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Autosomal dominant stapes fixation, syndactyly, and symphalangism in a family with 
NOG mutation: long term follow-up on surgical treatment

Short running title:
NOG mutation in family with facio-audio-symphalangism syndrome

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Abstract

Objective: Evaluation of clinical findings and audiological outcome after surgery in a Danish family with autosomal dominant facio-audio-symphalangism syndrome with stapes fixation, syndactyly and symphalangism.

Methods: Retrospective report on eight affected family members in a Danish family. Clinical investigation included X-ray, audiology and in one case video-recorded surgery. Main outcome measure was audiologic results after stapedectomy. Sanger DNA sequencing of NOG was performed on peripheral blood.

Results: Audiologic analysis showed that seven of eight affected family members had bilateral conductive hearing loss. Three patients were treated with stapedectomy, on one or both ears, due to fixation of stapes. All the affected members had syndactyly and symphalangism. A not previously reported mutation in the NOG gene (c.688_699del, p.Cys230_Cys232delins11) was found to segregate with the stapes fixation, syndactyly, and symphalangism. p.Cys230_Cysdelins11 was classified as likely pathogenic according to guidelines from the American College of Medical Genetics and Genomics.

Conclusion: The clinical presentation of the reported mutation corresponds with previous case reports of families with NOG mutation. In this family, surgery with stapedectomy had lasting effect without renewed fixation of the stapes in a follow up period of 18 months to 38 years.
1. Introduction

Syndromic congenital stapes fixation is rare, but can cause conductive hearing loss with early onset. In 1990, Teunissen and Cremers reported data on a family with conductive hearing loss caused by stapes ankyloses [1]. The affected family members had severe hyperopia, broad thumbs and first toes, brachytelephalangia and, in one case, symphalangism. The syndrome was reported within several other families and named Teunissen-Cremers syndrome [2, 3]. Nine years later, genetic analysis found a mutation in the NOG gene to be responsible for the syndrome [4].

Since the first presentation of a mutation in the NOG gene, more than 35 mutations have been identified causing overlapping variations of the syndrome: Teunissen-Cremers syndrome [1-3], Proximal symphalangism [5-8], Multiple synostoses syndrome/facioaudiosymphalangism syndrome [6-12], Tarsal-carpal coalition syndrome [8] and Bradydactyly type B [13]. In addition to heterogeneity among the syndromes, there are inter- and intrafamilial variations in phenotypes [8, 10, 14, 15]. Consequently, it can be difficult to distinguish between the syndromes and clinical diagnoses and an unifying term, NOG-related symphalangism spectrum disorder (NOG-SSD), has been introduced [16].

The NOG gene encodes the protein Noggin which inactivates bone morphogenetic proteins (BMPs). Absence of Noggin increase BMP-activity, resulting in recruitment of the cartilage cells, hyperplasia and bone growth [17]. Patients with stapes ankyloses caused by NOG mutation and morphological changes in Noggin may have an increased risk of refixation of stapes after surgery [18].

The aim of this study is to present long term audiological results after stapedectomy in a Danish family with a NOG mutation not earlier described.
2. Material and method

2.1. Ethics

The study was conducted in accordance with the Danish law for scientific ethics committee and approved by the Danish Data Protection Agency. Informed consent was obtained from all included patients or from parents of the children.

2.2. Subjects

The family was presented by P. Vase et al in 1975, where five family members had a syndrome with conductive hearing loss, syndactyly and symphalangism [19]. At this follow up study, there were eight affected family members in four generations. The pattern of inheritance was autosomal dominant as shown in the pedigree, figure 1.

Six of the seven living affected members gave consent to participate in the study, though one of the six patients did not consent to blood sample (patient IV:1). All available records, surgical reports, audiograms and imaging were reviewed. The individuals were examined by one of two otorhinolaryngologists with clinical examination, otomicroscopy and tympanometry. Audiologic examination consisted of pure tone audiometry (air and bone conduction) and speech audiometry with speech reception threshold (SRT) and discrimination score (DS). Patient III:1 and IV:1 underwent X-ray of hands and feet in connection to the study. X-ray was not repeated in patients were the diagnosis of symphalangism had been made with previous imaging.

