Published in final edited form as:

Am J Med Genet A. 2008 April 1; 146(7): 934–936. doi:10.1002/ajmg.a.32207.

Infrequency of Two Deletion Mutations at the DFNB1 Locus in Patients and Controls

Hsiao-Yuan Tang¹, Monica J. Basehore^{2,3}, Gregory L. Blakey^{2,3,4}, Sandra Darilek³, John S. Oghalai¹, Benjamin B. Roa^{2,3}, Ping Fang^{2,3}, and Raye Lynn Alford^{1,*}

¹Bobby R. Alford Department of Otolaryngology—Head and Neck Surgery, Baylor College of Medicine, Houston, Texas

²Medical Genetics Laboratories, Baylor College of Medicine, Houston, Texas

³Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas

⁴Department of Pathology, Baylor College of Medicine, Houston, Texas

To the Editor

Mutations in *GJB2*, encoding the gap junction beta-2 protein Connexin 26, are associated with autosomal recessive nonsyndromic sensorineural hearing loss (NSHL), autosomal dominant NSHL, and several forms of syndromic sensorineural hearing loss [Denoyelle et al., 1997, 1998; Kelsell et al., 1997; Richard et al., 1998, 2002, 2004; Maestrini et al., 1999; van Geel et al., 2002; van Steensel et al., 2002; Brown et al., 2003]. Mutations in *GJB6*, encoding the gap junction beta-6 protein Connexin 30, are associated with autosomal recessive NSHL, autosomal dominant NSHL, and hidrotic ectodermal dysplasia [Grifa et al., 1999; Lamartine et al., 2000; Lerer et al., 2001; Pallares-Ruiz et al., 2002; del Castillo et al., 2002].

GJB2 and *GJB6* lie adjacent to one another on human chromosome 13q12 and together comprise the autosomal recessive NSHL locus DFNB1 [Van Camp and Smith, 2007]. Autosomal recessive NSHL that maps to DFNB1 is caused by biallelic mutations in *GJB2*, biallelic mutations in *GJB6*, or compound heterozygous mutations in *GJB2* and *GJB6* [Denoyelle et al., 1997; Kelsell et al., 1997; Lerer et al., 2001; Pallares-Ruiz et al., 2002; del Castillo et al., 2002; Del Castillo et al., 2005]. DFNB1 mutations reported to date include more than 80 mutations in *GJB2* and two large deletions involving *GJB6* [Lerer et al., 2001; Pallares-Ruiz et al., 2002; del Castillo et al., 2002; Del Castillo et al., 2005; Ballana et al., 2007]. In this study, a cohort of hearing impaired patients and a multi-ethnic control group were evaluated for mutations in *GJB2* and the two known deletions involving *GJB6*.

Patients were identified through the Bobby R. Alford Department of Otolaryngology—Head and Neck Surgery, the Department of Molecular and Human Genetics, and the Baylor DNA Diagnostic Laboratory of Baylor College of Medicine (BCM) as previously described [Tang et al., 2005, 2006]. The patient group is comprised of 324 probands undergoing clinical evaluation for hearing loss. The nature, degree, laterality and age of onset of the hearing loss in patients were not always known. Information about the ethnicity of patients was not

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*Correspondence to: Dr. Raye Lynn Alford, Ph.D., FACMG, Bobby R. Alford Department of Otolaryngology—Head and Neck Surgery, Baylor College of Medicine, One Baylor Plaza, NA102, Houston, TX 77030. ralford@bcm.edu.

Gregory L. Blakey's present address is Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Benjamin B. Roa's present address is Myriad Genetic Laboratories, Inc., Salt Lake City, UT.

always provided. Controls were obtained from the Baylor Polymorphism resource (<http://www.cardiogene.org>) as previously described [Tang et al., 2005, 2006]. The control group used in this study includes 63 Caucasian, 74 Hispanic, 72 Asian, and 71 African American individuals. The hearing status of the controls is not known. This work was approved by the BCM Institutional Review Board.

