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Peripapillary Hyper-Reflective Ovoid Mass-like Structures in Stickler syndrome.

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Running head: Stickler Syndrome PHOMS optic nerve

Abstract:

Purpose: To report a previously undescribed finding of peripapillary hyper reflective ovoid mass-like structures (PHOMS) in Stickler Syndrome

Design: Non-comparative case series

Subjects, Participants, and/or Controls: 22 eyes with anomalous optic disc from 11 Stickler Syndrome patients were identified and imaged.

Methods, Intervention, or Testing: PHOMS were graded using enhanced depth imaging optical coherence tomography (EDI-OCT) according to the consensus recommendations of The Optic Disc Drusen Studies Consortium. All EDI-OCT scans were obtained using the Heidelberg Spectralis (Heidelberg Engineering, Heidelberg, Germany) with a dense horizontal raster (15 × 10°, 97 sections) centred on the optic nerve head and graded by two independent assessors. In case of disagreement, the image was graded by a third assessor. The presence of any co-existing optic disc drusen was also assessed using EDI-OCT and autofluorescence.

Main Outcome Measures: The presence of PHOMS, clinical characteristics and genetic mutations.

Results

A pilot sample of 22 eyes with phenotypic optic disc abnormalities from 11 Stickler Syndrome patients were identified and imaged. Eight patients were female and 3 were

male. The mean age was 31 years (13-58 years). PHOMS were present in 91% (n=20 eyes) of imaged eyes. 70% (n=14 eyes) were type 1 Stickler Syndrome and 30% (n=6 eyes) were type 2 Stickler Syndrome. Five percent (n=1 eye) developed retinal detachment and 75% (n=15 eyes) had undergone 360º prophylactic retinopexy. 41% (n=9) of eyes with PHOMS were present in patients with co-existing hearing loss and 13.6% (n=3) had orofacial manifestation of Stickler Syndrome in the form of a cleft palate. Seventy-five percent (n=15 eyes) of patients with PHOMS reported joint laxity or symptoms of arthritis. No co-existing optic disc drusen were identified and raised intracranial pressure was also excluded after neurological investigation.

Conclusion

These data suggest that PHOMS are a novel finding in Stickler Syndrome patients and should be considered when evaluating the optic nerves of these patients.

Introduction

Peripapillary hyper reflective ovoid mass-like structures (PHOMS) are a non-specific finding on OCT and have been reported in a variety of optic nerve head disorders and anomalies, including optic disc drusen[1], myopic tilted discs[2–4] and disc oedema[5]. The chronic axoplasmic stasis and distended pre-laminar axons associated with abnormal disc morphology or oedema result in nerve fibre displacement and herniation in the peripapillary region. This leads to the formation of hyper-reflective oval structures above Bruch's membrane that elevate the surrounding retinal layers. These are visible on direct observation, optic disc photographs or en-face OCT as a C-shaped structure or partial torus around the optic nerve head[1].

Stickler syndrome (hereditary progressive arthro-ophthalmopathy, (MIM 108300, 604841, 184840) is characterised by ophthalmic, auditory, craniofacial, articular and skeletal abnormalities. It was first described by Stickler and colleagues in a family with mid-facial hypoplasia, vitreoretinal degeneration, joint hypermobility, and premature osteoarthritis[6].

There is considerable variability in the clinical phenotype and underlying genetic mutation associated with Stickler Syndrome[7]. Currently, pathogenic variants in six collagen-type genes (*COL2A1, COL11A1, COL11A2, COL9A1, COL9A2* and *COL9A3*) and at least five non-collagen genes (*LRP2, GZF1, BMP4, PLOD3* and *LOXL3*) have been linked to the disease pathogenesis[8].

