Prevalence and audiological features in carriers of GJB2 mutations, c.35delG and c.101T>C (p.M34T), in a UK population study

Amanda Hall,1 Marcus Pembrey,2 Mark Lutman,3 Colin Steer,2 Maria Bitner-Glindzicz4

ABSTRACT

Objectives: To determine the carrier rate of the GJB2 mutation c.35delG and c.101T>C in a UK population study; to determine whether carriers of the mutation had worse hearing or otoacoustic emissions compared to non-carriers.

Design: Prospective cohort study.

Setting: University of Bristol, UK.

Participants: Children in the Avon Longitudinal Study of Parents and Children. 9202 were successfully genotyped for the c.35delG mutation and c.101>T and classified as either carriers or non-carriers.

Outcome measures: Hearing thresholds at age 7, 9 and 11 years and otoacoustic emissions at age 9 and 11.

Results: The carrier frequency of the c.35delG mutation was 1.36% (95% CI 1.13 to 1.62) and c.101>T was 2.69% (95% CI 2.37 to 3.06). Carriers of c.35delG and c.101>T had worse hearing than non-carriers at the extra-high frequency of 16 kHz. The mean difference in hearing at age 7 for the c.35delG mutation was 3.94 dB (95% CI 2.10 to 5.78) and 7.61 dB (95% CI 4.26 to 10.96) at age 9. Otoacoustic emissions were smaller in the c.35delG mutation carrier group: at 4 kHz the mean difference was −4.95 dB (95% CI −6.70 to −3.21) at age 9 and −3.94 dB (95% CI −5.78 to −2.10) at age 11. There was weak evidence for differences in otoacoustic emissions amplitude for c.101>T carriers.

Conclusion: Carriers of the c.35delG mutation and c.101>T have worse extra-high-frequency hearing than non-carriers. This may be a predictor for changes in lower-frequency hearing in adulthood. The milder effects observed in carriers of c.101>T are in keeping with its classification as a mutation causing mild/moderate hearing loss in homozygosity or compound heterozygosity.

INTRODUCTION

The human gap junction β-2 gene (GJB2) that encodes the protein connexin 26 was the first autosomal gene to be identified for non-syndromic deafness.1 Connexin 26 is involved in recycling of potassium ions in the endolymph of the cochlea and mutations in this gene are by far the commonest cause of autosomal-recessive non-syndromic sensorineural hearing loss (NSSNHL) worldwide.

The mutation c.35delG is the most common mutation causing severe–profound deafness in Caucasian populations and accounts for approximately 70% of autosomal recessive NSSNHL.3 4 The c.35delG carrier rate was evaluated by Mahdieh and Rabbani4 who pooled data from 41 studies, including a review by Gasparini et al5 in which they estimated the carrier rate to be highest in Europe with a mean rate of 1.89%. However within Europe, there was variation across countries with a higher rate of 2.48% in Southern Europe compared with 1.53% in Northern Europe.5 This variation highlights the importance of knowing the carrier rate for individual countries. There
are, however, relatively few data from the UK with Gasparini et al\(^4\) finding 0/119 carriers in the UK arm of their European study. The high carrier rate of \(GJB2\) mutations is of interest and some have suggested possible heterozygote advantage.\(^7\) Such an advantage would have to outweigh any negative biological effects on hearing.

There has also been interest in \(c.101T>C\) (p.M34T) the effect of which on hearing is the subject of contention. \(c.101T>C\) (p.M34T) has a higher carrier rate than c.35delG in Caucasian populations. A US study found 3/128 carriers,\(^9\) giving a rate of 2.3% and Houseman et al\(^10\) found a carrier rate of 4.8% in the UK based on a small sample of 630.

