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CLINICAL REPORT

A new syndromic case of hearing loss and ectodermal anomalies associated with a recurrent missense variation in *GJB6* gene

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Abstract

GJB2 and *GJB6* variants, encoding Cx26 and Cx30 respectively, are the most frequently involved genes commonly contributing to hereditary hearing loss either isolated or in combination with skin abnormalities. *GJB6* variations are classically associated with two distinct conditions: non-syndromic hearing loss and hidrotic ectodermal dysplasia, type Clouston, the latter typically not involving deafness.

Method: Whole genome sequencing (WGS) was used to find genetic variants after clinical features of a 13-year-old female patient were recorded.

Results: In this report, we describe the association of congenital hearing loss and ectodermal anomalies (palmoplantar keratoderma, knuckle pads, and nail dystrophy) in a female with the ENST00000647029.1 (*GJB6*): c.175G>A (p.(Gly59Arg)) *GJB6* variant. As a result, we report on the third case of individuals showing this same missense variant and syndromic hearing loss.

Conclusion: This study underscores the overlapping phenotypes observed in patients with the p.Gly59Arg variant in the *GJB6* gene. The findings suggest a continuum of phenotypic presentations for this variant, with the key clinical features being the combination of congenital hearing loss and hyperkeratosis.

KEYWORDS

GJB6 gene, palmoplantar keratoderma, syndromic hearing loss

1 | INTRODUCTION

The most frequently implicated genes in hereditary hearing loss (HL) are *GJB2* and *GJB6* coding respectively for Cx26 and Cx30. Phenotypically, connexins-related deafness can manifest as either isolated or associated with other defects, which we refer to as syndromic HL later on. One-third of deafness cases with *GJB2* and *GJB6* variants are associated with malformation syndromes (Kelsell et al., 2001) as connexins are broadly expressed in several organs, including cochlea and skin (Delmar et al, 2018).

GJB6 is generally associated with two distinguishable diseases either non-syndromic HL or hidrotic ectodermal dysplasia 2 (or Clouston syndrome) (HED2). HED 2 is characterized by a triad of major clinical features including partial-to-complete alopecia, nail dystrophy, and palmoplantar hyperkeratosis without any auditory symptoms. Interestingly the association of HL and palmoplantar keratoderma (PPK) has been scarcely reported with a rare recurrent *GJB6* heterozygous variant (p.Gly59Arg) (Duzkale et al., 2022; Nemoto-Hasebe et al., 2009).

We herein describe the third case of a girl who has been diagnosed with HL and ectodermal anomalies and in whom genetic analysis identified the p.Gly59Arg *GJB6* variant.

2 | MATERIALS AND METHODS

In this study, the proband was recruited from the Rennes University Hospital. The informed consent from human subjects has been obtained before beginning the report. This report was approved by the Ethics Committee of the Rennes University Hospital. The detailed methods of genetic studies are described in the Data S1.

3 | RESULTS

3.1 | Clinical characteristics of the affected individual

The patient is a 13-year-old female born from non-consanguineous healthy parents. Both her grandfather and one maternal uncle showed congenital bilateral deafness.

The pregnancy showed no particular events.

The auditory test was normal at birth at the age of 1 year because of the familial medical background, a second test was performed which gave negative results. Walking was acquired around 16 months, and some fall with balance problems, even some dizzy sensations, were noted. Once a delay of language was picked up. At the age of 26 months, an audiogram showed a bilateral hearing loss, which is more profound on the left side than on the right side.

Since birth, she also presented right lateralized pigmented patches affecting the scalp, lower face, right upper hemithorax, and right arm, stopping at the forearm. Later on, noninflammatory pigmented melanocytic lesions dispatched in the same area were noticed (Figure 1d). In addition, hyperkeratotic lesions on the backs of the toes were observed from birth, with the later appearance of similar lesions on the backs of the hands opposite the proximal interphalangeal joint (PIPJ) on the palmar surface of both hands. These lesions are symmetrical, with the presence of palmoplantar keratodermas (PPKs) and leukonychia (Figure 1).

