Fabry or not Fabry: From genetics to diagnosis
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CHAPTER 7

Cornea verticillata supports a diagnosis of Fabry disease in non-classical phenotypes: results from the Dutch cohort and a systematic review

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ABSTRACT

Background
Screening for Fabry disease (FD) increasingly reveals individuals without characteristic features and with a variant of unknown significance in the α-galactosidase A (GLA) gene. Cornea verticillata (CV) assessment, as a characteristic sign of FD, may be a valuable diagnostic tool to assess if these individuals have a non-classical phenotype or no FD at all.

Methods
We performed a systematic review to estimate the prevalence of CV in FD. Additionally, CV prevalence was assessed in the Dutch FD cohort. Data were stratified by gender and phenotype (classical, non-classical, uncertain, no-FD) using pre-defined criteria.

Results
CV was assessed in 21 cohorts (n=753, 330 males, age 0-85 years). Pooled prevalence was 69% (74% males, 66% females). In 6 studies, 77 (19 males) individuals with a non-classical or uncertain diagnosis were identified. Individual data were available in 4/6 studies (n=66, 16 males). CV was present in 24% (n=16, 2 males).

Hundred-and-one (35 males) subjects from the Dutch cohort were grouped as classical, of whom 86% (94% males, 82% females including 5 who used amiodarone) had CV. Of the 25 (11 males) non-classical patients, 4 (3 males) had CV. Subjects in the uncertain and no-FD groups did not have CV.

Conclusion
CV is related to classical or biopsy proven non-classical FD, with a very high sensitivity in classical males. Thus, presence of CV in an individual with an uncertain diagnosis of FD indicates a pathogenic GLA variant, in the absence of medication that may induce CV; if CV is absent, FD cannot be excluded.
INTRODUCTION

The lysosomal storage disorder Fabry disease (FD) is caused by variants in the X-chromosomal α-galactosidase A (GLA) gene. Due to impaired function of the lysosomal hydrolase α-galactosidase A (αGalA), globotriaosylceramide (Gb3) accumulates. Absent or near absent enzyme activity in males usually leads to classical FD which is characterized by neuropathic pain with childhood onset, angiokeratoma and cornea verticillata (CV). End organ complications of the heart, kidney and brain arise later in life. Due to the X-linked nature of the disease, females are usually less severely affected.

Screening for FD and individual case finding has resulted in the identification of an increasing number of individuals with a GLA variant. While the birth prevalence of FD was previously estimated to be between 1:40,000-170,000, screening studies in high risk populations (i.e. groups with heart, kidney or brain disease) reveal a much higher prevalence of up to more than 1%. These individuals have a non-specific sign such as left ventricular hypertrophy or chronic kidney disease that may be attributable to FD, but most do not have the characteristic clinical and biochemical features of FD that are required to confirm a definite diagnosis of FD. Thus, the majority of these individuals have an uncertain diagnosis of FD in the presence of a genetic variant of unknown significance (GVUS) in the GLA gene. They may have a non-classical FD phenotype, or a neutral, non-disease causing GLA variant.

Diagnostic tools are needed to confirm the presence of FD, but also to avoid unjustified labelling of individuals with a non-disease causing GLA variant and wrongful initiation of costly treatment.

CV assessment may be a useful, non-invasive tool in the diagnosis of FD, specifically for those with an uncertain diagnosis of FD, since it has a high prevalence among FD patients. The whorl like pattern of corneal opacities is specific for FD, with the exception of some medications that may induce a corneal whirling that cannot be distinguished from that in FD (among others amiodarone and chloroquine, for review see 6). With this study we aim to value the presence of CV in the diagnosis of FD by investigating the prevalence of CV in individuals with a classical or non-classical FD phenotype and in individuals with an uncertain diagnosis of FD or no FD.

METHODS

Systematic review

Search

Medline and EMBASE (1980 till January 2013) were searched for studies that assessed eye abnormalities in FD patients. Search terms used were ‘Fabry disease’ combined with ‘Eye’, ‘Ophthalmology’, ‘Cornea verticillata’, ‘Tortuous retinal veins’, ‘Corneal opacity’, and their synonyms, Mesh terms (Medline) and headings (Embase). In Embase, limits were used to exclude conference papers and abstracts.