\(c.101T>C\) (p.M34T) was first described as a dominant mutation\(^1\) and subsequently as a recessive mutation.\(^10-12\) In vitro studies have shown that \(GJB2\): p.M34T is correctly synthesised, locates to the cell membrane normally, but shows impaired intercellular coupling as judged by transfer of dyes between neighbouring cells through the gap junctions. There are also observations that there is disturbed oligomerisation of \(GJB2:p.M34T\) connexins.\(^13-15\) One would therefore expect an effect on the cochlea and on hearing. Some studies have demonstrated dominant negative effects of the mutant (p.M34T) on wild-type connexins\(^15\) \(^16\) and yet it is clear from human genetic studies that this is not a dominantly acting mutation. Indeed many examples exist where homozygosity for p.M34T/p.M34T or compound heterozygosity p.M34T/c.35delG is associated with normal hearing\(^17-19\) or a significantly milder hearing loss than that associated with truncating and even other non-truncating mutations of \(GJB2\).\(^16-12\)\(^20\)

Previous studies of hearing in \(GJB2\) mutation carriers identified through genetic testing have shown conflicting results, summarised in supplementary table S1. All of these are small studies often based on ascertainment of carriers as the parents or relatives of children with severe/profound deafness. Morell et al\(^8\) showed no/minor differences in the pure-tone audiograms of c.35delG carriers compared to controls, as did Engel-Yeger et al.\(^22\)\(^25\)

Conversely, Franzé et al\(^8\) showed worse high-frequency hearing thresholds in c.35delG carriers compared to controls. Using otoacoustic emissions (OAE), perhaps a more sensitive measure of hearing, carriers showed reduced amplitude OAE compared to controls, particularly for the high frequencies. Amplitude differences ranged from 5 dB across 1–4 kHz\(^21\) to 1–2 dB across 1–10 kHz.\(^22\)\(^25\) It has also been suggested that carriers of \(GJB2\) mutations may be at greater risk of susceptibility to noise damage.\(^25\)

However, a study of over 3000 participants found no increased susceptibility to age or noise exposure in c.35delG carriers compared to non-carriers.\(^26\)

The relationship between genotype and phenotype in those carrying \(c.101T>C\) (p.M34T) is even less clear, but of great interest given the high carrier rate. To our knowledge, there have been no population studies examining the hearing of \(c.101T>C\) (p.M34T) carriers. However, studies of families with hearing loss have shown a varying effect of \(c.101T>C\) (p.M34T) on hearing. Bicego et al\(^15\) studied seven families with \(c.101T>C\) (p.M34T) and hearing impairment. Within these families, there were 11 \(c.101T>C\) (p.M34T) heterozygotes of which 5 had hearing loss. However, in several of these families, \(c.101T>C\) (p.M34T) did not segregate with hearing loss and some families appeared to have had dominantly inherited hearing losses.

In addition, these studies were all conducted on adults and it is not clear as to when in the life course these differences might arise. There is thus a need to accurately determine the UK carrier rate for \(GJB2\) mutations and to further investigate the audiological profile of carriers within a population to determine whether previously found differences in hearing and OAE in adults are present in childhood. The aims of this study were first to determine the carrier rate of c.35delG mutations and \(c.101T>C\) (p.M34T) in a large UK prospective population, the Avon Longitudinal Study of Parents and Children (ALSPAC) and second to compare hearing thresholds up to 16 kHz and transient evoked OAEs in carriers of the \(GJB2\) mutations c.35delG and c.101T>C with non-carriers at age 7, 9 and 11 years age. These issues have not been adequately addressed in the literature using a prospective study design with a large sample of participants unselected for hearing or genetic status.

### MATERIALS AND METHODS

#### Avon Longitudinal Study of Parents and Children

This study uses data from ALSPAC, a large UK prospective population study of child development. ALSPAC recruited 14 541 pregnant women resident in Avon, UK with expected dates of delivery 1 April 1991–31 December 1992. A wide range of information was collected on the mothers and their offspring, including detailed information about the mother during pregnancy, birth and medical history of the child, repeat physical and psychological measures of the child and educational outcomes.\(^27\)

The profile of the cohort has been recently described;\(^28\) of relevance to this study is the ethnicity of the children enrolled within ALSPAC who were predominantly white (96.09%). The follow-up and attrition rate of the enrolled sample over time are also described.\(^28\)

For further general information about ALSPAC, see http://www.bristol.ac.uk/alspac/.

From age 7, the whole cohort was invited to attend a regular half day assessment ‘Focus clinic’ at the University of Bristol. As part of these clinics, blood samples were obtained for DNA analysis and at three clinics, hearing was assessed.

Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

#### DNA analysis

DNA was extracted from cord blood and blood samples collected at Focus clinics.\(^29\) DNA from the ALSPAC...
cohort was screened by KBioScience following successful ‘blind’ validation of the assay using known positive and negative controls. Single-nucleotide polymorphism genotyping for the presence of both c.101T>C and c.35delG was performed by competitive allele PCR (KASPar) and TaqMan genotyping assays (www.kbioscience.co.uk/genotyping/genotyping-chemistry.htm).

