European Journal of Human Genetics (2001) 9, 226–230

www.nature.com/ejhg

SHORT REPORT

# Sensorineural hearing loss and the incidence of *Cx26* mutations in Austria

Judith Löffler[1], Doris Nekahm[2], Almut Hirst-Stadlmann[2], Barbara Günther[1], Hans-Jürgen Menzel[1], Gerd Utermann[1] and Andreas R Janecke*[,1]

[1]*Institute of Medical Biology and Human Genetics, University of Innsbruck, Innsbruck, Austria;* [2]*Department of Hearing, Speech and Voice Disorders, University Hospital of Innsbruck, Innsbruck, Austria*

**A clinical evaluation and *Cx26* mutation analysis was performed in 92 consecutive patients with sensorineural hearing loss in order to delineate the spectrum of genetically caused hearing loss. Among patients of Austrian origin, 53% were classified with hereditary hearing loss. *Cx26* mutations were found in 26% of NSHL patients (40% of familial *vs* 18% of sporadic cases). The mutation 35delG accounted for 52.8% of all presumed GJB2 disease alleles. The second most frequent mutation was L90P (16.7%) having been reported with a prevalence of 0.7–3.5% in other populations. Three novel mutations were found. The novel mutation, R143Q, was associated with dominant high-frequency hearing loss. Pseudodominant transmission of NSHL was seen in four families with *Cx26* mutations. A mutation 35delG carrier rate of 0.9% was observed among 672 controls from West-Austria. *Cx26* mutations were found associated with mild to profound, and with asymmetric hearing impairment.** *European Journal of Human Genetics* (2001) **9,** 226–230.

**Keywords:** hearing loss; Austria; Tirol; connexin 26; GJB2; population screening

## Introduction

Hearing loss is the most common sensory deficit and comprises a broad spectrum of clinical presentations. Surveys carried out both in Europe and the US indicate that hearing loss (HL) has a genetic origin in about 20–60% of cases, an acquired cause in 30–40% and remains of undefined origin in 20–40% of patients.[1] Recent advances in molecular genetics indicate an unparalleled heterogeneity with more than 100 genes estimated to be involved in deafness.[2] Mutations in the gene of locus DFNB1 and DFNA3, the connexin-26 gene (*Cx26*, *GJB2*), have been demonstrated to be responsible for 34–50% of autosomal recessive NSHL and 10–37% of cases with sporadic NSHL in various ethnic populations, and in rare instances of dominant transmission.[3]

We undertook a clinical characterisation of patients with sensorineural hearing loss in Austria, then focused on the prevalence of *Cx26* mutations and determined the carrier frequency of the most common mutation 35delG in a control population from Tirol (part of West-Austria).

***Correspondence: Andreas R Janecke, Institute of Medical Biology and Human Genetics, University of Innsbruck, Schöpfstr. 41, A-6020 Innsbruck, Austria. Tel.: +43 512 507 3243; Fax: +43 512 507 2861; Email: Andreas.Janecke@uibk.ac.at**
**Received 30 June 2000; revised 1 November 2000; accepted 15 November 2000**

## Patients and methods

This 1-year study included 92, unrelated individuals who presented with sensorineural hearing impairment consecutively seen at the department of Hearing, Speech, and Voice Disorders in a region of west Austria. Seventy-six patients, 40 males and 36 females, were of Austrian origin. The parents of the other patients were from Bosnia (six) and Turkey (10). All but 16 cases had a prelingual onset of hearing loss. Clinical work-up included evaluation of motor and cognitive development, search for dysmorphism or integumentary disorders, proteinuria and haematuria, thyroideal disorders, as well as ophthalmological and cardiological examinations with special emphasis on possible indicators for an acquired hearing deficit. Individuals with NSHL were classified as (1) familial cases having at least one hearing impaired first degree relation or otherwise as, (2) sporadic cases. Assessment of age of onset, severity and pattern of hearing loss by pure-tone audiometry, tympanometry, auditory brainstem response and transient evoked otoacoustic emissions were

performed according to the recommendations of the European Workgroup on Genetics of Hearing Impairment.[4]

