DFNB1

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WGS DFNB1 deafness variants functional assays

1. Introduction

Hearing loss is the most common sensory pathology, affecting about 1–2 in every 1000 newborns, with a prevalence which increases with age [1][2][3][4]. In industrialized countries, congenital deafness has a genetic origin in 80% of cases [5]. Deafness can be syndromic or not (associated or not with other pathologies or malformations), respectively representing 10% and 90% of cases. More than 500 syndromes are associated with syndromic deafness and more than 100 genes have been described in non-syndromic hearing loss (NSHL) (Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage. https://hereditaryhearingloss.org, the 10 May 2021).

NSHL can be classified according to heredity. Generally 80–90% of those affected have autosomal recessive inheritance (DFNB), 10–15% have a dominant mode (DFNA), 1% of cases are associated with the X chromosome (DFNX), and others have a mitochondrial inheritance mode [5][6].

The predominant form is autosomal recessive non-syndromic hearing loss (*DFNB1*). Most *DFNB1* phenotypes are described as prelingual and bilateral non-syndromic hearing loss, this being severe to profound. This type of deafness affects all frequencies and is not associated with inner ear malformations. Vestibular function remains unaffected [1][2][3][4][5][7][8]. The *GJB2* (Gap Junction β 2-chr13:20,187,470–20,192,938 (hg38)) gene is mainly implicated in *DFNB1* with frequencies ranging from 20% to 40%, according to populations with the most frequent mutation, c.35delG [3][5][9][10][11].

Moreover, seven large *DFNB1* deletions have been described in *DFNB1* patients: del-920 kb [12], del-101 kb del(*GJB2*-D13S175) [13], del(*GJB6*-D13S1830) [14], del(*GJB6*-D13S1854) [15], del-131kb [16], del-179kb [17], and del-8kb [18]. This year, Brozkova et al. described a *DFNB1* deletion of 3 kb in one patient [11].

The genomic architecture of our chromosomes is now far better investigated and understood. Many studies have focused on the role of non-coding regions and on genetic variants they contain, opening up new research possibilities. However, a vast majority of coding and non-coding variants may remain of unknown clinical significance [19][20][21].

Almost 8% of the human genome is covered with candidate *cis*-regulatory elements (cCREs) [22]. The identification of distal acting regulatory elements has been the object of active research in recent years. Disruptions of such regulatory elements and/or chromatin conformation are likely to play a critical role in human genetic diseases [19][20][21].

Routine molecular diagnosis in the Molecular Genetics Laboratory at Brest University Hospital involves the testing of around 80 deaf patients each year, and among these patients, ~20% are *DFNB1* biallelic carriers. However, several genotypes remain incomplete; for monoallelic *DFNB1*, which represents fewer than 1% of the tested patients, most patients are carriers of the c.35delG heterozygous and some have rare variants.

The c.35delG heterozygous genotype may be related to the general population frequency with an overall frequency of 2% but an excess of the deletion has been shown in the deaf population [8].

2. GJB2 Mutations

WGS analysis identified a second mutation on the *GJB2* gene in patients P3, P4, P8, and P10 (**Table 1**).

Table 1. *DFNB1* mutations detected by WGS.

Patient	Gene	HGVSc	Chr.	Position (hg38)	HGVSp	Impact	Consequences	Variant Class	rs Number	Allele Frequency (GnomAD)
P3	*GJB2*	NM_004004.5:c.269dup	chr13	20189312	NP_003995.2:p.Val91SerfsTer11	HIGH	frameshift_variant	insertion	rs730880338	0.00002940
	GJB2	NM_004004.5:c.269T > C	chr13	20189313	NP_003995.2:p.Leu90Pro	MODERATE	missense_variant	SNV	rs80338945	0.001161
P4	*GJB2*	NM_004004.5:c.35del	chr13	20189546	NP_003995.2:p.Gly12ValfsTer2	HIGH	frameshift_variant	deletion	rs80338939	0.009802
	GJB2	NM_004004.5:c.269dup	chr13	20189312	NP_003995.2:p.Val91SerfsTer11	HIGH	frameshift_variant	insertion	rs730880338	0.00002940
P8	*GJB2*	NM_004004.5:c.139G > T	chr13	20189443	NP_003995.2:p.Glu47Ter	HIGH	stop_gained	SNV	rs104894398	0.0001176
	GJB2	NM_004004.5:c.-23 + 1G > A	chr13	20192782	.	HIGH	splice_donor_variant	SNV	rs80338940	0.0003236
P10	*GJB2*	NM_004004.5:c.139G > T	chr13	20189443	NP_003995.2:p.Glu47Ter	HIGH	stop_gained	SNV	rs104894398	0.0001176
	GJB2	G > C	chr13	20193022	-	UNKNOWN	upstream_gene_variant	SNV	rs1425012952	0.00001472
	GJB2	G > T	chr13	20183294	-	UNKNOWN	downstream_gene_variant	SNV	rs372782198	0.003099

In the routine molecular diagnosis, the Sanger sequencing of the *GJB2* gene of patient P3 allowed us to detect frameshift variation (rs730880338) at c.269. Then, a missense variation (rs80338945) was discovered by WGS analysis at the same position. Indeed, these variations in the same nucleotide complicated the interpretation of Sanger sequencing analysis. The duplication hid the other mutation so that the initial Sanger sequencing interpretation failed to detect the two mutations.

