriched in the Finnish population, and detected in this pediatric retinal degeneration cohort

Gene	Mode of inheritance	Mutation	Allele frequence in Finnish population from gnomAD	Allele frequence in European non-Finnish population from gnomAD	Original reference
CLRN1	ar	c.567T>G (p.Tyr189Ter)	0.006904 (178/25782)	0.0001187 (15/126344)	Joensuu et al. (2001)
EYS	ar	c.1155T>A (p.Cys385Ter)	0.006336 (163/25728)	0.0002780 (35/125884)	Avela et al. (2018)
TULP1	ar	c.148delG	0.002882 (36/12490)	0 (0/36614)	Avela et al. (2018), and this report
RPGRIP1	ar	c.2314C>T (p.Gln772Ter)	0.0002245 (1/4454)	0 (0/38550)	This report
TYR	ar	C.533G>A (p.Trp178Ter)	0.0001551 (4/25788)	0 (0/126648)	This report
RS1	X-linked	c.214G>A (p.Glu72Lys)	0.0001216 (2/16443)	0 (0/80126)	Huopaniemi et al. (1999)
RS1	X-linked	c.325G>C (p.Gly109Arg)	0.0001826 (3/16432)	0 (0/79858)	Huopaniemi et al. (1999)
CLN3	ar	2.8 kb deletion	NA	NA	The International Batten Disease Consortium (1995)
GUCY2D	ar	c.2944 + 1deIG	NA, accounts for 50% of LCA cases in this report	NA	Hanein et al. (2004), and this report

ar = autosomal recessive, NA = not available, X-linked = X-chromosomal.

causative change in entirely different gene.

Mutation c.148delG in *TULP1* was also found to be enriched in the Finnish population as the allele frequency was 0.002882 in Finland, and 0 in European, non-Finnish population (Table 3). A homozygous c.148delG mutation in *TULP1* gene was found in two siblings with early-onset RD/RCD (case 16).

One of the enriched alleles was c.2314C>T (p.Gln772Ter) in *RPGRIP1* gene. We detected it in a compound heterozygous state in two LCA siblings (case 6).

One patient with oculocutaneous albinism was a compound heterozygote for c.533G>A (p.Trp178Ter) and c.650G>A (p.Arg217Gln) in *TYR* gene (case 24). c.533G>A was found to be more frequent in Finland than elsewhere in Europe, whereas no difference in the occurrence of c.650G>A was detected between the populations of Finland and the rest of Europe.

Altogether, pathogenic mutations were found in 17 genes in 77% of the families (41/53) participating in the study. A variant of unknown significance (VUS) or a carriership of a RD mutation was found in seven families. No pathogenic mutation was detected in five families (Table 2).

Discussion

We present a cohort of 68 Finnish children born in 1993–2009 with inherited RD, based on our investigations for the genetic aetiology of their disease. This cohort can be considered as a representative sample about Finnish children with RD, born in 1993–2009 and registered at the FRVI, as 46% of such children participated in the study. 80% of the children were from the southern, western and central Finland, while only 20% came from the northern and north-eastern country reflecting the fact that most of the people live in the southern and south-western part of the Finland. There are still some sub-isolates in northern and north-eastern parts of the country which are under-represented in this cohort, and their RD alleles still remain to be discovered.

In this study, 29% of the patients had *XLRS* comprising the largest patient group. According to De La Chappelle et al. (1994) the XLRS prevalence is greater than 1:17 000 in Finland which is in line with our results, that is 20 cases, 46% coverage, and 1, 006, 006 births during 1993–2009 (Gissler et al. 2017). The high amount of males in this study is explained by the large number of XLRS patients in Finland.

X-linked juvenile retinoschisis is enriched in the Finnish population (Table 3) and is one of the around 40 diseases of the Finnish Disease Heritage (Norio 2003), which are more prevalent in Finland than elsewhere. The Finnish Disease Heritage includes other eye diseases as well namely choroideraemia and hyperornithinaemia with gyrate atrophy of choroid and retina (HOGA), which were not seen in this study. This could be due to regional differences or to sample size. Instead this cohort included two cases of Usher syndrome type 3, impairing both vision and hearing, and two cases of a severe neurological disorder, neuronal ceroid lipofuscinoses (INCL, $vLINCL_{Fin,}$ JNCL), all of them are Finnish diseases. The Finnish Disease Heritage has evolved as a consequence of the Finnish population history, especially small groups of original settlers, and geographical isolation have led to enrichment of certain disease mutations in the population. These common mutations are called founder mutations.