Blood samples from patients with NSHL were obtained following informed consent from each individual or his parents. Genomic DNA was prepared from dried blood spots.[5] *Cx26* mutation screening was performed as reported previously.[6,7]

DNA samples of 672 anonymous blood donors from Tirol (part of West-Austria) were distributed in 42 pools each containing DNA from 16 individuals. The carrier frequency of the mutation 35delG was determined by performing with each pool an allele-specific PCR for 35delG as described.[6] In positive pools, each individual DNA sample was then investigated for the presence and zygosity of the mutation 35delG by allele specific PCR.

## Results and Discussion

The classification of 92 sequential patients referred for sensorineural hearing loss is shown in Table 1. Among 76 patients of Austrian origin the aetiology of HL was determined to be hereditary in 53% of patients, either due to *Cx26* mutations (24%), to congenital genetically caused syndromes (9%) or to the presence of a positive family history (20%). No individual had a proven, acquired hearing loss. 'Pseudo-dominant' inheritance was revealed in four of 11 families with vertical transmission of NSHL.

### Distribution of Connexin-26 mutations

Our *Cx26* mutation detection rates of 40% in familial and of 18% in sporadic cases among Austrian NSHL patients suggest a north-south gradient of *Cx26*-related HL in connection with other studies.[2,3,8] Altogether, seven different *Cx26* mutations were observed (Table 2) with the mutations 35delG and L90P accounting for 52.8% and 16.7% of presumed GJB2 alleles. Regarding the subset of 12 patients with *Cx26* mutations from the federal country Tirol (part of West-Austria), 35delG and L90P accounted for 37.5% and 20.8% of disease alleles. The 35delG carrier frequency observed among control individuals from Tirol, 0.9%, is within the range of frequencies found in other European populations (0.5 – 4.4%)[9] but contrasts with carrier frequencies in neighbouring countries of Austria (Italy 1 : 32; Germany 1 : 50; Czech Republic 1 : 48.5) and with the predicted north-south gradient.[9]

The high prevalence of the mutation L90P (16.7 – 20.8% of disease alleles) among Austrian patients might reflect a founder effect in a central European country, having been reported previously to account for only 0.7 – 3.5% of disease chromosomes in Italian, Spanish and French patients.[2,3,7,8] Furthermore, the presence of L90P in other populations (ie Japan) has not been reported.[2,3]

The novel mutation c.428G→A results in an exchange of arginine at codon 143 to glutamine within the third transmembrane domain of *Cx26* affecting a residue highly conserved among most species and $\alpha$- and $\beta$-connexins. The co-segregation of the novel mutation R143Q with high-frequency hearing loss in one pedigree points towards a dominant effect of R143Q (Figure 1A). According to this interpretation, the severely affected child V.3 in this family carries a dominant and a recessive *Cx26* mutation. An arginine to tryptophan change at codon 143 (due to a mutation involving the same CpG dinucleotide) was observed in several patients with autosomal-recessive inherited HL from Spain and from Ghana.[3,10] While both highly non-conservative amino acid changes, R143W and R143Q, are expected to alter the secondary structure of the protein, the heterozygous R143Q mutation is likely to partially block the activity of evenly present wildtype *Cx26*.

