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Title: Description of a patient cohort with Hereditary Sensory Neuropathy Type 1 without retinal disease Macular Telangiectasia type 2 – implications for retinal screening in HSN1

Running title: HSN1 cohort without MacTel

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Data availability statement

The data that supports the findings of this study are available in the supplemental material of this article.

Conflict of Interest statement

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Abstract and Keywords

Abstract:

Background and Aims: Pathogenic variants in the genes encoding serine palmitoyl transferase (*SPTLC1* or *SPTLC2*) are the most common causes of the rare peripheral nerve disorder Hereditary Sensory Neuropathy Type 1 (HSN1). Macular telangiectasia type 2 (MacTel), a retinal disorder associated with disordered serine-glycine metabolism and has been described in some patients with HSN1. This study aims to further investigate this association in a cohort of people with HSN1.

Methods: Fourteen patients with a clinically and genetically confirmed diagnosis of HSN1 from the National Hospital for Neurology and Neurosurgery (NHNN, University College London Hospitals NHS Foundation Trust, London, United Kingdom) were recruited to the MacTel Registry, between July 2018 and April 2019. Two additional patients were identified from the dataset of the international clinical registry study (www.lmri.net). Ocular examination included fundus autofluorescence, blue light and infrared reflectance, macular pigment optical density mapping, and optical coherence tomography.

Results: Twelve patients had a pathogenic variant in the *SPTLC1* gene, with p.Cys133Trp in eleven cases (92%) and p.Cys133Tyr in one case (8%). Four patients had a variant in the *SPTLC2* gene. None of the patients showed clinical evidence of MacTel.

Interpretation: The link between HSN1 and MacTel seems more complex than can solely be explained by the genetic variants. An extension of the spectrum of *SPTLC1/2*-related disease with phenotypic pleiotropy is proposed. HSN1 patients should be screened for visual symptoms and referred for specialist retinal screening,

but the association of the two diseases is likely to be variable and remains unexplained.

Keywords:

Hereditary Sensory and Autonomic Neuropathies

HSAN 1

Macular telangiectasia type 2

SPTLC1 protein

SPTLC2 protein

Introduction

Serine is an amino acid involved in several metabolic pathways, including the common biosynthesis pathway of sphingolipids. The enzyme complex serine palmitoyltransferase (SPT) includes two large core subunits, encoded by the genes *SPTLC1* and *SPTLC2*[1], and catalyses the condensation of serine with palmitoyl-CoA to create sphinganine, the first and rate limiting step in sphingolipids synthesis.[2,3] Variants in the genes *SPTLC1* and *SPTLC2* cause reduced specificity of the enzyme complex serine palmitoyltransferase (SPT) and an increased usage of the amino acid alanine as a substrate instead of serine.[3,4] Deoxysphingolipids are created, which are known to be toxic to multiple cells, including neurons[5–10] and photoreceptors.[11]

Pathogenic variants in *SPTLC1* or *SPTLC2* are the most common causes of the rare disorder Hereditary Sensory Neuropathy Type 1[5,12,13], commonly abbreviated as HSN1 in the UK, and as HSAN1 in the USA. HSN1 has an autosomal dominant inheritance, and is characterized by a sensory neuropathy with variable autonomic and motor involvement.[14,15] Symptoms include loss of pain and temperature sensation, lancinating pain and distal weakness. The formation of ulcers at the feet and hands can occur. Typically, first clinical symptoms appear between the second and fifth decade[16,17] and despite the name of the disease, there is prominent motor involvement and weakness early on in the course of the disease.[18]

Patients with HSN1 were recently found to have coincident Macular telangiectasia type 2 (MacTel).[11] MacTel is a bilateral neurodegenerative disease of the central retina with an almost pathognomonic limitation to the so-called “MacTel area”, an oval area of approximately 3 mm across the temporal-nasal axis and 2 mm across the superior-inferior axis centered on the fovea.[19,20] It has an estimated

prevalence between 0.005% and 0.1% based on traditional fundus colour photography, though the true prevalence with modern multimodal imaging is likely to be higher, with no sex predilection.[21–23] Although age of onset may vary, symptoms typically start in the fifth or sixth decade of life with reading difficulties and central vision distortion[20,24,25]. Progression of vision loss is usually slow[19,24,25], but it has been shown to substantially interfere with quality of life.[26] Approximately 20% of patients lose driving quality vision.[20]

The diagnosis of MacTel is affirmed by characteristic findings on clinical examination and multimodal retinal imaging - in particular, optical coherence tomography (OCT) (a non-contact, cross-sectional in vivo imaging of the retina), fundus autofluorescence, blue light reflectance and macular pigment optical density mapping (Fig. 1 – bottom). A comprehensive review described the findings for each modality in detail.[19] A phase III trial is currently investigating the role of an ocular implant secreting ciliary neurotrophic factor in delaying disease progression (ClinicalTrials.gov Identifier: NCT03316300). There are no other treatment options currently available.

