Autosomal dominant hereditary sensory neuropathy with chronic cough and gastro-oesophageal reflux: clinical features in two families linked to chromosome 3p22–p24

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Autosomal dominant hereditary sensory neuropathy (HSN I) is a clinically and genetically heterogeneous group of disorders, and in some families it is due to mutations in the serine palmitoyltransferase (SPTLC1) gene. We have characterized two families with HSN I associated with cough and gastro-oesophageal reflux (GOR). From a large Australian family, 27 individuals and from a smaller family, 11 individuals provided clinical information and blood for genetic analysis. Affected individuals had an adult onset of paroxysmal cough, GOR and distal sensory loss. Cough could be triggered by noxious odours or by pressure in the external auditory canal (Arnold’s ear–cough reflex). Other features included throat clearing, hoarse voice, cough syncope and sensorineural hearing loss. Neurophysiological and pathological studies demonstrated a sensory axonal neuropathy. Gastric emptying studies were normal, and autonomic function and sweat tests were either normal or showed distal hypohidrosis. Cough was likely to be due to a combination of denervation hypersensitivity of the upper airways and oesophagus, and prominent GOR. Most affected individuals were shown on 24 h ambulatory oesophageal pH monitoring to have multiple episodes of GOR, closely temporally associated with coughing. Hoarse voice was probably attributable to acid-induced laryngeal damage, and there was no evidence of vocal cord palsy. No other cause for cough was found on most respiratory or otorhinological studies. Linkage to chromosome 3p22–p24 has been found in both families, with no evidence of linkage to loci for known HSN I, autosomal dominant hereditary motor and sensory neuropathy, hereditary GOR or triple A syndrome. These families represent a genetically novel variant of HSN I, with a distinctive cough owing to involvement of the upper aerodigestive tract.

Keywords: skin biopsy; neuropathy; hereditary neuropathy; epidermal nerve; autonomic dysfunction

Abbreviations: ANA = anti-nuclear antibody; BAERs = brainstem auditory evoked responses; CMAP = compound motor action potential; CMT = Charcot–Marie–Tooth; ENA = extractable nuclear antigens; ENFD = epidermal nerve fibre density; ENT = ear nose and throat; FESST = fibreoptic endoscopic evaluation of swallowing and sensory testing; GIT = gastrointestinal tract; GOR = gastro-oesophageal reflux; HMSN = hereditary motor and sensory neuropathy; HSAN = hereditary sensory and autonomic neuropathy; HSN = hereditary sensory neuropathy; LOS = lower oesophageal sphincter; NCS = nerve conduction studies; NGF = nerve growth factor; QST = quantitative sensory testing

Introduction

In this study, we identified two families with the combination of a dominantly inherited sensory axonal neuropathy with cough and gastro-oesophageal reflux (GOR), with intermittent throat clearing, hoarse voice and sensorineural hearing loss. These families have shown no linkage to any known loci for hereditary neuropathy or GOR, but linkage to chromosome 9q22–22.3 has been found in both families.

Hereditary sensory neuropathies (HSNs) are a heterogeneous group of disorders, which generally present with a sensory axonal neuropathy and a variety of other neurological deficits. As autonomic involvement is a feature of many HSN types, the term hereditary sensory and autonomic neuropathy (HSAN) is often used. In the Dyck classification of 1984 (HSAN types I–V), type I is the autosomal dominant adult-onset form (Dyck, 1993). HSN I is typified by distal sensory loss with acral ulceration and mutilation. Some patients experience lancinating pains, while others can have prominent dysesthesia and burning extremities (Denny-Brown, 1951; Dyck, 1993). The autonomic involvement in HSN I is usually confined to distal hypohidrosis. More severely affected HSN I patients also develop motor weakness and wasting. The additional features seen in some HSN I families have included sensorineural deafness, dementia, mental retardation, spasticity, ataxia, cataracts and skin lesions (Flynn and Aird, 1965; Horoupian, 1989; Dyck, 1993; Heckmann et al., 1995; Wright and Dyck, 1995; Hojo et al., 1999).

Most HSN I pedigrees have been found to be caused by mutations in the serine palmitoyl transferase type 1 (SPTLC1) gene on chromosome 3p22–24 (Nicholson et al., 1993; Heckmann et al., 1999). However, none of the previously published GOR families has had sensory neuropathy or cough. Cough has been reported as a feature of neurological disorders that affect the autonomic nervous system. These include the Holmes–Adie syndrome (Kimber et al., 1998), acute panautonomic neuropathy (Sannomiya et al., 1989), vagal mononeuropathy (Jardine et al., 2000) and a variant of neuropathy associated with the Thr124Met mutation in the peripheral myelin protein zero (MPZ) gene (Baloh et al., 2004).

As yet, two forms of dominantly inherited GOR have been identified: GOR with spastic paraparesis, amyotrophy and cataracts linked to chromosome 10q23–q24 (Ser et al., 1999), and a severe paediatric form linked to chromosome 13q14 (Hu et al., 2000). However, none of the previously published GOR families has had sensory neuropathy or cough. Cough has been described as a feature of neurological disorders that affect the sensory autonomic nervous system. These include the Holmes–Adie syndrome (Kimber et al., 1998), acute panautonomic neuropathy (Sannomiya et al., 1989), vagal mononeuropathy (Jardine et al., 2000) and a variant of neuropathy associated with the Thr124Met mutation in the peripheral myelin protein zero (MPZ) gene (Baloh et al., 2004).

