



Stereocilin gene variants associated with episodic vertigo: expansion of the DFNB16 phenotype

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Abstract

Vestibular disorders comprise a heterogeneous group of diseases with transient or permanent loss of vestibular function. Vestibulopathy is in most cases associated with migraine, Ménière disease, hereditary ataxias, or sensorineural hearing loss. We identified two brothers and their first cousin affected by hearing loss and episodic vertigo. The brothers were homozygous *STRC* nonsense variant [c.4027 C > T, p.(Q1343*)], whereas their first cousin was compound heterozygous for the *STRC* nonsense variant and a 97 kb deletion spanning the entire *STRC* gene. Clinical investigations confirmed pathological vestibular responses in addition to a characteristic DFNB16 hearing loss. The *STRC* gene encodes Stereocilin in the cochlea and in the vestibular organ where it ensheathes the kinocilium of the otolithic membranes. Stereocilin is associated with the gel overlaying the vestibular kinocilia, suggesting a role for the protein in sensing balance and spatial orientation. Our findings support such a function for Stereocilin in the vestibular organ and expand the phenotype associated with DFNB16.

Introduction

The vestibular organ comprises the semicircular canals to sense rotation, and the otolithic organs, the utricle and the saccule, required to sense linear acceleration. Vestibular disorders are usually episodic and may involve one or several symptoms such as vertigo, motion sickness, dizziness, nystagmus, blurred vision, and unsteadiness [1, 2]. Vestibulopathy is, in most cases, part of cochlear-

vestibular disorders with hearing loss, tinnitus, and ataxia [3–5].

Variants in *STRC* cause DFNB16B (MIM# 60372) representing at least 10% of cases with autosomal recessive, non-syndromic sensorineural hearing loss (SNHL) [6–8]. Deletions spanning the *STRC* gene has an estimated carrier frequency of 1.6% in a European population [9]. In mice, stereocilin is expressed in the kinocilia of the vestibular saccule and utricle, and to a lower extent in the ampullas of the semicircular canals in addition to the expression in the cochlea [10, 11]. However, the clinical significance of Stereocilin in the vestibular organ has remained elusive [6, 8, 11].

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Methods

Subjects

We identified a non-consanguineous family of Swedish-Norwegian origin segregating two brothers (III:2 and III:4) and their first cousin (III:6) with non-progressive and moderate SNHL (Fig. 1a, b). The affected members had a history of episodic vertigo from age 2 years (III:2), 5 years (III:4), and 11 years (III:6), respectively.

