

A PCR-RFLP Assay for the A716T Mutation in the *WFS1* Gene, a Common Cause of Low-Frequency Sensorineural Hearing Loss

THERU A. SIVAKUMARAN and MARCI M. LESPERANCE

ABSTRACT

Nonsyndromic low-frequency sensorineural hearing loss (LFSNHL) is an unusual type of hearing loss that affects frequencies at 2,000 Hz and below. Recently, we reported five different heterozygous missense mutations in the Wolfram syndrome gene, *WFS1*, found to be responsible for LFSNHL in six families. One of the five mutations, A716T, may be a common cause of LFSNHL, as it has been reported in three families to date (Bespalova *et al.*, 2001; Young *et al.*, 2001). We have developed a PCR-based restriction fragment-length polymorphism (RFLP) assay to detect the A716T mutation in a simple, specific test. This method was evaluated with DNA samples from a family in which the A716T mutation was segregating with LFSNHL. This simple assay successfully detected the presence of the A716T mutation in all of the individuals predicted to be affected, based on audiologic results. Therefore, this assay can be routinely used for initial screening of the A716T mutation in patients with LFSNHL, before screening the entire coding region of the *WFS1* gene.

INTRODUCTION

NONSYNDROMIC LOW-FREQUENCY SENSORINEURAL HEARING LOSS (LFSNHL) is an unusual type of hearing loss affecting the low frequencies, 2000 Hz and below, which worsens over time, without progressing to profound deafness (Vanderbilt Hereditary Deafness Study Group, 1968; Lesperance *et al.*, 1995; Kunst *et al.*, 1999). Although over 70 loci responsible for nonsyndromic sensorineural hearing loss have been mapped to date, only two chromosomal locations, 5q31 (DFNA1) (Lynch *et al.*, 1997) and 4p16 (DFNA6/14/38) (Lesperance *et al.*, 1995; Van Camp *et al.*, 1999; Young *et al.*, 2001) are associated with autosomal dominant LFSNHL. Recently, mutations in the Wolfram syndrome gene (*WFS1*), which is mutated in the recessively inherited Wolfram syndrome (an association of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness), were found in families with LFSNHL (Bespalova *et al.*, 2001; Young *et al.*, 2001).

One mutation, 2316G → A, also known as A716T (GenBank AF084481), has been found to segregate in three of seven LFSNHL families with heterozygous *WFS1* mutations. Because this mutation occurs in a CpG dinucleotide (Cooper and You-

soufian, 1988) and appears to have arisen independently in different families (Bespalova *et al.*, 2001), we hypothesize that this mutation may be a common cause of LFSNHL. This mutation can be detected by DNA sequencing or screened by methods such as denaturing high-performance liquid chromatography (DHPLC), or single-stranded conformation polymorphism analysis (SSCP). DNA sequencing alone is not optimal for detection of the A716T mutation, because the overlapping peaks of a heterozygous mutation are easily missed. Single nucleotide polymorphisms (SNPs) occur frequently in the coding region of the *WFS1* gene, with one variant every 66 bp, on average (T.A. Sivakumaran and M.M. Lesperance, unpublished data). Thus, DHPLC and SSCP are inefficient methods for screening *WFS1*, because multiple variants in addition to the one of interest will be identified. Using DNA sequencing to distinguish between polymorphisms and disease-causing mutations detected by SSCP or DHPLC adds time and expense to the mutation screening process.

Because the A716T mutation abolishes a site for the restriction endonuclease *Hae* II, a PCR-based RFLP assay can be used to identify the alteration in a single test. In this way, the expense, labor, and difficulties associated with interpretation of

DNA sequencing can be avoided. Therefore, we developed a PCR-RFLP assay to screen for the A716T mutation and evaluated its sensitivity in a LFSNHL family known to segregate this mutation.

MATERIALS AND METHODS

Subjects

DNA sequence analysis identifying the A716T mutation in affected individuals from Family 35 has been previously reported (Bespalova *et al.*, 2001). The current study included 26 family members (9 affected and 17 unaffected relatives).

