Original research

DNAJC30 disease-causing gene variants in a large Central European cohort of patients with suspected Leber's hereditary optic neuropathy and optic atrophy

Sinja Kieninger ,[1] Ting Xiao ,[1] Nicole Weisschuh,[1] Susanne Kohl,[1] Klaus Rüther,[2] Peter Michael Kroisel,[3] Tobias Brockmann,[4] Steffi Knappe,[4] Ulrich Kellner,[5,6] Wolf Lagrèze,[7] Pascale Mazzola,[8] Tobias B Haack ,[8,9] Bernd Wissinger,[1] Felix Tonagel[10]

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For numbered affiliations see end of article.

Correspondence to
Dr Felix Tonagel, Centre for Ophthalmology, University of Tübingen, Tübingen, Germany; Felix.Tonagel@med.uni-tuebingen.de

SK and TX contributed equally.

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ABSTRACT

Background Leber's hereditary optic neuropathy (LHON) has been considered a prototypical mitochondriopathy and a textbook example for maternal inheritance linked to certain disease-causing variants in the mitochondrial genome. Recently, an autosomal recessive form of LHON (arLHON) has been described, caused by disease-causing variants in the nuclear encoded gene *DNAJC30*.

Methods and results In this study, we screened the *DNAJC30* gene in a large Central European cohort of patients with a clinical diagnosis of LHON or other autosomal inherited optic atrophies (OA). We identified likely pathogenic variants in 35/1202 patients, corresponding to a detection rate of 2.9%. The previously described missense variant c.152A>G;p.(Tyr51Cys) accounts for 90% of disease-associated alleles in our cohort and we confirmed a strong founder effect. Furthermore, we identified two novel pathogenic variants in *DNAJC30*: the nonsense variant c.610G>T;p.(Glu204*) and the in-frame deletion c.230_232del;p.(His77del). Clinical investigation of the patients with arLHON revealed a younger age of onset, a more frequent bilateral onset and an increased clinically relevant recovery compared with LHON associated with disease-causing variants in the mitochondrial DNA.

Conclusion This study expands previous findings on arLHON and emphasises the importance of *DNAJC30* in the genetic diagnostics of LHON and OA in European patients.

INTRODUCTION

Leber's hereditary optic neuropathy (LHON, OMIM:535000), first reported by Theodore Leber in 1871,[1] is the most common disease linked to mitochondrial DNA (mtDNA) variants and a textbook example for maternal inheritance.[2]

Onset of LHON is usually in the second or third decade of life, but manifestations in childhood or at an older age have also been observed. Symptoms include initial unilateral acute or subacute painless vision loss with involvement of the second eye after a few weeks to months, accompanied by dyschromatopsia. In the acute phase of LHON, fundus examination shows a papillary hyperaemia of the optic disc without leakage in fluorescein fundus angiography. In addition, peripapillary microangiopathy is often observed at the beginning of the disease. Central or cecocentral scotomas are another typical finding.[2 3] In the chronic phase, best-corrected visual acuity in patients with LHON nearly always decreases to 20/200 or below. The temporal quadrant or all quadrants of the optic disc are pale, and optical coherence tomography shows the thinning of the retinal nerve fibre layer in the corresponding quadrants.[3 4] Penetrance is reduced in LHON but rarely related to the 'mutation load' since mtDNA variants are usually homoplasmic in LHON families.[5] Rather certain mtDNA haplogoups are predominantly observed in patients with LHON (eg, haplogroup J in patients with the m.11778G>A and the m.14484T>C variant) and are thought to increase the risk of visual loss.[6] In addition, penetrance is about 3–5 times higher in males.[7 8] Differences in the exposure to toxic factors (eg, tobacco or alcohol consumption), the presence of an X linked susceptibility factor and the protective role of oestrogens have been proposed to play a role in this gender bias.[7–10] Abuse of alcohol and cigarettes is known to worsen the symptoms and prognosis.[11 12] Idebenone, a synthetic coenzyme Q10 analogue initially developed for the treatment of Alzheimer's disease, has been proved safe and efficient in rescuing of visual acuity (VA) in patients with LHON,[13 14] although spontaneous visual recovery is sometimes also observed in non-treated patients with LHON.[15 16]