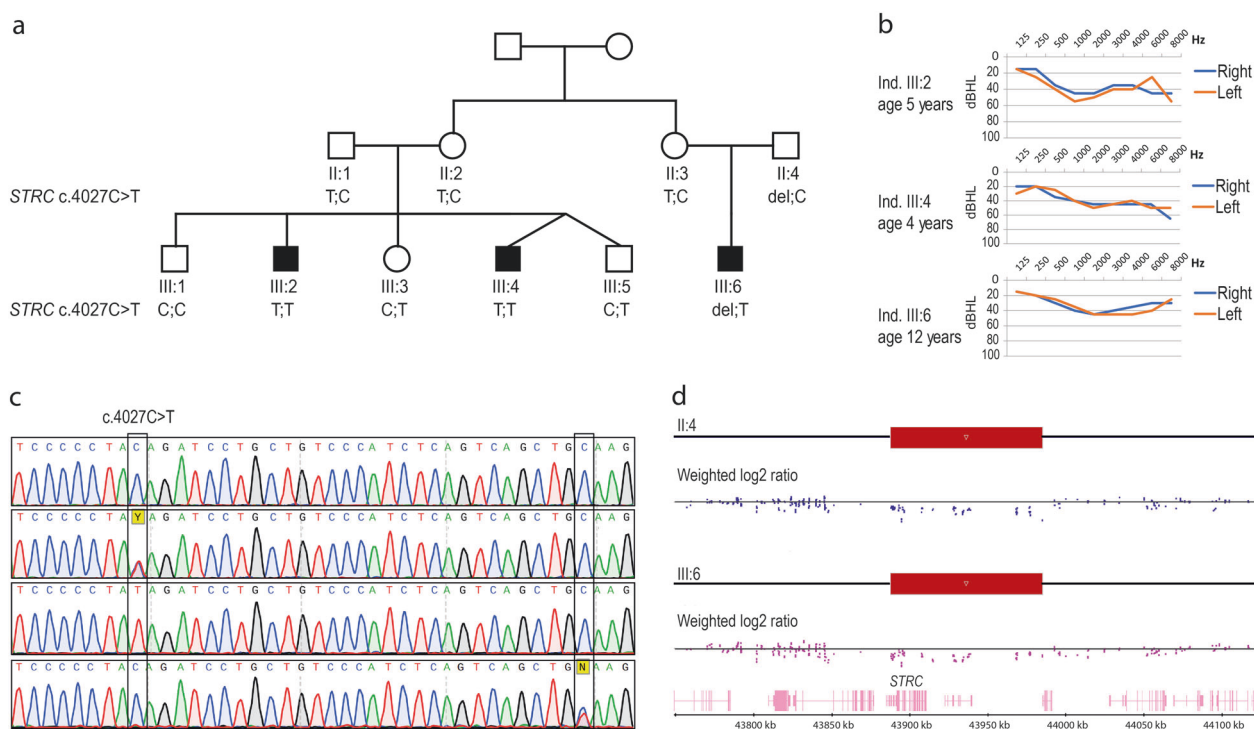


Fig. 1 Pedigree of the family segregating DFN16 and episodic vertigo. **a** Pedigree indicating three members affected by moderate sensorineural hearing loss and episodic vertigo. Segregation of the *STRC* variants is shown below each symbol. **b** Bilateral tone audiograms from the three affected individuals homozygous, or compound heterozygous, for the *STRC* variants. **c** Chromatograms showing part of *STRC* exon 20 using *STRC* specific primers from a non-carrier (top row), a heterozygous individual (second row), and an individual

homozygous for the *c.4027 C > T* nonsense variant (third row), respectively. The bottom chromatogram is from a non-carrier using primers that anneal to both *STRC* and *pSTRC*. The position with a sequence divergence between *STRC* and *pSTRC* (c.3931-81) is boxed. **d** Cytoscan HD Array analysis showing the heterozygous 97 kb deletion (red box) spanning *STRC* in ind. III:6 and his unaffected father (II:4). del: 97 kb deletion spanning the entire *STRC* gene

Vestibular tests

Vestibular function was assessed using bithermal caloric test, vestibular head impulse (vHIT), videonystagmogram, subjective visual horizontal (SVH) test, cervical (c) and ocular (o) vestibular-evoked myogenic potential (VEMP) according to standardized methods [12].

Exome sequencing and SNP array analysis

Exome sequencing was performed on DNA from two affected individuals (III:2 and III:4) on the Ion Proton System (Life Technologies, Carlsbad, CA, USA; Supplementary Information). CytoScan high-density array (Thermo Fisher Scientific, Waltham, MA) analysis was performed on DNA from III:6 and his unaffected parents using computer software Chromosome Analysis Suite version 3.3 (Thermo Fisher Scientific). The DNA variants identified in this study are accessible in the dbSNP: <https://www.ncbi.nlm.nih.gov/SNP/> and the ClinVar database: <https://www.ncbi.nlm.nih.gov/clinvar/>.

Results and discussion

Individual III:2 had an onset of episodic vertigo at 2 years of age characterized by imbalance, blurred vision, vomiting, and nausea. The frequency of episodes was one to four annually and became less frequent from 15 years of age. Individual III:4 had episodes of positional vertigo associated with vomiting since the age of 5 years. With age, the episodes became less intense. Individual III:6 had an onset of episodes of vertigo associated with nausea, vomiting, and blurred vision at 11 years of age. These episodes occurred frequently the first year but became rare from age 12 years. In all three affected individuals, the episodes lasted for one to several days and were triggered by food or sleep deprivation. No other neurological symptoms or cognitive abnormalities were identified.