Known ocular manifestations include congenital myopia, cataract, abnormalities of vitreous gel architecture and retinal detachment. The gene locus can contribute to the

124 phenotype observed, with type 1 Stickler syndrome pedigrees (*COL2A1* pathogenic
125 variant) associated with a high risk of blindness through Giant Retinal Tear (GRT) and
126 retinal detachment[9]. Patients with type 2 Stickler syndrome (*COL11A1* pathogenic
127 variants) are also prone to GRT detachment although the incidence appears to be
128 lower than Type 1 Stickler syndrome[10]. Phenotypic variation can result from
129 alternatively spliced exons, splice site mutations resulting in alternatively spliced
130 transcripts, differential effects of various amino-acid substitutions, mosaicism and
131 compound heterozygosity[11].
132
133 In this pilot observational study we assessed the presence of PHOMS in a cohort of
134 genetically confirmed Type 1 and Type 2 Stickler syndrome patients. These patients
135 were clinically observed to have anomalous discs, some of whom underwent extensive
136 investigation for suspected papilloedema. We hypothesized that PHOMS may be
137 present in this group of Stickler Syndrome patients with observed anomalous discs
138 due to the association with myopia.
139
140 We report to our knowledge for the first time the occurrence of PHOMS in patients with
141 Stickler Syndrome. PHOMS occurs in the presence of multiple different genetic
142 mutations responsible for Stickler Syndrome pathogenesis and does not appear to
143 relate to the presence or degree of myopia.
144
145 This novel finding should prompt those involved in the care of patients with Stickler
146 syndrome to be aware of this finding when evaluating the optic nerves of these
147 patients. Furthermore, the presence of PHOMS in this group of patients with

148 pathogenic variants in these collagen genes warrants further investigation into the
149 underlying contribution of collagen dysfunction to optic neuropathies.
150
151
152 **Methods**
153 Twenty-two eyes with anomalous optic discs from 11 genetically confirmed Type 1 and
154 2 Stickler Syndrome patients were identified from the Vitreoretinal Research clinic at
155 Cambridge University Hospitals NHS Foundation Trust that receives Stickler
156 Syndrome referrals from throughout the UK as part of the NHS England Highly
157 Specialised Services Commissioning Service. The study adhered to the Declaration
158 of Helsinki and was performed with approval of the local ethics committee (LREC
159 92/019 and 02/172). Informed consent was obtained from all patients. Diagnoses of
160 Stickler Syndrome were made according to published clinical criteria[12,13].
161
162 A two stage strategy for mutation screening depending upon the vitreous phenotype
163 was followed as previously described. The coding regions of genes pertinent to a
164 clinical indication of Stickler syndrome (PanelApp, Genomics England,
165 https://panelapp.genomicsengland.co.uk/), were screened by next generation
166 sequencing (NGS) using a custom Twist NGS targeted enrichment panel (Twist
167 Biosciences, San Francisco, USA). Variants were confirmed by Sanger sequencing in
168 comparison to normal controls. Putative splice variants were assessed by functional
169 analysis using an in vitro mini-gene assay, with Sanger sequencing to identify splicing
170 consequences.
171
172 **PHOMS identification**

The anomalous disc morphology in this series of Stickler syndrome patients was identified as part of their clinical examination and fundal assessment. The PHOMS were graded using enhanced depth imaging optical coherence tomography (EDI-OCT) according to the consensus recommendations of the Optic Disc Drusen Studies Consortium[14].

EDI-OCT scans were obtained using the Heidelberg Spectralis (Heidelberg Engineering, Heidelberg, Germany) with a dense horizontal raster (15 × 10°, 97 sections) centred on the optic nerve head and graded by two independent assessors. In case of disagreement, the image was graded by a third assessor. The presence of any co-existing optic disc drusen was also assessed using EDI-OCT and autofluorescence.

Results

PHOMS were present in 91% (n=20 eyes) of imaged eyes (Figures 1-4). All patients had PHOMS in at least one eye. 70% (n=14 eyes) were type 1 Stickler Syndrome and 30% (n=6 eyes) were type 2 Stickler Syndrome.

There was considerable variation observed in the optic disc phenotype associated with PHOMS (Figure 1). 360º elevation of the disc rim (Figure 1Ai-1Avii) giving rise to pseudopapilloedema prompted further extensive investigation for raised intracranial pressure which was subsequently excluded. Tilted myopic discs were also associated with PHOMS and a partial torus as previously reported[4,15] in our cohort (Figure 2Ai-2Avii).