2.3. Genetic analysis

A genetic analysis was performed on peripheral blood obtained from five of the six affected family members (patient III:1, II:2, III:2, IV:2 and IV:3). Genomic DNA was isolated from peripheral blood leucocytes. Sanger DNA sequencing of NOG was performed at Klinisch-Genetisch Centrum,
Nijmegen, KGCN, Nijmegen, Nederland. The familial mutation was analyzed by bidirectional sequencing using BigDye® Terminator v.3.2 cycle sequencing kit (Applied Biosystems, Denmark) and an AB13730XL capillary sequencer (Applied Biosystems, Denmark). Tertiary structure was evaluated based on the published structure of Noggin (1M4U).

3. Results

3.1. Otologic and audiologic findings

None of the individuals had a history of trauma or noise damage. Two of the affected patients (II:2 and IV:2) had an anamnesis with middle ear infections. Patient IV:2 had tubulation of both tympanic membranes at the age of 2 years. The tympanic membrane was healed at the time of examination and there was no suspicion of glue or infection in the middle ear. Three patients (III:1, IV:1 and III:2) had gone through stapedectomy on one or both ears at the time of examination. The ears (n=5) who had gone through surgery had postoperative thickening of the tympanic membrane. The preoperative and early audiograms consisted only of pure tones (air and bone conduction) and data on speech reception threshold were not available. The audiologic results are shown in table 1 in accordance to guidelines from Committee on Hearing and Equilibrium [20].

Patient 1, proband, III:1 (figure 1), 55 years of age at follow up, had a hearing loss documented at the age of 4 years. She underwent explorative tympanotomy at the age of 5 years, which revealed osseous fixation of stapes but no deformity of the bones of the middle ear. No further surgery was performed until bilateral stapedectomy at the age of 10 years. The preoperative audiograms at the age of 10 years showed bilateral conductive hearing loss of 50-60 dB. An improvement was obtained by stapedectomy and reduced the air-bone gap as shown in table 1 and figure 2.
At the follow up, 38 years postoperatively, the hearing had declined and audiologic treatment with hearing aids was required. The air-bone gap was 10 dB, but the hearing threshold was increased to 20-40 dB (figure 2 and table 1).

Patient 2, her son, IV:1, 30 years of age at follow up, was diagnosed with hearing loss at the same age as the mother. At the age of 10 years the audiogram showed severe bilateral conductive hearing loss with maximal air-bone gap, and he used bilateral hearing aids. After stapedectomy on the left ear (at the age of 14 years) and on the right ear (at the age of 15 years), hearing was improved and only a minor air-bone gap persisted in the audiogram 10 years after surgery (table 1).

Patient 3, the paternal aunt of the proband, II:2, 71 years of age at follow up, had not gone through any ear surgery. She had hearing loss since childhood, mainly on the left ear. At the follow up the audiogram showed severe mixed conductive and sensorineural hearing loss and no stapedius reflexes. She used hearing aids on both ears.

Patient 4, her daughter, III:2, 47 years of age at follow up, had a conductive hearing loss of 40-50 dB since childhood. After stapedectomy on the right ear at the age of 11 years, the hearing improved and persisted at the follow up 30 years later. Stapedectomy was performed on the left ear at the age of 41 years. Audiogram at the follow-up (18 month after surgery) showed a reduced hearing loss (table 1).

Patient 5, her oldest son, IV:2, 18 years of age at follow up, had noticed a slightly decreased hearing on his left ear at the age of 14 years. The audiogram showed a conductive hearing loss of 20-30 dB at the low frequencies on the left ear. He did not use hearing aids.

Patient 6, the younger son of patient 4, IV:3, 15 years of age at follow up, had no subjective hearing loss and the audiogram was normal at the age of 9 years.

The surgical report from the stapedectomies in patient 1, 2, and 4 consistently reported stapes ankyloses with osseous fixation of the stapes footplate and elongation of crus longum incudis in all
three patients. At the stapedectomy of III:2, left ear, the prosthesis measured 6 mm, which is relatively long for a woman. Malleus was normal in all cases.