Three point mutations in the mitochondrial genome (m.11778G>A in *MT-ND4*, m.3460G>A in *MT-NDI* and m.14484T>C in *MT-ND6*) account for about 90%–95% of the LHON disease cases and have been shown to cause dysfunction of complex I (CI) in the mitochondrial respiratory chain, a decrease of ATP synthesis and the increased production of reactive oxygen species, eventually leading to death of retinal ganglion cells.[2 17] In addition, some rare mtDNA variants have been recurrently associated with LHON.[18 19]

Recently, Stenton *et al* reported that certain variants in the nuclear gene *DNAJC30* result in

an autosomal recessive inherited form of LHON (arLHON, OMIM:619382). Notably, a strong geographic accumulation of *DNAJC30*-linked arLHON was reported with >85% of their 29 families originating from Eastern Europe (Russia, Ukraine, Poland, Romania).[20] The patients with arLHON manifested similar symptoms to classical LHON associated with disease-causing variants in the mtDNA (mtLHON), except one female patient who presented with Leigh syndrome. The DNAJC30 protein is a chaperone protein of CI interacting with mitochondrial complex V to promote ATP synthesis, and is mainly expressed in neurons.[20 21]

Given the unexpected report of pathogenic variants in a nuclear gene to cause LHON, we performed a retrospective screening of *DNAJC30* in a large series of genetically unsolved Central European patients with LHON, and patients diagnosed with inherited optic atrophy (OA). We compiled and compared the prevalence, patients' demography and clinical findings with those linked to mtDNA variants.

SUBJECTS AND METHODS

Study cohort

In this study, 1202 patients (1197 index patients and 5 affected family members) were retrospectively selected for *DNAJC30* screening, including 800 patients with a clinical diagnosis of LHON and 402 patients with OA. The cohort comprised 769 male and 431 female patients (1.8:1), the gender of two patients is unknown. Genomic DNA samples of patients were collected between 1992 and 2021 at the Institute for Ophthalmic Research, University Clinics Tübingen, Germany. Prior to this study, patients had undergone routine LHON or OA diagnostic or research-based genetic testing in which no likely pathogenic variants could be detected.

Identification of *DNAJC30* variants (Sanger sequencing)

Screening of the entire single exon *DNAJC30* gene (NM_032317.3) was performed using Sanger sequencing. Exon 1 and parts of the flanking untranslated region of *DNAJC30* were amplified by PCR from genomic DNA using forward primer 5′−3′: ggcacccggtttttatgtc, and reverse primer 5′−3′: gcaggggagtacagttcct. PCR products were purified by treatment with ExoSAP-IT reagent (Thermo Fisher Scientific, Darmstadt, Germany) and Sanger sequencing was performed using BigDye Cycle Sequencing V.1.1 chemistry (Thermo Fisher Scientific). Sequencing products were separated by capillary electrophoresis on an ABI 3130xl Genetic Analyzer (Applied Biosystems—Thermo Fisher Scientific).

The obtained sequences of exon 1 and the flanking intronic regions were analysed using Sequencing Analysis V.5.2 (Applied Biosystems—Thermo Fisher Scientific) and the SeqMan II (DNASTAR, Madison, Wisconsin, USA) software. Missense variants were evaluated for their pathogenic potential using the web-based tools MutationTaster (http://www.mutationtaster.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/). Variants were filtered for an allele frequency of <0.01 in the normal population. Allele frequencies were retrieved from the Genome Aggregation Database (gnomAD V.2.1.1, https://gnomad.broadinstitute.org/gene/ENSG00000176410). Moreover, for consistency with the autosomal recessive mode of inheritance, only variants in a homozygous or compound heterozygous state with a second likely pathogenic variant were considered. Likely pathogenic variants were finally validated through additional Sanger sequencing on independent DNA samples where available.

Confirmation of biallelism

Segregation analysis by means of Sanger sequencing was performed depending on availability of DNA of additional family members. Biallelism for compound heterozygous variants was assessed by allelic cloning. In brief, a fragment harbouring both variants was amplified by PCR from patient's genomic DNA using standard protocols and the resulting PCR product was cloned into a pMiniT2.0 vector using the PCR Cloning Kit (New England Biolabs, Frankfurt, Germany). Plasmid DNA isolated from individual clones were then used for Sanger sequencing.