First selection was done based on title and abstract. We selected full text articles and reports with original data on eye abnormalities in FD subjects in all languages (with an English abstract),
including papers that presented data from international Fabry disease registries (Fabry Registry, Genzyme, a Sanofi company and Fabry Outcome Survey, FOS, Shire HGT). Case reports, newborn screening studies, comments, reviews and book chapters were excluded. Subsequently, studies were screened and included based on full text if the inclusion criteria were met.

**Data collection and analysis**

Data were recorded on the type of study (registry, screening or cohort study), participating study center(s), number of subjects, gender and age groups (children and/or adults), together with the type of eye abnormalities assessed and therapy status at the time of ophthalmology assessment. A sub analysis was performed on subjects designated as non-classical or uncertain FD. For this purpose subjects were selected who were reported to have:

- a high residual enzyme activity
- non-classical FD disease manifestations such as a cardiac or renal variant
- a GLA variant that has frequently been associated with a non-classical FD phenotype
- a GLA variant that is generally considered non-pathogenic/neutral, or which pathogenicity is currently discussed in the literature (e.g. p.R112H, p.P389A, p.N215S, p.A143T, c.936+919G>A (IVS4+919G>A))

Raw prevalence data from all studies were combined for calculation of a weighed pooled prevalence, and specified for gender. Data from registry and screening studies were analyzed separately.

**Dutch cohort**

**Patient selection and groups**

The Dutch database, comprising data from all subjects that visited the outpatient FD clinic with any GLA variant was searched for all adults (>18 years of age). Data on the use of amiodarone and/or chloroquine at any time during the medical history were retrieved from the medical records.

Previously reported criteria were applied to classify subjects into 4 groups. Classical: The strict criteria for a definite diagnosis of Fabry disease were used to identify patients with a classical FD phenotype. Criteria include very low or absent enzyme activity in leukocytes (males), very high lysoGb3, FD specific clinical characteristics (neuropathic pain, cornea verticillata and angiokeratoma) and a family history which is positive for classical FD (see chapter 3, table 2). For this study an exception was made: CV was not used as a criterion to group subjects as ‘classical’, because CV is the feature under investigation in this study. Non-classical: Subjects who do not fulfill these detailed criteria for a definite diagnosis as described in chapter 3, table 2, were grouped as non-classical if this subject or a family member has FD characteristic storage on electron microscopy in a biopsy of an affected organ (i.e. heart or kidney). Uncertain: subjects who do not fulfill the strict criteria, and of whom a biopsy was not available, were grouped as uncertain. No FD: subjects were grouped as no FD (a neutral GLA variant) if a biopsy from a subject or from one of his/her family members did not show FD characteristic storage, or if the individual carried the well-known neutral variant p.D313Y. Subjects were excluded from the analyses if data were insufficient to apply the above criteria, and/or CV assessment was missing.
Age at time of database search was calculated for all groups, and stratified by gender. For deceased subjects, age at death was used.

**CV assessments**
Assessment of CV was performed as part of regular clinical care at adulthood or adolescence. IRB/Ethics Committee ruled that approval was not required for this study. A slit-lamp examination was performed by an experienced ophthalmologist or trained physician (LT, supervised by ophthalmologist MS) to assess the left and right cornea. CV was recorded as present, mild or absent. Corneal photographs were obtained in some illustrative cases, see figure 2. Data on lenticular changes and retinal vessel tortuosity were not available.

**Data analyses**
Prevalence of CV was calculated for ‘classical’, ‘non-classical’, ‘uncertain’ and ‘no FD’ groups and specified for gender. Ninety-five percent confidence intervals for proportions were calculated using the modified Wald method. Positive and negative predictive values were calculated, for individuals who initially presented with an uncertain diagnosis of FD (non-classical and no FD, uncertain cases were excluded).

**RESULTS**

**Systematic review**

**Search**
Four-hundred-and-sixty records were retrieved from Medline and Embase after duplicates were removed. Twenty-three studies were selected for data extraction. Two studies were subsequently added by the authors because of their interest for the research question (Whybra et al. 11), not selected with the search and Sher et al 12, initially excluded based on the publication date <1980). These studies included 21 cohort studies 13-31, 2 high risk group screening studies 32,33, and 2 registry studies.34,35 Details on selection and inclusion are presented in figure 1.

