# Ultrastructure of the Hair in Genetic Prelingual Deafness Associated with the 35delG Mutation in the Connexin 26 Gene (*GJB2*)

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Ultrastructure of the surface of long hair in 77 subjects with a phenotype of childhood prelingual deafness was evaluated by scanning electron microscopy. The subjects were homozygous or heterozygous carriers of the 35delG mutation in the connexin 26 gene (*GJB2*). The presence of severe abnormalities in the marginal layer of the cuticular plate (fracture-like defects) is pathognomonic for homozygous carriers of the 35delG mutation. Ultrastructural characteristics of the hair in subjects with connexin-associated deafness significantly differed from those in healthy volunteers (control group of the same age) and deaf people with nongenetic hearing impairment. Analysis of variance revealed no differences in hair thickness between deaf homozygous and heterozygous carriers of the 35delG *GJB2* gene mutation and healthy volunteers.

**Key Words:** *prelingual recessive deafness; 35delG mutation in the GJB2 gene; connexin 26; hair*

Connexins (Cx) belong to a group of proteins that form nonspecific intercellular gap junctions for passive transport of electrolytes, secondary messengers, and small molecules between adjacent cells [6]. The group of Cx 26, 30, 30.3, 31, 32, and 43 is the main risk factor for congenital hypoacusis and deafness in all ethnic populations [1,8]. Mutations in Cx genes (Cx 26 and Cx 30) are manifested in a variety of symptoms, including the development of persistent and severe auditory dysfunction in the prelingual period (below 1 year of age) [4]. Due to high expression of Cx in organs and tissues (*e.g.*, in epithelial structures) [3,5], genetic mutations in Cx are accompanied by a syndrome with the combined phenotype of hypoacusis, dysplasia of the teeth and intestine, dentofacial dysmorphism, peripheral neuropathy [8], and skin disorders (congenital chronic hyperkeratoses) [1,6-8]. The effect of mutation in Cx genes on skin derivatives (*e.g.*, hair) remains unknown.

A hair stigma was revealed in homozygous carriers of the 35delG mutation in the Cx 26 gene (*GJB2*). The children below 1.5 years of age are characterized by poor hair growth on the head (thin, short, fragile, and usually light hair) and typical alopecia in the frontotemporal and parietal regions. The parents of most children with connexin-associated deafness report that hair change occurs in the period of clinical manifestations of speech delay, which requires consultation with speech therapist or children's otolaryngologist. Audiological screening for congenital hearing impairment in infants by the method of otoacoustic emission is not widely used in Russia. This phenomenon is not related to the severity of rickets, hair color in parents, and type of infant nutrition (artificial or breast feeding). Cx 26 is expressed not only in cochlear structures, but also in the skin (*e.g.*, epithelial cells of the hair follicle) [3,9]. We hypothesized that the pathogenesis of

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this symptom and deafness is associated with dysfunction of the connexin gap junction due to the 35delG mutation in the *GJB2* gene.

Here we studied the ultrastructure (US) of long hair in deaf people that carry the 35delG mutation in the Cx 26 gene (*GJB2*).

## MATERIALS AND METHODS

We examined the people of the Caucasian race (Slavic ethnic group) living in the Northwestern region of Russia and aging 2-20 years. Hair samples were obtained from 77 subjects with sporadic non-syndrome recessive prelingual deafness, which carried the 35delG mutation in the Cx 26 gene (*GJB2*). The mutation was identified by means of three-primer PCR [10]. The control group consisted of hair samples from healthy volunteers of similar age without hearing loss or chronic diseases ($n$=24) and deaf people with non-genetic hearing impairment during the prelingual period (meningitis and ototoxicity; $n$=27).

The study was performed with the near-root hair fragment (length 1 cm). The thickness and surface US of the cuticle (smooth or defective) and crosscut area of the hair plate (integrity, breaks, and friable margin) were evaluated. The objects were examined and photographed on a JEOL JSM-6390 LA scanning electron microscope (×1000-5000). Microphotographs of 5 hairs from each subject were examined.

The state of hair surface was expressed in points. The number of scales with degenerative changes (in one field of view) served as an evaluation criterion: 0 points, intact surface of the cuticle; 1 point, marginal defects in some scales (5-10%); 2 points, marginal defects in 50% scales (in one field of view); and 3 points, total change in the hair surface.

Clinical trial was conducted according to the requirements of the Ethics Committee (I. P. Pavlov St. Petersburg State Medical University). The results were analyzed by nonparametric tests (Fisher exact test and analysis of variance).

## RESULTS

Scanning microscopy of the hair surface showed that US of the hair in deaf people with congenital hearing impairment differed from that in healthy volunteers. The surface of long hair in healthy subjects was presented by the cuticular layer. The layer consisted of densely positioned and overlapping (in a tail-like manner) flat keratinized cells, which produced a cross-striated pattern (Fig. 1, *b*).

The hair of deaf homozygous carriers of the 35delG mutation in the *GJB2* gene were characterized by the following ultrastructural signs of degeneration (Fig. 1, *a*); fragile or friable crosscut area of the hair plate; and rough surface with intrusions and extrusions. The analysis of variance revealed no differences in hair thickness between the groups of deaf homozygous and heterozygous carriers of the 35delG *GJB2* gene mutation and healthy volunteers (control).