Founder effect analysis for the *DNAJC30*: c.152A>G variant

Microsatellite marker analysis was done by PCR amplification with fluorescence-labelled primers using Qiagen Multiplex PCR Kit reagents (Qiagen, Hilden, Germany) and PCR conditions as recommended by the supplier. PCR fragments were separated on an ABI 3130xl capillary sequencer (Applied Biosystems—Thermo Fisher Scientific) along with a ROX-500 length standard and fragment sizes were determined using GeneMapper Software (Applied Biosystems—Thermo Fisher Scientific). PCR primer sequences are provided in online supplemental table 1.

Clinical investigations

Patients underwent ophthalmological examination according to the clinical standards of the recruiting centres, including VA measurement, slit lamp examination, perimetry and indirect ophthalmoscopy. Colour vision was examined using Ishihara plates and Farnsworth-Munsell Dichotomous D-15 test. VA is given in logMAR. A clinically relevant recovery (CRR) of visual impairment was defined as improvement of VA of 0.2 logMAR or greater.

RESULTS

Genetic findings

Sanger sequencing of 1202 LHON and OA genetically unsolved patients identified 35 individuals from 32 families with homozygous or compound heterozygous, putatively pathogenic *DNAJC30* variants (table 1). This corresponds to a detection rate of 2.9% in the entire cohort. More specifically, 29/800 (3.6%) of the clinically diagnosed LHON cases and 6/402 (1.5%) OA cases of our cohort were identified to carry putatively disease-causing *DNAJC30* variants. Of the 35 patients, 30 were males and 5 were females (ratio 6:1), demonstrating apparent male predominance in *DNAJC30*-linked arLHON given a male:female ratio of 1.8:1 in the entire cohort. Segregation analysis could be performed in eight patients from five families, allelic cloning was conducted in three patients from three families carrying two heterozygous variants. Segregation and allelic cloning confirmed the expected recessive mode of inheritance and true biallelism.

The most prevalent disease-associated variant identified in our study was the c.152A>G;p.(Tyr51Cys) variant already reported by Stenton *et al*.[20] Additionally, two novel most likely pathogenic variants were identified: a nonsense variant c.610G>T;p.(Glu204*) and a 3 bp inframe deletion c.230_232del,p.(His77del). All detected variants are located in the J domain of the DNAJC30 protein, with the exception of the nonsense variant which is located upstream of the transmembrane domain (figure 1).[21] The missense variant c.152A>G;p.(Tyr51Cys) was found in 30 patients with LHON (n=26) and OA (n=4) in homozygous state (figure 2D, online supplemental table 2). The nonsense variant c.610G>T;p.(Glu204*) was observed in three unrelated patients with LHON in compound heterozygous state with the common c.152A>G;p.(Tyr51Cys) variant, as confirmed

Table 1 Patients with bi-allelic variants in *DNAJC30*

Patient	Gender	Clinical diagnosis	Variant	Allele status	Method	Segregation analysis	Family relation
LHON 59 (1316)	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	Yes	Brother of patient LHON 59 (1824)
LHON 59 (1824)	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	Yes	Brother of patient LHON 59 (1316)
LHON 84	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 96	M	LHON	c.152A>G;p.(Tyr51Cys) c.610G>T;p.(Glu204*)	Compd het	SS	Allelic cloning	NA
LHON 210	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 238	F	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 246	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 286	F	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 347	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 377	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 380	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 466	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 507	F	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	Yes	NA
LHON 526	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 573	M	LHON	c.152A>G;p.(Tyr51Cys) c.610G>T;p.(Glu204*)	Compd het	SS	Allelic cloning	NA
LHON 582	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 600	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 606	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 612	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 749 (12040)	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	Yes	Twin of patient LHON 749 (14508)
LHON 749 (14508)	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	Yes	Twin of patient LHON 749 (12040)
LHON 760	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	Yes	NA
LHON 785	F	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 895	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 1076	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 1088	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 1089	F	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 1129	M	LHON	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
LHON 1149	M	LHON	c.152A>G;p.(Tyr51Cys) c.610G>T;p.(Glu204*)	Compd het	SS	Allelic cloning	NA
OAK 317	M	OA	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
OAK 559 (19776)	M	OA	c.230_232del;p.(His77del)	Hom	SS	Yes	Brother of patient OAK 559 (31530)
OAK 559 (31530)	M	OA	c.230_232del;p.(His77del)	Hom	WGS	Yes	Brother of patient OAK 559 (19776)
OAK 627	M	DOA	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
OAK 715	M	OA	c.152A>G;p.(Tyr51Cys)	Hom	SS	NA	NA
OAK 767	M	DOA	c.152A>G;p.(Tyr51Cys)	Hom	WGS	NA	NA

