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ORIGINAL ARTICLE

Opinions of hearing parents about the causes of hearing impairment of their children with biallelic *GJB2* mutations

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Abstract Hereditary hearing impairment (HI) caused by recessive *GJB2* mutations is a frequent sensory disorder. The results of the molecular-based studies of HI are widely used in various genetic test systems. However, the ethical aspects are less described than the genetic aspects. The concerns expressed by individuals from groups with genetic risks must be included in the counseling of patients and their families. For evaluation of subjective opinions of hearing parents about the presumed causes of HI of their children, we analyze the cohort of parents having children with confirmed hereditary HI caused by biallelic recessive *GJB2* mutations (in a homozygous or a compound heterozygous state). This study included 70 deaf children with HI due to mutations in the *GJB2* gene and 91 questionnaires about the presumed causes of their deafness filled by their parents. Most of the parents at 78% (CI 68.4–85.4%) attributed their children's HI to "non-hereditary" causes and 22% (CI 14.7–31.6%) to "hereditary" causes ($p < 0.05$). Therefore, the prior opinions of the parents did not correspond to positive *GJB2* genetic testing results. The subjective opinions of parents are probably partly based on family history, since respondents with deaf relatives in their pedigree more likely supposed hereditary causes for HI in their children than the respondents without deaf relatives ($p < 0.001$).

Keywords Hereditary hearing impairment · Opinions of the parents · Genetic testing · *GJB2* gene

Introduction

Congenital deafness is one of the most frequent sensory disorders with an incidence of about 1 in 1000 newborns, with approximately half of all cases having a genetic etiology (Marazita et al. 1993; Morton et al. 2006). Currently, more than 100 identified genes are associated with hearing impairment (HI), which are registered in the Hereditary Hearing Loss

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Homepage (http://hereditaryhearingloss.org) (February, 2017). However, one of the most common types of hereditary deafness is autosomal recessive deafness 1A (MIM 220290) caused by biallelic mutations (in a homozygous or a compound heterozygous state) in the *GJB2* (Cx26) gene. The results of molecular-based studies on HI are widely used in various genetic tests for *GJB2* gene mutations. However, problems that arise at the junction of rapidly advancing *GJB2* testing and the concerns of individuals from groups with genetic risks are less studied than the molecular genetic aspects of HI.

Previously, studies were mostly focused on the causes of HI (Gray 1989; Marazita et al. 1993; Derekoy 2000), attitudes to prenatal diagnosis for HI (Middleton et al. 1998; Brunger et al. 2000; Burton et al. 2006; Boudreault et al. 2010; Baldwin et al. 2012; Nahar et al. 2013), the impact of genetic testing on the beliefs and attitudes of parents of deaf children towards genetic testing (Palmer et al. 2009), and the effect of pre-test genetic counseling of deaf individuals on knowledge of genetic testing (Baldwin et al. 2012). It is known that 90–95% of deaf children are born to hearing parents (Schein and Delk 1974; Mitchel and Karchmer 2004). However, the opinions of hearing parents about the presumed causes of HI in their children before genetic testing have not been sufficiently studied (Li et al. 2007; Steinberg et al. 2007; Rodrigues et al. 2013). At the same time, this information might allow for better preparation for the counseling of patients and their families and anticipation of a parent's initial assumptions. This information will contribute to the better perception and acceptance of genetic testing results by parents.

Now, the search for mutations in the *GJB2* gene is the only available routine molecular method for the genetic testing of HI cases in most regions of Russia. However, the causes of many inherited HI cases often remain unclear due to known extreme heterogeneous genetic etiology of this disorder. The prevalence of *GJB2* mutations among deaf patients was recently described in three regions of Russia (the Sakha Republic, the Tyva Republic, and the Bashkortostan Republic) (Dzhemileva 2011; Bady-Khoo et al. 2014; Barashkov et al. 2016). For a clear evaluation of subjective opinions of hearing parents about the presumed causes of HI of their children, we analyze the cohort of parents having children with confirmed hereditary HI caused by biallelic recessive *GJB2* mutations (in a homozygous or a compound heterozygous state).