**Prevalence of cornea verticillata**
CV was assessed in 753 individuals (330 males) from 21 cohorts with an age range of 0-85 years, for details see table 1. Pooled prevalence of CV was 69% (range 26-96). Gender specific data were available for 18 out of 21 studies (n=685, 295 males) 11,12,14-16,18-24,26-31, revealing a pooled prevalence of 74% (range 14-94) for males and 66% (range 31-100) for females. Thirteen studies reported data on ERT administration, although in most cases the timing of CV assessment in relation to ERT administration was not specified. Therefore, further analysis of these data was not feasible.

In 6 cohort studies, 77 (19 males) individuals with a non-classical phenotype or uncertain FD could be identified, see table 2. Separate data for gender were available in 4 out of these 6 studies (n=66, 16 males). CV was present in 24% of non-classical or uncertain subjects (n=16, 2 males), mainly comprising of the GLA variant c.936+919G>A (IVS4+919G>A) (n=15, 1 male) from one study on this specific GLA variant.22 Allen at al reported mild CV in a 3.5 years old boy with a p.A143T variant, while his brother with the same GLA variant did not have CV at age 1.5 years (13 and personal communication).
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One of the registry studies reported a CV prevalence of 75% (males 73%, females 77%) \(^{30}\), while the second reported that in 11% of males and 12% of females CV was the presenting symptom \(^{34}\). The high-risk group screening studies revealed that CV was absent in all adult individuals \((n=29, 12\text{ males})\) who were identified with a GLA variant \(^{32,33}\).

Dutch cohort

**Patient selection and groups**

Hundred-and-ninety-four records of adults with a GLA variant were retrieved from the database, of whom 50 were excluded because data were not sufficient to fulfill the study criteria for disease groups and/or CV assessment was not (yet) performed due to lost to follow-up or because they had died before assessments were completed \((n=45)\), or patients were recently referred and investigations were ongoing at the time of the study \((n=5)\). Hundred-and-forty-four subjects \((56\text{ males, 4\ males deceased})\) were included in the analyses.

Most subjects fit the criteria for a definite diagnosis of FD \((\text{classical, } n=101, 35\text{ males})\) and 25 subjects \((11\text{ males})\) were grouped as ‘non-classical’. FD was excluded in 7 subjects \((\text{no FD, 5 males})\) and in 11 subjects \((5\text{ males})\) the diagnosis of FD was still uncertain.

One exceptions to the classification criteria was made. In three families \((n=5\text{ subjects})\), biopsies of an affected organ were lacking. Because other families in our cohort with the same GLA variant did have positive biopsies, these subjects were classified as non-classical, biopsy proven disease.

**Figure 1.** Selection of studies.
Cornea verticillata supports a diagnosis of Fabry disease in non-classical phenotypes