Some authors showed that "deaf" recessive mutations in *GJB2* (homozygous variants or heterozygous combinations with other mutations of this gene) are expressed by a single symptom (severe prelingual hearing impairment) [4]. The results of other investigations [2] and our study indicate that these mutations are responsible for the development of non-syndrome deafness and some non-cochlear symptoms of lower clinical significance. The range of these disorders will be widened in the future.

The signs for degeneration of hair surface were nonspecific (Table 1). Many factors contribute to disturbances in hair structure (type of nutrition, hormonal status, and genetic specificity of enzyme systems for collagen synthesis). These features determine moder-

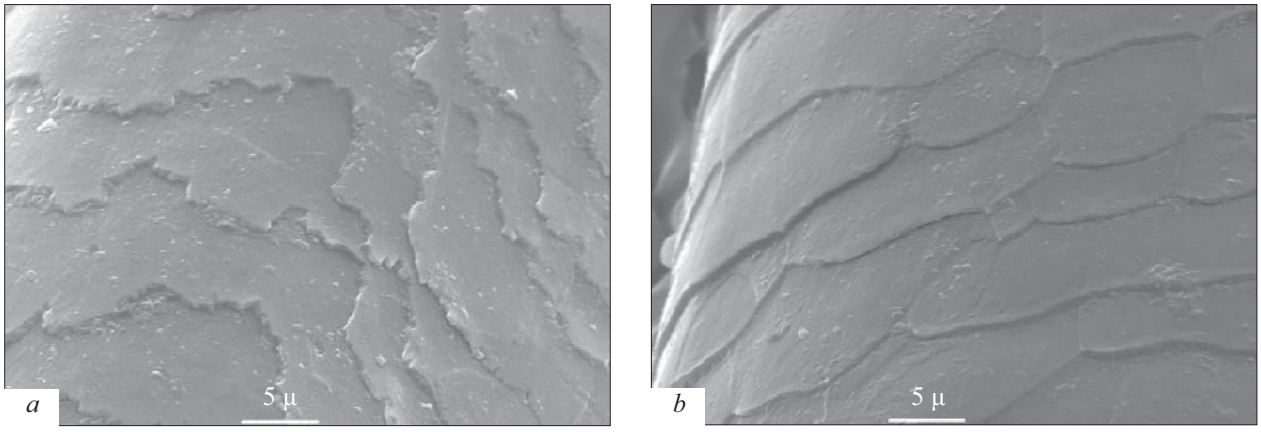


**Fig. 1.** US of the hair surface in a patient with congenital prelingual deafness (*a*, boy, 4 years of age, homozygous carrier of the 35delG mutation in the *GJB2* gene) and healthy subject of the same age (*b*). Microphotograph, scanning electron microscopy (×3300).

**TABLE 1.** US of the Hair Surface in Carriers of the 35delG Mutation in the *GJB2* Gene and Genetically Healthy Subjects

| Surface of the hair cuticle | Clinical and genetic status | | | | Total |
|---|---|---|---|---|---|
| | non-genetic deafness | deaf | | healthy | |
| | | 35delG heterozygotes | 35delG homozygotes | | |
| Total | 27 | 30 | 47 | 24 | 128 |
| Normal | 10 (37.0%) | 11 (36.7%) | 3 (6.4%) | 15 (50.0%) | 36 (28.1%) |
| Marginal defects in some scales | 8 (29.6%) | 9 (30.0%) | 11* (23.4%) | 6 (52.0%) | 34 (26.6%) |
| Marginal defects in 50% scales (one field of view) | 8 (29.6%) | 7 (23.3%) | 9* (19.1%) | 5 (20.8%) | 29 (22.7%) |
| Total marginal defect in horny scales | 1 (3.7%) | 3 (10.0%) | 24* (51.1%) | 1 (4.2%) | 29 (22.7%) |

**Note.** *$p<0.0001$ compared to healthy subjects (Fisher exact test).

ate degeneration of hair plates in some genetically healthy subjects (Table 1).

The homozygous mutation 35delG in the Cx 26 gene (one of the most important channels of cell-cell cooperation) is manifested not only in deafness, but also in total ultrastructural degeneration of the hair surface. The observed changes are probably associated with abnormalities in keratin maturation in proliferating cells of the hair papilla. Hearing impairment in deaf heterozygotes of 35delG is determined by a combination with another recessive mutation in the *GJB2* gene. Total degeneration of the hair surface is not observed in these subjects. The ultrastructural gradient of degeneration in 35delG homozygotes and heterozygotes corresponds to different severity of hearing loss. The degree of hearing impairment is highest in homozygous carriers of 35delG. By contrast, the combinations of 35delG are characterized by various degrees of poor hearing (II-IV) [11].

The natural acoustic analyzer is a complex structure, which provides advantages in survival. Therefore, the loss of this organ is evolutionarily inappropriate. The evaluation of clinically insignificant non-cochlear manifestations of recessive CX mutations will elucidate the biological importance of a wide distribution of major "deaf" mutations in *GJB2* among various ethnic groups. It probably provides some advantages for carriers and populational group.

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