**Hearing and middle-ear measures**

At age 7, 9 and 11 years, children were assessed with pure-tone audiometry using Kamplex AD12 and GSI 61 audiometers at age 7, and a GSI 61 audiometer at age 9 and 11, with TDH 39 headphones, calibrated to ISO 389. Air conduction thresholds were measured at 0.5–8 kHz, and bone conduction thresholds at 0.5–4 kHz according to the British Society of Audiology recommended procedure for audiometry. At age 7 and 9, extra-high-frequency hearing thresholds were measured at 16 kHz using the GSI 61 audiometer with circumaural HDA200 headphones. The 16 kHz threshold was only measured where time permitted. Tympanometry was measured using a Kamplex AT2 tympanometer at age 7 and a GSI 38 tympanometer at age 9 and 11 years.

Measures at age 7 were taken in a quiet room and at 9 and 11 in a sound-treated booth. All tests were carried out by qualified audiologists or testers specially trained for this purpose. All staff underwent regular audits to assess their reliability on audiometry. Testers were blind to the results of previous hearing tests when performing audiometry and to the genetic status of the children. If a hearing loss was measured at the Focus clinic, parents were given a copy of the results and advised to consult their child’s general physician.

**Otoacoustic emissions**

At age 9 and 11 years, transient evoked otoacoustic emissions were recorded using the Otodynamics ILO92 system. Click stimuli were presented at a gain of −10.5 dB and −19.5 dB (re: reference click at −80 dB sound pressure level (SPL)) and recordings made in the linear mode. These settings were used as lower-level stimuli may be more sensitive to changes and differences in cochlear function. Analysis of OAE waveforms here concentrates on the measure that is conventionally named as response, which is the SPL of the recorded components that are common to the two interleaved averages obtained during recording, conventionally denoted by A and B. Analogous to the way that the power of a signal is obtained mathematically by summing across frequency the product of the Fourier transform of the signal and its complex conjugate, the response measure is derived by summing across frequency the real part of the cross-product of the Fourier transform of A and the complex conjugate of the Fourier transform of B. The real part contains only those components that are in phase in A and B. This measure can simply be considered as an estimate of the OAE signal after removal of the noise. The response measure was obtained from the raw (unfiltered) recordings and also after filtering into frequency bands centred on 1, 2, 3 and 4 kHz. Each filter had a bandwidth of 1 kHz.

OAE amplitudes, as defined by the response measure, of the broadband wave and at 1, 2, 3 and 4 kHz were used as outcomes.

**Sociodemographic data**

Information on child sex (male/female), ethnicity (white/non-white), birthweight, gestation, maternal age and highest level of maternal education (<16, 16 and >16 years) was available from medical records, clinic visits and parental self-completion questions. These data were used to describe the sociodemographics of the sample and to compare to those with no genetic and hearing data available.

The relationship of child sex and ethnicity with hearing and carrier status was investigated for possible confounding.

**Statistical analysis**

Univariate linear regression analyses were performed to analyse associations between carrier status (carrier of c.35delG mutation/non-carrier; carrier of c.101T>C/ non-carrier) and hearing/OAE outcomes. Both genetic variables were included in the analyses to allow direct comparison of effect sizes. To increase the statistical power, right and left ear hearing thresholds and right and left OAE amplitude data were averaged for each participant. Analyses were performed using STATAV.11.0.

**RESULTS**

**Sample**

Genetic data were available on 9631 children of the whole cohort whose parents consented for biological samples to be taken at clinical visits. Of these 9631 samples, genotyping was successfully performed on 9202 samples (95.5%). Their characteristics and how they compare with the rest of the ALSPAC cohort are shown in table 1. As is typically seen in epidemiological studies the study sample was more advantaged than the rest of the cohort. There was an under-representation of non-white children and an over-representation of children from older, more educated mothers in the study sample. The children in the study sample also had a higher birthweight and a longer gestation period.