M, male; F, female; LHON, Leber's hereditary optic neuropathy; OAK, optic atrophy/Kjer type; DOA, dominant optic atrophy; OA, optic atrophy; Hom, homozygous; Compd het, compound heterozygous; SS, Sanger sequencing; WGS, whole genome sequencing; NA, not available.

by allelic cloning (figure 2A B, online supplemental table 2). And finally, two affected siblings clinically diagnosed with OA carry the deletion c.230_232del in homozygous state (figure 2C, online supplemental table 2). In 15 additional patients, only a single heterozygous variant could be identified: 12 patients with the common missense variant c.152A>G;p.(Tyr51Cys) and 3 cases with additional missense variants: c.292T>C;p.(Tyr98His), c.494A>G;p.(Asp165Gly) and c.278G>C;p.(Arg98Pro). All variants were predicted to be disease-causing. However, no second potential pathogenic variant to fulfil the requirements for an autosomal recessive mode of inheritance was identified in these patients.

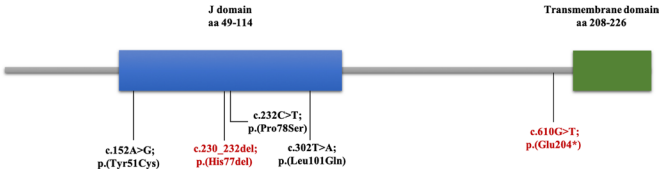

Figure 1 Scheme of the DNAJC30 protein domains and location of the variants. Variant p.(Tyr51Cys), p.(His77del), p.(Pro78Ser) and p.(Leu101Gln) are located in the J domain. The variant p.(Glu204*) is located upstream of the transmembrane domain. Novel variants detected in our study are indicated in red.

Given the high prevalence of the c.152A>G;p.(Tyr51Cys) variant in our cohort, we investigated a potential founder effect for this variant. For that purpose, we genotyped five microsatellite markers covering a region 2.26 Mb on chromosome 7q11.23 in close vicinity to *DNAJC30* (online supplemental table 1). Analysis of the allele spectrum at D7S809, the marker closest to *DNAJC30* locus (~80 kb telomeric), revealed a strong bias for a single marker allele (287 bp allele) on chromosomes bearing the c.152A>G variant (85%) in comparison with non-mutant controls (12.5%) (figure 3). This strongly suggests a founder

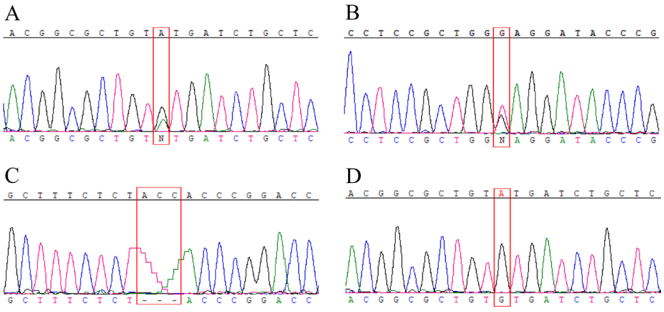

Figure 2 Representative electropherograms of detected pathogenic sequence variants. The upper sequence digits represent the wild-type sequence. The mutant sequence corresponds to the lower sequence digits. The red box highlights the position of the variant. (A, B) Patient LHON 96, LHON 573 and LHON 1149 carry the compound heterozygous variants c.152A>G and c.610G>T. (C) Patient OAK 559 (19776) and his brother OAK 559 (31530) carry the 3 bp deletion c.230_232del in homozygous state. (D) Thirty patients carry the missense variant c.152A>G in homozygous state. LHON, Leber's hereditary optic neuropathy.

effect and a common, eventually a single ancestral mutation event. For other more distant markers, the linkage between the c.152A>G variant in *DNAJC30* and the respective marker is much more 'eroded' (online supplemental table 1), arguing for a high age of the variant which is consistent with the high relative prevalence of the variant in the European population (0.001457 and 0.004765 in non-Finnish Europeans and Finnish Europeans, respectively; gnomAD V.2.1.1).

