Screening for Fabry disease and hereditary ATTR amyloidosis in idiopathic small fiber and mixed neuropathy

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Ethical Publication Statement

We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosure of Conflict of Interest

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Abstract

Introduction: We aimed to explore the value of genetic screening for Fabry disease (FD) and hereditary ATTR amyloidosis in patients with idiopathic small fiber neuropathy (SFN) or mixed neuropathy in a clinical setting.

Methods: This was a Nordic multicenter study with 9 participating centers. Patients with idiopathic SFN or mixed neuropathy were included. Genetic sequencing of the TTR and GLA genes was performed.

Results: 172 patients were enrolled. Genetic screening was performed in 155 patients. No pathogenic mutations in the TTR gene were found. A single patient had a possible pathogenic variant, R118C, in the GLA gene, but the clinical investigation showed no firm signs of FD.

Discussion: Screening for hereditary ATTR amyloidosis and FD in patients with idiopathic SFN or mixed neuropathy without any additional disease-specific symptoms or clinical characteristics in a Nordic population appears to be of little value in a clinical setting.

Keywords
Fabry disease; genetic screening study; hereditary ATTR amyloidosis; idiopathic polyneuropathy; small fiber neuropathy
INTRODUCTION

Polyneuropathy is a common disorder with an age-dependent prevalence of 1-3 % in the general population and up to 6-7 % in the elderly.\textsuperscript{1-2} The etiology often remains unclear, especially in patients with slowly progressive predominantly sensory axonal polyneuropathy and small fiber neuropathy (SFN).\textsuperscript{2-3}

Two treatable, possibly underdiagnosed hereditary diseases, known to cause painful polyneuropathy with involvement of small fibers, are Fabry disease (FD) and hereditary ATTR (hATTR) amyloidosis.\textsuperscript{4,5} The results of previous screening studies of the two conditions have been inconsistent.\textsuperscript{6-10}

In this study we aimed to further explore the value of genetic screening for FD and hATTR amyloidosis in patients with idiopathic SFN or mixed neuropathy (combined SFN and large fiber sensorimotor axonal polyneuropathy) in a clinical setting in the Nordic region.

METHODS

This retrospective and prospective multicenter study, performed during October 2015-February 2017, involved 9 participating neurology departments from 4 different Nordic countries.

The study was carried out according to the Helsinki Declaration and was approved by the local ethical committee in each country. All participants received written and oral information about the study, as well as genetic counseling, prior to inclusion and gave their written informed consent upon entering the study.
The inclusion criteria were age ≥ 18 years, with a diagnosis of SFN, consisting of patients with a pure SFN or mixed neuropathy (combined SFN and large fiber sensorimotor axonal polyneuropathy) without any identified etiology. The diagnosis of SFN was confirmed by clinical examination, abnormal warm or cold quantitative sensory testing (TSA-II NeuroSensory Analyzer, Medoc Ltd, USA, or Thermotest Somedic AB Type I, Sweden) and/or skin biopsy. The exclusion criteria consisted of a previously-established diagnosis of FD or hATTR amyloidosis, previously performed genotyping for transthyretin (TTR) and/or alpha-galactosidase A (GLA) mutations, or other conditions known to cause SFN, such as diabetes, uremia, thyroid disease, vitamin deficiency, immune mediated neuropathies, alcohol abuse, neurotoxic drugs and infections.

In the retrospective part of the study patients were identified through diagnosis registers at each center and a subsequent review of medical records. If the SFN had been judged as idiopathic by the responsible neurologist at the time for SFN diagnosis, the patient was contacted by letter and invited to participate in the study. The patients were thoroughly interviewed to exclude possible SFN associated-conditions. No further etiological investigation was performed. If possible SFN-associated co-morbidities started after the time-point of SFN diagnosis, a case-to case judgement regarding the possible association with SFN was performed. For the prospectively included patients the etiological investigation was performed according to each center’s clinical routine. The evaluations were not standardized.

A blood sample was collected from all patients who met the inclusion- and exclusion criteria.