DNA samples from parents were not available for segregation analysis, but single-strand NGS sequencing (see IGV (Integrative Genome Viewer) BAM visualization (**Figure 1**) confirmed that these variants are in *trans*.

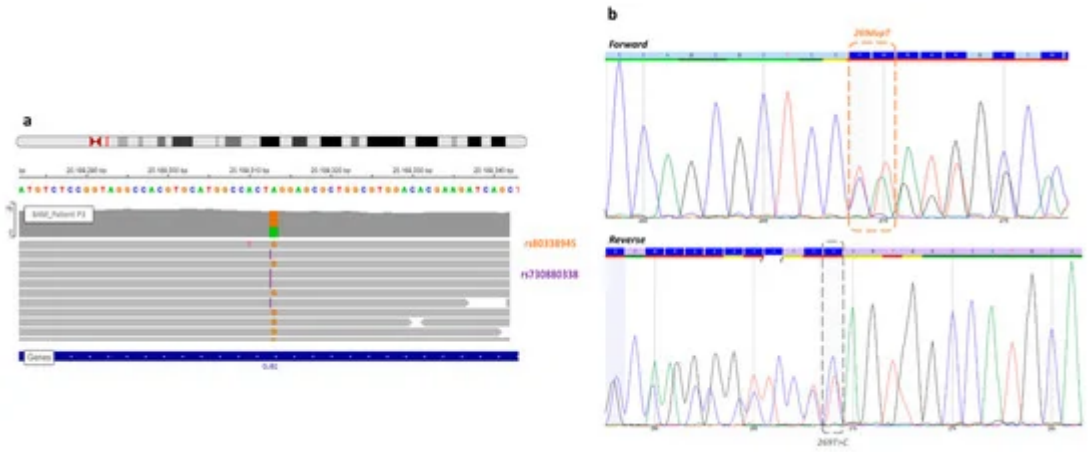

Figure 1. View of the missense variant and frameshift *GJB2* gene of patient P3. (**a**) In the Integrative Genome Viewer, the frameshift variant (rs730880338) at c.269 *GJB2* position, known before WGS (orange), and the missense variant (rs80338945) discovered by WGS analysis (purple). Each variant was on a different read, so this analysis confirmed a *trans* configuration. (**b**) A new Sanger sequencing in forward and reverse detected both mutations, but it still remains difficult to interpret.

Patient 4 carried two *GJB2* mutations, a recurrent mutation, in deaf population, only the c.35delG (rs8033893) have been detected by DHPLC (Denaturing High Performance Liquid Chromatography). The second mutation discovered by WGS analysis was a frameshift (rs730880338), c.269dup (**Figure 2**). Indeed, since no hetero- and homo-duplex was detected by HPLC we did not realize Sanger sequencing. This is why genotype was unresolved before WGS. Variant segregation of parents allowed the determination of variants transmission for their children (**Figure 3**).

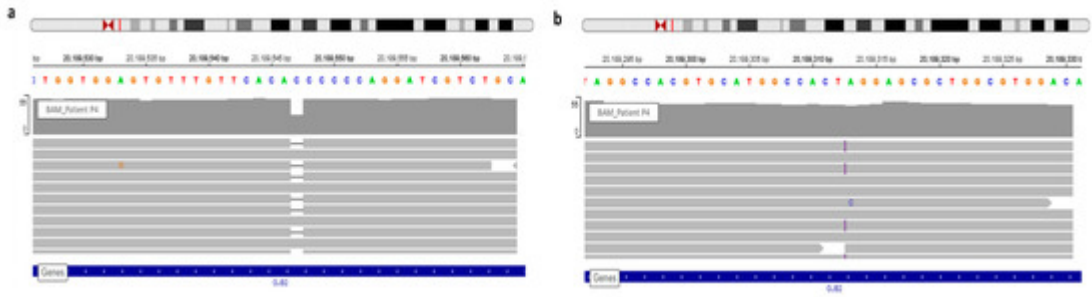

Figure 2. View of *GJB2* variants of patient P4 in IGV. (**a**) WGS confirmed the known c.35delG. (**b**) The frameshift variation, c.269dup, detected via WGS and observed in IGV.