The clinical examination reported a macrocephaly with advanced weight and height development (Figure 2). An ophthalmic examination with funduscopy showed no abnormality.

Brain MRI and cerebral scan were performed, showing no particular anomalies with normal morphological aspects of the cochlea-vestibular nerves, no signal abnormality in the brain parenchyma, and absence of anomaly of the aqueduct of the vestibule.

At first, she was equipped with bilateral hearing aids, but in the face of inadequate language development and the aggravation of the audiogram results, the audiogram checkup showed a profound HL on the right ear (threshold >90 dB) and severe to profound on the left ear (threshold at 85 dB on the 500Hz) at the age of four, where she was equipped with a cochlear implant on the left side, 1 year later with degradation of the hearing in her right ear, a cochlear implant was carried out at the age of five.

Based on the association of deafness, leukonychia, palmar hyperkeratotic lesions, and hyperkeratotic nodules on the dorsal surface of the joints with atypical signs both on the cutaneous exam, a Bart–Pumphrey syndrome usually linked with *GJB2* variations was hypothesized.

3.2 | Genetic findings

GJB2 exon 2 variants and the most frequently reported *GJB6* deletion *del*(GJB6-D13S1830) and *del*(GJB6-D13S1854) were conducted using Sanger sequencing and multiplex PCR respectively and, the results of both methods were normal. An array-CGH showed no chromosomal abnormalities.

WGS was performed in the genomic DNA from the affected child and her parents (see Section 2 in the Data S1). The variant analysis highlighted the presence of de novo

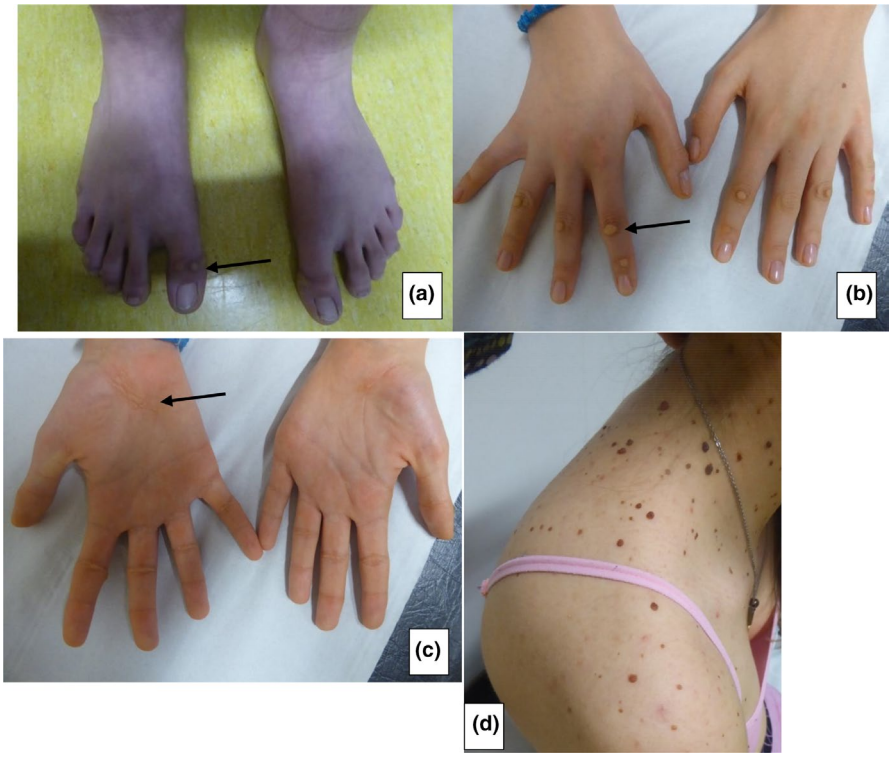

FIGURE 1 Skin clinical features. (a and b) Hyperkeratotic lesions on the backs of the toes and the hands (arrow). (c) Palmar keratoderma (arrow). (d) Pigmented patches of the right upper hemi thorax and right arm with non-inflammatory pigmented melanocytic lesions.