Figure 2 illustrates asymmetrical optic nerves with PHOMS present in the right eye (Figure 2Aii -Avii) and absent in the left eye (Figure 2Bi-Bvi) from a single patient with a specific genetic variant *COL2A1* pathogenic variant NM_001844.5(COL2A1): c.808G>C, p.(Gly270Arg). The pathogenic variant giving rise to Stickler Syndrome alone was not therefore sufficient to give rise to PHOMS in both eyes of this individual. Figure 3 depicts the variation in PHOMS phenotype for a given genetic variant *COL11A1* pathogenic variant NM_001854.4(COL11A1): c.2522G>A, p.(Gly841Glu). Figure 4 demonstrates the PHOMS phenotype in isolated unique mutations in this cohort, *COL11A1* pathogenic variant NM_001854.4(COL11A1): c.1694G>T, p.(Gly565Val), *COL2A1* pathogenic variant NM_001844.5(COL2A1): c.931_934delAGTA, p.(Ser311Valfs*317), COL2A1 pathogenic variant NM_001844.5:c.2679+5G>C and COL2A1 pathogenic variant NM_001844.5(COL2A1): c.1527+135G>A).

Clinical features are summarised in Table 1. Five percent (n=1 eye) developed retinal detachment and 75% (n=15 eyes) had undergone 360^{o} prophylactic retinopexy. 41% (n=9) of eyes with PHOMS were present in patients with co-existing hearing loss and 13.6% (n=3) had orofacial manifestation of Stickler Syndrome in the form of a cleft palate. Seventy-five percent (n=15 eyes) of patients with PHOMS reported joint laxity or symptoms of arthritis. All patients were myopic and the degree of myopia did not appear to affect whether or not PHOMS was present in this cohort.

Discussion

We report for the first time the presence of PHOMS in the optic nerve head in series of patients with genetically confirmed Stickler Syndrome. PHOMS occurred in the

presence of mutations affecting both *COL2A1* and *COL11A1* in our cohort but the underlying mechanism through which PHOMS occurs in these patients requires further research. The combination of congenital myopia and oblique insertion of the optic nerve causing axoplasmic stasis as previously reported[1], may be a contributory factor as may collagen dysfunction contributing to abnormalities at the vitreo-papillary interface altering the optic nerve head architecture and axoplasmic flow[16].

Depending on the affected gene, pathogenic variants can cause haploinsufficiency through the creation of a premature stop codon through nonsense or frameshift mutations or loss-of-function changes that prevent the mutant protein from interacting with other proteins. Pathogenic variants can also exert a dominant negative effect either through missense or nucleotide adding/deleting mutations that produce an in-frame transcript or nonsense variants that lead to exon skipping[17].

Careful characterisation of the pathogenic variants together with a detailed assessment of the anatomical variation of the optic nerve head in a larger cohort of Stickler Syndrome patients with PHOMS may help to improve our understanding of the underlying pathogenesis.

Longitudinal assessment would help to assess the relationship with disease severity and progression. It should be noted that a prospective study looking at the occurrence and development of PHOMS in patients with multiple sclerosis and healthy control subjects[18] found no association between the presence of PHOMS and the clinical disease course, duration or management with disease-modifying treatments. Longitudinal assessment would also help to distinguish between PHOMS

development in the congenital myopia characteristic of Stickler Syndrome (which is often stable), and those in progressive secondary developmental myopia associated with peripapillary atrophy[19].

Now that effective preventative treatment options exist for the most severely blinding complications of Stickler Syndrome[20,21], understanding the basis for other ocular pathologies that have the potential to cause visual impairment is crucial. Detailed vitreous phenotyping has been crucial to the classification and refinement of the genetic testing strategy in Stickler Syndrome patients[11,12] . It remains to be seen whether variations in the PHOMS phenotype may prove to be a useful biomarker of disease.