**Carrier rate**

The c.35delG and c.101T>C mutation rate is summarised in table 2. This shows a carrier rate of 1.36% (95% CI 1.13% to 1.62%) for c.35delG and 2.69% (95% CI 2.37% to 3.05%) for c.101T>C. Three cases, 0.03% (95% CI 0.006% to 0.09%) were homozygous for c.101T>C, see figure 1 for their audiograms. None of the participants carried both the c.35delG mutation and c.101T>C.
There was no evidence of disequilibrium for either the c.35delG mutation (p=0.510) or c.101T>C (p=0.334).

The sex and ethnicity characteristics of the sample were examined to determine whether these varied with both the carrier status and hearing thresholds, and thus could confound the results. Table 3 shows the sample characteristics according to carrier status. There was evidence of a weak association between child ethnicity and c.101T>C carrier status (p=0.026), but not between child ethnicity and average hearing thresholds at age 7 (left ear p=0.584; right ear p=0.207). There was no evidence of a sex difference between carriers and non-carriers for c.35delG (p=0.457) or c.101T>C (p=0.387), although there was a relationship between sex and average hearing threshold with females having worse hearing than males (age 7 results: left ear p=0.076; right ear p=0.000). As carrier status and hearing thresholds did not vary consistently with sex and ethnicity, confounding is unlikely and therefore unadjusted statistical results are presented. Analyses were also performed excluding non-white children from the sample, and these gave essentially the same results, see supplemental tables S2 and S3.

### Hearing thresholds

Hearing data from at least one time point were available on 97 out of 125 c.35delG carriers and 190 of 246 c.101T>C carriers. Figure 2 displays the mean audiometric hearing thresholds for carriers and non-carriers at age 11 showing that the mean thresholds of the carrier and non-carrier groups were all within the normal range.

At age 7, 7774 of the cohort had hearing thresholds measured, at age 9 it was 7379 and at age 11 it was 7111. As described for the genetic data, those children attending the Focus clinics were more likely to be advantaged compared with the rest of the cohort. Cases where both hearing threshold and genotype data were available were used in linear regression analysis to estimate the difference in hearing thresholds between carriers and non-carriers for each of the frequencies tested at age 7, 9 and 11. Fewer than half the children had data for 16 kHz due to time constraints. The c.35delG results of the regression analysis are shown in table 4 and the c.101T>C results in table 5. For the c.35delG mutation there was no evidence that the carriers had worse hearing thresholds at the conventional audiometric frequencies. However, at 16 kHz there was strong evidence of a difference between the two groups with carriers having mean thresholds 8 dB worse than non-carriers at age 7, increasing to 12 dB at age 9.

For c.101T>C there was no evidence of a difference in hearing threshold between carriers and non-carriers at the conventional audiometric frequencies. At 16 kHz, there was evidence of worse hearing in the carrier group at age 9 and 11, with hearing 3 dB and 7 dB worse, respectively.

There was weak evidence that carrying the c.35delG mutation had a larger effect than c.101T>C on 16 kHz hearing thresholds at age 9 (p=0.111) and 11 (p=0.079).

### Otoacoustic emissions

OAE results obtained at the two different stimulus levels were analysed which showed essentially the same results.

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**Table 1** Characteristics of the study sample compared with the remaining ALSPAC cohort

<table>
<thead>
<tr>
<th></th>
<th>Sample with genetic information (either c.35delG or c.101T&gt;C) (n=9202)</th>
<th>Rest of ALSPAC cohort (n=5321)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% males)</td>
<td>51.85</td>
<td>50.78</td>
<td>0.215</td>
</tr>
<tr>
<td>Child’s ethnicity (% non-white)</td>
<td>4.33</td>
<td>6.43</td>
<td>0.000</td>
</tr>
<tr>
<td>Mean birthweight (g) (SD)</td>
<td>3422.56 (543.25)</td>
<td>3339.68 (582.17)</td>
<td>0.000</td>
</tr>
<tr>
<td>Mean gestation (weeks) (SD)</td>
<td>39.44 (1.81)</td>
<td>39.30 (2.10)</td>
<td>0.000</td>
</tr>
<tr>
<td>Mean age of mother (years) (SD)</td>
<td>28.48 (4.83)</td>
<td>27.15 (5.06)</td>
<td>0.000</td>
</tr>
<tr>
<td>Maternal highest education qualification (%)</td>
<td>&lt;16 years: 26.43</td>
<td>39.96</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>16 years: 35.17</td>
<td>34.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;16 years: 38.43</td>
<td>35.38</td>
<td></td>
</tr>
</tbody>
</table>

ALSPAC, Avon Longitudinal Study of Parents and Children.