The MacTel Project (www.lmri.net) has contributed to the understanding of the mechanisms of the condition. Several studies suggested a strong genetic component in MacTel.[19,27–30] In particular, genome-wide association studies (GWAS) and metabolomic studies have suggested a role of the glycine-serine metabolism in the pathophysiology of MacTel.[31] GWAS have identified MacTel disease risk-associated single nucleotide polymorphisms (SNPs) within three genes that participate in the glycine-serine metabolism[31–33]: *PSPH* (encoding phosphoserine phosphatase), *PHGDH* (encoding phosphoglycerate dehydrogenase), and *CPS1* (encoding carbamoyl-phosphate synthase). Interestingly, MacTel patients seem to

have lower serum levels of serine and glycine when compared to controls and higher circulating deoxysphingolipid levels, which correlate negatively with each other[11,31,34].

Although patients with MacTel show high levels of deoxysphingolipids with a metabolic profile similar to HSN1, the majority of them do not carry an *SPTLC1* or *SPTLC2* variant. However, nine patients with HSN1 have recently been reported to have MacTel; seven patients had a variant affecting *SPTLC1* whereas two patients had a variant affecting *SPTLC2*.[11] Two patients of the same cohort with HSN1 (*SPTCL1* variant) were not found to have coincident MacTel.[11]

The aim of this work is to examine a larger cohort of patients with HSN1 for retinal signs of MacTel to further elucidate the link between these two disorders.

Materials and Methods

Patients with a clinical and genetically confirmed diagnosis of Hereditary Sensory Neuropathy Type 1 (HSN1) who are under the clinical care of the National Hospital for Neurology and Neurosurgery (NHNN, University College London Hospitals NHS Foundation Trust, London, United Kingdom) were recruited for screening to the MacTel Registry, between July 2018 and April 2019. Additional patients were identified from the dataset of the international clinical registry study (www.lmri.net).

Written informed consent for participation in the study was obtained for all patients. The study was approved by local Research Ethics Committees and performed in accordance with the Declaration of Helsinki.

Clinical follow-up data (including history and examination with or without nerve conduction studies) was collected prospectively during previous annual neurology clinic reviews between 2006 – 2018. This clinical information, along with genetic analysis, was obtained by reviewing the clinical records. Complete past general and ocular history as well as previous and current medication data was also collected at the registry study visit.

Ocular examinations included BCVA recording (using standard ETDRS protocols), biomicroscopy and dilated retinal examination. Ophthalmic imaging was performed with a confocal imaging system (HRA Spectralis, Heidelberg Engineering, Germany) and included autofluorescence (AF), blue light and infraredreflectance (BLR, IR), and OCT. Macular pigment optical density (MPOD) mapping by dual-wavelength auto-fluorescence (DWAF) was performed with prototype instruments provided by Heidelberg Engineering (Heidelberg, Germany), as described previously.[35]

The presence of MacTel signs was evaluated in the Moorfields Eye Hospital Reading Centre. The graders were masked to the diagnosis of HSN1.

Results

Sixteen patients with a clinical and genetically confirmed diagnosis of HSN1 were included in the study. Fourteen of these patients were under the care of University College Hospital (patients 1 to 14). Two patients were identified from data held at LMRI as part of the international registry study and had been assessed at Scripps Clinic, La Jolla, California (patient 15) and at the Centre for Eye Research, Melbourne, Australia (patient 16). Demographic, genetic and clinical features of all patients are summarized in Table 1. Table 2 shows the respective neurological and neurophysiological characteristics. The majority of these patients (11/16) carried the known p.Cys133Trp pathogenic variant in *SPTLC1*. One patient (patient 15) carried the pathogenic variant in *SPTLC1,* p.Cys133Tyr. The remaining four patients carried variants in *SPTLC2* (patient 12: p. Ser384Phe; patients 5 and 11: p.Ala182Pro; patient 6: p.Asn177His).