Several founder mutations were identified in this study. The mutations c.214G>A and c.325G>C in *RS1*, c.567T>G in *CLNR1* and 2.8 kb deletion in *CLN3* have been described previously (The International Batten Disease Consortium 1995; Huopaniemi et al. 1999; Joensuu et al. 2001). Hanein et al. 2002 have reported c.2944 + 1delG in *GUCY2D* in three Finnish LCA patients. We were able to confirm that c.2944 + 1delG is a founder allele, as it accounted for 70% (14/20) of the LCA-alleles in this cohort.

Recently, c.375C>G p.(Cys125Trp) in *CERKL* and c.1155T>A (p.Cys385Ter) in *EYS* were reported to be founder mutations in a Finnish cohort of adult RD patients (Avela et al. 2018). The total absence of *CERKL* patients and the relatively small number of *EYS* patients in this paediatric cohort most likely reflects the typical adolescence or adult onset of those diseases (Pierrottet et al. 2014, and Avela et al. 2018). EYS mutations have been reported in arRP in several European countries, and in Japan and China with variable phenotypes (Littink et al. 2010; Chen et al. 2015; Gu et al. 2016).

Table 4. The phenotypes of Finnish patients with EYS gene associated retinitis pigmentosa

ID	Family	Sex	Current age/age at onset	Clinical diagnosis	Mutations	Initial symptoms	BCVA OD/OS	Refraction OD/OS	Cataract OD/OS	WHO class	Night blindness	Photophobia	Visual field VF3	Fundus appearance
8	8	F	19/13	arRP	c.1155T>A (p.Cys385Ter) homoz., c.8648_8655del8 homoz.	Night vision	0.3/0.3	−1.0/−1.25	−/−	3	+/+	+/+	Central tubular 10° D (G II/4a) Central tubular 20° D (G V/4a)No peripheral fields (G V/4a)	Optic discs pale, surrouded by peripapillar RPE atrophy Moderate attenuation of retinal vessels Rather high cystic macular oedema Peripheral retinal atrophy with retinal pigment dots, but no bone spicule corpuscles Large choroidal vessels clearly visible under atrophic RPESlightly pale fundus in NIR-reflectance imaging
9	9	M	24/15	arRP	Compound heterozygote c.1155T>A (p.Cys385Ter) and c.8229delA	Night vision	1.2/1.0	−2.0 cyl+0.75 ax 150°/−2.5 cyl+1.25 ax 92°	−/−	3	+/+	+/+	Central tubular 15° D (G II/4) Pericentral ring scotoma from 5°–15° Peripheral scotomas (G V/4a)	Optic discs pale, surrounded by white temporal cuffs Moderate attenuation of retinal vesselsMacular foveal cups very shallow (Fig. 1) General chorioretinal atrophy, pigment migration with dots and bone spicule corpusclesLarge choroidal vessels clearly visible under atrophic RPESlightly pale fundus in NIR-reflectance imagingPhenotype is not typical to the 'classic RP', but it nicely resembles that of AMA 069

BCVA = best corrected visual acuity, VF = according to Goldmann perimetry II/4a, V/4a, 3WHO categories of visual impairment (1973) = WHO class 3 (profound visual impairment) means functional blindness.