The two brothers underwent a detailed vestibular examination at age 8 years and 16 years (III:2), and at age 12 years (III:4), respectively (Table 1, Supplementary Table 1). In III:2, bilateral bithermal caloric stimulation revealed a left-sided weakness with a ratio of 0.25 at 8 years of age. This was normalized at age 16 years. However, III:2 had a

Table 1 Vestibular test results in two individuals with DFNB16, who are homozygous for the *STRC* variant c.4027C>T

Subject	Test (normal ranges)									
	vHIT (0.8–1)		Caloric ratio (0–0.2)	SVH upright (–2.5 <i>M</i> >+2.5)	SVH tilt (>–3)	o-VEMP (Amplitude diff. 2.5)		o-VEMP (Amplitude diff. 2.5) ^c -VEMP (Amplitude diff. 2.5)		
III:2	R: 0.94	L: 0.95	0.25 ^a	–0.38	R: 2.5	L: 16 ^b	R: 3	L: 0	R: 1.1	L: 0.9
III:4	R: 0.87	L: 0.85	0.02	0.15	R: –8 ^c	L: –16 ^c	R: 2.4	L: 1.4	R: 0.7	L: 0.2 ^d

^aAccompanied with geotropic nystagmus.

^bRight vs. left difference indicates left-sided utricle asymmetry for tilt perception.

^cPathological values for tilt perception.

^dc-VEMP with acoustic stimulation revealed significant amplitude difference.

c-VEMP cervical vestibular-evoked myogenic potential, L left, o-VEMP ocular vestibular-evoked myogenic potential, R right, SVH subjective visual horizontal test, vHIT vestibular head impulse

persistent positional left-sided and slow geotropic nystagmus at both occasions suggesting a primary labyrinthine asymmetry and not an otolith detachment causing benign and paroxysmal positional vertigo. In supine position and after headshake, nystagmus was directed to the left. c-VEMP revealed similar amplitudes on both sides after correction, whereas o-VEMP amplitudes with acoustic stimulation showed a side difference [13, 14]. The vHIT test and SVH in upright position were within normal range ($-2.5 < M > +2.5$). However, when SVH was measured at tilted body positions, the mean value (*K*) on the right side was 2.5 and on the left side 16 (> -3 is normal), indicating a left-sided utricle asymmetry for tilt perception [15]. Furthermore, the left vertical vestibulo-ocular reflex was weak, consistent with an otolithic disorder. Caloric stimulations on III:4 revealed a normal ratio (0.02). The o-VEMP amplitudes were symmetrical, suggesting normal function of the superior vestibular nerve or utricle. However, c-VEMP with acoustic stimulation showed amplitude difference, indicating a weakness of the saccule or inferior nerve. The vHIT showed a normal bilateral gain. An SVH in upright position were within the normal range ($-2.5 < M > +2.5$), whereas investigation of tilt perceptions revealed a pathological mean value (*K*) on both sides (right: -8 ; left: -16). The left vertical vestibulo-ocular reflex was weak. In summary, the results from vestibular tests indicated a left-sided utricular weakness in III:2, whereas III:4 had a bilateral and predominantly left-sided dysfunction of the vestibular organ involving both the saccular and utricular parts. III:6 was reluctant to vestibular testing but his detailed description of episodes, confirmed by his parents, was consistent with the vestibular symptoms in III:2 and III:4.