Hoarse voice has been described in a number of hereditary neuropathies, in particular HMSN IIC. In this disorder, recently mapped to a locus on chromosome 12q23–24, hoarseness has been shown to result from vocal cord paralysis, and can be accompanied by diaphragmatic weakness and respiratory failure (Dyck et al., 1994). Other conditions involving vocal cord palsy include the autosomal recessive HMSN IVA, associated with mutations in the ganglioside differentiation, associated protein 1 (GDA1) gene (Cuesta et al., 2002; Sevilla et al., 2003), and autosomal dominant hereditary motor neuropathy type VII, also known as distal hereditary motor neuropathy with vocal cord paralysis (DHMNVP), which has been mapped onto chromosome 2q14 (Young and Harper, 1980; McIntagart et al., 2001).
Here we describe the clinical, neurophysiological and neuropathological findings in two families with a distinct variant of autosomal dominant HSN with GOR, cough, hoarse voice and sensorineural hearing loss, linked to chromosome 3p22–p24. Full details of genetic studies have been reported elsewhere (Kok et al., 2003).

Patients and methods
Two unrelated families have been identified with features of sensory neuropathy associated with cough and GOR. Both families are descendants of ancestors in England, and reside predominantly in eastern Australia, but with the majority of each family based in different states from one another. Subjects were classified as 'definitely affected' if they had evidence of a sensory neuropathy, and at least one of the features of chronic cough and/or significant GOR. Our minimal diagnostic requirement for sensory neuropathy was the presence of clinical signs of distal sensory loss, with an axonal neuropathy on nerve conduction studies (NCS) whenever these were performed and were abnormal; cough was required to have been present for at least 1 year, with no medication or condition found to account for the cough; and significant GOR was defined as symptoms of either heartburn, acid brash or regurgitation at least once per week. Individuals with cough or GOR, but no evidence of neuropathy were classified as 'possibly affected'. Assuming autosomal dominant inheritance, those with none of the above clinical features by the age of 35 years were classified as 'unaffected'.

Twenty-seven family members from Family 1 provided blood for genetic analysis, of whom eight were definitely affected and four were possibly clinically affected. This family is depicted in Pedigree 1 (Fig. 1A). All affected and possibly affected subjects and seven others were examined clinically (P.J.S., Sapphire, Medelec). The remainder provided written and/or telephone histories. Eleven family members from the second family, of whom two were affected, and two were possibly affected, also provided blood samples and clinical information. The second family is depicted in Pedigree 2 (Fig. 1B). Further investigations were undertaken as described below with the numbers studied from Families 1 and 2 given in brackets (1:2). All subjects provided written consent in accordance with the Declaration of Helsinki to be involved in this study, which was part of several larger projects approved by the Human Ethics Committees of the University of Sydney, and the Royal Prince Alfred and Concord Hospitals, Sydney. Approval had been granted for control or unaffected subjects to undergo clinical and neurological studies, and genetic testing.

Neurophysiology
NCS of upper and lower limb sensory and motor nerves were performed by one operator using standard techniques (P.J.S.; or for subjects in the state of Queensland, Medelec 100) (11:3). These included bilateral orthodromic sural sensory studies, ulnar sensory and motor studies, common peroneal and posterior tibial motor studies.

The following autonomic studies were performed: measurement of pulse and blood pressure responses to 60° tilt for 10 min, Valsalva manoeuvre and deep breathing (5:3). Tilt was achieved using a custom-built tilt table. Intermittent blood pressure recordings were taken with a Hewlett Packard automatic sphygmomanometer. Continuous beat-to-beat blood pressure and pulse were recorded with a Finapres device and custom-designed Labview program. A sympathetic skin response was recorded from one hand and foot (Saphhire, Medelec). Thermoregulatory sweat tests (5:3) were performed using

Fig. 1 (A) Family 1 Pedigree. (B) Family 2 Pedigree. Arrow = index case; Blackened: upper left quadrant = sensory neuropathy on nerve conduction studies; upper right = symptoms and signs of sensory neuropathy; lower left = GOR; lower right = cough.
a custom-made bed and a moisture sensitive powder (alizarin red, rice starch, sodium carbonate) applied to the body below the neck.

Quantitative sensory testing (QST) of cold detection threshold (CDT) and heat as pain threshold (HPT) were performed using the CASE IV device (Stillwater, MN) and a 3 cm thermode attached to the dorsum of the foot (9;3). Thresholds were compared with a database of stored normative data and expressed as a percentile score. Based on previous studies, the normal range was considered to be between the 5th and 95th percentiles (Periquet et al., 1999).

Hearing function was evaluated with audiometry (10;2) and brainstem auditory evoked responses (BAERs) (5;0).

Biopsies

A 3 mm skin biopsy was taken using a disposable punch device (KAI) from a site 10 cm above the lateral malleolus. Biopsies were fixed for 12–24 h with PLP solution (paraformaldehyde, lysine, sodium periodate), rinsed with 0.1 M Sorenson’s phosphate then stored for 1–5 days in a cryoprotectant solution (glycerol, 0.1 M Sorenson’s phosphate). Biopsies were cut into 50 μm sections on a freezing microtome (Cryotome E, Life Sciences International, Cheshire, UK) and stored at −1 to 20°C in an antifreeze solution containing glycerol and ethanediol. Skin sections were immunohistochemically stained with a polyclonal antibody to PGP 9.5 (Chemicon), a pan-neuronal marker, using a method as described by McCarthy et al. (1995).

