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Hair phenotype in non-syndromic deafness

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ABSTRACT

The GJB2 gene is located on chromosome 13q12 and it encodes the connexin 26, a transmembrane protein involved in cell–cell attachment of almost all tissues. GJB2 mutations cause autosomal recessive (DFNB1) and sometimes dominant (DFNA3) non-syndromic sensorineural hearing loss. Moreover, it has been demonstrated that connexins are involved in regulation of growth and differentiation of epidermal tissues. Hence, mutations in GJB2 gene, which is responsible for non-syndromic deafness, may be associated with an abnormal skin and hair phenotype.

We analyzed hair samples from 96 subjects: a study group of 42 patients with hearing impairments of genetic origin (38 with a non-syndromic form, 4 with a syndromic form), and a control group including 54 people, i.e. 43 patients with other, non-genetic hearing impairments and 11 healthy volunteers aged up to 10 years old. The surface structure of 49 hair samples was normal, whereas in 45 cases it was altered, with a damaged appearance. Two hair samples were considered unclassifiable: one from the patient heterozygotic for the pendrin mutation (Fig. 2C), the other from a patient from Ghana with a R134W mutation (Fig. 2D). Among the 43 altered hair samples, 31 belonged to patients with connexin mutations and the other 12 came from patients without connexin mutations.

1. Introduction

In 2005, the World Health Organization (WHO) estimated that 12.5 million people worldwide suffered from bilateral profound hearing loss.

Congenital hearing loss is the hereditary sensorial defect most commonly observed in newborns. No data are available on worldwide prevalence of congenital deafness. In Italy the overall prevalence is 0.78 per 1000 for males and 0.69 per 1000 for females [1].

Genetic factors are involved in the pathogenesis of congenital hearing loss in at least 50% of cases.

Despite an extraordinary genetic heterogeneity, up to 50% of patients with autosomal recessive non-syndromic hearing loss reveal mutations in one particular gene, *GJB2*, which encodes for the gap junction connexin 26, involved in inner ear homeostasis.

GJB2 mutations have been widely studied in recent years, but there are still some doubts about the precise mechanism responsible for the hearing impairment correlating with a mutated connexin 26 [2–4].

Our incomplete knowledge of the gap junction in the cochlea derives mainly from the fact that what we know about the distribution of connexin in the inner ear has been learned from animal models (mainly rodents). Our understanding of the physiology of connexin relies on the assumption that it would have a similar distribution in humans, but genetic studies have also identified phenotypic discrepancies between mouse and human ears [5,6].

Recent studies [6–9] have shown that mutations in the GJB2 gene responsible for non-syndromic deafness may be also associated with an abnormal skin and hair phenotype. It has been demonstrated that connexins are involved in regulation of growth and differentiation of epidermal tissues, and gap junctions are found abundantly both in inner ear and epidermal tissues.

GJB2 mutations have also been identified in syndromic disorders with hearing loss associated with various skin disease phenotypes. GJB2 mutations associated with skin disease are, in general, transmitted with a dominant inheritance pattern. Non-syndromic deafness is caused prevalently by a loss of function, while literature evidences suggest for syndromic deafness a mechanism based on gain of function. The spectrum of skin manifestations associated with some mutations seems to have a very high phenotypic variability. Why some mutations can lead to

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widely varying cutaneous manifestations is poorly understood and in particular, the reason why the skin disease-deafness phenotypes differ from each other thus remains unclear [10].

The aim of the present work was to establish whether the GJB2 gene mutations that cause a gap junction dysfunction in the cochlea may also affect gap junctions in the hair, resulting in particular hair phenotypes.

We used scanning electron microscopy to compare images of the morphology and mineral surface composition of hair from hearing-impaired patients with and without associated GJB2 mutations.

2. Materials and methods

We performed a case-control study on 107 patients undergoing cochlear implantation at the Sant'Anna University Hospital in the department of Audiology from March 2008 to January 2010.

For control purposes we considered hair samples obtained from 11 healthy volunteers of similar age with no hearing loss or chronic diseases, and no GJB2 mutations.

Patients' files were analyzed in accordance with Italian privacy law and the hospital's regulations.

The personal and clinical details considered to describe the sample of patients were as follows: age, gender, cause(s) of deafness, genetic mutations, family history of hearing impairments, parental consanguinity, hair color, thickness and shape and nationality.

