SHORT REPORT

New mutations, genotype phenotype studies and manifesting carriers in giant axonal neuropathy

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Giant axonal neuropathy (GAN; MIM 256850) is a severe childhood onset autosomal recessive sensorimotor neuropathy affecting both the peripheral nerves and the central nervous system. Bomont and colleagues identified a novel ubiquitously expressed gene they named Gigaxonin on chromosome 16q24 as the cause of GAN in a number of families. We analysed five families with GAN for mutations in the Gigaxonin gene and mutations were found in four families; three families had homozygous mutations, one had two compound heterozygous mutations and one family had no mutation identified. All families had the typical clinical features, kinky hair and nerve biopsy. We report some unusual clinical features associated with GAN and Gigaxonin mutations as well as confirm the heterogeneity in GAN and the identification of two families with manifesting carriers.

In 1972, Asbury and Gale[1] and Berg and colleagues[2] described the first case of giant axonal neuropathy (GAN) in a 6-year-old girl with kinky hair who had an age of onset of 3 years with progressive clumsiness, muscle weakness and atrophy, areflexia and sensory loss. Sural nerve biopsy revealed large axonal spheroids densely packed with neurofilaments in both myelinated and unmyelinated fibres. Since the original report that carefully defined GAN, many similar patients and families with this severe autosomal recessive motor and sensory neuropathy have been reported throughout the world.[3]

The majority of cases of GAN have an age of onset of approximately 3 years with developmental delay, patients have similar faces, usually kinky hair, an axonal neuropathy and CNS abnormalities.[3 4] Sural nerve biopsy shows giant axons, a pathological feature that is also found in other neuropathies associated with toxins, such as acrylamide, iminodipropionitrile, methyl-n-butyl ketone and n-hexane.[3] Giant axons have also been described in a litter of German shepherd kinky haired dogs and other forms of Charcot–Marie–Tooth disease, including families with neurofilament light chain[5] and KIAA1985[6] mutations.

The gene causing GAN was identified by Bomont and colleagues[7] and is called Gigaxonin. They identified seven homozygous mutations and eight compound heterozygous mutations in families with GAN. Three typical families with GAN based on clinical and pathological families had no mutation in the Gigaxonin gene,[8] suggesting possible heterogeneity. Mutations in the Gigaxonin gene have been identified in a number of other families.[9–13]

We have identified five families with GAN. Gigaxonin mutations were found in four families. Clinical and pathological details are described in these kindreds and we speculate on the genotype phenotype effect of these Gigaxonin mutations (table 1).

METHODS

Ethics approval was obtained from the joint medical and ethics committee. DNA was extracted from blood samples obtained with informed consent. DNA from family No 5 was obtained from a cell pellet from the Montreal Children's Hospital Research Institute. The 11 Gigaxonin exons and flanking introns were sequenced, as previously described.[7] Mutations were rechecked by sequencing in the proband and other family members. None of the mutations was identified in 100 control cases. The method and primer sequences are available on request.

RESULTS

GAN families (table 1) were identified from England (family No 1), Pakistan (family Nos 2 and 3), Scotland (family No 4) and Canada (family No 5). Sequencing of the Gigaxonin gene revealed three homozygous mutations in the Gigaxonin gene in family Nos 2, 3 and 4. Family No 1 had two different compound heterozygous mutations (table 1). These conserved mutations were frameshift, non-sense or missense and were not present in 100 mixed Caucasian or Asian controls.