Sections were examined under light microscopy for evaluation of epidermal nerve fibre density (ENFD) testing (5;3). Epidermal length was measured under low power using Zeiss KS400 3.0 (West Germany) Image Analysis software and epidermal nerve fibres were counted under high power (>×10 objective). ENFD was assessed for four non-consecutive sections per subject and expressed in nerves per millimeter. ENFD results were compared with published normative data (McArthur et al., 1998) and normative data from our laboratory, with a lower limit of normal (2 SD below control mean) at 8.0 nerves/mm (unpublished data, Spring PJ and Spies JM).

Sural nerve biopsies were performed on two individuals and processed according to standard techniques. Paraffin sections were prepared after fixation in PSA (picric acid/saline/acetic acid), then 5–6 μm sections were stained with haematoxylin and eosin (H&E). One nerve was also stained with Congo red. Toluidine blue stained sections were prepared by fixing sural nerve in Dalton’s Chrome Oxium solution (sodium chloride, potassium dichromate, osmic acid) then embedding in Spurr’s resin. Semi-thin 0.5 μm sections were cut and stained with toluidine blue. Teased fibre preparations were made after staining a section of formalin-fixed nerve with 1% aqueous osmic acid for 2 h, then transferring the nerve to 2:1 glycerol/water for 12 h.

Blood tests

Some individuals (4:1) had blood tests to exclude other causes of neuropathy including full blood count, electrolytes, liver function tests, calcium, phosphate, ESR, C-reactive protein, electrophoresis, immunoelectrophoresis, anti-nuclear antibody (ANA), anti-double-stranded DNA antibody, extractable nuclear antigens (ENA), thyroid function tests, vitamin B12 and folate levels.

Gastrointestinal, respiratory and laryngeal investigations

Previous GIT, respiratory and ear nose and throat (ENT) investigation results were reviewed. Some individuals underwent further studies including dual probe 24 h ambulatory oesophageal pH monitoring with a subject-triggered cough sensor (6;2) [A.J.I., using a previously described method (Ing et al., 1994)]. Episodes of acid reflux are detected by a reduction in pH to <4 in the distal or proximal oesophagus and these are temporally correlated with episodes of cough. Fibroptic endoscopic evaluation of swallowing and sensory testing (FEESST) (6;3) was also performed (using the method modified from Aviv et al. [1998b]) in order to identify impaired laryngeal sensitivity to air pressure, an indication of either sensory loss or laryngeal acid damage. This study also included examination of the larynx for vocal cord abnormalities and voluntary motor function during phonation and swallowing.

Other GIT investigations in some individuals included gastroscopy (5;2), radionuclide gastric emptying studies (3) and whole gut radionuclide transit study (1). Other causes for cough including asthma and sinusitis were investigated using chest radiographs, sinus computerized tomography (CT) scans and respiratory function testing. These routinely include forced expiratory volume in one second (FEV1), forced vital capacity (FVC), lung volumes, and either histamine or methacholine challenge for bronchial hyperresponsiveness (BHR) (6;3).

Genetic studies

Genomic DNA was extracted from peripheral blood leucocytes using standard techniques.

The families were screened and found to have no mutations in genes of known CMT 1 loci, CMT 1A, 1B and 1D as well as for mutations in the SPTLC gene. Initially, linkage to the following known CMT 2 loci was excluded—2A, 2B, 2D, 2E and 2F. No linkage was found for known GOR loci on chromosomes 10 or 13. A 10 cM genome screen with two-point analysis demonstrated linkage to chromosome 3p22–p24 in Family 1, with a maximum LOD score of 3.51. The second family showed linkage to the same locus, but with a different haplotype. No linkage to chromosome 12 was detected, excluding linkage to the recently identified loci for triple A syndrome, CMT 2C and CMT 2G (Nelis et al., 2004). Further details of the genetic studies are described elsewhere (Kok et al., 2003).

Results

Summaries of the clinical features and results of the above investigations in the two families are provided in Tables 1–4, and Figs 2 and 3. Representative case histories of some affected individuals at the time of entry to the study are summarized below.

Family 1

IV:3—Index case. This 34-year-old man had complained of a recurrent paroxysmal dry cough since age 16. It was often triggered by inhalation of strong odours such as perfume, cigarette smoke, pollen and by eating dry food. There were occasional episodes of cough syncope, and prolonged paroxysmal coughing spasms could be induced by otoscopic examination or pressure of objects such as a cotton bud in the external auditory canal on either side. He had frequent vomiting, at the end of some coughing attacks, post-prandially and at night, but no heartburn. A previous gastroscopy had shown reflux oesophagitis, and other problems included recurrent abdominal pain and diarrhoea diagnosed as irritable bowel syndrome, occasional impotence and dry eyes. From
the age of 31 he had experienced numbness and paraesthesia in the hands and feet with intermittent lancinating pains in the limbs. At age 34 he had a painless burn of the hand with no other history of ulceration or foot deformity. On examination, there was evidence of a predominantly small fibre, glove and stocking sensory neuropathy.