**Table 2** Summary of GJB2 mutation carrier rate in Avon Longitudinal Study of Parents and Children

<table>
<thead>
<tr>
<th></th>
<th>Homozygote (%) (n)</th>
<th>Heterozygote (%) (n)</th>
<th>Homozygote recessive</th>
<th>Total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.35delG</td>
<td>G:G</td>
<td>G:−</td>
<td>0 (0)</td>
<td>9139</td>
</tr>
<tr>
<td></td>
<td>98.63 (9014)</td>
<td>1.36 (125)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.101T&gt;C</td>
<td>T:T</td>
<td>C:T</td>
<td>C:C</td>
<td>9112</td>
</tr>
<tr>
<td></td>
<td>97.27 (8863)</td>
<td>2.69 (246)</td>
<td>0.03 (3)</td>
<td></td>
</tr>
</tbody>
</table>
for the lower and higher stimulus level settings. There was no evidence that lower stimulus settings were more sensitive to differences between groups and therefore only higher-stimulus-level results are shown.

Linear regression was used to compare the OAE amplitude across frequency for carriers and non-carriers. Table 6 shows the difference in amplitude at age 9 and 11 for the carriers and non-carriers (OAE were not recorded at age 7). At age 9 the c.35delG carrier group had smaller OAE amplitude compared to non-carriers across the frequency range, with the largest differences at 3 and 4 kHz. A similar pattern was observed at age 11, although the differences were slightly smaller.

For c.101T>C there was evidence of smaller OAE amplitude at 4 kHz in the carriers at age 9 but not at 11.

There was strong evidence that carrying the c.35delG mutation had a larger effect than c.101T>C on OAE amplitude at age 9 (1 kHz, p=0.008; 2 kHz, p=0.011;

Figure 1 Audiograms of the three cases homozygous for c.101T>C. The most recent and complete data are shown. (A) Case 1 at age 7, (B) case 2 at age 11, (C) case 3 at age 9.
3 kHz, p=0.001 and 4 kHz, p=0.001); the evidence was weaker at age 11 (1 kHz, p=0.295; 2 kHz, p=0.046; 3 kHz, p=0.141 and 4 kHz, p=0.009).

DISCUSSION
Carrier rate
This study is one of the largest of its type where children were unselected for hearing status and tested as part of a prospective population study of development. ALSPAC is broadly representative of the UK in terms of sociodemographics albeit with a lower proportion of ethnic minorities. The results reported here are likely to be broadly generalisable for a white UK population.

The prevalence of c.35delG carriers in the ALSPAC cohort was 1.36% (95% CI 1.13% to 1.62%) which is consistent with the figure of 1.53% (95% CI 1.26% to 1.83%, calculated from data provided in the paper) given by Mahdieh and Rabbani for Northern Europe.

The prevalence of c.101T>C in this study was 2.69% (95% CI 2.37% to 3.05%), which is double that of c.35delG and lower than the rate of 5.81% (95% CI 4.44% to 7.44%, calculated from data provided in the paper) found in Estonia based on a sample size of 998. There were no cases carrying both the c.35delG mutation and c.101T>C in ALSPAC. Three cases (0.03%) were homozygous for c.101T>C. There was weak evidence that c.101T>C was less likely to be present in non-white children.

Hearing thresholds and otoacoustic emissions in c.35delG carriers
There was no effect of carrier status on hearing at the conventional audiometric thresholds examined at 7, 9

Figure 2 Mean audiograms at age 11. (A) c.35delG non-carriers, (B) c.35delG carriers, (C) c.101T>C non-carriers and (D) c.101T>C carriers.
### Table 4 Differences in hearing threshold between c.35delG carriers at age 7 (n=80), age 9 (n=81) and age 11 (n=77) and non-carriers (unadjusted results)