No previous Pakistani, English or Scottish GAN families have been reported in the past. No mutations were identified in the Canadian GAN family; this family was clinically typical for GAN with severe childhood axonal neuropathy, age of onset in childhood and typical kinky hair. The fibroblasts from this case have been studied previously and found to have errors in the organisation of intermediate filaments.[14]

Clinical details

All four families with GAN were diagnosed prior to genetic studies. A clinical and electrophysiological, motor more than sensory, axonal neuropathy was present in all families with GAN, as were abnormal CNS findings. Family Nos 1 and 4 had particularly prominent cranial nerve abnormalities. Family No 2 had additional epilepsy and respiratory problems in the most severely affected sibling. Two cousins of the affected siblings in family No 2 also had a similar progressive neurological condition. One of the cousins had the distinctive hair and was found to be a heterozygous carrier of the mutation while the other had normal hair but died before testing was available. In family No 3, the most severely affected sibling had dysphagia, visual problems with dense optic atrophy, and problems with feeding and secretions. Family No 4 had an onset with delayed walking problems and developed early gastrointestinal reflux and regurgitation. Cranial nerve signs were prominent and she was hypotonic with brisk reflexes and scoliosis. MRI of the brain was carried out in family Nos 1, 2 and 4 and showed diffuse high T2 signal abnormalities.

The patient from family No 1 had an age of onset of 2–3 years and from then on developed progressive difficulties, with

Abbreviation: GAN, giant axonal neuropathy

Table 1 Families with giant axonal neuropathy and Gigaxonin mutations identified

Family No	Age of onset	Diagnosis	Affected	Origin	Hair	Nerve biopsy
1	3 y	GAN	1	England	Kinky	GAN (see fig 1)
2	II-1: 4 y II-5: 21 mo	GAN	5	Pakistan	Kinky	GAN
3	II-1: 5 y II-2: 5 y	GAN	2, 2 possibly affected	Pakistan	Kinky	GAN
4	<2 y	GAN	1	Scotland	Kinky	GAN
5	25 mo	GAN	1	Canada	Kinky	GAN
Family No	Exon/intron	Nucleotide change	Amino acid	Domain	Consanguinity	Mutation type
1 (proband and mother)	Exon 5	c.944 CCG to CTG	Pro 315 Leu	Kelch	No	Compound heterozygous
1 (proband and father)	Exon 10	1553/1554 Del TT	Phe 518Fs Probable NMD	Kelch	No	Compound heterozygous
2	Exon 1	c.151 GCC to CCC	Ala 51 Pro	BTB	Yes	Homozygous
3	Exon 10	c.1505 TGG to TAG	Trp 502 Stop Probable NMD	Kelch	Yes	Homozygous
4	Exon 2	c.213 TAT to TAA	Tyr 71 Stop Probable NMD	BTB	No	Homozygous
5	No mutation identified in the coding region or flanking introns	No				

c., cDNA nucleotide numbering; GAN, Giant axonal neuropathy; Fs, frameshift; NMD, non-sense mediated decay.

weakness of his feet and ankles and walking problems. His mother has a diagnosis of ''mild multiple sclerosis'', diagnosed after problems in her legs and eyes. Her MRI was consistent with demyelination and was typical of multiple sclerosis, and lumbar puncture showed unmatched oligoclonal bands. His father was normal and there was no family history of consanguinity. At the age of 24 years the patient had blond frizzy hair that was different to his parents, and he walked with