None of the eyes showed clinical evidence consistent with a diagnosis of MacTel. Representative multimodal imaging of age-matched subjects (all Caucasian) of a normal control (top), a HSN1 patient from the current cohort (patient number 3) (middle) and a patient with MacTel (bottom) are presented in Fig. 1. The macular foveal reflex is preserved in the non-affected control (Fig. 1 - top row). Both AF and BLR are centrally reduced, due to a peak in central macular pigment as seen on DWAF MPOD. IR shows a uniform reflectance pattern. The OCT shows regular foveal contour with preserved retinal lamination. Imaging of the HSN1 patient (Fig. 1 - middle row) reveals macular findings similar to the normal patient. The HSN1 patient shows incidental findings of small drusen temporal to the macula and away from the centre, with corresponding hypoautofluorescence and decreased BLR. Imaging was negative for MacTel typical findings, as described next. An example of

a non-diabetic patient diagnosed with MacTel (Fig. 1- low row) shows a blunt foveal reflex, macular greying, macular crystalline deposits and telangiectatic vessels on the colour fundus photo. Central AF and macular BLR are increased, while DWAF MPOD shows a lack of macular pigment with a surrounding ring-like enhancement. IR shows increased macular reflectance. IR shows increased macular reflectance. The OCT shows hyporeflective (neurodegenerative) spaces in the inner retina. Clinical and imaging features of the ocular examinations are summarised in Table 3. HSN1 patients imaging results per imaging modality are provided in Supplemental Figures 1-6.

Discussion

MacTel and HSN1 patients share a common elevation of atypical deoxysphingolipids which have been associated with both retinal and peripheral neural toxicity[5,6,9,11] The reason why elevated deoxyshingolipid levels seem to primarily affect primarily the retina or peripheral neurons is unknown.

Although a recent study strongly suggested an association between HSN1 and MacTel, we could not show the same association in our current cohort.[11]

Most of the patients in the previous study carried the p.Cys133Tyr variant in *SPTLC1*. Seven of those nine patients were found to have MacTel, whereas one patient was possibly affected and another unaffected.[11] The same study reported two unrelated HSN1 patients with the p.Cys133Trp variant in *SPTLC1* who did not show signs of MacTel.[11] This variant was the most common in our current cohort. Two related patients with the variant p. Ser384Phe in SPTLC2, aged 44 and 65, were previously found to be affected with MacTel[11], but the patient with the same mutation in the current cohort was not. To the best of our knowledge, there are no previous reports of MacTel status in HSN1 patients with the remaining two variants present in our cohort (*SPTLC2* variants p.Ala182Pro and p.Asn177His). All the pathogenic variants in the current cohort and previous cohort are known to be associated with increased deoxysphingolipid levels[14], with the exception of *SPTLC2* p.Asn177His variant which has not been reported before. Figure 2 summarises the findings of the original cohort[11] and current cohort (labelled as US/Australian cohort and UK cohort, respectively). It is noteworthy that two patients from the current cohort (patients number one and two) and one patient from the original cohort are under 40 and therefore younger than the typical age of onset for MacTel (fifth to sixth decade of

life)[19,24,36] Taking this into consideration,these have been labelled as “possibly affected” in Fig. 2, since they might still develop this condition in the future. Nevertheless, the age of onset in MacTel can vary, as exemplified in the previously published cohort, in which one of the patients with HSN1 and MacTel was 24 years old, while three additional affected patients were in the fourth decade of life.[11] Taking both cohorts into consideration (Figure 2), in a total of 29 HSN1 patients who were screened for MacTel, 9 patients were affected (31%), 5 were possibly affected (17.2%) and 15 were unaffected (51.7%). These findings may suggest an extension of the spectrum of *SPTLC1/2*-related disease but with phenotypic pleiotropy. Pleiotropy refers to the different phenotypic effects of a specific genetic variant and is governed by the underlying genetic background. Hypothetically, the variable co-occurrence of HSN and MacTel in patients carrying *SPTLC1/2* variants could either be explained by variant-specific effects on SPT activity, or other functions unrelated to SPT activity. Furthermore, the genetic background of modifiers may in turn be responsible for this phenotypic pleiotropy and thus determine which patients with HSN and *SPTLC1/2* variants may also go on to develop MacTel. Additional exploration of these possible modifiers in the current study population and recruitment of further HSN1 patients with diverse genotypes in larger international collaborative studies will be essential in addressing phenotypic pleiotropy in such a rare condition as HSN1.Plasma deoxyshingolipid-levels correlate significantly with disease severity in HSN1-patients.[37] A positive relationship between ellipsoid zone loss and deoxysphinganine levels has been shown in patients with MacTel type 2.[11] It is not known whether the severity of the macular changes and neuropathy are correlated in patients with HSN1.