Each patient underwent audiometric assessment and genetic analysis. Images of the patients' hair samples underwent scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS).

Direct DNA sequencing of the GJB2 gene (analyzing the whole coding region, including the promoter and non-codifying regions) was done at the molecular genetics laboratory in Ferrara on genomic DNA obtained from blood samples from all patients and controls. PCR products were screened for mutations by DHPLC (denaturing high-performance liquid chromatography) and SSCP (single strand conformational polymorphism). All variant profiles were characterized by sequencing the product of a second PCR amplification using an ABI PRISM 3130.

Hair samples were obtained from all patients and controls, and analyzed under the scanning electron microscope (SEM); a length of hair (1 cm long) was cut from near the root with stainless steel scissors. Subjects were asked to not wash their hair with any shampoo or soap and not to use any hair products for at least 3 days prior to the collection.

For each subject, we examined the microphotographs of two hair (considering the basal part in three cases and apical part in one).

We classified the surface of the hair as normal (intact cuticle) or changed.

Then, the elements in the hair were further analyzed using energy-dispersive X-ray spectroscopy (EDS), where the digital image of the intensity of the X-ray characteristic of the atomic structure of a given element provides a semiquantitative map of their distribution on the surface of the sample.

Correlations were drawn using the STATISTICA 7.1 software (Stat Soft Italy), which enabled us to measure the distribution of the sample, the significance of the outcomes of ANOVA, and the parametric (Pearson's r) and nonparametric (Spearman's) correlation indexes.

3. Results

We initially analyzed hair samples from 107 patients, but had to exclude the samples from 22 because the details emerging from their genetic analysis were incomplete.

The final sample consisted of 96 subjects, i.e. a study group of 42 patients with hearing impairments of genetic origin (38 with a non-syndromic form, 4 with a syndromic form), and a control group including 54 people, i.e. 43 patients with other, non-genetic hearing impairments and 11 healthy volunteers aged up to 10 years old.

The median age of the 96 participants was 9 years and 5 months (SD $\pm$ 11 years 6 months); 85% of the subjects were less than 10 years old and 15% were over ten; the youngest was 1 year and 8 months old, and the oldest was 59.

Most of the patients were Italian (78.8%), 12% came from other European countries, 4% from Asia, 3% from Africa and 1% from America.

With the exception of 4 cases of progressive hearing loss, all the patients had bilateral, symmetrical, congenital, profound hearing impairments. Just one out 4 patients with progressive form was homozygotic for the 35delG mutation. There were no cases of parental consanguinity, while 10 patients had a family history of deafness with at least one member of their family suffering from hearing loss.

No correlations emerged between hair color or shape and the hair phenotypes except for the fact that 45% of the patients under 10 years old with a connexin mutation had very thin hair.

Among the 96 subjects, 38 had a GJB2 gene mutation. All of the 38 patients with non-syndromic form had a mutation on GJB2 gene. Among the 4 patients with the syndromic form (one related to the Waardemburg syndrome, one to the Cockayne sindrome one to the Opitz syndrome and one to the DOOR syndrome) no one had a GJB2 mutation. Among the 58 who had no GJB2 gene mutations, there was 1 deaf patient with a pendrin gene mutation (G334A) in heterozygosis, not previously described in the literature.

We found 11 different genotypes for the GJB2 allele. The most frequent mutation was 35delG; homozygotic patients with

Table 1
Different mutations in GJB2 and GJB6 genes and their relative frequency in the sample.

	Mutations in GJB2	No. of patients and relative frequency	Total
Homozygosis	35delG/35delG	26 (30.6%)	27 (28.1%)
	R143W/R143W	1 (1.2%)	
Compound heterozygosis	35delG/M34T	1 (1.2%)	6 (8.8%)
	35delG/R184P	2 (2.4%)	
	35delG/V95M	1 (1.2%)	
	35delG/L90P	1 (1.2%)	
	35delG/ΔConnexin30	1 (1.2%)	
Simple heterozygosis	M34T	1 (1.2%)	5 (5.2%)
	IVS1+1G->A	2 (2.4%)	
	S139C	1 (1.2%)	
	T55N	1 (1.2%)	
Total			38 (39.6%)