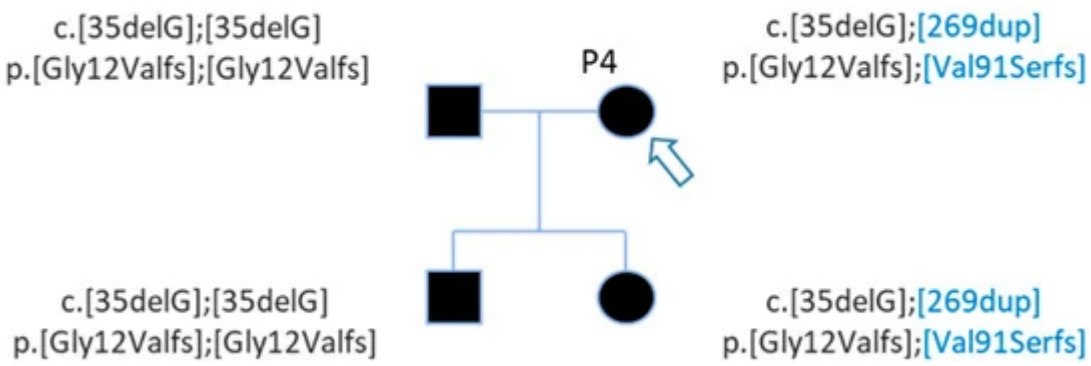

Figure 3. Pedigree of patient P4 with 2 *GJB2* variants. Patient P4 (arrow) carries two *GJB2* mutations, the c.35delG known before WGS and the c.269dup discovered by WGS analysis (in blue). WGS analysis detected mutations for her daughter also.

The first Sanger sequencing of Patient P8 was performed in 2002; only the nonsense mutation of *GJB2* was detected (rs104894398—c.139G > T) (**Figure 4**).

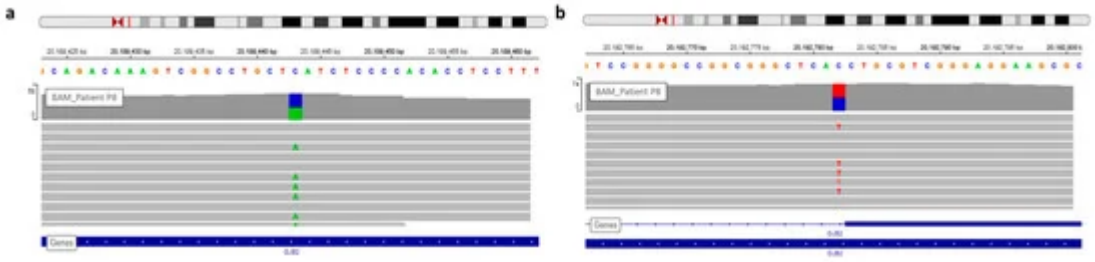

Figure 4. View of *GJB2* variants of patient P8 in IGV. (**a**) The nonsense variation, c.139G > T, discovered during routine care. (**b**) The second *GJB2* variation detected by WGS analysis is a splice site mutation.

WGS analysis allowed identification of a second *GJB2* mutation, a splice site mutation (rs80338940—c.-23 + 1G > A) on intron 1 of the *GJB2* gene (**Figure 4**). This variant had not been found earlier because the sequencing of exon 1 has only been routinely done in the laboratory since 2005.

After WGS, Sanger sequencing confirmed this second mutation, and segregation confirmed that these mutations are in *trans* (**Figure 5**). The genotypes of patient P8 and her brother are c.[-23 + 1G > A];[139G > T] p.[?];[Glu47Ter] (**Figure 5**).

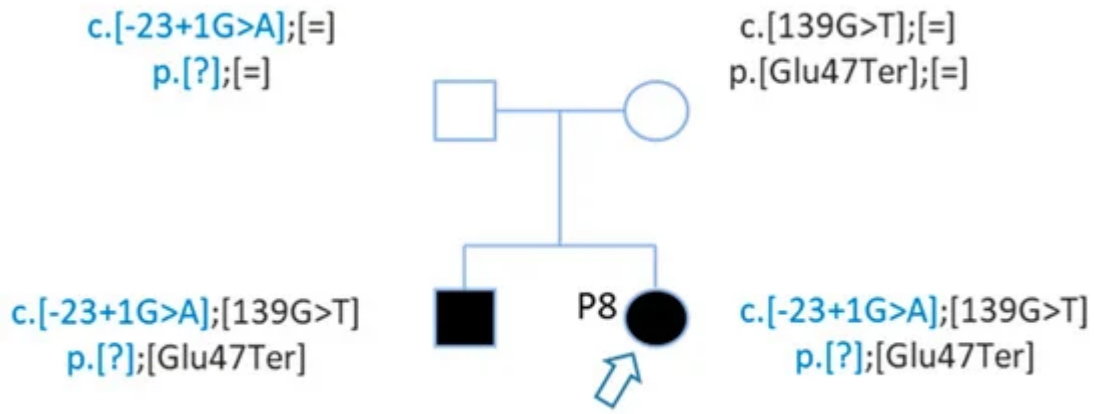

Figure 5. Pedigree of patient P8 with 2 *GJB2* mutations in trans. Patient P8 and her brother carried 2 mutations in trans. Mutations were inherited from each parent.

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