III:17. This 39-year-old woman is the paternal aunt of the index case. She had experienced a worsening paroxysmal dry cough since age 20, often triggered by cigarette smoke and eating. Episodes occurred up to four or five times per day often leading to a choking sensation, and she frequently cleared her throat. There was only infrequent heartburn but she occasionally regurgitated undigested food with postprandial coughing. Tactile stimulation of the left external auditory canal could trigger a coughing spasm. From the age of 35 she had noticed hyperaesthesia over her fingertips and numbness of the toes, mainly on the right. At that stage she had a normal neurological examination apart from subtle reductions in pin prick, temperature and vibration sensation to the wrists and mid-shins with anaesthesia over the right great toe. Reflexes and power were intact and NCS were normal apart from absent sural responses. Fifteen months later she had begun to develop painless superficial injuries of the hands and feet, and had severe lancinating pains in the hands. There was extensive sensory loss for all modalities up to the shoulders (more extensive on the right than left) and upper thighs with normal reflexes and mild distal hand and foot weakness. Repeat nerve conductions were unchanged apart from low amplitude (1.2 mV) common peroneal motor amplitudes. Refer to Table 2.

III:9. This 57-year-old man is the paternal uncle of the index case. He had a history since age 34 of paroxysmal cough, with similar triggers to his affected relatives, occurring at least four times per day, in addition to frequent throat clearing. Prolonged episodes were associated with presyncope and one had caused a retinal detachment. He had a history of reflux symptoms since age 46 and a previous gastroscopy had shown moderate reflux oesophagitis with Barrett’s metaplasia, subsequently treated with acid suppression. At age 55, although neurologically asymptomatic, he was found to have reduced ankle jerks with signs of a mild distal sensory neuropathy. NCS demonstrated an axonal neuropathy with very small sural sensory amplitudes (1–2 μV). Over the next 2 years he noticed mild paraesthesia in the hands and feet with the
sensation of an ‘elastic band’ around the ankles, and developed glove and stocking sensory loss.

**Family 2**

III:3. This 59-year-old man had a history of severe paroxysmal dry cough since the age of 40 with triggers including cigarette smoke, perfume and insertion of cotton buds in the external auditory canal. He experienced frequent voice hoarseness and throat clearing but no heartburn. From the age of 52 he had been experiencing almost daily episodes of cough-induced syncope or presyncope, resulting in a motor vehicle accident and retinal detachment. Neuropathy symptoms had developed from the age of 49 with burning and tightness in the feet, progressing at age 56 to include the hands. Potency had been reduced for 5 years. On examination he had marked sensory loss to the knees bilaterally, the wrist on the left and elbow on the right.

Additional results are described below.

**Neurological clinical features and NCS**

All of the patients who were classified as affected had symptoms and signs of a sensory neuropathy as summarized in Tables 1 and 4. NCS demonstrated a pure sensory axonal neuropathy in all but one of these subjects (1-III:15), who was one of the youngest in his generation and had a suspected small fibre neuropathy. Motor studies were all normal apart from the reduced common peroneal compound motor action potentials (amp = 0.5 mV, CV = 47 m/s and DL = 5.1 ms). NCS results are presented in Table 2.

**Sural nerve biopsies**

There was evidence of a chronic axonal neuropathy on sural nerve biopsy in two members of Family 1 (III:9, IV:3) (see Figure 1). In one (IV:3), there was a moderately severe loss of myelinated fibres, with the remainder of the fibres being of medium diameter, with occasional small myelinated fibres present. There was no active axonal degeneration or segmental demyelination evident on teased fibre preparations. H&E sections showed no vasculitis or infiltrates, and Congo red staining showed no amyloid deposits. The sural nerve from the other individual (III:9) showed similar, but more severe findings (Fig. 2A).

**Skin biopsies and quantitative sensory testing**

All six subjects with a neuropathy who underwent skin biopsy had markedly reduced or absent ENFD. Some dermal nerves
Table 3 Results of other investigations in the two families