3.2. Clinical and radiologic findings

All patients had malformations on both hands and feet, though the clinical findings varied slightly among the individuals. Consistent findings were symphalangism of the proximal interphalangeal (PIP) joints and soft tissue syndactyly on the fingers and toes.

Patient 1, III:1 had impaired movement of the wrists with limited dorsiflexion. X-ray examination confirmed the clinical findings with ankyloses of PIP joints of the 4th and 5th finger and toe and ankyloses of several of the carpal and tarsal bones (figure 3).

Patient 2, IV:1 had cutaneous syndactyly of the four ulnar fingers and four lateral toes and had gone through surgery to remove skin-webbing on both hands. X-ray confirmed the clinical finding of ankyloses of the PIP joint of the 5th finger and toe. He was diagnosed with Möbius syndrome at birth, which per se can lead to soft tissue symphalangism, deformations on hands and feet and conductive hearing loss.

Patient 3, II:2, only had cutaneous syndactyly of the 2nd and 3rd finger and of the four lateral toes. The symphalangism included the PIP joint of 4th and 5th finger and toe.

Patient 4, III:2, had bilateral pes equino varus at birth and went through surgical treatment of both feet. She had syndactyly of the 2nd-4th finger and of the four lateral toes and ankyloses of PIP joints of the 3rd-5th finger on both hands and the corresponding toes.

Patient 5 and 6, IV:2 and IV:3, both had ankylosis of the PIP joints of all the four ulnar fingers. IV:2 had cutaneous syndactyly of all four ulnar fingers, whereas in his younger brother, IV:3, the syndactyly did not involve the 5th finger.

None of the individuals had broad thumbs or toes and no facial dysmorphism was seen.
3.3. Genetic findings

The insertion/deletion mutation c.688_699del resulting in p.Cys230_Cys232delins11 was observed in a heterozygous state in the family. This variant has not been described in Genome Aggregation Database (genomAD), Exome Aggregation Consortium (ExAC), or dbSNP database, and has not previously been reported in Human Gene Mutation Database [21-24]. Due to absence in population databases, p.Cys230_Cys232delins11 can be classified as pathogenic moderate 2 (PM2) according to guidelines from the American College of Medical Genetics and Genomics (ACMG) [25]. The mutation represents a deletion of the last 3 C-terminal amino acids including the termination codon. It extents the protein coding region into the 3'-untranslated part of the NOG loci resulting in a new termination codon 11 amino acids later. Cysteine 230 and Cysteine 232 are highly conserved even within the cytokine-Knot cytokines gene superfamily. Cysteine 230 is forming an intramolecular disulfide bridge to Cysteine 184. Cysteine 232 is part of an intramolecular disulfide bridge to Cysteine 232 of the other peptide in the noggin dimer. It can be predicted that deletion of these two cysteines will result in large conformational changes both on the inter- and intramolecular level. Both Cysteine 230 and Cysteine 232 participate in disulfide bonding within the C-terminal part of NOG hence the mutation can be classified as pathogenic with moderate evidence of pathogenicity (PM1) [25]. The variant can be further classified as PP1 due to co-segregation with disease in multiple affected family members, and PP4 since the phenotype and family history is highly specific for the gene [25]. Figure 4A show the C-terminal part of NOG affected by the mutation in patient III:1, and Sanger sequence of a normal gene compared to the sequence with the NOG mutation (patient III:1) is shown in Figure 4B.
4. Discussion

Congenital or early onset of conductive hearing loss is rare. However, mutations in \textit{NOG} can result in stapes ankyloses and conductive hearing loss. A wide range of mutations in \textit{NOG} have been described, all resulting in autosomal dominant syndromes with various affection of joints and hearing loss [2, 3, 6, 14, 15, 26, 27]. The variety of phenotypes combined with relatively few patients in all ages complicates studies on epidemiology, treatment and prognosis.