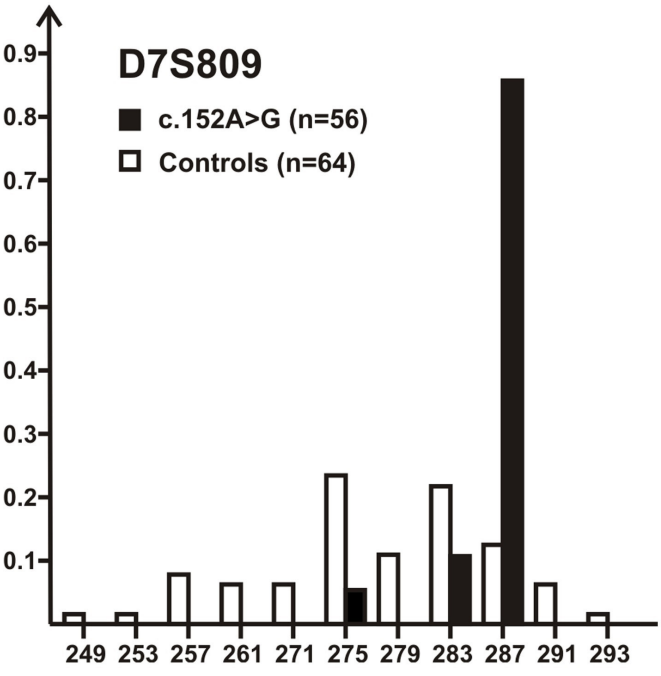

Figure 3 The c.152A>G variant is a founder variant. Allele spectrum (x-axis=allele size, y-axis=relative frequency) of marker D7S809 on chromosomes bearing *DNAJC30*: c.152A>G variant (black bars) in comparison to non-mutant chromosomes from controls (white bars). The 287 bp allele is strongly over-represented on disease-linked chromosomes (85% vs 12.5% on control chromosomes) indicating a founder effect.

Clinical findings

The cumulated demographic and clinical findings in the 35 patients with bi-allelic *DNAJC30* variants are compiled in table 2 and online supplemental table 2. The vast majority of patients (>85%) were of Central European origin (ie, Germany and Austria). Clinical data were available for 28 of the 35 patients. Follow-up data were available for 20 patients. The median age at onset of the disease was 18.5 years (range 9.5–45.1). All patients showed involvement of both eyes. Bilateral onset was observed in 40% (n=8). In cases with bilateral onset, a median of 3.5 weeks (range 1–17) elapsed between the first eye and the second eye. Initial papillary microangiopathy (figure 4) was common and occurred in 94.1% (n=16). In the course of the disease, mostly temporally accentuated papillary atrophy was observed in 91.7% (n=22). Visual field defects (figure 4) were central or cecocentral in 96.6% (n=28). Colour vision disturbances were observed in 68.8% (n=11) and were non-specific. Median VA at nadir was 1.3 logMAR (1.9–0.7) and 0.5 logMAR (1.9–0) at last visit. CRR was seen in 45% (n=9) at a median of 19 months (range 1–58) after onset. Final VA in patients with CRR was 0.15 logMAR (0.8–0) and 1.0 logMAR (range 1.9–0.2) in patients without CRR. Only one patient received idebenone over a period of 6 months. He developed a CRR even before the start of idebenone therapy.

DISCUSSION

To the surprise of many researchers, Stenton *et al* recently reported that variants in the nuclear gene *DNAJC30* can cause an autosomal recessive form of LHON.[20] We here report the results of a first independent replication study confirming the existence of arLHON associated with bi-allelic variants in *DNAJC30* overall. Taking advantage of a very large cohort of patients clinically diagnosed with LHON or OA but still unsolved with respect to the genetic aetiology, we identified 35 patients from 32 families with putatively disease-causing homozygous or compound heterozygous variants in *DNAJC30*. While Stenton *et al* reported a strong geographic accumulation of *DNAJC30*-linked arLHON in Eastern Europe (Russia, Ukraine, Poland, Romania),[20] our study which included in its majority German patients or patients with residency in Germany demonstrates that *DNAJC30*-linked arLHON is not a regional peculiarity and not uncommon in Central Europe.