Our data help to expand the knowledge on the complex relationship between two rare disorders, HSN1 and MacTel type 2. There appears to be a divergence between the retinal and peripheral neuropathy phenotypes, and the link between both disorders seems more complex than can solely be explained by the genetic variants mentioned. Exploring the current cohort's genetic data and metabolic data would expand on our results interpretation and provide further elucidation on the link between the two conditions. We are planning on performing Genome Wide Association Studies on these patients. In this context, it will be valuable to explore the presence of MacTel disease risk-associated SNPs within PSPH, PHGDH and CPS1.[31-33] Furthermore, the cohort has already undergone metabolomics analysis, including serine, glycine and deoxysphingolipid levels assessment (manuscript in preparation).

The implications of our study and previous studies for people with HSN1 are that clinicians should ask for a history of visual symptoms and if necessary, refer to a specialist centerfor screening for MacTel, given the nearly 50% chance of having MacTel in the HSN1 population. However, the chance of having the disorder appears to depend on the underlying genetic variant and factors that are still to be determined and in some genotypes is likely to be very low. Similarly, screening people with MacTel for peripheral nerve symptoms would also be prudent while the exact relationship between the two diseases remains enigmatic.

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Tables

Table 1 – Summary of demographic, genetic and clinical features of patients with Hereditary Sensory and Autonomic Neuropathy Type 1

Family	Patient	Sex	Age	Ethnicity	Variant affecting SPT	Genetic testing method	HSN1 age of onset	Diabetes status	Serine supplementation	Macular Telangiectasia Type 2
1	1†	M	38	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	10	No	No	No
1	2†	F	35	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	17	No	No	No
2	3º	F	53	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	16	No	No	No
2	4º	F	46	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	19	No	No	No
	5	F	71	White/Caucasian	*SPTLC2* p.Ala182Pro	Exome sequencing and confirmed by Sanger	10	No	No	No
	6	M	56	White/Caucasian	*SPTLC2* p.Asn177His	CMT NGS panel sequencing	20s	No	No	No
	7	M	52	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	20s	No	No	No
	8	M	43	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	13	No	Yes - for 12 months prior to observation	No
	9	M	69	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	18	No	No	No
	10	M	52	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	25	No	No	No
	11	F	62	White/Caucasian	*SPTLC2* p.Ala182Pro	CMT NGS panel sequencing	40	No	No	No
	12	M	62	White/Caucasian	*SPTLC2* p.Ser384Phe	Exome sequencing and confirmed by Sanger	late 30s	No	No	No
	13	F	46	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	24	No	No	No
	14	M	50	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	late teens	No	No	No
	15	M	69	White/Caucasian	*SPTLC1* p.Cys133Tyr	Bi-directional Sanger sequencing of gene	47	No	Briefly; stopped prior to assessment	No
	16	M	47	White/Caucasian	*SPTLC1* p.Cys133Trp	Bi-directional Sanger sequencing of gene	18	No	Twice/week for 12 months	No

Abbreviations (Alphabetical order): F: female; HSN1: Hereditary Sensory and Autonomic Neuropathy Type 1; M: male; NGS: Next-generation sequencing

† Patient 1 and 2 are brother and sister

º Patient 3 and 4 are sisters.