Materials and methods

Survey participants

Data on individuals with HI were obtained from the special residential schools for deaf and hard-of-hearing children located in the towns of Yakutsk, Kyzyl, and Ufa (the Sakha, Tyva, and Bashkortostan Republics of Russia, respectively). The *GJB2* status of all participants and their family members was previously unknown; children with HI were tested for *GJB2* gene mutations for the first time. Overall, 157 students were tested for mutations in the *GJB2* gene, and special questionnaires (n = 314) were sent to their parents at their place of residence, 183 of which were returned. Genetic testing revealed that 70 out of 157 deaf children have biallelic recessive *GJB2* mutations (in a homozygous or a compound heterozygous state). For a clear evaluation of subjective opinions of hearing parents about the presumed causes of HI of their children, we analyze 91 questionnaires filled by parents who have children with confirmed genetic etiology of HI due to *GJB2* mutations. Some questionnaires that had been filled by other relatives (not the parents) were excluded. The data on surveyed parents are given in Table 1, and the data on deaf children with biallelic recessive *GJB2* mutations are presented in Table 2.

The questionnaire

We have developed a questionnaire including two main questions to ascertain the views of hearing parents on the presumed causes of HI in their children. The parents of deaf or hard-of-hearing children subjected to genetic testing for the *GJB2* gene mutations were surveyed before the announcement of the genetic testing results. The first question in the questionnaire was about presumed causes of children's HI and parents had to choose one of two options: "hereditary" or "non-

Table 1 The data on surveyed hearing parents

Hearing parents (n = 91)	Number
The related status	
Mother	61 (67.0%)
Father	30 (33.0%)
Age (mean age 39.8 ± 8.7)	
Unknown	3 (3.3%)
21–30	15 (16.5%)
31–40	32 (35.1%)
41–50	25 (27.5)
51–60	16 (17.6%)
Educational level[a]	
High	17 (18.7%)
Medium	74 (81.3%)
Place of residence	
Urban	18 (19.8%)
Rural	73 (80.2%)
Regions of study (Russian Federation)	
The Sakha Republic	74 (81.3%)
The Tyva Republic	9 (9.9%)
The Bashkortostan Republic	8 (8.8%)

[a] High—completed degree(s) at university/college/other tertiary educational institution. Medium—completed secondary school/high school

Table 2 The data of deaf children with biallelic recessive *GJB2* mutations

Deaf children (n = 70)	Number
Age (mean age 13.7 ± 5.3)	
2–5	7 (10.0%)
6–10	14 (20.0%)
11–15	19 (27.1%)
16–20	25 (35.7%)
21–25	5 (7.2%)
GJB2 genotypes	
c.[−23+1G>A];[−23+1G>A]	47 (67.2%)
c.[35delG];[35delG]	11 (15.7%)
c.[516G>C];[516G>C]	5 (7.2%)
c.[235delC];[516G>C]	2 (2.9%)
c.[−23+1G>A];[35delG]	1 (1.4%)
c.[−23+1G>A];[516G>C]	1 (1.4%)
c.[35delG];[299_300delAT]	1 (1.4%)
c.[516G>C];[299_300delAT]	1 (1.4%)
c.[313_326del14];[313_326del14]	1 (1.4%)
Manifestation of hearing loss	
Congenital	62 (88.6%)
Pre-lingual	8 (11.4%)

hereditary" causes. If they chose option non-hereditary, they had to specify the presumed reason of HI in their child (Fig. 1). The second question was about the presence of deaf close relatives, and the respondents were asked to answer "yes" or "no" (Fig. 1). We interpreted a yes as a positive family history concerning HI. The answers of both parents were analyzed independently of each other.

Mutation analysis of the *GJB2* gene

The genomic DNA was extracted from the lymphocytes of the peripheral blood. Amplification of the coding exon 2 and flanking intronic regions was performed using the following primers: Cx26A-U/Cx26U-L (5′-TCTTTTCCAGAGCAAACCGC-3′, 5′-GACACGAAGATCAGCTGCAG-3′) (Kelsell et al. 1997), Cx342U/Cx739-L (5′-AGGCCGACTTTGTCTGCAACA-3′, 5′-GTGGGCCGGGACACAAAG-3′) (Kelley et al. 1998), and 5 -TATGTCATGTACGACGGCT-3′/5′-TCTAACAACTGGGCAATGC-3′ (Zelante et al. 1997). Amplification of the non-coding exon 1 and flanking intronic regions was performed using primers Ex1-F/Ex1-R (5′-CCGGGAAGCTCTGAGGAC-3′, 5′-GCAACCGCTCTGGGTCTC-3′) with 10% Betaine (Sigma, USA) (Sirmaci et al. 2006). The PCR products were sequenced using the same primers on ABI PRISM 3130XL (Applied Biosystems, USA).