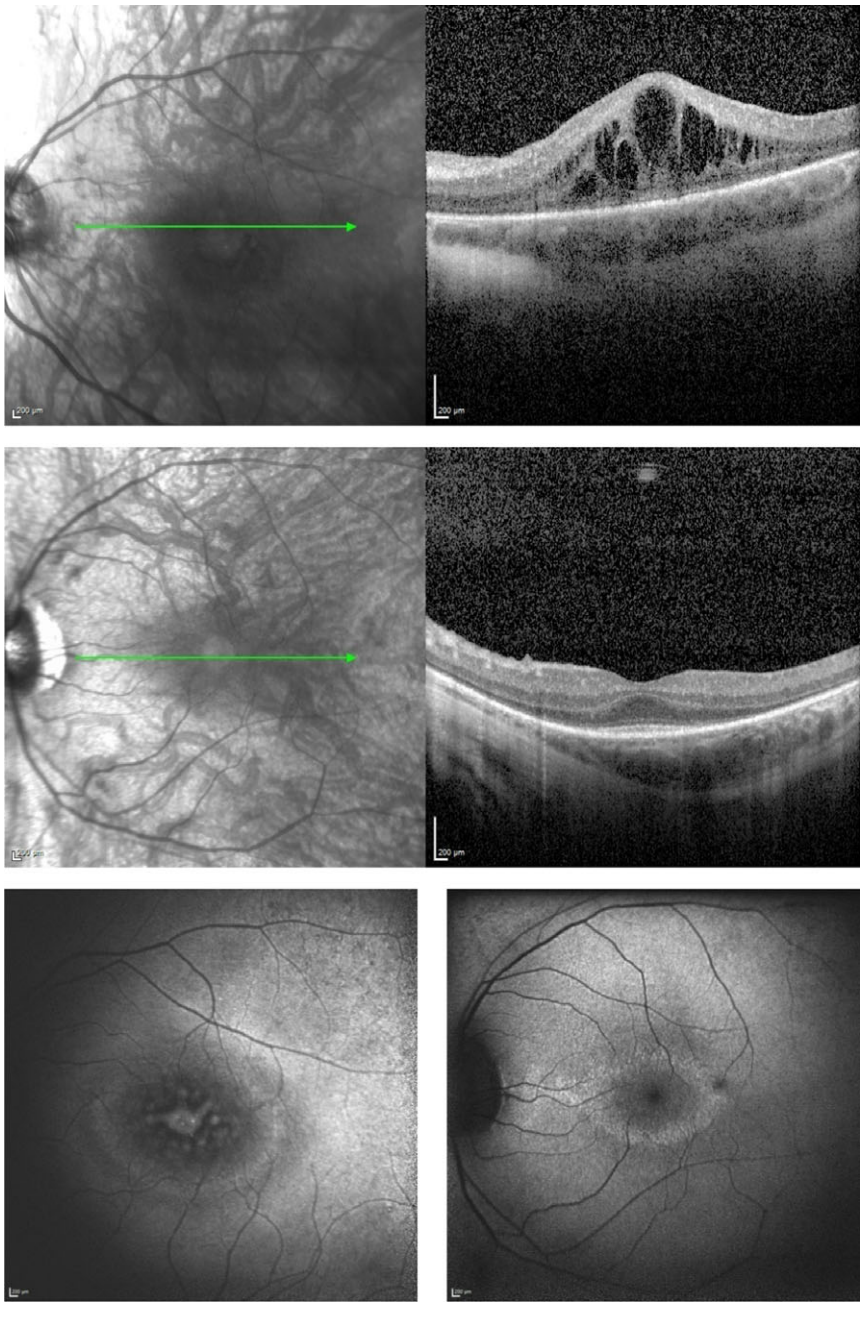

Fig. 1. Spectral fundus images of two patients with retinitis pigmentosa associated with homozygous *EYS* gene mutations. First line: case 8 with severe degeneration in foveal–perifoveal photoreceptors, RPE and choriocapillaris, and with high cystic macular oedema (IR 30 & OCT images). Second line: case 8 with degeneration in foveal and perifoveal photoreceptors, RPE and choriocapillaris, but no cystic macular oedema (IR 30 & OCT images). Third line: case 8 and case 9 with a hyperfluorescent ring of lipofuscin accumulated in the central RPE of both patients, in consequence of RPE degeneration (BAF images).

The two cases with *EYS* mutations in this study presented with rather similar phenotypes, except for the severe central macular oedema (CME), which was seen in the fundus of the female patient. Rather high prevalences of CME have been reported in RP (e.g. Hajali et al. 2008). In this study group, CME was present in 41% (7/17) of the patients with RP and Usher syndrome, being consistent with recent scientific reports.

Interestingly, one mother of two visually impaired siblings without a diagnosis was homozygous for c.1155T>A *EYS* mutation. She has a so-called 'classic' RP phenotype with typical bone spicule pigmentations. The father of these siblings was unavailable for the examinations, but he or anyone else in the family was not known to be visually impaired. The phenotypes of our paediatric *EYS* cases (8 and 9) differed from the mother's classic one, in part due to the longer duration of the mother's RP with heavy pigment migration from her atrophic retinal pigment epithelium (RPE) into retinal layers. However, by MultiColour Green and Infrared Reflectance imaging (30°), the fundi of cases 8 and 9 were more reflective than expected (Fig. 1), probably due to notable shortage or loss of pigment in the course of RPE atrophy. Further studies are needed for clarifying the diversity and evolution of phenotypes of *EYS* mutations in the Finnish population.