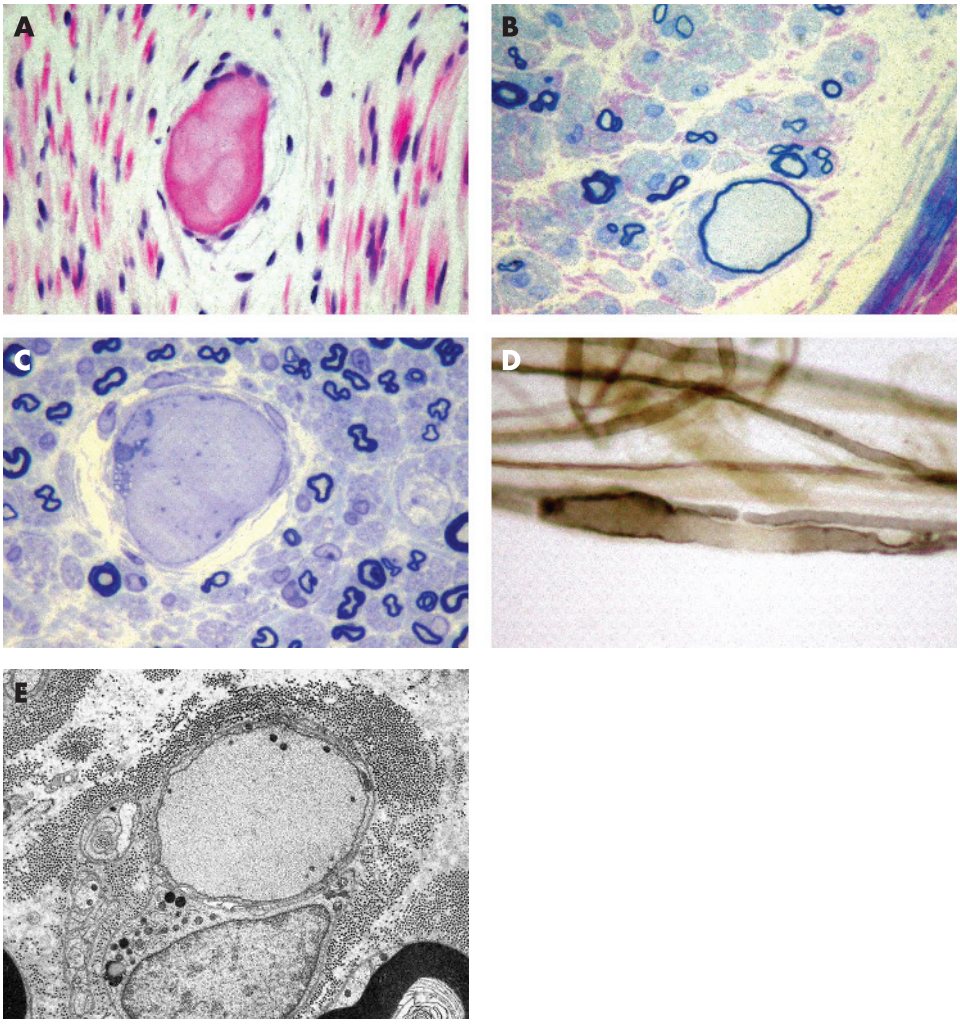

Figure 1 Sural nerve biopsy from the proband of family No 1. Images of paraffin sections (A), resin sections (B, C), teased fibres (D) and electron microscopy (E) of the sural nerve biopsy. (A) Axonal swellings show variable eosinophilia in haematoxylin and eosin stained paraffin sections. (B, C) At least one ''giant'' axon was present in each fascicle: some were surrounded by an attenuated myelin sheath (B) while others were de- or non-myelinated (C). Teased fibres showed secondary demyelination in the region of the axonal swellings (D). Electron microscopy showed that the ''giant'' axons were filled with closely packed neurofilaments and organelles, and that unmyelinated axons may also be involved (E). Scale bar 20 μm (A–D) or 5 μm (E).

bilateral foot drop and an ataxic gait. He had a left ptosis, horizontal nystagmus in all directions, mild bilateral facial weakness and a cerebellar dysarthria. He had a kyphoscoliosis with weakness, wasting and sensory loss in his upper and lower limbs, distal more than proximal.

MRI of the brain showed symmetrical high T2 signal change within the medulla and dorsal pons but no supratentorial parenchymal lesions. Electromyography showed chronic denervation, and nerve conduction studies showed a moderately severe sensory motor axonal neuropathy. The father in family No 1 had normal nerve conduction studies, the mother had an absent response from the extensor digitorum brevis and rather small responses from the abductor pollicis brevis and abductor hallucis, suggesting a slightly patchy peripheral neuropathy. Nerve biopsy showed greatly swollen axons caused by accumulation of neurofilaments. The appearances were consistent with GAN (sural nerve biopsy from the proband shown in fig 1), as was the proband's tight kinky hair that was characteristic of GAN.

Neuropathology

Nerve biopsies were carried out in all five families and were consistent with the diagnosis of GAN. The most striking feature in the nerve biopsies was the abnormally enlarged axons with homogeneous axoplasm and thin or absent myelin sheath. There was no apparent reduction in the number of myelinated fibres (fig 1). Ultrastructural examination showed that the giant axons consisted of closely packed and irregularly oriented neurofilaments, with focal areas of increased density. Both myelinated and unmyelinated axons were affected. Aggregates of neurotubules with mitochondria, vesicles and dense bodies were sometimes confined to the centre or edges of the axoplasm (fig 1E, family No 1 sural nerve biopsy).

DISCUSSION

We have reported four families with previously unidentified Gigaxonin mutations. The majority of Gigaxonin mutations are non-sense or frameshift changes that lead to a premature termination codon. This was the case for three of the mutations identified here (table 1). One family with GAN (family No 5) did not have a Gigaxonin mutation. This confirms the genetic heterogeneity in GAN.

A number of studies have been carried out on the normal functional role and interactions of Gigaxonin and the consequences of mutations and features of null mice.[15] Disruption of the Gigaxonin interacting genes (TBCB, MAP1B and MAP8) varied depending on the location of the mutation in Gigaxonin, suggesting that defects in Kelch repeats caused the greatest TBCB protein disruption compared with the BTB domain or null mutations that caused the greatest MAP1B and MAP8 protein disruption and toxic neuronal accumulation.[15]

The mutations in family Nos 1 and 3 caused a less severe phenotype and later age of onset as opposed to the more severe phenotype and earlier onset in family Nos 2 and 4. There are still relatively few mutations reported to draw significant genotype phenotype conclusions, but predicting complications is important for clinicians and families. Mutations in the BTB domain seem to cause an earlier age of onset, earlier age at loss of ambulation and/or death compared with mutations in the Kelch repeats or non-domain areas. This is shown in family Nos 2 and 4 from this study and from other reported studies (family 19[10]) and the single case from Brockmann and colleagues.[11] An unusual family (Tunisian family II) had a homozygous mutation in the BTB domain (R15S) but the phenotype was distinct, with a progressive multisystem degeneration with giant axons but lack of kinky hair.[7]

Two reports have suggested the possibility of manifesting heterozygous carriers.[9 13] In our series of patients, likely manifesting carriers were identified in family Nos 1 and 2 where the carriers had a point mutation in the Gigaxonin gene, one in the BTB and one in the Kelch region. The reported manifesting carriers[9 13] had a mutation in the Kelch domain (Arg201Stop) and one between the BTB and Kelch domains (Arg293Stop), suggesting that a Gigaxonin mutation may act as a risk factor for axon damage by either a haploinsufficiency or by causing a dominant negative effect and interfering with the normal allele in Gigaxonin carriers.

In summary, we have reported five novel Gigaxonin mutations and their associated clinical phenotypes. Genetic heterogeneity was confirmed and a genotype phenotype effect was suggested, based on the particular domain the mutation affected. We also identified a further two families who had manifesting heterozygous carriers. Further mutation reports and clinical analysis of heterozygous carriers will enable a more detailed evaluation to define the penetrance of Gigaxonin mutations.

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NEUROLOGICAL PICTURE

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The sonographic pitfall of carotid collateralisation via the vasa vasorum

A 52-year-old man with obesity, type 2 diabetes mellitus and hypertension was admitted because of sudden onset of left hemiparesis and somnolence. Brain MRI disclosed a recent right thalamic stroke and multiple encephaloclastic lesions, suggestive of vascular sequelae. Carotid ultrasound demonstrated occlusion of the right internal carotid artery (ICA) and residual flow in the periphery of the left ICA lumen (fig 1A, B). Subocclusive stenosis was suspected. Carotid arteriography confirmed atherosclerotic occlusion of both bulbar ICAs. On the left side, however, filiform collateralisation with a spiral configuration was seen, extending from the ICA bulb to the cavernous segment (fig 1C). The patient was offered medical treatment.

