

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Bitner-Glindzicz M, Pembrey M, Duncan A, et al. Prevalence of mitochondrial 1555A→G mutation in European children. *N Engl J Med* 2009;360:640-2.

The ALSPAC cohort

ALSPAC recruited 14 541 pregnant women with an estimated date of delivery between 1 April 1991 and 31 December 1992. 13 988 children survived for at least 12 months. About 85% of eligible expectant mothers participated; recruitment, dropout, and other methodologies have been described elsewhere (<http://www.alspac.bris.ac.uk/>). Resources include data from questionnaires completed by the parents and children themselves at regular intervals, biological samples including DNA, and direct measurements (including pure tone audiometry, and tympanometry on children). Data is also available regarding admission of children to neonatal intensive care units (NICU). All of this data has been collected prospectively.

Consent

The study had the approval of the ALSPAC Ethics and Law Advisory Committee and local ethics committees. Detailed information is available on the ALSPAC website <http://www.alspac.bris.ac.uk/>.

Maternal consent was obtained for audiological testing to which the study child assented. Maternal consent was obtained for undisclosed genotyping of her child's DNA.

Ancestral Origin

Over 95% of the study parents are described as white. Ancestral origin was obtained by maternal self-reporting on the following question at 32 weeks gestation and also at 97 months of child's age. 'How would you describe the race or ethnic group of yourself, your live-in husband or partner and your parents?

(i) Yourself (ii) Partner (iii) Your mother (iv) Your father: White, Black/Caribbean, Black/African, Black/other (please describe), Indian, Pakistani, Bangladeshi, Chinese, any other ethnic group (please describe).

<http://www.bristol.ac.uk/alspac/documents/ques-m13-mother-and-family.pdf>

Genotyping

Table 1.

Genotyping results for m.1555A>G

	Frequency	Valid percent
Genotype A	9353	99.8
Genotype G	18	0.2
Total	9371	100.0

SNP genotyping for the presence of m.1555A>G was performed by competitive allele polymerase chain reaction (PCR, KASPar)

(<http://www.kbioscience.co.uk/genotyping/genotyping-chemistry.htm>) following successful 'blind' validation of the assay using known positive and negative controls. Blanks and duplicate samples were included in all plates for quality assurance purposes. The 3% of samples which were duplicated all returned the same genotype. No blanks returned a result. For the m.1555A>G mutation, homoplasmic wild type genotype is A, and homoplasmic mutant is G. "Homoplasmic" indicates a variant present in every mt DNA molecule in the cell. Genotype A:G would indicate heteroplasmy, which was not observed but would be detectable at levels of 25% of mutant by this assay. Therefore it is possible that heteroplasmy below the level of

25% would return a genotype of A and that prevalence of this mutation in a low level heteroplasmic state could be even greater than we have estimated here. The penetrance of hearing loss in individuals with low level heteroplasmy for m.1555A>G is unknown.

Hearing Measures

A summary of hearing results for the cohort aged 7 are presented for each frequency, 0.5 to 8 kHz, for the right ear and left ear respectively in tables 2 and 3, along with tympanometry results in tables 4 and 5. Not all children were able to complete a full audiogram, which is why numbers of children tested at each frequency varies slightly.

Hearing results for the entire cohort aged 9 are presented (tables 6 and 7) for each frequency, 0.5 to 8 kHz, for the right ear and left ear respectively. The decrease in the number of children with abnormal hearing thresholds from age 7 to 9 years across the whole cohort is likely to be a result of improved concentration and a reduction in minor middle ear dysfunction with age and also due to the improved testing conditions used at age 9.

Table 2.
Hearing in the right ear for each frequency at age 7 years for the entire cohort

	Frequency (percent)				
	500Hz	1kHz	2kHz	4kHz	8kHz
<20dBHL	6828 (91.6)	7357 (94.6)	7464 (97.0)	7407 (95.3)	6674 (86.3)
>20dBHL	628 (8.4)	417 (5.4)	233 (3.0)	363 (4.7)	1062 (13.7)
Total	7456	7774	7697	7770	7736

Table 3.
Hearing in the left ear for each frequency at age 7 years for the entire cohort

	Frequency (percent)				
	500Hz	1kHz	2kHz	4kHz	8kHz
<20dBHL	6839 (91.8)	7413 (95.4)	7443 (96.7)	7391 (95.1)	6700 (86.7)
>20dBHL	610 (8.2)	359 (4.6)	251 (3.3)	377 (4.9)	1025 (13.3)
Total	7449	7772	7694	7768	7725

Table 4.
Tympanometry results for the right ear at age 7 years for the entire cohort

	Frequency (percent)
A/C1	6951 (88.9)
B/C2	784 (10.0)
Grom/Perf/Infection	87 (1.1)
Total	7822

Table 5.

Tympanometry results for the left ear at age 7 years for the entire cohort

	Frequency (percent)
A/C1	6972 (89.2)
B/C2	739 (9.5)
Grom/Perf/Infection	107 (1.4)
Total	7818

Table 6.
Hearing in the right ear for each frequency at age 9 years for the entire cohort tested

	Frequency (percent)				
	500Hz	1kHz	2kHz	4kHz	8kHz
<20dBHL	7038 (96.9)	7181 (97.3)	7233 (98.0)	7187 (97.4)	6637 (91.8)
>20dBHL	225 (3.1)	198 (2.7)	144 (2.0)	189 (2.6)	593 (8.2)
Total	7263	7379	7377	7376	7230

Table 7.
Hearing in the left ear for each frequency at age 9 years for the entire cohort tested

	Frequency (percent)				
	500Hz	1kHz	2kHz	4kHz	8kHz
<20dBHL	7028 (96.8)	7178 (97.3)	7218 (97.9)	7140 (96.8)	6588 (91.2)
>20dBHL	236 (3.2)	199 (2.7)	157 (2.1)	233 (3.2)	636 (8.8)
Total	7264	7377	7375	7373	7224

Table 8.
Tympanometry results for the right ear at age 9 years for the entire cohort

	Frequency (percent)
A/C1	6721 (93.1)
B/C2	468 (6.5)
Grom/Perf/Infection	29 (0.4)
Total	7218

Table 9.
Tympanometry results for the left ear at age 9 years for the entire cohort

	Frequency (percent)
A/C1	6712 (93.1)
B/C2	471 (6.5)
Grom/Perf/Infection	29 (0.4)
Total	7212

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