Interestingly, we found three novel founder mutations (Table 3). One of them was c.148delG in *TULP1* causing ar-LCA and early-onset RD (EORD). In *TULP1*-associated LCA and EORD, rods and cones are affected very early, due to prenatal retinal maldevelopment and rapid postnatal degeneration. Functional rods are usually not found at birth, and macular cones are insensitive (Jacobson et al. 2014). Nystagmus and night blindness appear during the first weeks of life and are important signs for initiating ophthalmological examinations.

Our patients with *TULP1* homozygous mutation (cases 16a and 16b, Table 2) were two male siblings with RCD/early-onset RD, aged 6 years and 4 years at the onset of examinations. Their ages of clinical diagnosis were 3 years and one year, respectively, and their phenotypes very much like previously reported (Ajmal et al. 2012). The older sibling (case 16a) had severe low vision with BCVA 0.20 OD and 0.12 OS, tritan colour vision defects, peripheral constrictions in the visual fields (VFs), large central-pericentral scotomas and small functional spots remaining in the VF centre. Thin retinal blood vessels with some occluded fibrosed vessels were seen in the fundi. Optic discs with white peripapillar cuffs, general RPE atrophy, retinal thinning with granular pigmentation and pigment dots, and maculopathy with some foveal dysplasia, light perifoveal annular rings and large choroidal vessels well discernible under thinned retinal layers were the

prevailing features of the phenotype. The ERGs were undetectable.

RPGRIP1 mutations lead into a severe nonsyndromic retinal ciliopathy, typical to Leber congenital amaurosis (Khan et al. 2013). The phenotypes of our patients (cases 6, Table 2) were similar to those in previous reports. Signs of poor vision and nystagmus had been noticed within the first 3 months in both siblings, and ERG responses had been nonrecordable since birth. The ocular fundi had looked normal during the first year of life, but thereafter, rapidly progressive retinal degeneration led into most severe atrophy and functional blindness. The reported BCVAs were at the level of light perception (LP).

TYR mutations cause oculocutaneous albinism type1 (OCA1) characterized by nystagmus, hypopigmentation of skin and hair, iris translucency, low levels of retinal pigment, foveal hypoplasia and choroidal blood vessels well discernible through light retinal layers during ophthalmoscopy. As most Finnish children have white hair and pale skin, the diagnosis of ocular or oculocutaneous albinism is not as obvious in Finnish as in some other populations. One of our patients was suspected to have oculocutaneous albinism and was shown to have c.650G>A (p.Arg217Gln) and c.533G>A (p.Trp178Ter) in *TYR*. The latter of the mutations is enriched in the Finnish population according to gnomAD database (Table 3). At the age of 9 years, our female patient (case 24) had horizontal nystagmus since birth, BCVA of 0.08 OD and 0.08 OS, rather high binocular hyperopia and astigmatism, alternating esotropia, photophobia, yellowish fundi, some macular dysplasia and foveal hypoplasia with central and perisentral scotomas by Goldmann perimetry. Slight brown pigment was discernible in her eyebrows, eyelashes, irises and fundi. Obviously, she has some melanin synthesis in her RPE melanosomes, with low function of tyrosinase enzyme, and thus the diagnosis can be further defined to OCA1B.

Despite many founder mutations, nonfounder gene defects were also diagnosed (*RDH12*, *RHO*, *PRPF8*, *RPGR*, *ABCA4*, *CRX*, *CEP290*, *CNGB3*) indicating some genetic heterogeneity among Finnish children with retinal degeneration, even though to a lesser extent than elsewhere in Europe.

To conclude, the most common clinical diagnoses for retinal degeneration in Finnish children in this cohort were XLRS, RP, LCA, EO-RCD and CRD. The unusual population history of Finland is reflected in the findings of this study. Several founder mutations were identified, including three new founder mutations, c.148delG in *TULP1*, c.2314C>R (p.Gln772Ter) in *RPGRIP1*, and c.533G>A (Trp178Ter) in *TYR*. We also confirmed the previous tentative finding of c.2944 + 1delG being a frequent cause of LCA in Finland. The genetic aetiology was identified in 77% of the families. This is higher than reported from other populations, likely due to the founder mutations.