Table 2 – Non-Ocular Clinical Data

Patient	Presenting symptoms	Progression	Sensory symptoms	Age of loss of ambulation	Neuropathic agents	Hx of ulceration/ osteomyelitis/ amputations	Neurophysiology assessment: age and findings	other PMHx
1	Numbness in feet	Progr. LL numbness and progressive LL weakness since age 15; Progr. UL weakness and numbness since age 18	Numbness; occasional shooting pains in legs	Still ambulant (ankle foot orthoses since 18)	Nil	Foot ulcers	37 yo. Absent/very small SNAPs in UL and very small CMAPs in UL	Nil
2	Stabbing pains in feet	Slowly progr. numbness; metatarsal stess fractures in 20s	Numbness; stabbing pains; pins and needles	Still ambulant	Gabapentin previously, pregabalin	No	31 yo. Normal SNAPs and CMAPs in UL; small CMAPs in LL	Nil
3	Painless toe ulcer	Progr. numbness in LL from onset; progr. numbness in hands since 20s; LL weakness in late 20s; hand weakness in 30s	Numbness; electric-shock like pains; burning pain	Still ambulant	Amitriptyline previously	Ulcers and OM in left foot	46 yo. SNAPs and CMAPs absent	Nil
4	Black toe: from damage from whenever wearing shoes	Progr. numbness in LL; progressive numbness in hands in 30s; weakness in LL in late 20s and hands in 30s.	Numbness; shooting pains	Still ambulant	Pregabalin	Ulcers with x3 foot surgeries	39 yo. SNAPs and CMAPs absent	Depression
5	Numbness in feet	Progr. LL numbness; progressive LL weakness (distal, around ankles) noticed from age 53; painful tingling and progressive weakness in hands from 55	Numbness; painful tingling; electric shock-like pains	Still ambulant (rigid ankle-foot orthoses)	Gabapentin, pregabalin	No	65 yo. Absent SNAPs in all 4 limbs; absent CMAPs in LL and reduced in UL	Nil
6	Numbness in feet after minor injury	Progr. LL numbness from 40 onwards	Numbness; pain	Still ambulant	Amitriptyline, pregabalin, gabapentin (previously)	Ulcers; osteomyelitis; toe amputation on right foot	52 yo. Absent SNAPs in all 4 limbs; reduced CMAPs in LL and normal in UL	Cervical myelopathy, HTN, hypercholesterolaemia, psoriasis
7	Numbness	Progr. LL and UL numbness and weakness	Numbness	Still ambulant	Nil	Recurrent ulcers, chronic osteomyelitis requiring debridemen reconstruction of left foot/heel	47 yo. Absent SNAPs in all 4 limbs; absent CMAPs in LL and very small in UL	Atrial Fibrillation
8	Stabbing pain in feet	Progr. numbness and weakness in LL and UL since 20s	Numbness; shooting pain	Still ambulant (ankle foot orthoses)	Nil	Foot ulcers	39 yo. Absent SNAPs in UL and LL; absent CMAPs in LL	Nil

9	Numbness in feet and minor painless injuries	Progr. numbness since 20s; UL numbness since 30s; LL weakness since late 40s; autonomic neuropathy	Numbness; stabbing pain	62	Nil	Recurrent ulcers, ankle fusion	63 yo. Absent SNAPs and CMAPs in UL and LL	Autonomic neuropathy; HTN
10	Numbness in feet	Progr. LL numbness; bilateral foot drop since mid-20s and progressive weakness; UL burning and weakness since 30s	Numbness; burning pain	Still ambulant (Push acqui splints)	Gabapentin	Ulcer	47 yo. Absent SNAPs all 4 limbs; CMAPs very small in LL and in the UL CMAPs showed marginal slowing (32-33)	Renal calculi
11	Sharp foot pain	Progr. LL numbness; UL tingling and shooting pains since 50; LL weakness since age 50	Numbness; burning pain; shooting pain	Still ambulant	Amitriptyline and gabapentin previously	Nil	61 yo. Absent SNAPs; absent CMAPs in LL and normal CMAPs in UL	Nil
12	Numbness in feet	Progr. LL numbness; (no motor weakness into 50s)	Numbness; electric shocks	Still ambulant	Nil	Nil	54 yo. Normal UL SNAPs and CMAPs; in LL normal CMAPs but SNAPs were reduced/absent	Nil
13	Non-healing foot ulcer	Progr. numbness in LL since 28; progressive LL weakness since 30s; progr. hand weakness since age 34-35	Numbness; occasional shooting pain	Still ambulant	Nil	Needed skin graft operation for ulcer	Not available	Borderline HTN
14	Painless toe burn on radiator	Progr. numbness and weakness since 20s	Numbness; shooting pain; pins and needles; tingling	Still ambulant	Nil	Foot ulcer	SNAPs absent; small/absent LL CMAPs and small ulnar CMAPs	Nil
15	Decreased temperature sensation in feet	Foot drop shortly after diagnosis; at age 49 developed pain in feet, hands and wrists that was progressing proximally	Decreased temperature sensitivity	Still ambulant - uses leg braces	Gabapentin, duloxetine, oxycodone	History of finger "infections"; digit amputation down to second joint	Not available	AF, CHF, hypothyroidism, endocarditis, DVTs, mitral insufficiency
16	Numbness in feet	Progr. numbness and weakness in LL and UL with muscle wasting since age 30s	Numbness	Still ambulant (ankle foot orhtrosis leg braces)	Nil	Foot/leg ulcers	Not avaliable	Nil