PCR

Genomic DNA was prepared using standard methodology. PCR reactions consisted of 40 ng of genomic DNA, 1× PCR buffer II (Perkin Elmer, Foster City, CA), 1.5 mM MgCl₂ (Perkin Elmer, Foster City, CA), 0.2 mM of each dNTP (Gibco-BRL, Rockville, MD), 0.5 μM of each forward and reverse primer, and 0.25 U of Amplitaq Gold DNA Polymerase (Perkin Elmer, Foster City, CA) in a total volume of 10 μl. A 573-bp fragment of exon 8 was amplified using the following primers (Strom *et al.*, 1998):

Forward primer 8.5F: ATCCTGGTGTGGCTCACG
Reverse primer 8.6R: GCTGAACCTCGATGAGGCTG

PCR reactions were carried out in a thermal cycler (MJ Research, Waltham, MA) with an initial denaturation step at 95°C for 10 min, then 35 cycles of 93°C for 1 min, 56°C for 30 sec, and 72°C for 30 sec followed by extension at 72°C for 10 min.

Restriction enzyme assay

Detection of the A716T mutation was carried out by digestion of the PCR products with *Hae* II. The PCR fragment amplified from the wild-type allele contains two *Hae* II sites at positions 242 and 455, whereas the mutant A716T has only one restriction site at position 455. The second restriction site in the fragment serves as an internal control for incomplete digestion. The enzymatic digestion consisted of 10 μl of PCR product, 2.5 U of restriction endonuclease *Hae* II (Gibco-BRL, Rockville, MD), and 1× reaction buffer in a total volume of 15 μl reaction incubated at 37°C for 6 hr. The products were then resolved on a 2% agarose gel, stained with ethidium bromide and detected on a UV transilluminator.

RESULTS

Hae II digestion of the PCR product amplified from the wild-type allele yields three bands of 242, 213, and 118 bp (Fig. 1). The A716T mutation abolishes the restriction site for *Hae* II at position 242, and the digestion reaction results in two fragments of 455 bp and 118 bp. DNA samples from individuals heterozygous for the A716 mutation yield four bands of 455, 242, 213, and 118 bp. As predicted, all unaffected individuals and controls were homozygous for the normal allele, while affected individuals were heterozygous for the A716T mutation.

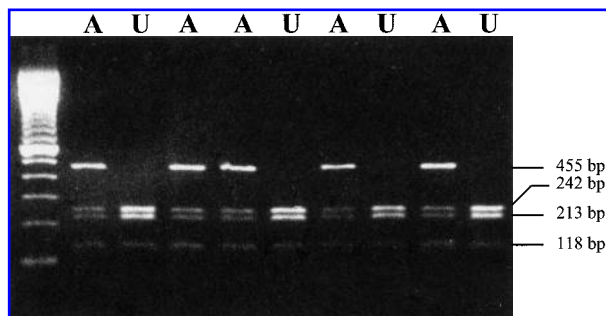


FIG. 1. Restriction enzyme analysis of PCR products for the detection of A716T missense mutation in *WFS1* gene in a family with LFSNHL. The A716T mutation abolishes one of the two restriction sites for *Hae* II. As a result, DNA samples from all the affected individuals (A) yield four bands (455, 242, 213, and 118 bp), while DNA samples from the unaffected individuals (U) yield three bands (242, 213, and 118 bp).

DISCUSSION

The A716T mutation has been described in 3 of 7 families reported to date with LFSNHL attributable to *WFS1*, a common cause of LFSNHL (Bespalova *et al.*, 2001; Young *et al.*, 2001). DNA sequencing may miss heterozygous mutations when overlapping peaks are difficult to detect and/or interpret on the chromatogram. SSCP and DHPLC may be efficient when the number of variants is low, but these methods are not as useful for screening the *WFS1* gene, which has a large number of SNPs in its coding sequence. While none of the >100 SNPs found so far affect this site, it is theoretically possible that a mutation other than A716T in the 4 bases of the *Hae* II site could abolish the restriction site. DNA sequencing can be used to confirm positive results on the PCR-RFLP assay in the same way that recognition of the variant sequence by a restriction endonuclease confirms the DNA sequence interpreted from the chromatogram.

The PCR-RFLP assay described herein is a simple and rapid test that successfully identified the presence or absence of the A716T mutation in all the family members analyzed. The second obligatory restriction site serves as an internal control, such that incomplete digestion will not be misinterpreted as heterozygosity for A716T. Therefore, this assay may be useful for initial screening of the A716T mutations in families with LFSNHL before screening the entire gene for mutations.

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Address reprint requests to:

Dr. Marci M. Lesperance

Division of Pediatric Otolaryngology

Department of Otolaryngology—Head and Neck Surgery

F6905 Mott, Box 0241

1500 East Medical Center Drive

Ann Arbor, MI 48109-0241

E-mail: lesperan@umich.edu

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