The detection rate of significant pathogenic variants would have been somewhat higher by whole exome sequencing or by additional special molecular laboratory techniques, like copy number variant analysis, long range PCR amplification of hard-to-sequence targets or mutation-specific analysis of deep intronic mutations, which were not possible to use in this cohort.

Exact genetic aetiology allows accurate genetic counselling for the benefit of involved families. This knowledge also allows us to recognize the families who can take advantage from future gene therapy treatments.

References

Ajmal M, Khan MI, Micheal S et al. (2012): Identification of recurrent and novel mutations in TULP1 in Pakistani families with early-onset retinitis pigmentosa. Mol Vis **18**: 1226–1237.

Arnaud C, Baille MF, Grandjean H, Cans C, du Mazaubrun C & Rumeau-Rouquette C (1998): Visual impairment in children: prevalence, aetiology and care, 1976-85. Paediatr Perinat Epidemiol **12**: 228–239.

Avela K, Sankila EM, Seitsonen S, Kuuluvainen L, Barton S, Gillies S & Aittomäki K. (2018): A founder mutation in CERKL is a major cause of retinal dystrophy in Finland. Acta Ophthalmol **18**: 3–191.

Bowles K, Cukras C, Turriff A, Sergeev Y, Vitale S, Bush RA & Sieving PA (2011): X-linked retinoschisis: RS1 mutation severity and age affect the ERG phenotype in a cohort of 68 affected male subjects. Invest Ophthalmol Vis Sci **52**: 9250–9256.

Brancati F, Barrano G, Silhavy JL et al. (2007): CEP290 mutations are frequently identified in the oculo-renal form of Joubert syndrome-related disorders. Am J Hum Genet **81**: 104–113.

Chen X, Liu X, Sheng X et al. (2015): Targeted next-generation sequencing reveals novel EYS mutations in Chinese families with autosomal recessive retinitis pigmentosa. Sci Rep **5**: 8927.

De La Chappelle A, Alitalo T & Forsius H (1994): X-linked juvenile retinoschisis. In: Wright AF & Jay B (eds). Molecular genet inherited eye disorders. Switzerland: Harwood Academic Publishers 339–357.

Duno M, Schwartz M, Larsen PL & Rosenberg T (2012): Phenotypic and genetic spectrum of Danish patients with ABCA4-related retinopathy. Ophthalmic Genet **33**: 225–231.

Ellingford JM, Barton S, Bhaskar S et al. (2016): Molecular findings from 537 individuals with inherited retinal disease. J Med Genet **53**: 761–767.

Farrar GJ, Carrigan M, Dockery A et al. (2017): Toward an elucidation of the molecular genetics of inherited retinal degenerations. Hum Mol Genet **26**: R2–R11.

Foster A & Gilbert C (1992): Epidemiology of childhood blindness. Eye **6**(Pt 2): 173–176.

Giebel LB, Musarella MA & Spritz RA (1991): A nonsense mutation in the tyrosinase gene of Afghan patients with tyrosinase negative (type IA) oculocutaneous albinism. J Med Genet **28**: 464–467.

Gissler M, Vuori E & Heino A (2017): Perinatal statistics 2016. Helsinki: National Institute for Health and Welfare (THL).

Gu S, Tian Y, Chen X & Zhao C (2016): Targeted next-generation sequencing extends the phenotypic and mutational spectrums for EYS mutations. Mol Vis **22**: 646–657.

Hajali M, Fishman GA & Anderson RJ (2008): The prevalence of cystoid macular oedema in retinitis pigmentosa patients determined by optical coherence tomography. Br J Ophthalmol **92**: 1065–1068.

Hanein S, Perrault I, Olsen P et al. (2002): Evidence of a founder effect for the RETGC1 (GUCY2D) 2943DelG mutation in Leber congenital amaurosis pedigrees of Finnish origin. Hum Mutat **20**: 322–323.

Hanein S, Perrault I, Gerber S et al. (2004): Leber congenital amaurosis: comprehensive survey of the genetic heterogeneity, refinement of the clinical definition, and genotype-phenotype correlations as a strategy for molecular diagnosis. Hum Mutat. **23**(4): 306–317.

den Hollander AI, Koenekoop RK, Yzer S et al. (2006): Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. Am J Hum Genet **55**: 6–61.