<table>
<thead>
<tr>
<th>Person</th>
<th>ENT larynx</th>
<th>Fibroptic motor study larynx</th>
<th>FEESST-laryngeal threshold (mm Hg)</th>
<th>pH study—time pH &lt;4 (% of study)</th>
<th>pH study—no. of distal GOR episodes</th>
<th>pH study—no. of proximal GOR episodes</th>
<th>Respiratory studies</th>
<th>Other</th>
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<td>Family 1</td>
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<td>II:2</td>
<td>N</td>
<td>N</td>
<td>&gt;10</td>
<td>13.8</td>
<td>39†</td>
<td>NP</td>
<td>CXR, N</td>
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<tr>
<td>III:5</td>
<td>N</td>
<td>N</td>
<td>&gt;10</td>
<td>13.8</td>
<td>39†</td>
<td>NP</td>
<td>Gastroscopy +ve</td>
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<td>N</td>
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<tr>
<td>III:8</td>
<td>N</td>
<td>N</td>
<td>&gt;10</td>
<td>10.9</td>
<td>70†</td>
<td>NP</td>
<td>Gastroscopy—N</td>
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<tr>
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<td>N</td>
<td>&gt;10</td>
<td>10.9</td>
<td>70†</td>
<td>NP</td>
<td>Sinus CT—N</td>
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<tr>
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<td>N</td>
<td>&gt;10</td>
<td>7.7</td>
<td>7.7</td>
<td>NP</td>
<td>Gastroscopy—Barrett’s Barium swallow—N</td>
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<td>III:12</td>
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<td>N</td>
<td>&gt;10</td>
<td>2 (d) &lt; 0.1 (p)</td>
<td>62†</td>
<td>2</td>
<td>Gastroscopy—N</td>
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<td>&gt;10</td>
<td>2 (d) &lt; 0.1 (p)</td>
<td>62†</td>
<td>2</td>
<td>Sinus CT—N</td>
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<tr>
<td>III:17</td>
<td>N</td>
<td>N</td>
<td>&gt;10</td>
<td>2 (d) &lt; 0.1 (p)</td>
<td>62†</td>
<td>2</td>
<td>Sinus CT—N</td>
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<tr>
<td>IV:3</td>
<td>N</td>
<td>N</td>
<td>&gt;10</td>
<td>2.6 p.p.i.</td>
<td>44†</td>
<td>NP</td>
<td>Gastroscopy +ve Gastric emptying study—N Whole gut scintigraphy—IBD Oesophageal manometry—Mild peristaltic defect</td>
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<td>IV:8</td>
<td>N</td>
<td>N</td>
<td>&gt;10</td>
<td>9.5</td>
<td>4 (14+)</td>
<td>NP</td>
<td>Mild BHR</td>
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<td></td>
<td>Gastroscopy +ve Oesophageal manometry—mild peristaltic defect</td>
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<td>Family 2</td>
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<td>II:2</td>
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<td>N</td>
<td>&gt;10</td>
<td>13.8</td>
<td>39†</td>
<td>NP</td>
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<tr>
<td>III:3</td>
<td>N</td>
<td>N</td>
<td>&gt;10</td>
<td>98 (d) 4.2 (p) &gt;100†</td>
<td>76</td>
<td>Mild BHR-15% reversibility</td>
<td>Gastroscopy +ve Oesophageal manometry—Hiatus hernia</td>
<td></td>
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<tr>
<td>III:7</td>
<td>N</td>
<td>N</td>
<td>&gt;10</td>
<td>8.5</td>
<td>8.5</td>
<td>8.5</td>
<td>N</td>
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N = normal; ENT larynx = visual examination of larynx by ear, nose and throat surgeon; FEESST = fibreoptic endoscopic evaluation of swallowing and sensory testing; laryngeal threshold = air pressure required to initiate laryngeal adductor reflex (normal = 2.5–4 mm Hg, >4 = impaired laryngeal sensitivity); pH study = ambulatory oesophageal pH study for 24 h; definite evidence of GOR = pH <4 in distal oesophagus for >3% of 24 h period, and/or pH <4 in proximal oesophagus for >0.3% of 24 h period, and/or number of distal GOR episodes >31 in 24 h; (d) = distal, (p) = proximal, p.p.i. = study performed while patient taking proton pump inhibitor e.g. omeprazole; NP = not performed; Respiratory studies = chest X-ray (CXR) and lung function tests—forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), FEV1/FVC ratio, lung volumes and methacholine or histamine challenge for bronchial hyperreactivity (BHR); Regarding gastroscopy: +ve = oesophagitis; regarding whole gut scintigraphy: IBS = increased transit rate consistent with irritable bowel syndrome. † = cough temporally related to GOR in at least 50% of coughing episodes; (*) = extrapolated number per 24 h—patient pulled out probe at 7 h.

Table 4 Summary of typical features of the syndrome

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>Description</th>
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<tr>
<td>Cough</td>
<td>Onset between second and fourth decade paroxysmal, dry, with episodes up to 60 times per day; often precipitated by inhalation of fumes, eating dry food, lying flat or tactile stimulation of external auditory canal; Intermittent hoarse voice or throat clearing frequently; Attributable to GOR as confirmed oesophageal pH monitoring.</td>
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<td>GOR</td>
<td>Generally mild heartburn only, or no typical GIT symptoms; Regurgitation and acid brash in more severe cases; Distal and often proximal GOR on pH study.</td>
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<td>Sensory neuropathy</td>
<td>Onset between 5 and 39 years after onset of cough, i.e. fourth, sixth decade; Variable presentations including; small fibre symptoms e.g. burning; Almost asymptomatic sensory loss and superficial painless injuries; Lancing pains, or truncal neuropathy; No acral ulcers, mutilation, joint deformity, pes cavus or gait abnormality; Mainly distal pain and temperature loss with normal reflexes in most; Pure sensory axonal neuropathy on nerve conduction studies; Autonomic studies normal apart from sweating &amp;/or mild possible adrenergic impairment in a minority; Bilateral high frequency sensorineural hearing loss in 50%, with no other cranial nerve dysfunction.</td>
</tr>
</tbody>
</table>
Fig. 2  (A) Sural nerve biopsy from subject I–III:9, toluidine blue stain, ×40 magnification, demonstrating severe axonal loss with some cluster formation (arrow); (B–D) Skin biopsies stained with antibody to PGP 9.5, 40× magnification, showing epidermal (arrow) and dermal (arrowhead) nerve fibres.  (B) Subject 1–IV:3—reduced epidermal nerve fibre density of 2.4 nerves/mm.  (C) Subject 1–III:5—epidermal nerve fibre density 0.1 nerves/mm, but dermal fibre only in this section.  (D) Control subject—epidermal nerve fibre density normal at 14.8 nerves/mm.  Scale bar = 20 μm.