How does its frequency compare to that of mtDNA variant-linked LHON? The 35 patients from 32 families with *DNAJC30* variants are opposed by 346 patients from 265 families with one of the common mtDNA variants (m.11778G>A; m.3460G>A; m.14484T>C) in our database. Thus, the relative proportion of patients with LHON in our entire database based on known genetic aetiology is: 66.4% (m.11778G>A), 16% (m.3460G>A), 9.8% (m.14484T>C) and 7.7% (bi-allelic *DNAJC30* variants). This ranks *DNAJC30*-linked arLHON behind the three common mtDNA variants but in its prevalence not far below that of the m.14484T>C variant in our population.

The previously reported *DNAJC30* variant c.152A>G;(p.Tyr51Cys) is also by far the most common variant in our patients with arLHON and accounts for 90% of all disease alleles. This is due to a founder effect as we demonstrated by microsatellite marker analysis and corroborating the SNP-based analysis in Eastern European patients by Stenton *et al*. The reduced fraction of common alleles for microsatellite markers more distant to *DNAJC30* (online supplemental table 1) argues for an old age of the c.152A>G;(p.Tyr51Cys) variant consistent with its considerable frequency in the European population. This frequency

Table 2 Aetiology and clinical results of patients with *DNAJC30*-associated LHON

	No. of patients/mean or median (range)	Percentage of documented cases (%)
Average age of onset, years (range)	18.5 (9.5–45.1)	NA
Female	5	14.3
Origin		
Central European	30	85.7
Eastern-Europe	2	5.7
Turkey	2	5.7
Arabia	1	2.8
Follow-up, weeks (range)	246 (3–1291)	NA
Presentation		
Bilateral*	28	100
Unilateral*	0	0
Onset		
Bilateral*	8	40
Unilateral*	12	60
Onset of subsequential eye*, weeks (n, range)	Median 3.5 (10, range 1–17)	NA
Initial papillary hyperaemia		
Absent*	5	29.4
Present*	12	70.6
Initial peripapillary microangiopathy		
Absent*	1	5.9
Present*	16	94.1
Papillary atrophy		
Absent*	2	8.3
Present*	22	91.7
Temporal quadrant	15	68.2
Global quadrant	2	8.3
Fraction not specified	5	20.8
Visual field defects		
Central and cecocentral*	28	96.6
Others*	1	3.4
Colour vision disturbance		
Absent*	2	12.5
Unspecific*	11	68.8
Protan/Deutan*	1	6.3
Tritan*	2	12.5
Median VA		
At nadir (n, range)	1.3 (37, 1.9–0.7)	NA
Of all patients at last visit (n, range)	0.5 (42, 1.9–0)	NA
Of CRR patients at last visit (n, range)	0.15 (18, 0.8–0)	NA
Of non-CRR patients at last visit (n, range)	1.0 (20, 1.9–0.2)	NA
Interval onset—nadir*, weeks (n, range)	7.5 (12, 2–28)	NA
CRR		
Absent*	11	55
Present*	9	45
Interval onset—CRR*, months (n, range)	19 (9, 1–58)	NA

VA is given in logMAR. All individual eyes were included in the calculation of the median VA. CRR was defined as an increase of at least 0.2 logMAR.
*Clinical parameters which were not available from all patients. Therefore, the sum of patients in the different parameter categories is <35.
CRR, clinically relevant recovery; LHON, Leber's hereditary optic neuropathy; NA, not available; VA, visual acuity.

may also explain the occurrence of subjects being single heterozygous carriers of the c.152A>G;(p.Tyr51Cys) variant in our large cohort, although we cannot fully rule out the possibility of missed disease-linked variants in remote parts of the gene or in distant regulatory sequences.

In addition to the three *DNAJC30* variants in arLHON reported so far, we also identified in our study two further novel *DNAJC30* variants, c.230_232del;(p.His77del) and c.610G>T;(p.Glu204*). The latter was found in *trans* with the common c.152A>G;(p.Tyr51Cys) variant in three patients, while the c.230_232del;(p.His77del) variant was found in homozygous state in two affected brothers of Turkish origin.