Table 1. Cornea verticillata (CV) prevalence in reviewed studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Center</th>
<th>Patients n (m/f)</th>
<th>Total %</th>
<th>Males %</th>
<th>Females %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen*</td>
<td>2010</td>
<td>UK (Cambridge)</td>
<td>26 (12/14)</td>
<td>50%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Barba Romero</td>
<td>2004</td>
<td>Spain</td>
<td>14 (14/0)</td>
<td>36%</td>
<td>36%</td>
<td>-</td>
</tr>
<tr>
<td>Beltran-Becerra</td>
<td>2012</td>
<td>Mexico</td>
<td>13 (7/6)</td>
<td>46%</td>
<td>57%</td>
<td>33%</td>
</tr>
<tr>
<td>Borgwardt</td>
<td>2012</td>
<td>Denmark</td>
<td>10 (6/4)</td>
<td>90%</td>
<td>83%</td>
<td>100%</td>
</tr>
<tr>
<td>Choi</td>
<td>2008</td>
<td>Korea</td>
<td>11 (8/3)</td>
<td>82%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Falke</td>
<td>2009</td>
<td>Germany (Rostock)</td>
<td>22 (6/16)</td>
<td>46%</td>
<td>50%</td>
<td>44%</td>
</tr>
<tr>
<td>Gupta*</td>
<td>2005</td>
<td>USA, Maryland</td>
<td>57 (0/57)</td>
<td>82%</td>
<td>-</td>
<td>82%</td>
</tr>
<tr>
<td>Kaminsky</td>
<td>2013</td>
<td>France (Nancy)</td>
<td>108 (41/67)</td>
<td>54%</td>
<td>51%</td>
<td>55%</td>
</tr>
<tr>
<td>Kobayashi</td>
<td>2008</td>
<td>Japan</td>
<td>36 (0/36)</td>
<td>50%</td>
<td>-</td>
<td>50%</td>
</tr>
<tr>
<td>Lin*</td>
<td>2010</td>
<td>Taiwan</td>
<td>52 (7/45)</td>
<td>29%</td>
<td>14%</td>
<td>31%</td>
</tr>
<tr>
<td>Nguyen</td>
<td>2006</td>
<td>Australia</td>
<td>66 (34/32)</td>
<td>83%</td>
<td>94%</td>
<td>72%</td>
</tr>
<tr>
<td>Orssaad</td>
<td>2003</td>
<td>France (Paris)</td>
<td>32 (32/0)</td>
<td>56%</td>
<td>56%</td>
<td>-</td>
</tr>
<tr>
<td>Pitz</td>
<td>2009</td>
<td>Germany (Mainz)</td>
<td>31 (15/16)</td>
<td>81%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rákóczi*</td>
<td>2007</td>
<td>Hungary</td>
<td>31 (15/16)</td>
<td>65%</td>
<td>67%</td>
<td>63%</td>
</tr>
<tr>
<td>Reisin</td>
<td>2010</td>
<td>Argentina</td>
<td>54 (31/23)</td>
<td>96%</td>
<td>94%</td>
<td>100%</td>
</tr>
<tr>
<td>Ries*</td>
<td>2003</td>
<td>Italy (Milan), Sweden, UK</td>
<td>33 (15/18)</td>
<td>76%</td>
<td>73%</td>
<td>78%</td>
</tr>
<tr>
<td>Ries*</td>
<td>2005</td>
<td>USA, Maryland</td>
<td>24 (24/0)</td>
<td>88%</td>
<td>88%</td>
<td>-</td>
</tr>
<tr>
<td>Sher</td>
<td>1979</td>
<td>USA (Minnesota)</td>
<td>62 (37/25)</td>
<td>92%</td>
<td>95%</td>
<td>88%</td>
</tr>
<tr>
<td>Sodi</td>
<td>2013</td>
<td>Italy (Florence), Belgium (Charleroi), UK (London), Germany (Mainz)</td>
<td>35 (17/18)</td>
<td>89%</td>
<td>94%</td>
<td>83%</td>
</tr>
<tr>
<td>Tøndel</td>
<td>2008</td>
<td>Norway</td>
<td>16 (9/7)</td>
<td>94%</td>
<td>89%</td>
<td>100%</td>
</tr>
<tr>
<td>Whybra</td>
<td>2001</td>
<td>Germany (Mainz)</td>
<td>20 (0/20)</td>
<td>70%</td>
<td>-</td>
<td>70%</td>
</tr>
</tbody>
</table>

TOTAL** | 753 (330/423) | 69% | 74% | 66% |

* Non-classical or uncertain cases were reported, see table 2 for details ** Only studies who reported gender specific data were used for male (m) and female (f) prevalence - not applicable or missing data.

Cornea verticillata prevalence and details

CV prevalence in the Dutch cohort is depicted in table 3. Nearly all males in the classical group had CV (94%). The two subjects without CV had received more than 9 years of treatment with ERT at the time of CV assessment. These 2 males had the p.D136Y and p.R342Q GLA variant causing complete absence of αGalA activity in leukocytes, very high lysoGb3 in plasma, acroparesthesia, white matter lesions and left ventricular hypertrophy. Eighty-two percent of females in the classical group had cornea verticillata, of whom 5 had used amiodarone for cardiac rhythm abnormalities before or at the time of CV assessment. See figure 2A. for an example of characteristic CV.
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Of the 25 non-classical subjects, 4 (16%, 3 males and 1 female) had CV. One female and 2 males had the p.P389A variant, and 1 male had the p.R112H variant. The clinical and biochemical characteristics of the subject and family members with the p.R112H variant are described in detail by Smid et al.36. The corneal changes in these patients were subtle and limited to one or two small sub epithelial deposits, thereby differing from the typical whorl like pattern that is seen in classical FD patients (figure 2B. and C). These subjects did not use medication that is associated with CV.