Fig. 3  Ambulatory oesophageal pH study.  This graph is from the 24 h study performed on subject III:5 from Family 1.  Time of day is represented on the x-axis and pH on the y-axis.  The subject pressed a trigger during each episode of cough, as indicated by vertical lines marked C.  Episodes of cough were closely temporally related to episodes of GOR (pH <4).
were usually seen, but the innervation of sweat glands appeared qualitatively to be somewhat reduced compared with controls. In these subjects, QST confirmed the marked small fibre dysfunction with a majority (n = 5) having elevated CDTs. Two individuals, (1-III:11, 2-III:7), who were considered possibly affected due to cough and/or GOR without neuropathy, had skin biopsies and QST. All of these studies, as well as QST in the family control subject, were normal. Results of skin biopsies and quantitative sensory testing are shown in Table 2, and skin biopsies are depicted in Fig. 2B and C, with a control skin biopsy in Fig. 2D.

**Autonomic function and thermoregulatory sweat tests**

Autonomic studies were performed on eight individuals, of whom two were possibly clinically affected (1-III:11, 2-III:7), and the remainder were definitely affected. In general, autonomic studies were normal apart from a decreased or absent late Phase 2 of the Valsalva profile, indicative of possible mild peripheral adrenergic impairment. Thermoregulatory sweat testing was normal apart from two patients with a marked sensory neuropathy. In one (1-III:17), asymmetrical reduction in sweating over the right arm corresponded to an area of marked sensory loss, and the other (2-III:2) had distal hypohidrosis. The sympathetic skin response was abnormal in only one affected individual (1-III:5). See Table 2 for further details.

**Hearing studies**

Bilateral high frequency sensorineural hearing loss was evident on audiograms in six definitely affected individuals, but BAERs were generally normal.

**‘Neuropathy screen’ blood tests**

The neuropathy blood workup was negative in all patients tested apart from a positive ANA in one individual (1-IV:3, titre 1:1280), but all other autoimmune and inflammatory markers were normal. Serum electrolytes were normal.

**Ocular studies**

As two subjects (III:5, IV:3) had complained of sicca symptoms with a normal ENA, one (IV:3) underwent a Schirmer’s test and was found to have markedly impaired lacrimation (1, 0.5 mm). Ocular examination demonstrated no evidence of tonic pupils in any affected or unaffected subjects.

**Other neurological studies**

One individual (1-IV:3) later underwent investigation of proximal muscle pain and a mildly elevated creatine kinase level (689, range 5–200 U/L). Needle EMG and a quadriceps muscle biopsy were normal including staining to exclude a mitochondrial myopathy and metabolic muscle disease. This subject (1-IV:3) also had a fluctuating reduction in luteinizing hormone level but a cerebral MRI scan demonstrated no abnormalities of the pituitary or cerebral brain parenchyma. A second patient (2-III:2) also had a normal lower limb EMG.

**Gastrointestinal respiratory and laryngeal studies**

Of the patients with cough who were tested with 24 h oesophageal pH studies, two were considered possibly affected (1-III:11, 1-IV:8), and the remainder were classified as definitely affected. All but one of the studies demonstrated evidence of significant GOR. The remaining study was inconclusive, as the possibly affected individual (1-IV:8) was unable to tolerate the nasal tube for longer than 7 h. On FEESST testing, all patients tested had markedly elevated laryngeal thresholds for reflex vocal cord adduction in response to applied air pressure, indicative of impaired laryngeal sensitivity. The majority of affected and possibly affected individuals underwent either an ENT laryngeal inspection or formal laryngeal motor studies. None of them had any visible changes in the vocal cords or any motor dysfunction with phonation or swallowing. No underlying cause for cough, other than GOR, was found in seven subjects tested as summarized in Table 3, and two individuals had a persistent cough despite optimal medical treatment of mild asthma or bronchial hyperresponsiveness (2-III:2 and III:3).

**Discussion**

We have described two families with a genetically novel form of autosomal dominant HSN, with prominent GOR-induced cough.

With regard to the sensory neuropathy, this was quite variable between individuals, even within one generation. The sensory neuropathy bears some resemblance to the other dominant HSNs, the common form of HSN I and HMSN IIb, but it is distinguished by the lack of motor involvement in most cases and also of acral mutilation and ulceration (Hicks, 1922; Dyck, 1993; De Jonghe et al., 1997; Elliott et al., 1997; Auer-Grumbach et al., 2000a). The latter also distinguishes it from most of the recessive HSANs (II, IV and V), although, the predominance of small fibre involvement is similar to that seen in HSAN III, IV and V (Dyck, 1993; Hilz, 2002). Table 5 compares in detail the features of HSN with cough/GOR with the most closely related HSNs—those that are dominantly inherited, as well as the recessive HSANs with predominantly small fibre involvement but no generalized autonomic neuropathy, HSANs IV and V. Of particular note, the lancinating pains, sensorineural deafness and a degree of asymmetry of the sensory loss or pattern of hypohidrosis (subjects 1-III:17 and 2-III:3) were reminiscent of features seen in HSN I, sometimes described as ‘hereditary sensory radicular neuropathy’ (Denny-Brown, 1951; Wallace, 1970).