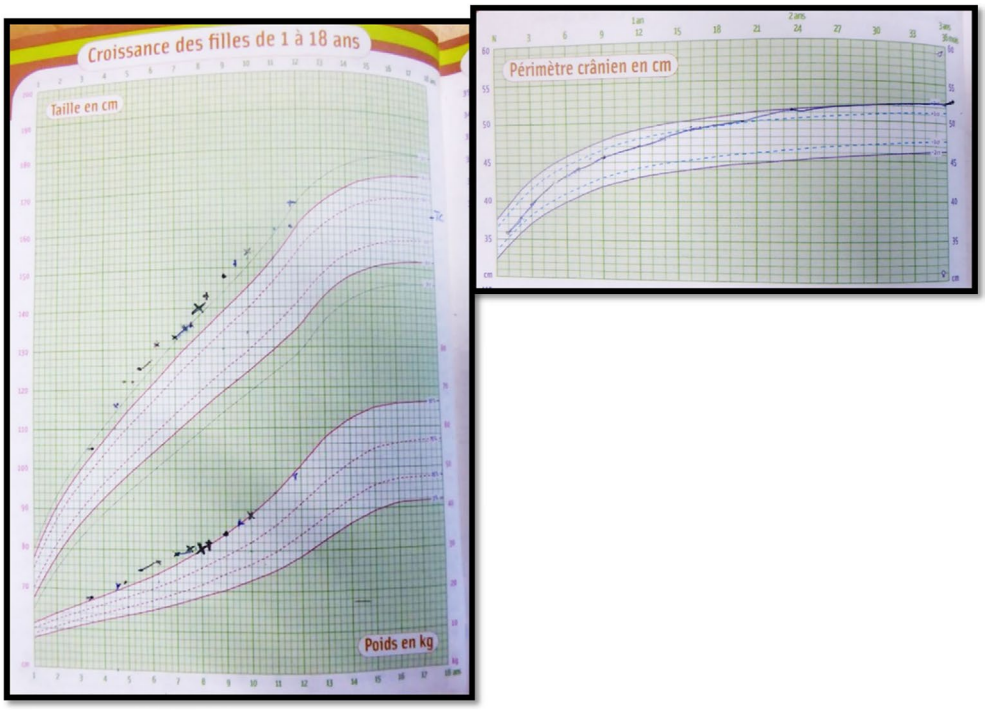

FIGURE 2 Height, weight head and circumference charts showing tall stature and mild macrocephaly.

heterozygous variant ENST00000647029.1(GJB6):c.175G>A (p.(Gly59Arg)) for diagnosis of ectodermal dysplasia associated with hearing loss (Table S1). The variant occurs at a highly conserved residue and has been interpreted as pathogenic (ACMG classification: PS1, PS2, PM5, PM2, and PP3). Further, it was predicted to be deleterious by multiple pathogenicity scores (Table S1). This variant is not reported in the GnomAD database (Table S1).

4 | DISCUSSION

According to our knowledge, the case described in the present investigation is the third occurrence of *GJB6*-related syndromic deafness (OMIM: 604418), that has been linked to a recurrently reported variant (p.(Gly59Arg)) that affects the E1 domain of Cx30.

Connexin 30 is a member of the connexin family of proteins, which makes up some gap junction channels. Connexin hemichannels (HCs) and gap junction channels (GJCs) are essential for cellular communication as they permit the flow of ions and small molecules between cells (Meşe et al., 2007).