Table 2. CV prevalence in non-classical/uncertain cases in the literature.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>n (m/f)</th>
<th>GLA variant</th>
<th>CV prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen</td>
<td>2010</td>
<td>9 (6/3)</td>
<td>7x p.N215S 2x p.A143T</td>
<td>11% 1 17% 1 0 0 Mild CV in 3.5 year old male with p.A143T (personal communication)</td>
</tr>
<tr>
<td>Gupta</td>
<td>2005</td>
<td>7 (0/7)</td>
<td>4x p.R112H 3x p.N215S</td>
<td>- - - - - -</td>
</tr>
<tr>
<td>Lin</td>
<td>2010</td>
<td>52 (7/45)</td>
<td>c.936+919G&gt;A (IVS4+919G&gt;A)</td>
<td>29% 15 14% 1 31% 14</td>
</tr>
<tr>
<td>Ries</td>
<td>2003</td>
<td>2 (2/0)</td>
<td>p.A143T</td>
<td>0 0 0 0 - -</td>
</tr>
<tr>
<td>Ries</td>
<td>2005</td>
<td>3 (3/0)</td>
<td>2x p.R112H 1x p.A97V</td>
<td>0 0 0 0 - -</td>
</tr>
<tr>
<td>Rákóczi</td>
<td>2007</td>
<td>4 (1/3)</td>
<td>p.N215S</td>
<td>- - - - - -</td>
</tr>
</tbody>
</table>

- not applicable or missing data, CV: cornea verticillata.

Of the 25 non-classical subjects, 4 (16%, 3 males and 1 female) had CV. One female and 2 males had the p.P389A variant, and 1 male had the p.R112H variant. The clinical and biochemical characteristics of the subject and family members with the p.R112H variant are described in detail by Smid et al.36. The corneal changes in these patients were subtle and limited to one or two small sub epithelial deposits, thereby differing from the typical whorl like pattern that is seen in classical FD patients (figure 2B. and C). These subjects did not use medication that is associated with CV.

Figure 2A. Cornea verticillata (arrow) in a 45 year old untreated female with a classical FD phenotype. Arrow: origin of the pigmented verticillata.

B. and C. Subtle cornea deposits (arrow) in a 36 year old male with a non-classical FD phenotype.
Cornea verticillata supports a diagnosis of Fabry disease in non-classical phenotypes

**DISCUSSION**

Screening for FD is often performed in high-risk groups, e.g. among individuals with chronic kidney disease, left ventricular hypertrophy or stroke, which may be attributed to FD. If a genetic variant in the GLA gene is found, the diagnosis of FD can be uncertain since characteristic FD signs or symptoms are often lacking 4. CV assessment may be helpful in these cases. Our study revealed a high prevalence of 94% in classically affected males, and there were no false positives. In FD patients with a classical phenotype CV is usually diffuse with a typical whorl-like pattern. In patients with the non-classical phenotype subtle changes were identified, which confirms previous findings of CV in individuals with a non-classical phenotype with the c.936+919G>A (IVS4+919G>A) variant 22. Our findings suggest that the presence of CV, in the absence of medication that may induce CV, confirms the diagnosis of classical or non-classical FD. The absence of CV, however, does not exclude FD. Especially in cases with a non-classical phenotype, CV may be absent, even if characteristic storage is present in an affected organ, such as the heart or kidney.

The prevalence of CV among FD patients in the literature is variable and generally lower in comparison to the Dutch cohort. This discrepancy is probably caused by the inclusion of subjects with a non-classical phenotype or even subjects with a neutral GLA variant in the reviewed