Abbreviations (Alphabetical order): AF: atrial fibrillation; BMI: body mass index; CMAPs: compound muscle action potentials; CHF: congestive heart failure; DM: diabetes mellitus; DVTs: deep venous thrombosis; HTN: arterial hypertension; Hx: History; LL: lower limbs; OM: osteomyelitis; PMHx: past medical history; Progr: progressive; SNAPs: sensory nerve action potentials; UL: upper limbs; yo: years old.

Table 3 – Ocular clinical and imaging data

Patient	BCVA RE (ETDRS letters)	BCVA LE (ETDRS letters)	Fundus examination	AF	DWAF MPOD	BLR	IR	OCT	Other relevant ophthalmologic findings
1	92	94	N	N	N	N	N	N	Mild anterior subcapsular cataract
2	90	90	BE Non-specific RPE changes in the periphery; perifoveal mildly RPE granular aspect	BE Granular focal hyperAF	BE Regular MPOD peak with minor perifoveal irregularity, not in a MacTel pattern	N	N	N	Nil
3	82	79	BE drusen away from the centre	HypoAF corresponding to drusen	N	Decreased BLR corresponding to drusen	N	N	Nil
4	84	80	RE juxtafoveal hypopigmentary changes. BE granular non-specific pigmentary changes	HypoAF corresponding to pigmentary changes area	N	N	N	N	Nil
5	91	90	BE temporal non-specific hypopigmentary changes	N	N	Increased BLR corresponding to pigmentary changes	N	N	BE Pseudophakia
6	83	84	N	N	not performed	N	N	N	Nil
7	90	84	LE foveal RPE changes	N	N	N	N	N	Mild nuclear cataract
8	90	94	N	N	N	N	N	N	Nil
9	91	84	Myopic fundus and peripapillary atrophy	N	N	N	N	N	Nil
10	89	92	N	N	N	N	N	N	Nil
11	67	69	N	N	N	N	N	N	BE anterior and posterior subcapsular cataract
12	84	88	N	N	N	N	N	N	Nil
13	87	82	N	Reduced central masking of central AF, no temporal versus nasal asymmetry	Generally reduced MPOD with central peak. BE discrete reduction in the superior parafovea	N	N	N	Nil
14	88	90	N	N	N	N	N	N	Nil
15	79	81	BE small drusen	N	n/a	N	N	BE small drusen	BE Pseudophakia, LASIK
16	86	84	LE extra-foveal PED	N	n/a	N	N	RE normal, LE extra-foveal PED	Nil

Abbreviations (Alphabetical order): AF: Autofluorescence; BCVA: Best Corrected Visual Acuity; BE: both eyes; BLR: Blue Light Reflectance; DWAF MPOD: dual-wavelength fundus autofluorescence macular pigment optical density measurement; ETDRS: Early Treatment Diabetic Retinopathy Study; IR: Infrared imaging; LE: left eye; N: normal; OCT: Optical Coherence Tomography; PED: Pigment Epithelium Detachment RE: right eye.

Figure legends

Figure 1 – Imaging study results, comparing the right eyes of age-matched subjects (all Caucasian) - normal (top), HSN1 (middle) and a patient with MacTel (bottom). Image modalities from left to right: Color fundus photo (CFP), Autofluorescence (AF), macular pigment optical density measurement by dual-wavelength fundus autofluorescence (DWAF MPOD); Blue Light Reflectance (BLR), Infrared imaging (IR) and Optical Coherence Tomography (OCT).

Figure 2 – Comparison of genetic data and MacTel status in patients with a confirmed HSN1-diagnosis in the original US/Australian cohort (Gantner et al.[11]) versus the current cohort, in which 14 of the 16 patients are from the UK. In both cohorts, patients below 40 with negative findings for MacTel were labelled as “possibly affected", since they might still develop MacTel in the future. Two patients in the US/Australian cohort carrying the p.Cys133Tyr pathogenic variant showed possible signs of MacTel on advanced retinal imaging.