In literature, the *GJB6* gene has not been associated with a syndromic form of HL, and it has two distinguishable presentations, a non-syndromic deafness and a skin disorder referred to as hidrotic ectodermal dysplasia 2 (type Clouston), in which the diagnosis criteria does not include deafness (Kelsell et al., 2001).

The locus of the *GJB6* gene in the human genome is chromosome 13q12.11, the same chromosomal locus as the *GJB2* gene, which encodes for Cx 26 another gap junction channel.

Expression of Cx26 and Cx30 overlap in palm skin and the cochlea. The two encoded proteins have been proved to interact in vitro and represent a high percentage of identity, about 76% of Cx30 protein identity when compared to Cx26 (Xu & Nicholson, 2013). Each connexin protein includes four transmembrane domains (designated as TM1-TM4) connected by two extracellular loops (ECL) and one intracellular loop (ICL). The amino terminus (NT) and the carboxyl terminus (CT) segments are cytoplasmic. Despite Cxs sharing high homology, the amino acid sequence of the ICL and CT differs significantly. These segments contain motifs for regulatory kinases and cytoskeletal binding proteins (Meşe et al., 2007).

The ECL 1 domain of connexins is important for the assembly, stability, and function of gap junction channels (García et al., 2016). The NT and NT/ECL1 boundaries of Cxs have been hypothesized to have a role in voltage sensing in GJCs and are located in regions that may be important in determining size restriction and pore selectivity (Rabionet et al., 2002).

Oligomerization between suited isoforms also contributes to the assortment of Cx-based channels. These combinations may produce GJCs with particular functional and regulatory properties (García et al., 2016).

In the cochlea, Cx 30, along with Cx26, proteins are expressed in supporting cells and sensory hair cells, where they are thought to play a critical role in maintaining the proper ionic and fluid environment for normal auditory function (Rabionet et al., 2002; Wingard & Zhao, 2015).

In the epidermis, Cx30 proteins form gap junctions between keratinocytes. There is a very specific distribution of Cx in the different epidemic layers. They show a dynamic change of function and distribution during development. In postnatal, Cx30 substitutes for Cx26 in the supporting cells surrounding the outer hair cells (Dieters' and Hensen's cells) (Martin et al., 2014; Srinivas et al., 2018).

The current reported variation glycine 59 is located in the first extracellular loop in Cx30, a domain that exhibits a high sequence conservation in homologous connexins. Variations in *GJB2* and *GJB6* can disrupt the function of gap junctions in the cochlea, leading to impaired ion and fluid exchange between cells and resulting in sensorineural HL (Rabionet et al., 2002; Wingard & Zhao, 2015).

There are three variants in *GJB2*, p.Gly59Ala, p.Gly59Trp, and p.Gly59Ser, located at glycine 59 which is orthologous to glycine 59 in *GJB6*, all implicated in syndromic forms of deafness involving different skin disorders (Alexandrino et al., 2005; Avshalumova et al., 2014).

Cx variants can be responsible for skin barrier lesions through different mechanisms, reduced channel density, altered gating characteristics, and alterations in the selectivity of ion permeation. They can also influence the trafficking, assembly, and stability of gap junctions causing interactions' modifications between nearby cells.

At last, a functional test demonstrates that this p.Gly59Arg variant in the *GJB6* gene impairs the trafficking of connexin 30 to the cell surface, with faulty connexin oligomerization, causing plaque to form in the Golgi apparatus, besides the reduction in the number of functional channels and decreased intercellular communication (Berger et al., 2014). Other studies have shown that this variant not only impairs the expression of Cx26 through a dominant negative effect but also alters the trafficking of connexin 26 to the cell surface, as these both connexin co-localize in intracellular compartments. Many studies concluded that these two genes can influence each other's expression and function (Scott et al., 2012).

