Lamination of the outer plexiform layer in optic atrophy caused by dominant *WFS1* mutations

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Running title

OPL lamination in optic atrophy secondary to dominant *WFS1* mutations

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Wolfram syndrome, or DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy (OA), and deafness), is a neurodegenerative disorder with heterogeneous clinical manifestations caused by homozygous or compound heterozygous recessive mutations in the *WFS1* gene (OMIM 606201).[1] More recently, the phenotypic spectrum has expanded to include patients with dominant inheritance and limited clinical features, in particular OA in association with diabetes mellitus and/or sensorineural deafness.[1] *WFS1* encodes for an endoplasmic reticulum transmembrane protein, Wolframin, which is highly expressed in retinal tissues, including retinal ganglion cells, the photoreceptor inner segments, and the inner nuclear layer (INL) of the human eye, and mouse Müller cells.[2,3] Thinning of the peripapillary nerve fibre and macular retinal ganglion cell layers are typical features of *WFS1*-related optic atrophy, but careful genotype-phenotype correlations have not yet been established. Here, we report on a comprehensive macular OCT imaging study of 14 patients with OA secondary to mutations in *WFS1* and the identification of a distinct outer plexiform layer (OPL) abnormality, which associates exclusively with dominant missense *WFS1* mutations (**Table 1**, available at www.aaojournal.org).

Averaged B-scans of 26 eyes from 14 patients with confirmed pathogenic *WFS1* mutations were retrospectively reviewed from our Spectralis™ (Heidelberg Engineering Ltd., Heidelberg, Germany) databases. A prominent feature in the macular SD-OCT images of 15 eyes from 8 patients harbouring dominant *WFS1* mutations (Patients 1-8) was an abnormal reflectivity of the OPL, which was not observed in 11 eyes from 6 patients with recessive *WFS1* mutations (**Figure 1** and **Table 1**, available at www.aaojournal.org). The OPL was comprised of three distinct laminae: an innermost highly reflective lamina; a middle non-reflective lamina; and an outermost highly reflective lamina. To a certain extent, OPL thickness varied depending on the angle between the OCT laser beam and the retinal layers. Beam positions that were more perpendicular to the Henle's fibre tracks resulted in a thicker OPL compared with beam positions that were parallel to these tracks, as illustrated by

the left eye of Patient 5 (**Figure 1**). However, in several eyes, an abnormally thick OPL was visualized even with straight OCT beam positions, as for Patient 6 (**Figure 1**). In the majority of patients, the middle OPL lamina formed a nearly confluent ring centred at the fovea and extending 1-1.5 mm from the foveolar centre up to the optic disc. Perifoveal volumetric retinal scans were obtained for 22 eyes from 12 patients in our *WFS1* cohort. Automated segmentation and retinal layer thickness analysis were carried out using the automated retinal Heidelberg Engineering segmentation tool included in the Spectralis Glaucoma Module software (version 6.0) after manual confirmation of each layer (**Table 2**, available at www.aaojournal.org). Marked thinning of the GCL-IPL complex was observed consistent with OA. All 11 eyes with abnormal OPL lamination had significantly thicker OPL and, more importantly, combined OPL and ONL complex compared with control eyes, or the eyes of patients without OPL lamination. Compared with control eyes, INL thickness was increased in 4 eyes (Patients 2, 5, 8) with INL cystoid changes (**Figure 1**), but also in 5 other eyes with OPL lamination and in 3 eyes without OPL abnormality (**Table 2,** available at www.aaojournal.org). Our study had ethical and institutional approval and its design complied with the Declaration of Helsinki.

We have identified a previously unreported macular OCT feature in patients with Wolfram-related optic atrophy that is associated with dominant, but not recessive, *WFS1* mutations. The lamination of the OPL was characterised by three distinct sublayers: (1) an innermost highly-reflective lamina, similar to what is frequently identified as the OPL in normal eyes; (2) a non-reflective cleft-like middle lamina; and (3) an outermost highly-reflective lamina with characteristics of Henle's fibre layer. Our retrospective study did not enable a systematic evaluation of the effect of angle changes on the OPL reflectivity. Further work is needed to clarify whether the outermost lamina has truly abnormal reflectivity and whether the middle lamina represents an additional spatial structure or merely abnormal OCT reflectivity. However, as the thickness of combined OPL and ONL complex should not be sensitive to OCT angle changes, its significantly increased thickness

in eyes with abnormal OPL lamination suggests that an additional space could indeed account for the laminated OPL structure observed in this patient subgroup with dominant *WFS1* mutations.