Whole exome sequencing was performed on DNA from two affected individuals (III:2 and III:4). The analysis identified two rare homozygous variants (1% cutoff) shared by the two brothers: A nonsense *STRC* variant (NM_153700.2; rs144948296, ClinVar accession RCV000516500.1, c.4027

C>T, p.(Q1343*)) and a missense *PCNT* variant (NM_006031.5; rs35044802, c.3580 G>A, p.(A1194T)). A non-processed pseudogene (*pSTRC*) with a 99.6% coding homology to the structural *STRC* gene complicates the identification of variants. A natural sequence divergence between *STRC* and *pSTRC* [NG_011636.1(NM_153700.2): c.3931-81 T>C, rs2467434] was used to design *STRC* specific primers (Fig. 1a, c; Supplementary information). Analysis of the first cousin (III:6) revealed compound heterozygosity for the *STRC* variant c.4027 C>T inherited from the mother and a deletion of approximately 97 kb [ClinVar submission ID SUB4337996, chr15:g.(438868571_43888004)(43984930_43992627)del(hg19) spanning the *STRC*, *CKMT1B* and *CATSPER2* genes, inherited from the father (Fig. 1d). The *PCNT* variant is present in dbSNP (rs35044802) and the Genome Aggregation Database (gnomAD) [16]. The variant is predicted to be benign (PolyPhen-2 score 0.016 benign; PROVEAN score -0.02 neutral; Sift 0.718 tolerated) and was not further considered. The *STRC* variant c.4027 C>T was predicted to be damaging by Mutation Taster and has previously been identified in a heterozygous state in combination with a *STRC* deletion on the second allele in a DFNB16 patient without reported vestibular symptoms [17].

Balance problems, but not vestibulopathy, have been observed in a few reported DFNB16 families [18, 19]. In these studies, vestibular function was either not mentioned or investigated. It cannot be excluded that infrequent and/or mild vestibular symptoms have remained undiagnosed. The sense of balance depends on information from vestibular organs, proprioceptive sensors, and visual impression to the cerebellum. In unilateral canal paresis (lateral vestibular semicircular canal), detected as an asymmetry in the caloric response, a central compensation will occur [20]. With time, the vestibular symptoms fade away and symmetry in caloric response is restored (Supplementary Table 2, II:2). The time span for central compensation in vestibular dysfunction is

shorter in children than in adults and probably holds for other semicircular canals and the otolith organs. In mice vestibular organ, *Stereocilin* is predominantly expressed in the saccule and utricle. A similar expression pattern in humans may explain the effect on the otolith organs in our patients. Both siblings are right-handed consistent with central projections on the right vestibular organ. The recorded aberrations on the left side may be explained by a less effective left-sided central compensation [20]. Balance or behavior abnormalities have not been reported in *Strc*^{-/-} mice. However, vestibular testing in the mouse model has not been performed and a mild, transient, or compensated vestibulopathy cannot be excluded. Furthermore, phenotypes in mice may be strain-specific and with compensatory mechanisms that are not present in humans. In humans, symptoms from vestibular dysfunction are heterogeneous including vertigo, dizziness, visual and balance symptoms, problems of emotion, memory, and self-perception. This may be difficult to describe, especially for children. In addition, strong compensatory mechanisms for vestibular deficiency in humans are well known [20]. Consequently, the episodic phenotype identified in our patients may have been overlooked in other DFNB16 patients. More extensive analysis of the *Strc*-null mouse and DFNB16 patients are now required to understand the role for *Stereocilin* in vestibular function.

In conclusion, we diagnosed episodic vertigo segregating DFNB16 and *STRC* variants affecting protein function in two brothers and their first cousin. Clinical investigations confirmed vestibular dysfunctions primarily from the saccula and utricle but less prominent in the semicircular canals, consistent with the distribution of *Stereocilin* in the vestibular organ [10]. *Stereocilin* is expressed in kinocilia of the vestibular hair cells and at the tips of individual hair cells, suggesting a function for the protein in the coupling of vestibular kinocilia to the otoconial membrane, required to sense both orientation and motion. Our report thus expands the DFNB16 phenotype and provides an additional mechanism for episodic vertigo.

Ethical approval

This study was approved by the regional ethical committee of Uppsala, Sweden (Dnr 2004:M-270).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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