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**Table 3. Dutch cohort.**

<table>
<thead>
<tr>
<th>Group (age median, range)</th>
<th>n</th>
<th>Percentage CV (n, 95% CI)</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males classical (40, 19-65)</td>
<td>35</td>
<td>94% (33, 80-99)</td>
<td>2 subjects without CV received &gt;9 years of treatment with ERT</td>
</tr>
<tr>
<td>Females classical (48, 19-81)</td>
<td>66</td>
<td>82% (54, 71-89)</td>
<td>5 subjects with CV received amiodarone treatment before or during CV assessment</td>
</tr>
<tr>
<td>TOTAL classical</td>
<td>101</td>
<td>86% (87, 78-92)</td>
<td></td>
</tr>
<tr>
<td>Males non-classical (64, 36-74)</td>
<td>11</td>
<td>27% (3, 9-57)</td>
<td>In all patients with CV, corneal changes were minimal</td>
</tr>
<tr>
<td>Females non-classical (39, 18-78)</td>
<td>14</td>
<td>7% (1, &lt;0.01-34)</td>
<td></td>
</tr>
<tr>
<td>TOTAL non-classical</td>
<td>25</td>
<td>16% (4, 6-35)</td>
<td></td>
</tr>
<tr>
<td>Males uncertain (49, 45-71)</td>
<td>5</td>
<td>0 (0-48)</td>
<td></td>
</tr>
<tr>
<td>Females uncertain (57, 30-68)</td>
<td>6</td>
<td>0 (0-44)</td>
<td></td>
</tr>
<tr>
<td>TOTAL uncertain</td>
<td>11</td>
<td>0 (0-30)</td>
<td></td>
</tr>
<tr>
<td>Males no FD (41, 23-70)</td>
<td>5</td>
<td>0 (0-48)</td>
<td></td>
</tr>
<tr>
<td>Females no FD (49, 52-46)</td>
<td>2</td>
<td>0 (0-71)</td>
<td></td>
</tr>
<tr>
<td>TOTAL no FD</td>
<td>7</td>
<td>0 (0-40)</td>
<td></td>
</tr>
</tbody>
</table>

FD: Fabry disease, CV: cornea verticillata, ERT: enzyme replacement therapy.

There were no false positive cases (none of the subjects in the no-FD group had CV). For individuals who presented to our clinic with an uncertain diagnosis (groups non-classical and no-FD), the positive predictive value of CV is 1, and the negative predictive value is 0.25.
studies. It was not possible to correct for this bias, because the required clinical, biochemical and genetic details were most often not provided.

Additionally, age may have affected the prevalence of CV, because of the inclusion of children in several studies. Borgwardt et al described two boys who started ERT treatment at the age of 10 and 12, in whom CV was absent at baseline, but who developed CV after 1 year of follow-up. These cases suggest that CV may not always be present from birth. Although in the above cases ERT did not seem to influence the development of CV, the effect of ERT on CV has not been studied systematically, and may have influenced the data. In the Dutch cohort, 2 males did not have CV at adulthood, while clinical and biochemical evaluation as well as the family history demonstrated a classical FD phenotype. As previously suggested by Sodi et al, we postulate that long-term ERT may have corrected the corneal changes in these subjects.

This study focused on CV, and did not study other ocular changes that are related to FD. Posterior lens cataract has previously been described as a specific feature in FD. This type of cataract has been reported in a few studies only with a prevalence up to 53% of FD males. Another FD associated ocular feature is retinal vascular tortuosity. This feature has not often been reported in the literature. Importantly, and in analogy with tortuosity and dilatation of the cerebral basilar artery, the specificity is yet uncertain, as tortuous and dilated retinal vessels are reported to be present in other diseases that affect the vasculature such as diabetes, and may be subjected to age. Because our study was observational and cataract and retinal vessels are not assessed routinely, these data were not available for the Dutch cohort.

In addition, we did not strive to study the nature of FD related ocular changes, but we pursued to assess the diagnostic applicability of an assessment that is non-invasive and worldwide applicable, in order to discern patients with a classical and non-classical FD phenotype from those without FD. We are confident that CV assessment, as the current most extensively studied and understood ocular feature in FD and with the highest prevalence, is the best suitable ophthalmological assessment to use for diagnostic purposes.

Data from the Dutch cohort show a correlation between CV and a classical FD phenotype or biopsy proven non-classical FD. But, the number of subjects who were classified as ‘no FD’ in this cohort is small. Further studies are needed to confirm that CV is not present in subjects with a neutral GLA variant (no FD), and thus, that the presence of CV predicts a classical or non-classical FD phenotype. We are confident, however, that the presence of diffuse CV with a whorl like pattern can substitute the gold standard for FD (a biopsy of an affected organ), in patients with an uncertain diagnosis of FD. Whether this also applies to the more subtle changes that are usually seen in non-classical FD patients should be subject of further studies.

In conclusion, in individuals with an uncertain diagnosis of FD, when no medication is used that can cause CV, the presence of CV provides evidence for FD.
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