Variants p.(Tyr51Cys) and p.(His77del) are located in the J domain of the DNAJC30 protein, whereas p.(Glu204*) is located upstream of the transmembrane domain. The J domain is a conserved domain in the protein, belonging to the family of chaperone proteins with important roles in various functional interactions.[22] So far, little is known about the underlying

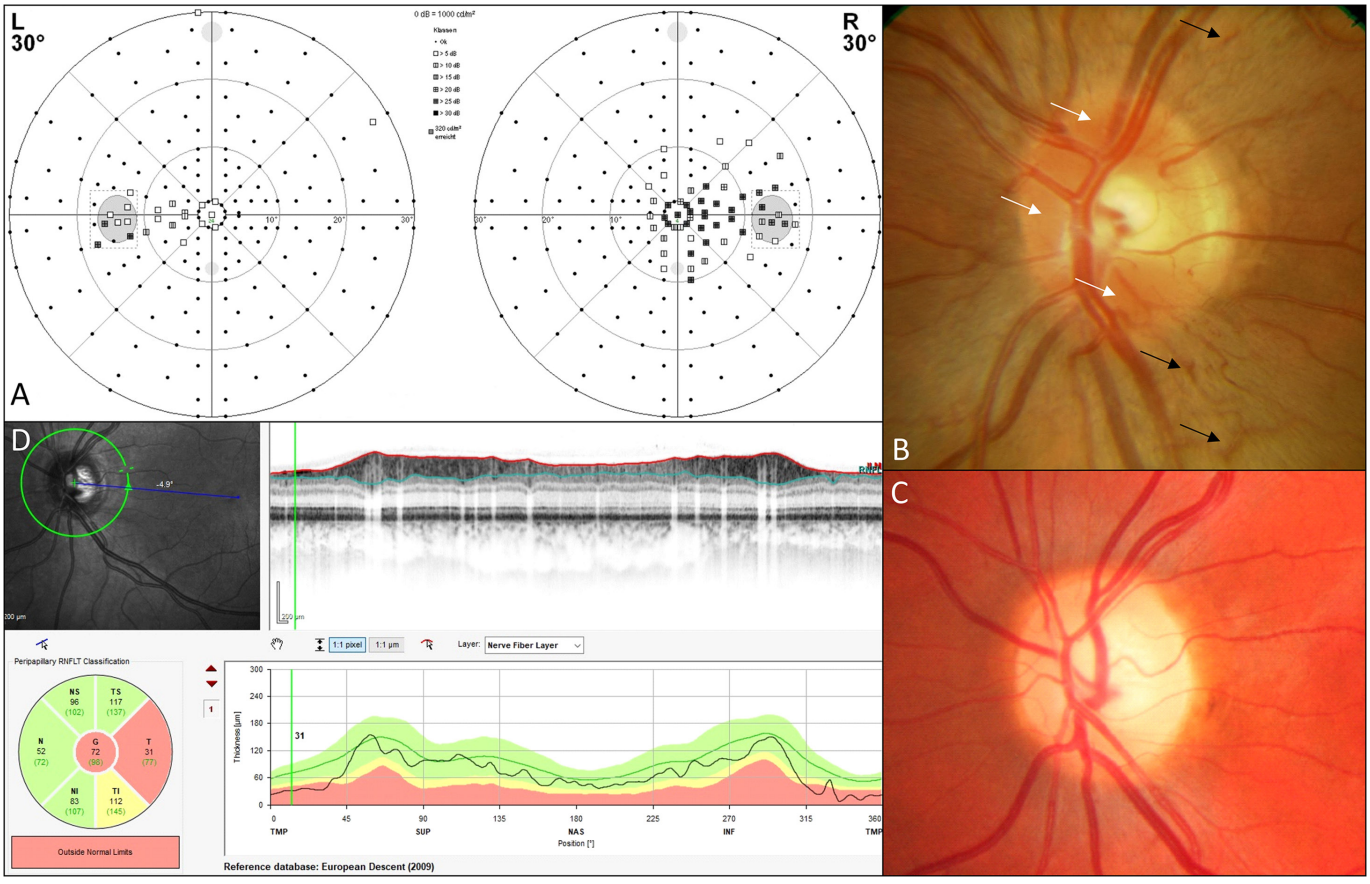

Figure 4 Exemplary clinical findings of *DNAJC30*-associated arLHON-affected patients. (A) Cecocentral visual field defect in patient LHON 1088. (B) Papillary hyperaemia (white arrows) and peripapillary microangiopathy (black arrows) shortly after onset of the disease in patient LHON 1089. (C) Temporally accentuated papillary atrophy occurring in the further course of the disease (in patient OAK 767). (D) Optical coherence tomography scan of the RNFL showing a temporal decrease of thickness in OAK 767. arLHON, autosomal recessive inherited form of LHON; LHON, Leber's hereditary optic neuropathy.