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Table 5 Comparison of HSN with cough + GOR with other inherited sensory neuropathies

<table>
<thead>
<tr>
<th></th>
<th>HSN with cough + GOR</th>
<th>HSN I</th>
<th>HMSNIIB</th>
<th>HSAN IV</th>
<th>HSANV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inheritance</td>
<td>AD</td>
<td>AD</td>
<td>AD</td>
<td>AR</td>
<td>AR</td>
</tr>
<tr>
<td>Onset first decade</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lancinating pains</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hyperthermia/fevers</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Painless injury</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Acral ulcers</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Acral mutilation</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Mental retardation</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Sensory loss</td>
<td>SF, or SF &gt; LF</td>
<td>SF &gt; LF</td>
<td>SF + LF</td>
<td>SF</td>
<td>Pain or No</td>
</tr>
<tr>
<td>Motor signs</td>
<td>Usually no</td>
<td>Yes (&lt; sensory)</td>
<td>Yes (prominent, early)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Ankle jerk reflexes</td>
<td>N, or reduced in minority</td>
<td>Often reduced</td>
<td>Reduced or absent</td>
<td>Usually N</td>
<td>N</td>
</tr>
<tr>
<td>Pes cavus</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cranial nerves</td>
<td>+/- SN deafness</td>
<td>+/– SN deafness</td>
<td>No</td>
<td>Reduced sensation of tongue</td>
<td>No</td>
</tr>
<tr>
<td>NCS</td>
<td>Usually SAN, rarely N or mild motor changes</td>
<td>Elevated CT, HPT variable</td>
<td>Elevated CT / HPT</td>
<td>Elevated CT, Insensate to pain</td>
<td>Normal CT Insensate to pain</td>
</tr>
<tr>
<td>Autonomic</td>
<td>Fibre loss—small &gt; large myelinated</td>
<td>Fibre loss—small &gt; large myelinated</td>
<td>Fibre loss—small and large myelinated; onion bulbs (de/remyelination)</td>
<td>Fibre loss—small only: thinly myelinated &gt; unmyelinated</td>
<td>N/increased sweating</td>
</tr>
<tr>
<td>Sural nerve biopsy</td>
<td>Fibre loss—small &gt; large myelinated</td>
<td>Fibre loss—small &gt; large myelinated</td>
<td>Fibre loss—small and large myelinated; onion bulbs (de/remyelination)</td>
<td>Fibre loss—small only: thinly myelinated &gt; unmyelinated</td>
<td>Fibre loss—small only: thinly myelinated &gt; unmyelinated</td>
</tr>
<tr>
<td>Skin biopsy</td>
<td>Reduced or absent EF, reduced DF/SG innervation</td>
<td>?</td>
<td>?</td>
<td>Absent EF, absent or severely reduced DF/SG nerves</td>
<td>?</td>
</tr>
<tr>
<td>References</td>
<td>(Hicks, 1922; Denny-Brown, 1951; Wallace, 1970; Dyck, 1993; Hilz, 2002)</td>
<td>(De Jonghe et al., 1997; Elliott et al., 1997; Auer-Grumbach et al., 2000b)</td>
<td>(Swanson, 1963; Goebel et al., 1980; Itoh et al., 1986; Rosenberg et al., 1994; Nolano et al., 2000; Verze et al., 2000; Hilz, 2002)</td>
<td>(Dyck et al., 1983; Indo et al., 1996; Hilz, 2002; Houlden et al., 2004; Einarsdottir et al., 2004; Minde et al., 2004)</td>
<td>(Dyck et al., 1983; Indo et al., 1996; Hilz, 2002; Houlden et al., 2004; Einarsdottir et al., 2004; Minde et al., 2004)</td>
</tr>
</tbody>
</table>

AD = autosomal dominant; AR = autosomal recessive; SF = small fibre modalities (pain and temperature sensation); LF = large fibre modalities (light touch; vibration and proprioception); N = normal; SN = sensorineural; SAN = sensory axonal neuropathy; SMAN = sensorimotor axonal neuropathy; CDT = cold detection threshold; HPT = heat pain threshold; EF = epidermal fibres; DF = dermal fibres; SG = sweat gland; ? = no published results available for genetically proven cases.
sudomotor fibre pathology, and minor peripheral adrenergic disturbance (McLeod, 1992). These findings are similar to those in HSN I and HSAN IV (Dyck, 1993; Hilz et al., 1999). Symptoms of impotence, urinary urgency, impaired lacrimation and lower gastrointestinal disturbances were seen in a minority of cases and may or may not have been related to the neuropathy. However, the index case in the MPZ family with cough, tonic pupils and autonomic features (Baloh et al., 2004) had a very similar combination of symptoms, raising the possibility that these may also be a component of the disorder in our subjects. Certainly, the extent of autonomic involvement was much more limited in our families than that seen in the HSANs with GIT disturbance (II, III) (Axelrod et al., 1991; Hilz, 2002).

With regard to the paroxysmal cough, the stereotyped features of attacks being triggered by inhalation of noxious odours and by tactile stimulation of the external auditory canal (in seven subjects) were remarkably similar to the descriptions of the MPZ mutation family members (Baloh et al., 2004). The latter phenomenon of the vagally-mediated ‘Arnold’s ear–cough reflex’ occurs in 1.7–4.2% of the general population, but its occurrence in multiple family members would be unusual (Bloustitne et al., 1976; Gupta et al., 1986; Feldman and Woodworth, 1993; Tekdemir et al., 1998). Vagal somatic sensory afferents from the thoracic structures and ear synapse in the nucleus of the tractus solitarius, with connections to the vagal efferent supply to respiratory muscles, forms the cough reflex pathway (Canning, 2002). Vagal afferents from the oesophagus also synapse in the medial part of the nucleus of the tractus solitarius via the paratrigeminal nucleus (Suwanprathes et al., 2003). Baloh et al. hypothesized that a prominent ear–cough reflex may result from impaired C fibre sensory innervation of either the upper airways or oesophagus, leading to ‘denervation hypersensitivity of the secondary neurons in the nucleus solitarius’ (Baloh et al., 2004), and the latter may be the case in our families. Coughing episodes were often very violent and prolonged, causing syncope, retinal detachment and driving impairment, and may be another example of reflex hypersensitivity. Syncope has been reported to occur, however, with various types of paroxysmal cough, including in a case of sporadic GOR-induced cough syncope (Puetz and Vakil, 1995).