Variants in Cx30 that are linked to skin disease and non-syndromic HL exhibit several distinct cellular pathologies which may indicate that the mutant protein acquires an aberrant function in specific cell types (Scott et al., 2012). The first clinical report was a 32-year-old Japanese woman with mild palmoplantar keratoderma, knuckle pads, and severe sensorineural HL. This clinical sign is usually reported in patients with Vohwinkel syndrome, a syndromic deafness usually related to a variation in Cx26 (Nemoto-Hasebe et al., 2009).

The second case was from a Turkish family, with the expression in three generations of deafness with hyperkeratosis (Duzkale et al., 2022). Diagnosis was made first within a 1-year-old girl with no consanguineous parents. Her prenatal and perinatal periods had been normal, but

TABLE 1 Various clinical findings in the reported cases.

	Vohwinkel syndrome (Online Mendelian Inheritance in Man, n.d.-b)	Bart–Pumphrey syndrome (Online Mendelian Inheritance in Man, n.d.-a)	Our case	Nemoto-Hasebe et al. (2009)	Duzkale et al. (2022)
Gene	*GJB2*	*GJB2*	*GJB6*	*GJB6*	*GJB6*
Genomic variation			c.175G>A	c.175G>C	c.175G>A
Amino acid change			p.Gly59Arg		
Deafness	Mild to moderate	Severe to profound	Severe to profound	At the age of 3, profound	At the age of 1 year, severe
Hyperkeratosis	+	+	+	+	– (reported in mother and grandfather)
Pseudoainhum	+	–	–	+	–
Macrocephaly	–	–	+	–	–
Leukonychia	–	+	+	+	+
Knuckle pads	–	+	+	+	–
Papular keratoderma	+	–	–	–	–

bilateral HL was found on an audiogram at the age of 1 month. Her physical examination findings and growth charts were normal. Both her mother and grandfather had been previously diagnosed with prelinguistic HL and could only speak sign language. And they had mild diffuse palmar and plantar hyperkeratosis. These lesions had begun to appear in her mother around the age of 6 years, but no clear information regarding the age at onset in her grandfather could be gained. The variant was detected in the girl and the proband's mother and grandfather.

The variant is absent from genomic population data, absent from Gnomad 3.1, and has not been reported in healthy populations. This finding confirmed the dominant effect of the mutation with complete penetrance. An ophthalmic evaluation demonstrated no signs of ophthalmic participation reported in the Turkish family. Table 1 summarizes the clinical data.

We reported in our case the presence of macrocephaly (+2 SD) and tall stature (+3 SD) that were not described in any of the other cases in the literature. This macrocephaly and excessive growth have been noticed since birth. We have not identified in the WGS other variations that could explain the HL or macrocephaly. We suggest this finding might be a new symptom to be included and widen the spectrum of the *GJB6*-related gene phenotype.

Finally, we highlight the different patients' phenotypes overlap, with specificity for each patient. This may suggest the presence of a continuum phenotypic presentation for this p.Gly59Arg *GJB6* variant, with the major clinical symptoms being the association of deafness and hyperkeratosis. However, the phenotype's determinism may be influenced by unidentified genes or epigenetic variables that are not detectable by NGS (Serra et al., 2021).

AUTHOR CONTRIBUTIONS

Badreddine Elmakhzen and Paul Rollier: Data analysis, figures, original manuscript writing and editing, clinical data collection, and manuscript drafting. Isabelle Fajardy: Data analysis, WGS analysis, and interpretation. Sylvie Odent: Investigation, manuscript review, and editing. Clémence Saillard, Benoit Godey, Cédric Le Marechal, Paul Gueguen: Conceptualization and resources and clinical data collection. Laurent Pasquier: Manuscript review and editing, conceptualization, resources, and supervision. All authors provided valuable feedback on earlier versions of the manuscript. The final manuscript was reviewed and approved by all authors.

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ETHICS STATEMENT

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Rennes University Hospital. Informed consent was obtained from the patient's parents for the genetic testing and for the use of the patient's medical data for research and publication purposes.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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