Taking into consideration the spatial relationships between the three OPL laminae and previously published histological studies,[4] we propose that the middle cleft-like lamina is located between the synaptic and pedicles sublayers of the OPL. This region has also been shown to possess a particular retinal architecture with bending Müller cells.[5] Wolframin is highly expressed in Müller cells and this cell type has been implicated in the development of OPL and INL edema, and changes within Henle's fibre layer.[4,5] The Müller cell is, therefore, an attractive candidate for the development of the observed OPL lamination.

Interestingly, OPL lamination was only associated with dominant missense *WFS1* mutations. It was not identified in any of the 6 patients harbouring recessive homozygous or compound heterozygous *WFS1* mutations. This distinct OPL abnormality has not previously been reported in the context of optic atrophy and it could represent a specific deleterious effect of dominant *WFS1* mutations. The missense variants identified in our patients are predicted to result in only a minor reduction in wolframin protein level.[1] Depletion of the native wild-type wolframin protein, which is thought to be the major pathophysiological mechanism in recessive Wolfram syndrome, is therefore unlikely to be implicated in the aetiology of the OPL lamination. Instead, dominant missense *WFS1* mutations could result in a dysfunctional aberrant wolframin protein.

WFS1 is a highly polymorphic gene and determining the mode of inheritance or the pathological significance of a specific *WFS1* variant is not always straightforward, especially in singleton cases with no access to other family members. Our study has revealed an interesting association between dominant missense *WFS1* mutations and distinct OPL lamination on SD-OCT, which was not observed in patients with recessive *WFS1* mutations. Future work in other independent patient cohorts with Wolfram syndrome will confirm whether this OCT feature could be used to distinguish between dominant and recessive forms of the disease.

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A figure legend:

Figure 1. Macular SD-OCT images of Patient 1 showing outer plexiform layer (OPL) lamination and of Patient 13 without this defect (upper panel). The lower panels show OCT images of Patients 2-8 with OPL lamination, with the right eye on the left and the left eye on the right. The localization of the layers that were not imaged through the optical axis are indicated within the inserts. The yellow arrows indicate the cleft-like middle structure of the OPL. The red arrows highlight areas of cystoid inner nuclear layer (INL) edema.

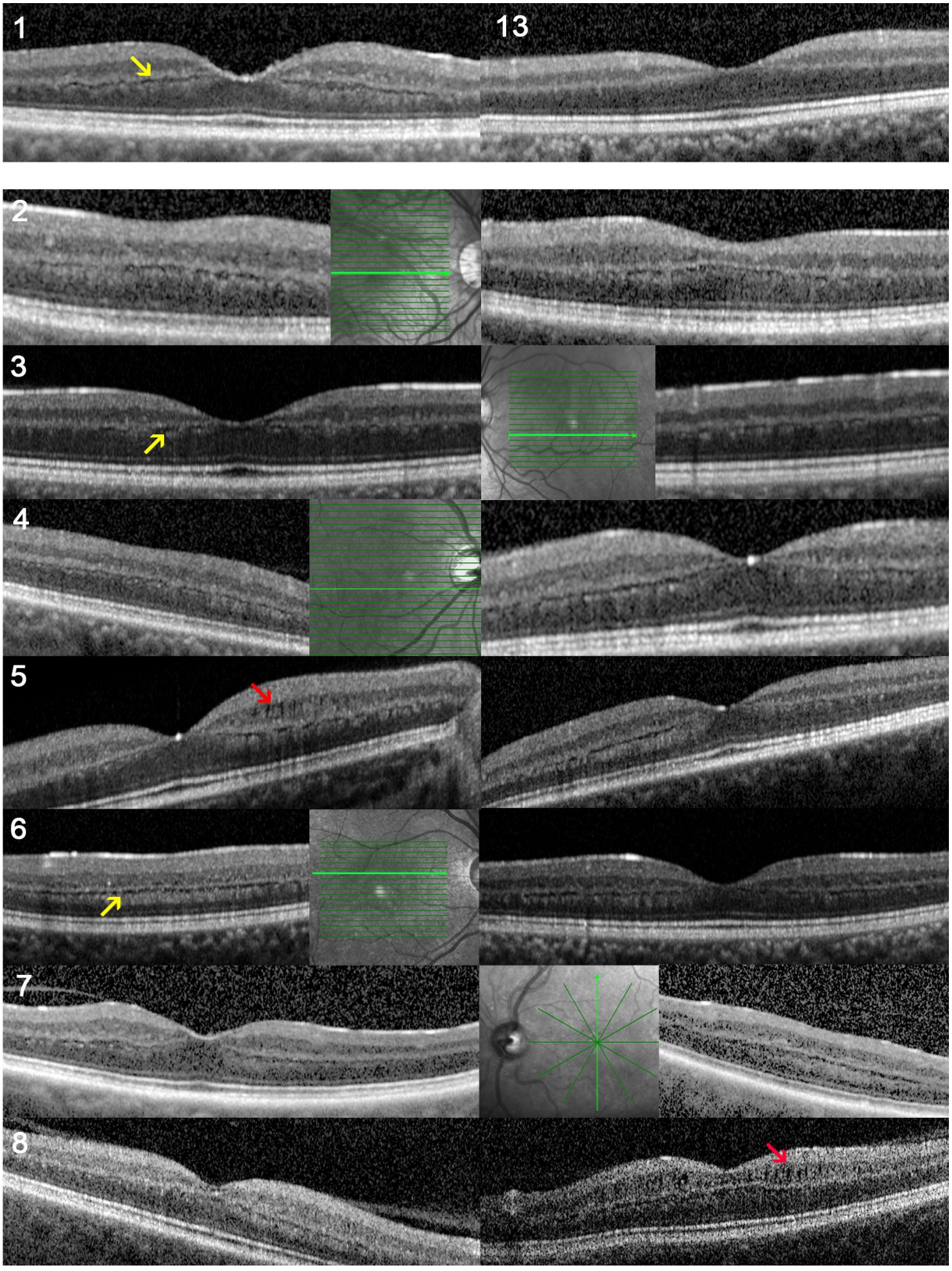
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Table 1. *WFS1* genotype and clinical characteristics of the patients in our study cohort.