In our families, we believe that another major contributing factor to the cough was GOR. Cough, as a manifestation of GOR, is well-recognized but uncommon, and can be identified by oesophageal pH monitoring demonstrating a close temporal relationship between episodes of low pH and coughing (Irwin and Richter, 2000). The most likely causes for this type of cough are thought to be either triggering of an oesophageal–tracheo–bronchial reflex via acid in the distal oesophagus, microaspiration of acid into the upper airways or a combination of the two mechanisms (Ing et al., 1991).

The presence of frequent throat clearing and an intermittently hoarse voice was indicative of impaired vocal cord function. This was also likely to be directly related to proximal reflux-induced laryngitis as, unlike the changes seen in several hereditary neuropathies with vocal cord palsy (HMSN IIC, HMSN IVA and DHMNVP), there was no evidence of motor dysfunction of the vocal cords on laryngoscopic examination nor any respiratory muscle weakness (Young and Harper, 1980; Dyck et al., 1994; Ylitalo et al., 2001; McEntagart et al., 2002; Sevilla et al., 2003). Studies such as FEESST have shown that subjects with GOR-induced laryngitis have a pure sensory deficit with an elevated laryngeal threshold to air pressure compared with controls probably secondary to acid-induced damage (Jacob et al., 1991; Aviv et al., 1998a). In our subjects, however, the possibility that this sensory deficit was instead a primary phenomenon could not be excluded.

With regard to the correlation between disease phenotype and haplotype, a comparison of the clinical features and the genetic results (Kok et al., 2003) produced several observations. In the two families, all 10 subjects with neuropathy also had the disease haplotype as well as the typical throat clearing, paroxysmal cough and GOR. The severe cases of cough also only occurred within this group of 10. However, in Families 1 and 2, a small number of individuals without neuropathy, but with either only cough and/or reflux (1:III-12, 1:IV-8, 2:III-7) or no symptoms (1:IV-10), still carried the disease haplotype. The absence of neuropathy in these individuals is most likely to be explainable on the basis that in all other cases with the full phenotype the neuropathy always presented later than the cough and GOR with a time delay of up to ~40 years. As subject I:IV-10 is in her early twenties, she may still develop clinical features later in life, as may the other three individuals currently without neuropathy. Conversely, a small number of cases of GOR with milder cough occurred in otherwise unaffected individuals without the disease haplotype. This would not be surprising, given the high rates of GOR in the general community, with at least weekly symptoms in up to 21% (Nebel et al., 1976). Several sporadic GOR cases could be expected in any large family.

Overall, there is still a small possibility that cough/GOR and neuropathy may be occurring by chance in affected individuals. Against this are the unexpectedly large number of cough/GOR cases, and the very close correlation between these features, the neuropathy and the presence of the haplotype in two different families. The two distinct haplotypes in the families also make a founder effect very unlikely (Nicholson et al., 2001). For future diagnostic purposes, evidence of a sensory neuropathy would be the most specific predictor of the presence of the haplotype, but the onset of the typical cough would be the earliest and most sensitive.

The explanation for the apparent association between a sensory neuropathy and GOR remains unclear. In several individuals with normal gastric emptying, oesophageal manometry demonstrated no abnormality apart from minor reductions in the amplitude of the primary peristaltic waves after swallowing. Combined with a possible alteration in the pattern of lower oesophageal sphincter (LOS) relaxations, this may be the mechanism for GOR in our families (Dent, 1997). Control of LOS function involves a complex
interaction between the extrinsic (mainly vagal parasympathetic and sympathetic) innervation and the enteric nervous system (Kahrilas, 1995). A possible length-dependent degeneration of the vagal sensory supply to the distal oesophagus and LOS could contribute to dysregulation of LOS control and a sensory deficit with reduced perception of acid as painful. This may account for the unusual propensity of the family members to have minimal heartburn despite severe proximal reflux. Further studies of the innervation or sensory function of the lower oesophagus in these families may help to elucidate the nature of this defect.

In these two families with a dominantly inherited HSN, linkage to known HSN I and CMT loci was excluded, and linkage to a novel site on chromosome 3p22–p24 was found. We hypothesize that, like the recent report of the family with an MPZ mutation (Baloh et al., 2004), cough and GOR may be associated with a variety of hereditary neuropathies, particularly those with prominent sensory involvement.

The future identification of further families is of particular relevance for several reasons. Further work is required to identify the underlying genetic defect in our families, and this may lead to improvements in knowledge of neural structure or function. In addition, effective therapies for GOR are available, such as acid suppression or surgery, and the commencement of these may be delayed if this association with cough and neuropathy is not recognized. As seen in one of our cases, prolonged untreated GOR can also lead to Barrett’s oesophagus, a potentially premalignant condition. Some hereditary neuropathies, if associated with an increased frequency of GOR, may represent an inherited risk factor for cancer, making early diagnosis and treatment very important.

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References

Clinical features of HSN with GOR and cough