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Figure Legends

Figure 1: Variation in optic disc phenotype. Ai: Overview of COL11A1 at the cDNA (a) and protein levels (b). NHR (non helical region), residues 230-419; IHR (interrupted helical region), residues 420-508; THD (triple helical domain), residues 529-1542; NC1 (C-terminal non-collagenous domain), residues 1577-1805. **Aii – v:** Multispectral fundus imaging **Aii**: composite **Aiii**: blue wavelength **Aiv:** green wavelength **Av:** near infrared wavelength **Avi:** PHOMS (white arrow) on EDI-OCT **Avii:** blue-light fundus

271 autofluorescence **Bi:** Overview of COL2A1 at the cDNA (a) and protein levels (b).
272 NPP (N propeptide amino acid residues 26-181); 181-Procollagen N-endopeptidase
273 cleavage site; THD (triple helical domain 201-1214); NHC (non helical c terminal
274 region 1215-1240); 1241-Procollagen C-endopeptidase cleavage site; NC1 (C-
275 terminal non-collagenous domain 1253-1487).**Bii – iv**: Multispectral fundus imaging
276 **Bii:** composite **Biii:** blue wavelength **Biv:** green wavelength **Bv:** near infrared
277 wavelength **Bvi:** PHOMS (white arrow) on EDI-OCT **Bvii:** blue-light fundus
278 autofluorescence
279
280 **Figure 2: Genetic mutation alone does not correlate with PHOMS phenotype**
281 **Ai – iv:** Multispectral fundus imaging **Ai:** composite **Aii:** blue wavelength **Aiii:** green
282 wavelength **Aiv:** near infrared wavelength **Av:** PHOMS (white arrow) on EDI-OCT
283 **Avi:** blue-light fundus autofluorescence **Bi – iv:** Multispectral fundus imaging **Bi:**
284 composite **Bii:** blue wavelength **Biii:** green wavelength **Biv:** near infrared wavelength
285 **Bv:** PHOMS absent on EDI-OCT **Bvi:** blue-light fundus autofluorescence
286
287 **Figure 3: PHOMS phenotype for a given specific mutation** (COL11A1 pathogenic
288 variant c.2522G>A). **Ai – iv:** Multispectral fundus imaging **Ai:** composite **Aii:** blue
289 wavelength **Aiii:** green wavelength **Aiv:** near infrared wavelength **Av-vi:** PHOMS
290 (white arrow) on EDI-OCT **Avii:** blue-light fundus autofluorescence **Bi – iv:**
291 Multispectral fundus imaging **Bi:** composite **Bii:** blue wavelength **Biii:** green
292 wavelength **Biv:** near infrared wavelength **Bv-vi:** PHOMS (white arrow) on EDI-OCT
293 **Bvii:** blue-light fundus autofluorescence **Ci – iv:** Multispectral fundus imaging **Ci:**
294 composite **Cii:** blue wavelength **Ciii:** green wavelength **Civ:** near infrared wavelength
295 **Cv:** PHOMS (white arrow) on EDI-OCT **Cvi:** blue-light fundus autofluorescence **Di –**

296 **iv:** Multispectral fundus imaging **Di:** composite **Dii:** blue wavelength **Diii:** green
297 wavelength **Div:** near infrared wavelength **Dv:** PHOMS (white arrow) on EDI-OCT
298 **Dvi:** blue-light fundus autofluorescence
299
300 **Figure 4: PHOMS phenotype in isolated unique mutations. A-Hi – iv:** Multispectral
301 fundus imaging **A-Hi:** composite **A-Hii:** blue wavelength **A-Hiii:** green wavelength **A-**
302 **Hiv:** near infrared wavelength **A-Hv:** PHOMS (white arrow) on EDI-OCT **A-Hvi:** blue-
303 light fundus autofluorescence **B-Hi – iv:** Multispectral fundus imaging **B-Hi:** composite
304 **B-Hii:** blue wavelength **B-Hiii:** green wavelength **B-Hiv:** near infrared wavelength **B-**
305 **Hv:** PHOMS (white arrow) on EDI-OCT **B-Hvi:** blue-light fundus autofluorescence
306
307 **Table 1: Clinical features and specific genetic mutation**
308
309 **References**
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