**Genotyping**

The TTR gene exons 1-4 and GLA gene exons 1-7 and part of intron 4 were Sanger sequenced. GLA copy number variation was determined by multiplex ligation-probe assay.
Statistics

The Kruskal-Wallis multiple comparison test for non-parametric values was used for comparison between the countries. For comparison of binary data between countries Chi-square testing was used.

RESULTS

A total of 172 patients were enrolled. Seventeen were subsequently excluded (Fig. 1).

Clinical Description

Patient characteristics are shown in Table 1. The patients from Norway were significantly younger than the patients from the other countries, with a median age of 54 years. There was no significant difference regarding sex, duration of symptoms or the distribution between isolated SFN or mixed neuropathy between the different centers. All included patients from Denmark and Norway had pain, which significantly differed from the patients from Finland and Sweden, among whom 89.7% and 71.7% had pain, respectively. Approximately 60% of the included patients reported autonomic symptoms, the most common being gastrointestinal, hypo- or hyperhidrosis and orthostatic hypotension.

Genetic results

We found no pathogenic mutations in the TTR gene. In the GLA gene we found a single patient harboring an earlier described possible pathogenic variant, p.Arg118Cys, also referred to as R118C.

Patient with a possible pathogenic R118C mutation in the GLA gene

This 69-year old male from Sweden, had a 10 year history of slowly progressive, mildly painful isolated SFN with symptoms restricted to his feet. He had no family history of polyneuropathy. Dried blood spot showed normal α-galactosidase (2.6 µmol/L/h, reference...
value: 2.3-17) and lyso-globotriaosylceramide (lyso-Gb3) levels (1.2 ng/ml, cut off values 0.0-3.5). Further clinical investigation showed no typical FD deposits in the eyes and no typical skin changes. The MRI of the heart showed no hypertrophy and normal global function. The brain MRI showed no typical FD features. He had a mildly impaired kidney function (estimated glomerular filtration rate (eGFR) 55 mL/min/1.7, reference value >60 mL/min/1.7) probably due to a postrenal obstruction secondary to an earlier urinary tract malignancy.

DISCUSSION

In this multicenter study of 155 Nordic patients with idiopathic small fiber neuropathy and mixed neuropathy, no definite FD and hATTR amyloidosis cases were identified. This result is concordant with earlier screening studies for FD and hATTR amyloidosis in patients with idiopathic SFN.\textsuperscript{7-9}

The idea of FD as an underdiagnosed disease in patients with symptoms from an isolated organ system arose when Spada et al published their screening study of newborns in Italy and found a high frequency of late-onset GLA mutations.\textsuperscript{11} Screening studies of patients with cryptogenic stroke, hypertrophic cardiac disease and isolated kidney dysfunction have similarly shown a higher frequency of FD than expected.\textsuperscript{12-14} The same hypothesis drove the German pilot study which identified one case of FD in a group of 24 patients with idiopathic SFN.\textsuperscript{6} However, this was not confirmed in a small controlled study of patients with idiopathic polyneuropathy,\textsuperscript{7} nor in a large genetic screening study of 440 Dutch patients with isolated SFN.\textsuperscript{8} In a recent Chinese study one case of FD was identified in 100 patients with idiopathic SFN.\textsuperscript{10}
The variant R118C was first described as a possible pathogenic late-onset mutation by Spada et al.\textsuperscript{11} It has been considered pathogenic in patients with cryptogenic stroke and chronic kidney failure.\textsuperscript{12,14} In a large screening study of Dutch patients with SFN, the R118C variant was identified in one woman. She had normal biochemistry and showed no FD manifestations during 3 years of follow-up.\textsuperscript{8} Recently, a review of clinical, biochemical and histopathological data of 22 patients with the R118C variant was published.\textsuperscript{15} The patients had normal life time expectancy and no major organ complications, however some had angiokeratoma. The authors suggest the variant to be non-pathogenic or of low pathogenicity and not to warrant enzymatic treatment.\textsuperscript{15} However, in another recent publication, R118C was suggested to be a late-onset mutation.\textsuperscript{16} Our patient with R118C had no clinical manifestations of FD and his enzyme level was normal. With a slowly progressive isolated SFN, very mild kidney dysfunction probably due to postrenal obstruction, and negative biomarkers, there is no firm evidence supporting the diagnosis of FD in this patient.