In this study, we presented a family with stapes ankyloses causing conductive hearing loss, symphalangism of the PIP joints on hands and feet and syndaktyly of fingers and toes. The mutation \textit{NOG} (c.688\_699del, p.Cys230\_Cys232delins11) has not previously been described, but the clinical features is similar to other \textit{NOG} mutations [6, 11, 12, 14, 15, 27-30]. We did not carry out genetic studies in unaffected individuals, but we confirmed that genetic variant had not previously been reported in Population databases (genomAD, ExAC or dbSNP). The affection was seen through more than four meiosis (family members of three generations), which may also confirm the pathogenicity. Substitution of Cysteine with tyrosine at position 230 has previously been reported as pathogenic [31] but in this study, we found a deletion and not a substitution. Bayat et al presented the novel mutation in \textit{NOG} resulting in substitution of Cysteine 230 in a Danish family, but none of the affected family members had hearing loss [31]. Another study reported a mutation in Cysteine 184 causing proximal symphalangism without hearing impairment [6]. In this family, Cysteine 184 and Cysteine 230 were affected resulting in stapes ankyloses with hearing loss, syndaktyly and symphalangism. These findings confirm that mutations in the same coding sequence of \textit{NOG} can lead to different phenotypes with interfamilial variability, which is also shown in other reports [8, 10, 14, 15]. It is suggested, that phenotypes can be independent of the location and the type of mutation in the \textit{NOG} gene [14, 15]. Combining ACMG classification criteria, p.Cys230\_Cysdelins11 can be classified as a likely pathogenic variant (PM1, PM2, PP1 and PP4) [25].
The results of otologic surgery in patients with stapes fixation caused by the mutant Noggin protein vary in the different reports. In this study, surgical treatment with stapedectomy in five ears (three patients) resulted in lasting hearing improvement with postoperative follow up period of 18 months to 38 years. One patient had a postoperative 15 dB decrease in pure tone average air conduction on the left ear and 20 dB on the right ear after 38 years. Similar results have been presented in several reports [5, 14], while other studies describe a gradual hearing loss due to regrowth of bone 2-5 years postoperatively [2, 8, 13]. The small number of patients makes it difficult to calculate statistics or suggest treatment recommendations. Results of stapedectomy may also depend on the genetic variant. In this study, we only present results from pure tone audiograms, because the early audiograms did not contain speech reception. Speech reception threshold is however also an important parameter in the audiometry and should be taken into consideration in evaluation of outcome of surgery in the middle ear.

5. Conclusion

Though conductive hearing loss in children is most likely due to otitis media serosa or other affections in the middle ear, \textit{NOG} mutations can cause early onset of conductive hearing loss. Family history and genetic testing may be useful in discriminating in these cases. Clinical findings and a family history of conductive hearing loss should lead to further evaluations. The literature is not consistent on the longterm effect of surgery, but results of this study indicates that stapedectomy may be successful and result in lasting improvement of the hearing. Still, additional long term follow up on patients with stapes ankyloses caused by mutation in \textit{NOG} gene is recommended.

6. Acknowledgement
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References

Table 1, Audiologic results after surgery.

<table>
<thead>
<tr>
<th>Patient</th>
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<td>PTA\textsubscript{bone} dB</td>
<td>Air-bone gap dB</td>
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<td>0</td>
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</tr>
</tbody>
</table>

PTA\textsubscript{air}: pure tone average of air conduction, PTA\textsubscript{bone}: pure tone average of bone conduction, NA: not available
Figure 1, Pedigree

Arrow denotes proband, patient III.1. Affection follows an autosomal dominant pattern.
Figure 2. Audiogram patient III, 1

Conductive hearing loss before surgery, after surgery and at the follow up
Figure 3, Clinical photo and X-ray of hands, patient III, 1

Symphalagism and cutaneous syndactyly on hands and feet.
A: NOG gene mutation

Figure 4, Genetics

B: Sanger sequence

A: Illustration of the disulfide bonding in the cystein knot of the C-terminal part of one peptide in the Noggin dimer. In blue and salmon the beta3 and strands. In yellow the conserved cysteines.

B: Sanger sequencing results. The upper line represents the consensus NOG sequence. Below, N-terminal amino acid sequence is presented as a green bar and the deletion indicated by the red markings.