Patients and controls were evaluated for DNA sequence variations in *GJB2* as previously described [Tang et al., 2005, 2006] and for deletions in *GJB6* using a triplex PCR test based on the assay of Del Castillo et al. [2005]. The triplex PCR test incorporated the following primers from Del Castillo et al. [2005]: GJB6-1R and BKR-1 to amplify across the breakpoints of the 309 kb deletion; and, Cx30Ex1A and Cx30Ex1B to amplify *GJB6* exon 1. To complete the triplex PCR reaction and amplify across the breakpoints of the 232 kb deletion, primers were designed with sequences 5'-TAGATACGCTTAGCTTCTGCAG-3' (delBK-1A) and 5'-GTCAGTGTGCATGTGAAAA-GATG-3' (delBK-1B). PCR was conducted with 70 ng genomic DNA, 15 pmol of primers GJB6-1R, BKR-1, delBK-1A and delBK-1B, 10 pmol of primers Cx30Ex1A and Cx30Ex1B, 0.25 mM each dNTP, 1.25 U Taq DNA Polymerase (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) and 1 × PCR Buffer as provided by the manufacturer, in a total volume of 20 µl. PCR was conducted as follows: 94°C × 2 min; 40 cycles of 94°C × 30 sec, 58°C × 30 sec, and 70°C × 1 min; and 70°C × 5 min. PCR conditions were modified slightly by the Baylor DNA Diagnostic Laboratory to facilitate incorporation of the assay into the laboratory work flow. PCR reactions were resolved on 1% agarose gels and visualized by ethidium bromide staining and Polaroid photography.

The *GJB2* genotypes of 135 of the patients and all 280 controls in this study were previously reported [Brown et al., 2003; Tang et al., 2005, 2006] and are summarized in Table I, together with the *GJB2* genotypes of the additional 189 patients included in this study. Many patients included in this study were selected because they carried mutations or variants of unknown or controversial clinical significance in *GJB2*.

Among the 648 patient and 560 control chromosomes analyzed in this study, the 232 kb deletion involving *GJB6* [Del Castillo et al., 2005] was not found. The 309 kb deletion involving *GJB6* [Lerer et al., 2001; Pallares-Ruiz et al., 2002; del Castillo et al., 2002] was observed only once, in a patient of unknown ethnicity who is heterozygous for a p.Gln57X (p.Q57X) mutation in *GJB2* (data not shown). In this patient, detection of heterozygous mutations in *GJB2* and *GJB6* supports an etiologic diagnosis of DFNB1-associated hereditary hearing loss.

These data support and extend the findings of prior studies which have suggested that the 232 and 309 kb deletion mutations in *GJB6* may not be common in all populations. It is likely that founder effects, population stratification, and assortative mating at least partially explain the variable frequency of these deletions in different populations [Lerer et al., 2001; Pallares-Ruiz et al., 2002; del Castillo et al., 2002; Fitzgerald et al., 2004; Gualandi et al., 2004; Roux et al., 2004; Dalamon et al., 2005; Del Castillo et al., 2005; Seeman et al., 2005].

It is curious that prior studies have consistently demonstrated larger than expected numbers of heterozygous *GJB2* mutation carriers in patient cohorts [Estivill et al., 1998; Green et al., 1999; Roux et al., 2004; Hutchin et al., 2005; Tang et al., 2006; Putcha et al., 2007]. Although some hearing impaired patients would be expected to be *GJB2* carriers by chance, the cumulative data support the existence of additional, as yet unknown, mutations at the DFNB1 locus. Alternatively, it is possible that carriers of recessive mutations in *GJB2* are at an increased risk for hearing loss from other causes [Estivill et al., 1998; Green et al., 1999;

Roux et al., 2004; Tang et al., 2006; Putcha et al., 2007]. The observations of this and prior studies suggest that further investigation of the DFNB1 locus is needed. Such studies may ultimately permit etiologic diagnoses for many patients currently lacking an explanation for their hearing loss.

Acknowledgments

Grant sponsor: Allbritton-Alford Fund; Grant sponsor: Alkek Foundation; Grant sponsor: Brown Foundation.

The authors thank Laura Molinari, Susan D. Fernbach, John W. Belmont, and the Baylor Polymorphism Resource (<http://www.cardiogene.org>) for the control population used in this work; Ignacio del Castillo and Heidi Rehm for positive controls for the deletion assays; Weihong Jin for technical support; and Aletta Moore for critical review of this manuscript. This work was supported by the Allbritton-Alford Fund, the Alkek Foundation, and the Brown Foundation (HYT, RLA).

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TABLE I

Summary of *GJB2* Genotypes

<i>GJB2</i> genotype ^a	Patients	Controls
Wild type ^b	225	200
One or more benign polymorphisms	43	62
One or more variants of unknown/controversial clinical significance	16	14 ^d
Single pathogenic mutation	32 ^c	4
Two pathogenic mutations	8	0
Total	324	280

^aExcept for the IVS1 + 1G >A mutation (alternatively described as U43932.1:g.1632G >A or c.-23 + 1G >A) only coding region sequence variations are included.

^bCompared to reference sequence: GenBank Accession Numbers M86849, U43932, and XM_007169.

^cOne of these 32 patients carries a heterozygous *de novo* mutation presumed to be dominant [Brown et al., 2003]. Of the remaining 31 patients, 3 also carry a variant of unknown/controversial clinical significance and 7 also carry one or more benign polymorphisms.

^dSeven of these 14 controls also carry one or more benign polymorphisms.