<table>
<thead>
<tr>
<th>Air or bone conduction</th>
<th>Frequency* (kHz)</th>
<th>N</th>
<th>Coefficient dB† (95% CI)</th>
<th>p Value</th>
<th>N</th>
<th>Coefficient dB† (95% CI)</th>
<th>p Value</th>
<th>N</th>
<th>Coefficient dB† (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0.5</td>
<td>5892</td>
<td>0.01 (−1.64 to 1.67)</td>
<td>0.985</td>
<td>5697</td>
<td>0.04 (−1.39 to 1.47)</td>
<td>0.953</td>
<td>5429</td>
<td>−0.34 (−1.81 to 1.13)</td>
<td>0.650</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6147</td>
<td>−0.16 (−1.81 to 1.48)</td>
<td>0.848</td>
<td>5783</td>
<td>0.68 (−0.79 to 2.14)</td>
<td>0.364</td>
<td>5473</td>
<td>−0.79 (−2.23 to 0.64)</td>
<td>0.277</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6091</td>
<td>−0.58 (−2.18 to 1.01)</td>
<td>0.472</td>
<td>5782</td>
<td>−1.16 (−2.59 to 0.26)</td>
<td>0.112</td>
<td>5472</td>
<td>−1.28 (−2.76 to 0.18)</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td>3†</td>
<td>6144</td>
<td>0.14 (−1.67 to 1.97)</td>
<td>0.872</td>
<td>5782</td>
<td>−0.46 (−2.10 to 1.17)</td>
<td>0.578</td>
<td>5431</td>
<td>−1.26 (−2.76 to 0.24)</td>
<td>0.100</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6119</td>
<td>0.63 (−1.43 to 2.70)</td>
<td>0.547</td>
<td>5675</td>
<td>0.34 (−1.63 to 2.33)</td>
<td>0.731</td>
<td>5468</td>
<td>−0.75 (−2.33 to 0.83)</td>
<td>0.352</td>
</tr>
<tr>
<td></td>
<td>6‡</td>
<td>2860</td>
<td>8.53 (2.99 to 14.07)</td>
<td>0.000</td>
<td>4166</td>
<td>12.57 (8.10 to 17.04)</td>
<td>0.000</td>
<td>5427</td>
<td>−0.67 (−2.53 to 1.19)</td>
<td>0.480</td>
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<tr>
<td>Bone¶</td>
<td>0.5</td>
<td>6001</td>
<td>−0.33 (−1.86 to 1.19)</td>
<td>0.669</td>
<td>5753</td>
<td>−0.75 (−2.18 to 0.66)</td>
<td>0.297</td>
<td>5388</td>
<td>−1.04 (−3.00 to 0.91)</td>
<td>0.297</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5581</td>
<td>0.18 (−1.27 to 1.65)</td>
<td>0.800</td>
<td>5473</td>
<td>−0.74 (−2.36 to 0.59)</td>
<td>0.613</td>
<td>5444</td>
<td>−0.92 (−2.38 to 0.53)</td>
<td>0.213</td>
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<tr>
<td></td>
<td>2</td>
<td>5581</td>
<td>0.18 (−1.27 to 1.65)</td>
<td>0.800</td>
<td>5473</td>
<td>−0.74 (−2.36 to 0.59)</td>
<td>0.613</td>
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<td>0.213</td>
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<td></td>
<td>4</td>
<td>5581</td>
<td>0.18 (−1.27 to 1.65)</td>
<td>0.800</td>
<td>5473</td>
<td>−0.74 (−2.36 to 0.59)</td>
<td>0.613</td>
<td>5444</td>
<td>−0.92 (−2.38 to 0.53)</td>
<td>0.213</td>
</tr>
</tbody>
</table>

*Right and left ear average.
†A positive coefficient means that hearing threshold is higher (ie, worse) in the carrier group compared to the non-carriers.
‡Thresholds at 3 and 6 kHz were measured at age 11.
§Thresholds at 16 kHz were measured at age 7 and 9.
¶Bone conduction thresholds were measured at 1 and 4 kHz at age 7, and at 0.5, 1 and 2 kHz at age 9 and 11.