Statistical methods

To compare the answers of two groups of respondents (with or without deaf relatives) on the question of the causes of HI of their children, we calculated 95% confidence intervals for the distribution of the answers of respondents using the sampling

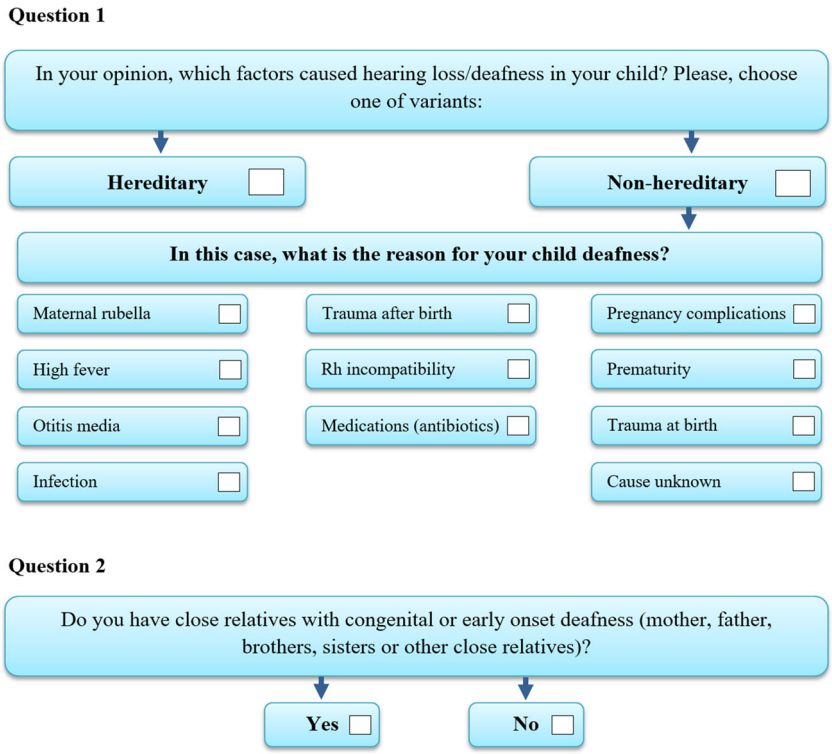

Fig. 1 Example of questionnaire. Note: List of deafness causes is according to Marazita et al. (1993)

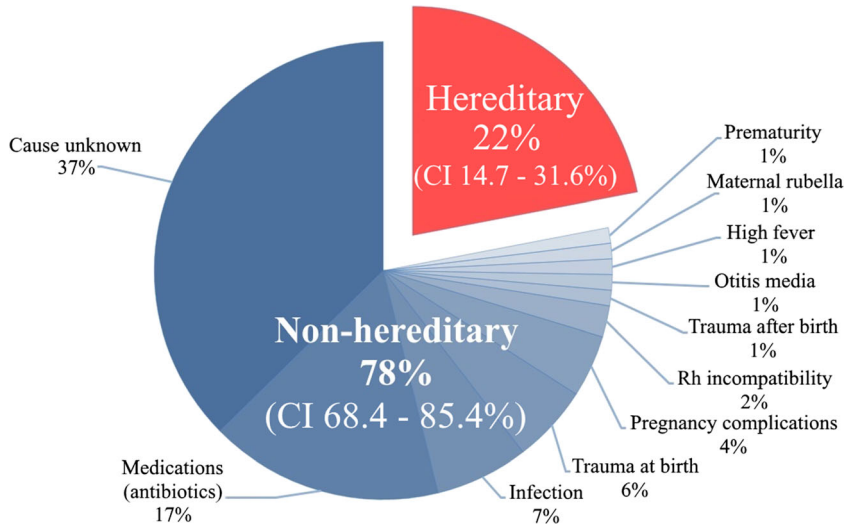

Fig. 2 The opinions of hearing parents about the causes of HI in their children with positive *GJB2* genetic testing results

software which was kindly provided by V. Macaulay and adapted by M. Metspalu (Estonian Biocentre, Tartu, Estonia). The statistical correlation between the opinions of the respondents about the possible causes of their children's HI and the presence of deaf relatives was evaluated using the chi-square test with the Medstat software (McGraw-Hill, Inc.Version 3.03). Differences were considered statistically significant for $p < 0.05$.

Results

Obtained results are presented in Fig. 2 and Table 3. On the first question "In your opinion, which factors caused hearing loss/deafness in your child? Please, choose one of variants," 71 (78%, CI 68.4–85.4%) of parents answered non-hereditary and 20 (22%, CI 14.7–31.6%) hereditary ($p < 0.05$). Parents who answered non-hereditary were asked to respond to the following question: "In this case, what is the reason for deafness of your child?" The most frequent answers were the following: "cause unknown," "medication," "infection," "trauma at birth," "pregnancy complications," and others (Fig. 2).

On the second question, "Do you have close relatives with congenital or early onset deafness (mother, father, brothers, sisters, or other close relatives)?" Twenty-five (27%) parents answered yes and 66 (73%) parents answered no.

Analysis of answers for both questions revealed that the respondents who had deaf close relatives answered hereditary significantly more often than the respondents without deaf relatives ($p < 0.001$). On the contrary, the answer hereditary was less likely among respondents without deaf close relatives ($p < 0.001$) (Table 3).

Discussion

In this paper, we analyzed the responses of 91 normal hearing parents about the presumed causes of HI of their children ($n = 70$) before the announcement of the positive *GJB2* genetic testing results (presence of biallelic recessive *GJB2* mutations) that confirmed genetic etiology of HI in their children. Most parents (78%) considered that the HI of their children was due to non-hereditary causes and only 22% of parents chose to answer hereditary (Fig. 2). Therefore, the prior opinions of the parents did not correspond to positive *GJB2* genetic testing results. We suppose that parents may underestimate the role of hereditary factors in HI. Our results demonstrate that most of the parents prefer the more obvious (medication, infection, trauma, pregnancy complications, and etc.) reasons for them for the deafness of their children than genetic causes. Therefore, it may be assumed that different exogenous risk factors can mask the true causes of HI.

The subjective opinions of parents are probably partly based on family history, since respondents with deaf relatives in their pedigree more likely supposed hereditary causes for HI in their children (Table 3). Similar results were obtained in a study aimed at assessing deaf people's attitude towards genetic testing and their awareness of the inheritability of HI (Baldwin et al.

Table 3 Opinions of respondents about possible causes of HI of their children on presence or absence of deaf relatives

Answer	The respondents having deaf close relatives ($n = 25$)	Respondents without deaf close relatives ($n = 66$)	χ^2	p
Non-hereditary	13 (52%)	58 (88%)	13.61	<0.001
Hereditary	12 (48%)	8 (12%)		

2012). In this study, most respondents without a family history of HI denied hereditary causes of deafness (Baldwin et al. 2012). Therefore, when announcing the results of genetic testing for *GJB2* gene mutations, it is necessary to consider that hearing parents of deaf children may have subjective preliminary opinions about the potential causes of their children's HI, which may not correspond to the positive results of the genetic testing. Therefore, it is very important to improve genetic knowledge of parents having deaf children to increase their awareness about possible risks of HI in their families.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This work and questionnaire were approved by the local bioethics committee at the Yakut Science Centre of Complex Medical Problems (Yakutsk, Protocol 16, on April 16, 2009), the Bioethics Committee at the Institute of Biochemistry and Genetics of the Ufa Scientific Centre of the Russian Academy of Sciences (Ufa, Protocol 23, on February 26, 2010), and the Bioethics Commission at the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Protocol 9, on April 24, 2012).

Informed consent All testing procedures were conducted with a written informed consent signed by the parents of deaf children.

References

Bady-Khoo MS, Bondar AA, Morozov IV, Zytsar MV, Mikhalskaya VY et al (2014) Study of hereditary forms of hearing loss in the Republic of Tyva. II. Evaluation of the mutational spectrum of the *GJB2* (Cx26) gene and its contribution to the etiology of hearing loss. Meditsinskaya Genetika 13:30–40 (Article in Russian)

Baldwin EE, Boudreault P, Fox M, Sinsheimer JS, Palmer CG (2012) Effect of pre-test genetic counseling for deaf adults on knowledge of genetic testing. J Genet Couns 21(2):256–272. doi:10.1007/s10897-011-9398-1

Barashkov NA, Pshennikova VG, