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Exome sequencing establishes a gelsolin mutation as the cause of inherited bulbar-onset neuropathy

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Abstract

Introduction—Progressive bulbar motor neuropathy is primarily caused by bulbar-onset ALS. Hereditary amyloidosis type IV also presents with a bulbar neuropathy that mimicks motor neuron disease. The disease is prevalent in Finland only and is not commonly included in the differential diagnosis of ALS.

Methods—We studied 18 members of a family in which some had bulbar motor neuropathy, and we performed exome sequencing.

Results—Five affected family members were found to have a D187Y substitution in the *GSN* gene known to cause hereditary amyloidosis type IV.

Conclusions—This American family presented with progressive bulbar neuropathy due to a gelsolin mutation not found in Finland. Hereditary amyloidosis type IV presents with bulbar motor neuropathy and not with peripheral neuropathy as occurs with common forms of amyloidosis. This report demonstrates the power of exome sequencing to determine the cause of rare hereditary diseases with incomplete or atypical phenotypes.

Keywords

amyloidosis; exome sequencing; gelsolin; corneal dystrophy; amyotrophic lateral sclerosis; motor neuron disease; bulbar neuropathy

INTRODUCTION

The amyloidoses are a disparate group of conditions caused by aggregation of misfolded proteins into insoluble forms that deposit in tissues to produce a collection of symptoms and findings. Amyloidosis can be acquired or hereditary, and the clinical presentation is highly variable in the age and location of onset. Hereditary amyloidosis is caused by mutations that lead to misfolding of the protein and produce abnormal aggregation of fibrils into sheets that in turn are deposited into numerous tissues including blood vessels, peripheral nerves, and the myocardium. The distribution of this deposition explains the clinical symptomatology associated with each type of amyloidosis.

The most common form of hereditary amyloidosis is caused by mutations in the transthyretin gene (*TTR*) and usually presents with a painful sensory and autonomic peripheral neuropathy¹. A less common form of amyloidosis (hereditary amyloidosis-IV) presents with bulbar motor findings that mimic motor neuron disease² and is usually due to a D187N mutation in *GSN*, the gene that codes for gelsolin protein. This form of hereditary amyloidosis-IV is seen nearly exclusively in southern Finland³, but there is a rarer D187Y mutation that has a wider geographic distribution⁴.

Here we describe an American family with a dominantly inherited bulbar motor neuropathy. Because distinguishing features of familial amyloidosis were not present in the initial

patients evaluated (particularly neuropathy and corneal dystrophy), the diagnosis was unclear, and atypical forms of bulbar-onset amyotrophic lateral sclerosis (ALS), Fazio-Londe syndrome, and Brown-Vialetto-van Laere syndromes were considered. The incomplete phenotype and broad differential diagnosis led us to undertake whole exome sequencing to determine the cause of bulbar motor neuropathy in this family.

Materials and Methods

Participants

All participants in the study provided written informed consent for clinical and genetic analysis. This American family from western North Carolina is of non-Finnish, European ancestry based on their understanding of the family's heritage (Figure 1). We specifically set out to examine blood relatives of the previously evaluated or suspected affected individuals who were old enough to be likely to have findings if disease was present.

Genetic analysis

Exome sequencing was performed on DNA from Individuals III-1 and III-12. Library preparation using the Nimblegen SeqCap EZ Human Exome Library v2.0 kit (Roche Applied Science, Indianapolis, IN) followed by 50 base pair paired end next generation sequencing on an Illumina HiSeq2000 was performed according to the manufacturer's protocol. Sequence alignment and variant calling were performed against the reference human genome (UCSC hg 18) using the Genome Analysis Toolkit (GATK version 1.0.5336, www.broadinstitute.org/gsa/wiki/index.php/The_Genome_Analysis_Toolkit). PCR duplicates were removed prior to variant calling using Picard software (version 1.25, <http://picard.sourceforge.net/index.shtml>). Based on the hypothesis that the mutation underlying this rare familial disease was not present in the general population, SNPs identified in the 1000 Genomes project (www.1000genomes.org/) or in dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>, Build 132) were removed. Variants that were not shared by both patients were then excluded. Next, synonymous changes were identified and filtered from the variant list. Variants detected by exome sequencing were Sanger-sequenced using the Big-Dye Terminator v3.1 chemistry (Applied Biosystems, CA), run on an ABI 3730xl analyzer, and analyzed using Sequencer software version 4.2 (Gene Codes, MI).

RESULTS

Participants

Of 18 subjects examined, 5 individuals were definitely affected and are described below.

Patient III-1—The proband, a 63 year-old woman, was healthy until age 56, when she began to experience facial muscle twitching. This was followed within a few years by difficulty swallowing and mild dysarthria. At age 57, she had blepharoplasty for excess tissue below the eyes and “droopy” eyelids. Her optometrist had treated her for dry eyes with drops presumed to be due to incomplete eyelid closure. Later, an ophthalmologist evaluated her for complaints of dry eyes and photophobia and diagnosed her with inherited corneal dystrophy and lagophthalmos as a complication of the blepharoplasty. The significance of

the inherited corneal dystrophy was not explained to the patient. For the complaints of facial twitching and tongue atrophy, an MRI of the brain was performed and showed scattered, non-specific deep white matter lesions but no abnormalities in the brainstem. On inspection of her face, there were bilateral but asynchronous brief contractions of facial muscles, and continuous small contractions seen in the orbicularis oculi muscles. These contractions involved parts of facial muscles and did not close the eye or move the corner of the mouth so would not be characterized as hemifacial spasm. Eye and lip closure were moderately weak, but extraocular movements and jaw strength were normal. Facial sensation was spared, but marked tongue atrophy and fasciculations were noted (Figure 2). The remainder of the neurologic examination was normal without signs of hyperreflexia or distal neuropathy. Subtle sagging of the skin under the eyes and “heavy” lids were present.

Nerve conduction studies and EMG of the upper and lower limbs was normal. On needle electrode examination, the tongue and facial muscles revealed fibrillation potentials and enlarged motor unit potentials with reduced recruitment consistent with a severe, chronic neurogenic process, but masseter and sternocleidomastoid muscles were normal. The spontaneous facial muscle contractions were shown to be brief bursts of myokymic discharges. Previously performed genetic testing in a commercial laboratory for X-linked spinobulbar muscular atrophy and familial ALS genes (*SOD1*, *TARDBP*, *ANG*, *FIG4*, *FUS*) was negative.

Patient II-2—This 83 year-old aunt of the proband had onset of facial twitching at age 52 and had undergone blepharoplasty at age 63 for ptosis. Also around age 63, she had underwent cardiac pacemaker placement for symptomatic bradycardia. At the time of our evaluation, she had undergone bilateral corneal transplant surgery for “dry eyes”, but her age at that time is unknown. It is unknown if lattice corneal dystrophy (LCD) was diagnosed. She complained of mild problems with speech articulation and occasional choking on dry foods. Her exam was notable for paralysis of the frontalis muscle with marked bifacial weakness, but less facial twitching than the other members of her family. Limb strength and reflexes were normal, but severe loss of vibratory sense was present in the feet.

Patient II-3—This 85 year-old aunt of the proband underwent blepharoplasty for ptosis at age 76 and reported onset of speech and swallowing problems around age 80 with progression to the point that a feeding tube was placed at age 84 to prevent aspiration. On examination, facial twitching and weakness were present, and there was mild sensory loss in the feet.

Patient III-12—This 56 year-old woman, a cousin of the proband, noted facial twitching at age 52 and complained of dry eyes. Her exam revealed facial twitching, but only mild facial weakness and no definite tongue abnormalities. Otherwise, strength, reflexes, and sensation were normal.

Patient III-13—This 53 year-old man, a cousin of the proband, developed facial twitching in his late 20's, but he had no other symptoms until noting subtle indistinct speech in his late 40's. He did not complain of dysphagia. His examination revealed similar facial twitching

and weakness with tongue atrophy and fasciculations. Limb strength, reflex, and sensory examinations were unremarkable.

Three individuals (III-7, III-8, IV-1) were classified as possibly affected. III-7 was possibly affected based on history of bilateral blepharoplasty for ptosis at age 45 and use of eye drops for dry eyes but, at age 64, did not have the expected findings of facial weakness or tongue fasciculations. III-8 was age 63 years at examination and was classified as possibly affected due the finding of subtle ptosis but did not have other facial weakness or tongue fasciculations. The offspring of the proband, (IV-1) was age 37 years at the time of examination and was considered possibly affected due to the findings of rare facial fasciculations and subtle facial asymmetry, indicating early weakness. This person also noted rare cramping in the jaw while chewing but had no complaints of dry eyes.

Three other individuals (I-1, II-1, II-4) were deceased at the time of the examinations but were classified as definitely affected based on the history reported by the family. None of the definitely or possibly affected patients underwent a biopsy or had other tissue examination that demonstrated amyloid deposition.

Genetic analysis

Next generation sequencing of individuals III-1 and III-12 provided adequate coverage of the exome (>91.0% and 90.8% of the exome showed 10× depth of coverage). After alignment and filtering, 21 heterozygous single nucleotide variants and 2 insertion/deletions (indels) were identified that were not described in online databases of human genetic polymorphisms and were shared by both patients. Sanger sequencing of these 21 variants and 2 indels in 3 other affected individuals from the same family reduced the list to a single variant in the gelsolin gene (NM_000177.4: c.640G>T; NP_000168.1: p.D214Y, chr9:123112918G>T in build hg18, rs121909715). This amino acid change is historically referred to as p.D187Y in the literature www.omim.org/entry/137350#0002, and we will maintain this convention to avoid confusion. This variant segregated with disease in the family in that all tested definitely affected individuals carried the mutation, whereas all tested unaffected individuals did not. Two of the 3 questionably affected individuals with very subtle exam findings (III-7 and III-8) did not carry the GSN mutation, and IV-1 had the mutant variant indicating she is pre-symptomatic.

DISCUSSION

Mutations in *GSN* are known to cause hereditary amyloidosis type IV, a rare autosomal dominant form of amyloidosis that can start with xerophthalmia due to LCD, followed by a slowly progressive bulbar motor neuropathy, and later, a mild distal neuropathy⁵. Pronounced drooping of the facial features, known as cutis laxa, is also seen frequently⁶. Individuals affected with hereditary amyloidosis type IV are overwhelmingly found in southern Finland⁶. Only 2 pathogenic mutations have been described: a common variant found in Finland (c.640G>A, p.D187N) and a rarer variant involving the same nucleotide position (c.640G>T, p.D187Y) found outside of the Finnish population. It is this rarer mutation that was identified in the family reported here and has previously been reported in only 3 families of French, Czech, and Danish heritage^{2,4}.

Although the 2 mutations affect the same amino acid residue, the clinical phenotypes are different. Patients who carry the rarer D187Y variant exhibit more severe bulbar dysfunction and an earlier age of onset than the mostly Finnish families with the D187N variant, who present after age 70 and do not have severe bulbar signs⁴. The clinical pattern in the family reported here confirms that observation. Cardiac conduction abnormalities are occasionally reported with both mutations⁴, and in family reported here, patient II-2 had a pacemaker placed for bradycardia in her early 60s. Other affected family members did not have a history of cardiac problems.

GSN encodes gelsolin, a ubiquitously expressed amyloid protein^{7,8} that may bind, sever, cap, and uncap actin filaments, processes that are critical for proper cytoskeletal dynamics⁹. In order to perform these functions, gelsolin must undergo a variety of conformational changes upon binding of calcium and actin. The 2 inherited mutations give rise to aberrant conformational changes, which block binding of calcium to its second domain and render gelsolin more sensitive to proteases than wild type. Therefore the protein is cleaved into amyloidogenic peptides, resulting in widespread tissue deposition^{10,11,12}. The amyloid has a tendency to adhere to basement membranes, small arterioles, nerves, cutaneous adnexa, and elastic fibers, resulting in the clinical features of hereditary amyloidosis type IV¹³.

The 3 affected family members initially examined (III-1, III-12, III-13) did not have peripheral neuropathy, thus the full clinical syndrome was not apparent and led to diagnostic uncertainty. The rarity of hereditary amyloidosis type IV in the United States also means that most clinicians would not recognize these bulbar signs as the neurological presentation of amyloidosis. Instead, the most striking clinical finding in each of these patients was tongue fasciculations, which would lead the clinician to consider the diagnosis of familial ALS. Goyal and Mozaffar recently reported 2 unrelated patients who presented with progressive sensorimotor neuropathy and were found to have tongue atrophy and fasciculations mimicking ALS¹⁴. These patients were shown to have the common *TTR* form of amyloidosis, thus it is surprising that neither hereditary amyloidosis type IV (gelsolin) nor transthyretin-related amyloidosis is not listed in the differential diagnosis of ALS or bulbar motor neuron disease in many major textbooks or frequently used online tools^{15,16,17}. Omitting hereditary amyloidosis, particularly type IV which presents with isolated bulbar motor neuropathy, from this differential diagnosis may contribute to under-diagnosis or misdiagnosis of the condition¹⁸.

The significance of LCD seems to have been overlooked in this family. Corneal dystrophies are a common hereditary corneal disease and are typically distinguished by the location and formation of the opacities. LCDs all show amyloid deposition in the stroma, and types I and III are not associated with systemic amyloidosis¹⁹. Type II LCD is seen with gelsolin amyloidosis and can be distinguished from the other types by the peripheral predominance of the corneal changes²⁰. It is unclear why none of the family members understood the hereditary nature of the eye disease, but perhaps their ophthalmologists believed this to be the more common type I LCD without systemic features and did not impart this information.

Our experience with this family shows that whole exome sequencing can be a powerful tool in establishing a genetic and neurological diagnosis. It is also a cheaper alternative to

traditional Sanger sequencing genetic testing panels when the differential diagnosis is broad and necessitates the sequencing of several candidate gene groups. Despite these advantages, whole exome sequencing is an imperfect technology, and clinicians must be aware of the associated pitfalls. Typically 10–15% of protein-coding regions are not sequenced, which can lead to the causative mutation being entirely missed²¹. Improvements in sequencing technology will likely overcome this issue in time. Also of concern is that exome sequencing generates numerous sequence variants, but the functional consequences of these variants can be ambiguous. The standard approach to this problem has been to sequence several affected members from the same family and look for the variants that segregate with disease. However, additional DNA samples may not be available, especially in rapidly fatal diseases such as ALS. The completion of large-scale projects such as the 100,000 Genomes Projects in the United Kingdom²² and President Obama's Precision Medicine Initiative²³ will improve our knowledge of variants in the general population and allow better interpretation of the pathogenic potential of variants in the future.

In summary, this report of hereditary amyloidosis type IV resulting from the D187Y *GSN* mutation in the United States confirms earlier reports that have suggested this mutation results in a more severe bulbar dysfunction phenotype than the common D187N mutation seen in Finland. Exome sequencing was used to identify the mutation in relatively younger family members who did not yet exhibit the complete phenotype. This demonstrates the power of the technique to determine the cause of hereditary disease in situations where the phenotype is incomplete or atypical.

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Abbreviations

LCD	Lattice Corneal Dystrophy
ALS	Amyotrophic Lateral Sclerosis

Figure 1.

Pedigree. Mutant alleles are indicated by mt, whereas wild-type alleles are indicated by wt. Brackets around the genotypes indicates that the genotypes were inferred. The proband is indicated by an arrow. The genders of pedigree members are obscured to protect privacy. A diagonal slash through a symbol indicates that person is deceased. Black filled diamonds indicate individuals who were definitely affected based on examination or report from the family. Gray filled diamonds indicate members of the family who were questionably affected. Numbers within the diamonds correspond to the number of individuals who match these parameters. The chromatogram shows the wild-type allele (top panel) and the mutant transversion (bottom panel) corresponding to p.D187Y in an affected pedigree member.

Figure 2.
Tongue atrophy in the proband.