pathological mechanism. Stenton *et al* observed a CI deficiency in skeletal muscle biopsy of patients with arLHON and reduced turnover of CI N-module proteins in patient-derived fibroblast cells.[20] Since p.(His77del) is located in the J domain, it could affect the function of the domain, thus impairing the turnover CI and resulting in the accumulation of low functional CI. Variant p.(Glu204*) is located upstream of the transmembrane domain and is expected to result in a shortened protein. Although not experimentally tested for *DNAJC30*, we would not expect that mutant transcripts undergo nonsense-mediated mRNA decay due to the single exon structure of the gene.

The phenotypic and clinical characteristics of patients with arLHON are similar in the basic features to those of mtLHON. These include the increased incidence in males compared with females at a ratio of 6:1 (male:female ratio in the entire cohort is 1.8:1), the initial peripapillary microangiopathy, the cecocentral visual field defects and also the optic atrophy that develops in the medium term. No extra-ocular manifestation were found in our *DNAJC30*-linked patients with arLHON.[20 21]

Although relevant clinical parameters were not available for all patients in this retrospective study, the large number of patients enabled us to give insights into the clinical characteristics and phenotypic spectrum of *DNAJC30*-linked arLHON. While the age of onset of mtLHON is given as 19–29 years in previous publications,[23] our patients were considerably younger with a mean age of 18.5 years. A bilateral onset in mtLHON was described as 25%[24]; in arLHON it was present in 40% in our cohort. If the eyes were affected consecutively, an interval of 8 weeks for mtLHON was reported in previous studies,[23 25] but it was much shorter, on average only 3.5 weeks in our patients with arLHON. For clinical management and patients' prognosis, the appearance of a CRR is of particular interest: In mtLHON, occurrence of CRR varies according to the disease-causing variant type and publication, ranging from 4% to 25% for m.3460G>A and m.11778G>A and from 37% to 58% for m.14484T>C.[23] In our study, a CRR was observed in 45% of the patients for which follow-up data were available. The young average age of our patients may have had an influence on this, as it has been reported that a younger age at onset may be associated with a better prognosis.[25] The young age of onset, more frequent bilateral onset and more frequent occurrence of CRR compared with mtLHON are basically consistent with and corroborate the findings of Stenton *et al*,[20] who also studied a cohort of patients with arLHON.

In conclusion, our findings confirm that variants in the nuclear encoded gene *DNAJC30* are causative for an autosomal recessively inherited form of LHON clinically similar and overlapping with the presentation of classical mtDNA variant-associated LHON. Moreover, patients with *DNAJC30* variants are not an Eastern European peculiarity but do represent a decent portion of the entire LHON patient population in Central Europe. Furthermore, we expanded the genetic variant spectrum of *DNAJC30*. Therefore, our study strongly

emphasises that *DNAJC30* should be included in prospective genetic diagnostics of patients with any form of hereditary optic neuropathies.

Author affiliations
[1]Molecular Genetics Laboratory, Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tübingen, Tübingen, Germany
[2]Facharztpraxis für Augenheilkunde, Berlin-Mitte, Germany
[3]Diagnostic & Research Institute of Human Genetics, Diagnostic & Research Centre for Molecular BioMedicine, Medical University of Graz, Graz, Austria
[4]Department of Ophthalmology, Universitätsmedizin Rostock, University of Rostock, Rostock, Germany
[5]Zentrum für Seltene Netzhauterkrankungen, AugenZentrum Siegburg, MVZ Augenärztliches Diagnostik- und Therapiecentrum Siegburg GmbH, Siegburg, Germany
[6]RetinaScience, Bonn, Germany
[7]Eye Centre, Medical Centre - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany
[8]Institute of Human Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany
[9]Centre for Rare Diseases, University of Tübingen, Tübingen, Germany
[10]Centre for Ophthalmology, University of Tübingen, Tübingen, Germany

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ORCID iDs
Sinja Kieninger http://orcid.org/0000-0002-8656-3239
Ting Xiao http://orcid.org/0000-0001-9853-9474
Tobias B Haack http://orcid.org/0000-0001-6033-4836

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