Allele specific oligonucleotide analysis of the common deafness mutation 35delG in the connexin 26 (GJB2) gene

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Abstract
Despite the large number of genes that are expected to be involved in non-syndromal, recessive deafness, only a few have been cloned. One of these genes is GJB2, which encodes connexin 26. A frameshift mutation in this gene has been reported to be common in several populations and a carrier frequency of about 1 in 30 people has been detected in Italy and Spain. Mutation 35delG is difficult to detect because it lies within a stretch of six guanines flanked by thymines, so the deletion of one G does not create or destroy a restriction site and mutagenesis primers are not useful for this mutation. We have generated an allele specific oligonucleotide method that uses 12-mer oligonucleotides and easily discriminates between the normal and 35delG alleles. The method should permit a rapid analysis of this mutation in congenital cases (recessive or sporadic), including diagnosis and carrier detection of 35delG in the population.

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The most common form of hereditary hearing loss is non-syndromal, autosomal recessive deafness. Although a large number of genes are expected to be involved in recessive deafness, only three have been identified to date, and these are the genes MYO7A (responsible for DFNB2, DFNA11, and Usher syndrome), PDS (Pendred syndrome and a recessive form of isolated deafness linked to 7q31), and GJB2, responsible for DFNB1 and DFNA3. In the Italian and Spanish populations, a large proportion (70-80%) of the families with non-syndromic deafness is linked to GJB2 (35delG) explains most of these cases. Patients with 35delG have profound, bilateral, congenital deafness and phenotypic variation has been described. This mutation has also been identified in patients from other countries. The 35delG mutation has also been found in sporadic cases (with initially unknown genetic basis) of congenital deafness, accounting for between 10 and 40% of all cases of congenital deafness, depending on the population. Finally, the frequency of this mutation has been determined in the general population of Italy and Spain, and about 1 in 30 subjects of the general population are asymptomatic carriers of 35delG.

A rapid method to detect this mutation would be very useful in the diagnosis of deafness and in the assessment of carrier status. This mutation, a deletion of a guanosine, in a stretch of six, cannot be detected with restriction enzymes, neither can a restriction site be introduced with a mutagenesis primer. Detection by SSCP (single strand conformational polymorphism) is not easy and difficult to reproduce. Indeed, different SSCP conditions were tested (10 and 12% commercial acrylamide gels, 5 and 15°C, 10% non-commercial gels, different times and voltages, and various sizes of PCR fragments) and the mutation was not seen, while other mutations in this gene were easily detected. Sequencing, of course, detects the mutation, but it is slow, quite expensive, and not feasible for routine diagnosis and carrier detection. Other methods, such as the determination of fragment sizes which differ by only 1 bp (for example, 50 bp and 51 bp) on acrylamide gels are not as reproducible as would be desired and need further confirmation of the result.

We present here an ASO (allele specific oligonucleotide) analysis of the 35delG mutation which has proved to be reproducible and allows rapid detection of the mutation. The oligonucleotides used are especially short (12 bp), allowing high specificity in the analysis of the stretch of six Gs where the 35delG mutation lies. Their sequences are 5'-TCCTGGGGGTTGTGA-3' for 35delG (mutated) and 5'-CCTGGGGGTTGTG-3' for 35G (normal). Both have a hybridisation temperature of 42°C.

The samples were PCR amplified with a pair of primers that flank the coding region of GJB2 and dot blotted onto a Hybond N+ filter in duplicate. Filters were then prehydrised in a solution of 7% SDS-phosphate buffer for at least 20 minutes at 42°C and hybridised with the corresponding 32P labelled probe (35delG or 35G) overnight at 42°C. Washing consisted of a quick wash at room temperature with 2 x SSC/0.1% SDS followed by 10 minutes with a fresh washing solution and 10 minutes at 42°C, and then increasing the stringency by using a washing solution of 0.2 x SSC/0.1% SDS for 10 minutes. The final wash was at 52°C for five minutes. The filters were exposed on x ray film for two hours at −80°C.

We have used this method for the detection of carriers and affected subjects in deaf families carrying the 35delG mutation, and in the detection of 35delG carriers in the general population.
The analysis of the 35delG mutation should allow the early detection of deaf children (homozygotes or heterozygotes) in families at risk and in sporadic cases and also permit confirmation of the diagnosis in affected subjects and carrier detection in the population.

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