Patient	*Sex*	*Inheritance (Family)*	*WFS1 mutation(s)**	*Extraocular findings*	OA age (y)**	*BCVA (logMAR)*		*Macular SD-OCT*	
						RE	*LE*	Age (y)**	OPL cleft***
1	F	AD (1)	c.2390A>T: p.Asp797Val	SNHL, DM2	45	1.10	NI	69	+
2	F	AD (2)	c.2051C>T: p.Ala684Val	SNHL, epilepsy, hypertension	5	0.60	0.60	46	+
3	M	AD (2)	c.2051C>T: p.Ala684Val	SNHL	23	0.48	0.60	23	+
4	F	AD (3)	c.968A>G: p.His323Arg	SNHL	U	0.30	0.43	44	+
5	F	AD (3)	c.968A>G: p.His323Arg	SNHL	4	0.00	0.00	8	+
6	M	*de novo* (4)	c.937C>T: p.His313Tyr c.-170-9_14dupTGCCCC	SNHL, DM1, short stature	7	0.10	0.20	10	+
7	F	AD (5)	c.2051C>T: p.Ala684Val	SNHL	78	1.98	1.98	78	+
8	F	AD (6)	c.2161A>T: p.Asn721Tyr	SNHL	50	1.98	1.98	51	+
9	M	AR (7)	c.505G>A: p.Glu169Lys c.874C>A: p.Pro292Thr	DM1, DI, NB	9	2.28	2.28	45	-
10	M	AR (7)	c.505G>A: p.Glu169Lys c.874C>A: p.Pro292Thr	DM1, DI, NB	U	1.30	NI	46	-
11	F	AR (8)	c.2648_2651delTCTT: p.Phe883SerfsX68 c.1597C>T: p.Pro533Ser	DI UC	23	0.60	0.60	44	-
12	F	AR (8)	c.2648_2651delTCTT: p.Phe883SerfsX68 c.1597C>T: p.Pro533Ser	DI UC	37	0.78	0.78	52	-
13	F	AR (9)	c.2643_2644delC: p.Phe883LeufsX56 (homozygous)	SNHL, DM1, DI PS, NEU	15	1.30	1.30	25	-
14	F	AR (10)	c.409_424dup1: p.Val142GlyfsX10 c.2262_2263delCT: p.Cys755SerfsX3	SNHL, DM1	31	1.00	1.00	35	-

* *WFS1* genetic testing was conducted by the West Midlands Regional Genetic Laboratory, Birmingham, UK.

** Age at the time of diagnosis of optic atrophy (OA) and when macula SD-OCT imaging was performed. Images were retrieved from the Spectralis™ (Heidelberg Engineering Ltd., Heidelberg, Germany) databases of Moorfields Eye Hospital, London, UK and the Newcastle Eye Centre, Royal Victoria Infirmary, Newcastle upon Tyne, UK. Our study had local ethical and institutional approval, and its design complied with the Declaration of Helsinki.

***The presence (+) or absence (-) of an outer plexiform layer (OPL) lamination is indicated.

Abbreviations: DI, Diabetes insipidus; DM1, Diabetes mellitus type 1; DM2, Diabetes mellitus type 2; LE, left eye; NB, Neurogenic bladder; NEU, intermittent myoclonus, occasional balance problem, postural hypotension; NI, not included into the study; NP, not performed; OA; optic atrophy; OPL; outer plexiform layer; PS, bipolar affective disorder RE, right eye; RNFL, peripapillary retinal nerve fiber layer thickness; SNHL, sensorineural hearing loss; U, Unknown; UC, urinary and faecal incontinence.