### Table 5 Differences in hearing threshold between c.101T>C carriers at age 7 (n=171), age 9 (n=150) and age 11 (n=135) and non-carriers (unadjusted results)

<table>
<thead>
<tr>
<th>Air or bone conduction</th>
<th>Frequency* (kHz)</th>
<th>N</th>
<th>Coefficient dB† (95% CI)</th>
<th>p Value</th>
<th>N</th>
<th>Coefficient dB† (95% CI)</th>
<th>p Value</th>
<th>N</th>
<th>Coefficient dB† (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0.5</td>
<td>5892</td>
<td>0.30 (−0.82 to 1.42)</td>
<td>0.602</td>
<td>5697</td>
<td>−0.02 (−1.08 to 1.03)</td>
<td>0.961</td>
<td>5429</td>
<td>0.33 (−0.76 to 1.44)</td>
<td>0.550</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6147</td>
<td>0.64 (−0.49 to 1.78)</td>
<td>0.266</td>
<td>5783</td>
<td>−0.40 (−1.49 to 0.68)</td>
<td>0.466</td>
<td>5473</td>
<td>−0.23 (−1.32 to 0.85)</td>
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<td>6091</td>
<td>0.38 (−0.71 to 1.49)</td>
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<td>5782</td>
<td>−0.36 (−1.44 to 0.67)</td>
<td>0.474</td>
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<td>5782</td>
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<td>5675</td>
<td>0.02 (−1.44 to 1.49)</td>
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<td>5468</td>
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<td>6‡</td>
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<td>4166</td>
<td>7.61 (4.26 to 10.96)</td>
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<td>0.33 (−1.06 to 1.72)</td>
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<td>6001</td>
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<td>5753</td>
<td>0.02 (−1.02 to 1.07)</td>
<td>0.961</td>
<td>5449</td>
<td>−0.28 (−1.35 to 0.78)</td>
<td>0.604</td>
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<td>0.26 (−0.81 to 1.33)</td>
<td>0.635</td>
<td>5473</td>
<td>0.02 (−1.02 to 1.07)</td>
<td>0.961</td>
<td>5449</td>
<td>−0.28 (−1.35 to 0.78)</td>
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<td>5449</td>
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<td>0.604</td>
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</table>

*Right and left ear average.
†A positive coefficient means that hearing threshold is higher (ie, worse) in the carrier group compared to the non-carriers.
‡Thresholds at 3 and 6 kHz were measured at age 11.
§Thresholds at 16 kHz were measured at age 7 and 9.
¶Bone conduction thresholds were measured at 1 and 4 kHz at age 7, and at 0.5, 1 and 2 kHz at age 9 and 11.
and 11 years, consistent with the smaller study of Engel-Yeager et al.\textsuperscript{22} Unlike the study by Franzé et al.\textsuperscript{23} we did not show a difference in hearing at 6 and 8 kHz. Their study selected participants from a clinical caseload and were older than the participants in this study, which may account for the different results.

However at age 7 and 9 years, a measure of extra-high-frequency hearing at 16 kHz was available and comparison of the hearing thresholds at this frequency showed c.35delG carriers had hearing thresholds approximately 5–10 dB worse than non-carriers. There was a larger difference at age 9 than at age 7, suggesting a worsening in extra-high-frequency hearing between these ages. This provides evidence of early changes to the hearing of the carrier group, which although not detectable on conventional audiology may be a predictor of later changes in hearing in adulthood.

In addition, there was also evidence that OAE amplitude is lower in the c.35delG carrier group: at age 9, the mean OAE amplitude of carriers was 2–5 dB lower than non-carriers with the largest differences observed at 4 kHz. At age 11, these differences were still apparent, although smaller than at age 9, possibly as a result of fewer cases in the analysis at 11. The poorer hearing thresholds at 16 kHz in the carrier group may explain the lower OAE amplitude, as variation in high- and extra-high-frequency hearing has been shown to explain differences in lower frequency OAE amplitude.\textsuperscript{34–36} The results could also be explained by subclinical damage to the lower-frequency regions of the cochlea not yet detectable on the audiogram.

### Hearing in carriers of c.35delG and c.101T>C

Hearing thresholds and OAE amplitude were examined for the c.101T>C carriers. There was no evidence of an effect of carrier status on hearing thresholds at the conventional frequencies at age 7, 9 or 11 years. Examination of the 16 kHz extra-high-frequency hearing threshold showed that those carrying c.101T>C had worse hearing at age 7 and 9. The size of the effect increased from 3 to 7 dB between these ages. These results were similar to those obtained for the c.35delG carriers, although with weak evidence of a smaller effect.