Fabry disease has no specific geographic distribution, but a high prevalence and awareness of hATTR amyloidosis (predominantly the Val50Met type) is expected as a possible cause of SFN in endemic areas such as the north of Sweden.\textsuperscript{17} In non-endemic areas one could suspect hATTR amyloidosis to be underdiagnosed, though screening for TTR mutations in countries with more genetic diversity implies a higher probability of negative results. In this multicenter study with a Nordic cohort of patients with idiopathic polyneuropathy we found no new cases of hATTR amyloidosis, which is consistent with the findings in a small genetic screening study of patients with idiopathic SFN from the US.\textsuperscript{9} However, Hsu et al found 3 cases (Ala97Ser) of hATTR amyloidosis in a cohort of 100 Chinese patients with an idiopathic pure SFN.\textsuperscript{10}
The major strength of this study is the multicenter approach including a large cohort of patients from different Nordic countries. However, due to the multicenter approach and retrospective design, the extent of etiological investigation of SFN varied between centers. By including only patients with a seemingly idiopathic SFN, we are aware that we may have ruled out patients with a known possible SFN-associated disease who might also have hATTR amyloidosis or FD. However, since the Dutch study included patients with other disease-associated etiologies of SFN, wider inclusion criteria probably would not have affected our results. Likewise, the exclusion of patients with previously-performed screening of the GLA and/or the TTR genes or known mutations in either of the genes introduced a bias with the risk of missing cases with either or both of the diseases.

Screening for hATTR amyloidosis and FD in patients with idiopathic SFN without any additional disease-specific symptoms or clinical characteristics in a Nordic population thus appears to be of little value in a clinical setting. Nevertheless, the divergent results in earlier genetic screening studies in patients with idiopathic SFN suggest that in some populations screening for these disorders might be worthwhile.6,10
ABBREVIATIONS

FD = Fabry Disease

GLA = alpha-galactosidase A gene

hATTR = hereditary ATTR

SFN = small fiber neuropathy

TTR = transthyretin gene
REFERENCES


Table 1. Demographics and clinical characteristics of included patients

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<th>Mean</th>
<th>Median</th>
<th>Range</th>
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<tr>
<td>Age (years)</td>
<td>60.9</td>
<td>63</td>
<td>21-86</td>
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<tr>
<td>Duration of symptoms (years)</td>
<td>9.7</td>
<td>8</td>
<td>0.5-40</td>
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<td>Female sex (%)</td>
<td>49</td>
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<td>Isolated SFN (%)</td>
<td>64.5</td>
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<tr>
<td>Mixed neuropathy* (%)</td>
<td>35.5</td>
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<tr>
<td>Neuropathic pain (%)</td>
<td>89</td>
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<td>QST performed (%)</td>
<td>73.5</td>
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<tr>
<td>Skin biopsy performed† (%)</td>
<td>63.2</td>
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GLA, alpha-galactosidase A gene; QST, quantitative sensory testing; SFN, small fiber neuropathy; TTR, transthyretin gene.

* Based on nerve conduction studies or in 2 cases where nerve conduction studies were lacking, clinical examination indicating large fiber involvement.

†Skin biopsy was performed in Denmark, Norway and Finland only.
Figure legend.

**Figure 1. Flowchart of included and excluded patients in each country**

The exclusions were due to:

a. Still’s disease, not SFN phenotype and hypothyroidism

b. Active ulcerative colitis, hypothyroidism diagnosis coincidental with start of SFN symptoms, earlier screened GLA mutation, alcohol abuse

c. Coeliac disease, systemic connective tissue disorder

d. Diabetes mellitus less than 2 years after onset of SFN, rheumatic disorder, Sjögren’s syndrome, not SFN phenotype, active thyroid disease (Graves’ disease), polyneuropathy associated with hematologic conditions (MGUS and cold agglutinin antibodies), alcohol abuse (two patients)