Appendices

Supplemental Figures – Legends

Supplemental Figures 1-6 – HSN1 patients imaging results per imaging modality. Please refer to Table 3 for interpretation results.

Supplemental Figure 1 - Optical Coherence Tomography (OCT) imaging of all 16 HSN1 patients. Abbreviations - RE: right eye; LE: left eye.

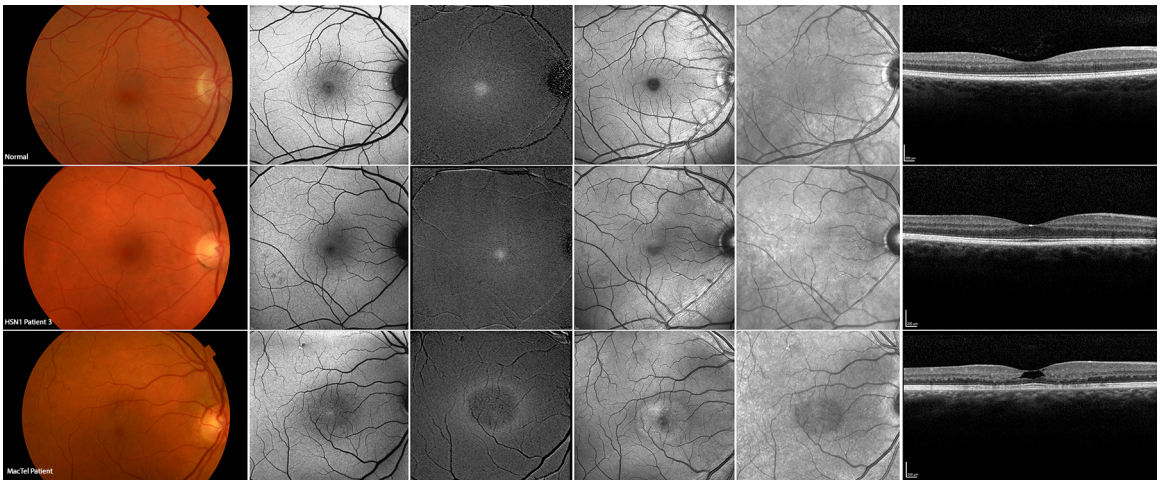
Supplemental Figure 2 – Autofluorescence imaging of the 16 HSN1 patients. Abbreviations - RE: right eye; LE: left eye.

Supplemental Figure 3 – Maps of the spatial distribution of macular pigment optical density obtained by dual-wavelength fundus autofluorescence in both eyes of 13 HSN1 patients (not obtained for patients 6, 15 and 16). Abbreviations - RE: right eye; LE: left eye.

Supplemental Figure 4 – Mean Macular Pigment Optical Density profile (together with standard deviations and extreme values) for all eccentricities in both eyes of 13 HSN1 patients (not obtained for patients 6, 15 and 16). The diagram displays the radial distribution of the optical density around the centre of evaluation. The radius is displayed in degrees. The green bars show the standard deviation at the given radius and the blue bars show maximum and minimum optical densities at this radius. Abbreviations - RE: right eye; LE: left eye.

Supplemental Figure 5 – Blue light reflectance imaging of the 16 HSN1 patients. Abbreviations - RE: right eye; LE: left eye.

Supplemental Figure 6 – Infrared imaging of the 16 HSN1 patients. Abbreviations - RE: right eye; LE: left eye.



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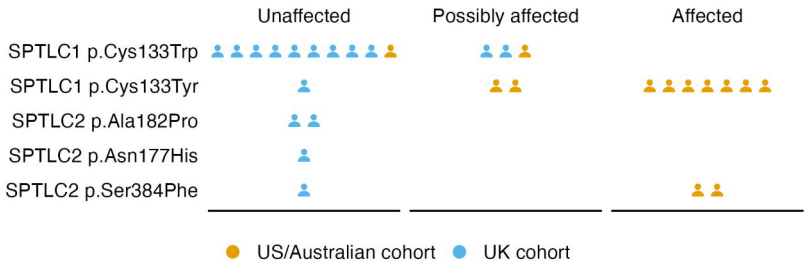

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