Huopaniemi L, Rantala A, Forsius H, Somer M, de la Chapelle A & Alitalo T (1999): Three widespread founder mutations contribute to high incidence of X-linked juvenile retinoschisis in Finland. Eur J Hum Genet **7**: 368–376.

Jacobson SG, Cideciyan AV, Huang WC et al. (2014): TULP1 mutations causing early-

onset retinal degeneration. Preserved but insensitive macular cones. Invest Ophthalmol Vis Sci **55**: 5354–5364.

Joensuu T, Hämäläinen R, Yuan B et al. (2001): Mutations in a novel gene with transmembrane domains underlie Usher syndrome type 3. Am J Hum Genet **69**: 673–684. Erratum in: Am J Hum Genet 69:1160.

Khan AO, Abu-Safieh L, Eisenberger T, Bolz HJ & Alkuraya FS (2013): The RPGRIP1-related retinal phenotype in children. Br J Ophthalmol **97**: 760–764.

Lek M, Karczewski KJ, Minikel EV et al. (2016): Analysis of protein – coding genetic variation in 60, 706 human. gnomAD database. Nature **536**: 285–291.

Lewis RA, Shroyer NF, Singh N et al. (1999): Genotype/Phenotype analysis of a photoreceptor-specific ATP-binding cassette transporter gene, ABCR, in Stargardt disease. Am J Hum Genet **64**: 422–434.

Littink KW, van den Born LI, Koenekoop RK et al. (2010): Mutations in the EYS gene account for approximately 5% of autosomal recessive retinitis pigmentosa and cause a fairly homogeneous phenotype. Ophthalmology **117**: 2026–2033.

McKie AB, McHale JC, Keen TJ et al. (2001): Mutations in the pre-mRNA splicing factor gene PRPC8 in autosomal dominant retinitis pigmentosa (RP13). Hum Mol Genet **10**: 1555–1562.

Norio R (2003): The Finnish Disease Heritage III: the individual diseases. Hum Genet **112**: 470–526.

Oetting WS & King RA (1993): Molecular basis of type I (tyrosinase-related) oculocutaneous albinism: mutations and polymorphisms of the human tyrosinase gene. Hum Mutat **2**: 1–6.

Pierrottet CO, Zuntini M, Digiuni M et al. (2014): Syndromic and non-syndromic forms of retinitis pigmentosa: a comprehensive Italian clinical and molecular study reveals new mutations. Genet Mol Res **13**: 8815–8833.

Rudanko SL & Laatikainen L (2004): Visual impairment in children born at full term from 1972 through 1989 in Finland. Ophthalmology **111**: 2307–2312.

Sankila EM, Joensuu TH, Hämäläinen RH, Raitanen N, Valle O, Ignatius J & Cormand B (2000): A CRX mutation in a Finnish family with dominant cone-rod retinal dystrophy. Hum Mutat **16**: 94.

Sohocki MM, Daiger SP, Bowne SJ et al. (2001): Prevalence of mutations causing retinitis pigmentosa and other inherited retinopathies. Hum Mutat **17**: 42–51.

Sundin OH, Yang JM, Li Y, Zhu D, Hurd JN, Mitchell TN, Silva ED & Maumenee IH (2000): Genetic basis of total colourblindness among the Pingelapese islanders. Nat Genet **25**: 289–293.

The International Batten Disease Consortium (1995): Isolation of a novel gene underlying Batten disease, CLN3. Cell **22**: 949–957.

The Retinoschisis Consortium (1998): Functional implications of the spectrum of mutations found in 234 cases with X-linked juvenile retinoschisis (XLRS). Hum Mol Genet **7**: 1185–1192.

Thompson DA, Janecke AR, Lange J et al. (2005): Retinal degeneration associated with RDH12 mutations results from decreased 11-cis retinal synthesis due to disruption of the visual cycle. Hum Mol Genet **14**: 3865–3875.

World Medical Association General Assembly (2001): World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects (revised October 7, 2000). HIV Clin Trials **79**:92–95.

Received on December 18th, 2018.
Accepted on April 10th, 2019.

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The study was supported by Silmäsäätiö, Sokeain Ystävät ry, Päivikki & Sakari Sohlberg Foundation, Retina Finland and Finnish Medical Foundation. We are deeply grateful to all patients and their families for participating in the study and their excellent co-operation during all phases of the study.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. List of genes in the NGS Panel of 105 RP genes.