**Table 1** Genetic hearing loss among 92 sequential patients referred for sensorineural hearing loss

| *Type of hearing loss among patients of Austrian origin* (n=*76*) | *Diagnosis/syndrome (frequency)* |
|---|---|
| Non-syndromic HL (*n*=69) | Connexin-26 mutations (26%) |
| Familial (*n*=25) | Connexin-26 mutations (40%) |
| Non-familial (*n*=44) | Connexin-26 mutations (18%) |
| (including three cases of unilateral HL and 11 cases of Meniere-like symptoms) | |
| Syndronic HL (*n*=7) | Jervell and Lange-Nielsen syndrome (1%) |
| | Waardenburg syndrome type 1 (2%) (1 PAX3 gene mutation) |
| | t(5;18)-translocation syndrome (1%) |
| | Branchio-Oto-Renal syndrome (1%) |
| | Ectrodactyly-Ectodermal-Dysplasia and Cleft Lip/Palate (EEC) syndrome (1%) |
| | Usher syndrome type 2 (1%) |
| | Skeletal dysplasia (1%) |
| *Type of hearing loss among patients of foreign origin* (n=*16*) | *Diagnosis/syndrome (frequency)* |
| Non-syndromic HL (*n*=16)* | |
| Turkish patients (*n*=10) | Connexin-26 mutation (one case) |
| Bosnian patients (*n*=6) | Connexin-26 mutations (two cases) |

*No further classification due to small sample size.

**Table 2** *GJB2* mutations and clinical findings (including three sib pairs)

| *Proband* | *Origin (federal countries of Austria or other countries)* | *Family history* | *Genotype*[a] | *Onset* | *Hearing loss*[c] *Severity; configuration of audiogram* |
|---|---|---|---|---|---|
| FJ-2682 | Steiermark | Autosomal recessive case | 35delG/35delG | Prelingual | Profound; gently sloping |
| SJ-2827 | Tirol | Sporadic case | 35delG/35delG | Prelingual | Profound/severe; progressive; gently sloping/flat |
| SJ-2875 | Tirol | Sporadic case | 35delG/35delG | Perilingual | Moderate/severe; gently sloping |
| LK-2927 | Niederösterreich | Autosomal recessive case | 35delG/35delG | Prelingual | Profound; gently sloping |
| LM-2928 | Niederösterreich | Brother of LK-2927 | 35delG/35delG | Prelingual | Profound; gently sloping |
| AS-3037 | Kärnten/Oberösterreich | Autosomal recessive case | 35delG/35delG | Prelingual | Profound; gently sloping |
| SM-3011 | Kärnten | Autosomal recessive case | 35delG/35delG | Prelingual | Profound; gently sloping/flat |
| MV-3432 | Tirol/*Germany* | Sporadic case | 35delG/35delG | Prelingual | Profound/severe; gently sloping |
| VM-2734 | Niederösterreich/*Czech Rep.* | Autosomal dominant case | L90P/R143Q (428G>A)[b] | Prelingual | Profound; gently sloping |
| OS-2954 | Tirol | Sporadic case | L90P/314del14 | Perilingual | Mild; steeply sloping |
| WK-3062 | Tirol | Autosomal recessive case | L90P/35delG | Prelingual | Moderate; steeply sloping |
| WE-3238 | Tirol | Sister of WK-3062 | L90P/35delG | Prelingual | Profound/moderate; gently sloping/flat |
| MK-3133 | Tirol | Autosomal dominant case | L90P/35delG | 1st decade | Moderate/severe; progressive; steeply/gently sloping |
| SM-3393 | Tirol | Sporadic case | L90P/I20T (59T>C)[b] | 1st decade | Moderate; steeply sloping |
| KM-3058 | Tirol | Sporadic case | 35delG/V84L | Prelingual | Profound; gently sloping |
| SA-2996 | *Bosnia* | Autosomal recessive case | 35delG/IVS1+1G>A | Prelingual | Moderate; flat |
| SD-2998 | *Bosnia* | Brother of SA-2996 | 35delG/IVS1+1G>A | Prelingual | Moderate; flat |
| GC-3072 | Tirol | Autosomal dominant case | 35delG/? | 1st decade | Moderate; progessive; steeply sloping/flat |
| LJ-3297 | Tirol | Sporadic case | 35delG/? | Prelingual | Moderate; gently sloping |
| ZG-3124 | Tirol | Sporadic case | L90P/? | Prelingual | Profound; gently sloping |
| JS-3038 | *Bosnia* | Sporadic case | R127H/? | Prelingual | Profound; gently sloping |
| SI-3257 | *Turkey* | Sporadic case | Y155X (465T>A)[b]/? | Prelingual | Mild; gently sloping |
| EA-3463 | Tirol | Autosomal dominant case | G160S/? | 4th decade | Mild; U-shaped |
| KC-2959 | Tirol | Autosomal dominant case | G150S/? | Prelingual | Severe; gently sloping |

[a]References to mutations: 35delG (Zelante *et al.*[11]); L90P (Denoyelle *et al*[7]); 314del14; V84L (Kelley *et al*[12]); R127H (Estivill *et al*[13]); IVS8+1G>A (Denoyelle *et al*[7]). [b]Novel mutations associated with NSHL. [c]Patients had no other associated symptoms, only MK-3133 had a history of recurrent vertigo and neuritis. In all individuals otoacoustic emissions were negative. In all 19 patients the results on evoked potentials testing confirmed the degree of hearing loss measured by pure tone audiometry. Nine individuals underwent a CT of the temporal bone which revealed no inner ear malformations.

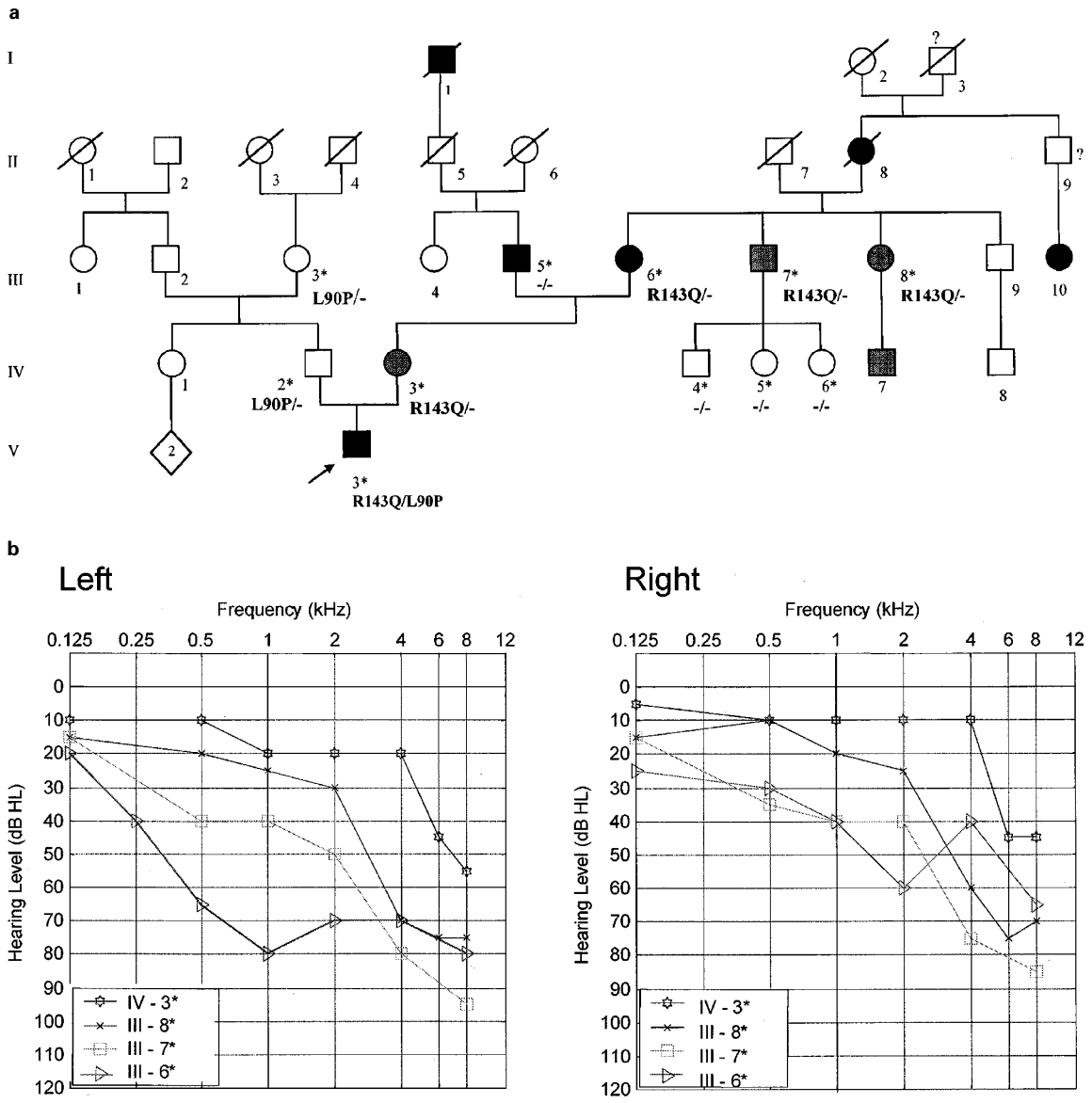


**Figure 1** Co-segregation of the *GJB2* mutation R143Q with profound or high frequency hearing loss. (**A**) The 7-year-old index patient (arrow) presented with profound hearing loss and compound heterozygosity for R143Q and L90P. Family members indicated by an asterisk were available for audiometric and molecular investigation. Black symbols denote severe or profound hearing loss, grey symbols denote high frequency hearing loss (starting at 2 kHz) and white symbols indicate family members with normal audiometric or clinical phenotype. In maternal family members carrying the mutation R143Q no second mutation in the coding-region of *Cx26* was found. (**B**) Audiograms of patients heterozygous for R143Q showing high frequency hearing loss suggest a dominant effect of this mutation. The mildest affected family member (IV.3*) is the youngest one (29 years). The severity of hearing loss is increased in the older family members pointing towards a progressive type of hearing loss.

Another novel missense mutation, I20T (c.59T>C) affecting a highly conserved residue within the N-terminal domain of GJB2, was identified in trans with L90P in an adult with apparently sporadic congenital HL originating from Tirol. The mutation Y155X (c.465T>A) was found in a patient of Turkish origin as the sole mutation. The affected girl presented isolated, mild hearing impairment without a family history compatible with autosomal recessive transmission of this mutation.

The pathogenicity remains unclear for the mutation G160S, identified as the sole *Cx26* mutation in this study in two Austrian NSHL patients with a history of autosomal

dominant hearing loss. In one case, both parents, and in the other case, two daughters, were reported having hearing impairment (not available for molecular analysis). Originally this sequence variation was considered as a polymorphism, having been observed twice in 100 random individuals from the Midwestern US whose hearing status was probably unknown.[6] While the amino acid change of glycine to serine is conservative, the affected residue is invariant in connexins 26, 30, 31, 32 and 44 of different species.

The fact that our analyses involved the coding and only part of the non-coding region of the *Cx26* gene might account for the failure to identify a presumed partnering *Cx26* mutation in seven hearing impaired individuals. At the moment, we can not exclude the possibility that some of these patients are heterozygous carriers, with another genetic or non-genetic cause accounting for their hearing loss.

### Genotype-phenotype study

The hearing impairment in 24 patients carrying homozygous or compound heterozygous *Cx26* mutations (including two sib pairs) was bilateral in all cases, severity ranged from mild to profound (from 25 to 115 dB), and differed markedly between individuals with the same genotype (Table 2). Contrary to other studies,[7,8] several patients showed asymmetric hearing loss which can not yet be explained. The cochlear deficit associated with *Cx26* mutations involved all frequencies, except in heterozygotes for R143Q who showed only impaired hearing starting at 2–4 kHz (Figure 1B).

Interestingly, all NSHL patients heterozygous for 35delG with unknown second mutation had a moderate audiometric phenotype. Our mildest affected patients with *Cx26* mutations had 35/36dB hearing loss with genotype L90P/511del14b and 35/25dB hearing loss with only the heterozygous mutation Y155X detected. The latter patient was born prematurely and formerly suspected of a perinatal infection. We identified *Cx26* mutations in three adults with a postlingually detected moderate audiometric phenotype. We therefore suggest extending the *Cx26* mutation analysis to those groups of patients.

This study had direct clinical impact for genetic counselling of the families with HL, and might improve the educational process of some hearing impaired children. The challenge that remains is to identify the underlying genetic cause in familial cases not yet clarified.

### Acknowledgements

*We wish to thank Prof K Welzl-Müller, Prof P Zorowka, Dr B Utermann, Dr A Schmidt, Dr ML Rouhbakhsh, Dr E Zimmermann, Dr H Herka, Dr V Weichbold, Mrs I Senn, K Egger, and all the families who participated in this study for their co-operation and support. The skilful technical assistance of Susanne Rauchenwald is gratefully acknowledged.*

### References

1 Cohen Jr MM, Gorlin RJ: Epidemiology, aetiology and genetic patterns; in Gorlin RJ, Toriello HV, Cohen Jr MM (eds): *Hereditary hearing loss and its syndromes*. New York: Oxford University Press, 1995, pp 9–21.
2 Van Camp G, Smith RJ: 1998; Hereditary Hearing Loss Homepage. World Wide Web http://hgins.uia.ac.be/dnalab/hhh/
3 Rabionet R, Gasparini P, Estivill X: Connexins and deafness Homepage. World wide web URL: http://www/iro.es/Cx26deaf.html
4 Martini A: The European Concerted Action Project On Genetic Hearing Impairment. World wide web URL:http://hear.unife.it
5 Verlingue C, Mercier B, Lecoq I *et al*: Retrospective study of the cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in Guthrie cards from a large cohort of neonatal screening for cystic fibrosis. *Hum Genet* 1994; **93**: 429–434.
6 Scott DA, Kraft ML, Carmi R *et al*: Identification of mutations in the connexin 26 gene that cause autosomal recessive nonsyndromichearing loss. *Hum Mutat* 1998; **11**: 387–394.
7 Denoyelle F, Marlin S, Weil D *et al*: Clinical features of the prevalent form of childhood deafness, DFNB1, due to connexin-26 gene defect: implications for genetic counselling. *Lancet* 1999; **353**: 1298–1303.
8 Rabionet R, Zelante L, Lopez-Bigas N *et al*: Molecular basis of childhood deafness resulting from mutations in the GJB2 (connexin 26) gene. *Hum Genet* 2000; **106**: 40–44.
9 Gasparini P, Rabionet R, Barbujani G *et al*: High carrier frequency of the 35delG deafness mutation in European populations. Genetic Analysis Consortium of GJB2 35delG. *Eur J Hum Genet* 2000; **8**: 19–23.
10 Brobby GW, Muller-Myhsok B, Horstmann RD: Connexin 26 R143W mutation associated with recessive nonsyndromic sensorineural deafness in Africa. *N Engl J Med* 1998; **338**: 548–550.
11 Zelante L, Gasparini P, Estivill X *et al*: Connexin 26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNBI) in Mediterraneans. *Hum Mol Genet* 1997; **6**: 1605–1609.
12 Kelley PM, Harris DJ, Comer BC *et al*: Novel mutations in the connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1) hearing loss. *Am J Hum Genet* 1998; **62**: 792–799.
13 Estivill X, Fortima P, Surrey S *et al*: Connexin 26 mutations in sporadic and inherited sensorineural deafness. *Lancet* 1998; **351**: 394–398.