Collateralisation through the vasa vasorum of an atheromatous occlusion of the ICA is a rare finding. Growth of collaterals in the wall of the vessel is a slow process, stimulated by the proangiogenic properties of the plaque.[1]

Differentiating carotid occlusion with collateralisation from pre-occlusive stenosis is of the utmost importance because there is no benefit of endarterectomy or angioplasty in the first situation.

Carotid ultrasound is a reliable method in the assessment of ICA stenosis and occlusion. However, the findings of residual flow with normal velocities and waveform may erroneously be attributed to subocclusive carotid disease[2] in which it has been shown that velocity measurements start to decrease. Therefore, corroboration with arteriography is recommended. High resolution transverse colour coded sections showing segments of flow within the ICA wall itself, outlining the circumference of the vessel may be a clue to the correct diagnosis.

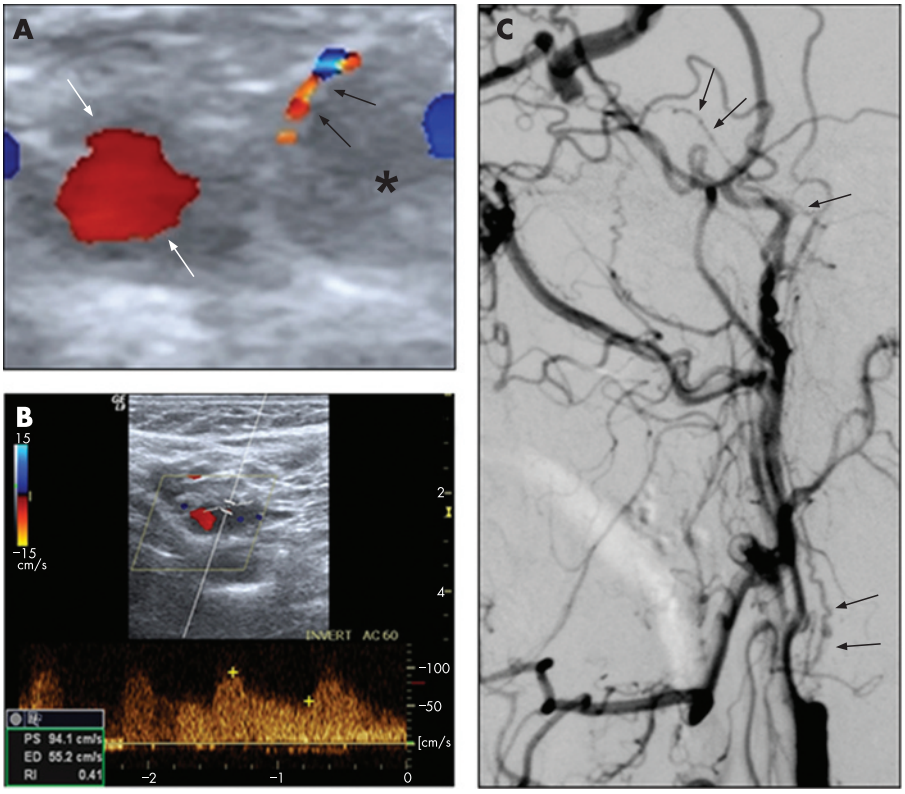

Figure 1 (A) Colour coded duplex ultrasonography, transverse section, 1 cm above the carotid bifurcation, shows the occluded internal carotid artery (ICA) lumen (*) and a thin segment of flow within its wall (black arrows), outlining the perimeter of the artery. External carotid artery is normal (white arrows). (B) Spectral tracing from the same segment as in (A), reveals a normal waveform and velocity. (C) Contrast arteriogram of the left ICA, arterial phase, lateral view, demonstrates a rounded proximal stump. Spiral vasa vasorum (black arrows) originate from the bulb and contribute to the filling of the cavernous segment. The true lumen of the ICA is never filled.

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