For the OAE results, the evidence was generally weak that amplitude was lower in the carrier group. At age 9, the 4 kHz amplitude was 1–2 dB smaller in the carrier group but this effect was reduced at age 11. These results suggest that the smaller differences in extra-high-frequency hearing have a negligible effect on the lower-frequency OAE and suggest that c.101T>C has a weaker effect on hearing than carrying c.35delG, which would be consistent with its effects in individuals homozygous for c.101T>C. There has been controversy in the literature as to whether c.101T>C has an influence on hearing and many c.101T>C homozygotes and compound heterozygotes have milder hearing loss than that observed with truncating mutations such as

<table>
<thead>
<tr>
<th>Table 6</th>
<th>Differences in otoacoustic emissions (OAE) amplitude between c.35delG carriers at age 9 (n=131) and age 11 (n=99) and non-carriers, and c.101T&gt;C carriers at age 9 (n=61) and age 11 (n=55) and non-carriers</th>
<th>Carrier</th>
<th>OAE frequency* (kHz)</th>
<th>N</th>
<th>Coefficient</th>
<th>(95% CI) p Value</th>
<th>Coefficient†</th>
<th>(95% CI) p Value</th>
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<td>Broadband</td>
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<td>c.35delG</td>
<td>Age 9</td>
<td>4810</td>
<td>-0.20</td>
<td>(-3.32 to 2.91) 0.476</td>
<td>0.35</td>
<td>(0.19 to 0.53) 0.000</td>
<td>0.25</td>
<td>(0.16 to 0.33) 0.000</td>
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<tr>
<td></td>
<td>Age 11</td>
<td>4810</td>
<td>-0.30</td>
<td>(-5.45 to 4.85) 0.978</td>
<td>0.20</td>
<td>(0.08 to 0.33) 0.011</td>
<td>0.14</td>
<td>(0.05 to 0.23) 0.011</td>
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<tr>
<td>c.101T&gt;C</td>
<td>Age 9</td>
<td>4810</td>
<td>-0.30</td>
<td>(-4.95 to 4.35) 0.978</td>
<td>0.16</td>
<td>(0.07 to 0.26) 0.011</td>
<td>0.07</td>
<td>(0.00 to 0.14) 0.040</td>
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</tr>
</tbody>
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*Right and left ear average. A negative coefficient indicates that OAE amplitude is smaller in the carrier group compared to non-carriers.
c.35delG\textsuperscript{20} or even normal hearing. The milder effects we observe here might also suggest reduced penetrance and/or a later age of onset on hearing as suggested by others.\textsuperscript{11}

**Study limitations**

The sample was more advantaged than the whole of the ALSPAC cohort, as is typical with longitudinal studies of health and development\textsuperscript{37} but this should not distort specific genotype–phenotype associations. Owing to the low numbers of non-white children within ALSPAC,\textsuperscript{28} the results are generalisable to the white UK population only.

The weaknesses of this study include sample attrition and thus possible loss of statistical power to detect differences between the carriers and non-carriers, particularly for the smaller c.35delG carrier group. Not all the carrier group had hearing tests performed at each of the time points and so it is possible that we did not have the power to detect differences at the conventional audiometric frequencies. The number of cases available for the OAE analysis was smaller than those available for the hearing analysis, and thus power is equally an issue for these data.

**CONCLUSIONS**

We have shown that carriers of the c.35delG mutation and c.101T\textsuperscript{C} have subtle differences in their audiological profile at age 9 compared to non-carriers at the extra-high frequencies. This adds further evidence that c.101T\textsuperscript{C} is a mild but functional variant with the effect larger in the c.35delG group. It will be interesting to observe whether effects become more pronounced with advancing age.

**Acknowledgements**

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**Contributors**

MP, MB-G and AH designed the study. AH took the lead in analysis, writing and submission. MP and MB-G provided genetics expertise. ML analysed the otoacoustic emission data and advised on audiological measures. CS advised on statistical analysis. All authors were involved in drafting, revising and agreeing the final version of the article.

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**Competing interests**

None.

**Ethics approval**

ALSPAC Law and Ethics Committee and Local Research Ethics Committees.

**Provenance and peer review**

Not commissioned; externally peer reviewed.

**REFERENCES**


Hearing in carriers of c.35delG and c.101T>C


Prevalence and audiological features in carriers of GJB2 mutations, c.35delG and c.101T>C (p.M34T), in a UK population study

Amanda Hall, Marcus Pembrey, Mark Lutman, Colin Steer and Maria Bitner-Glindzicz

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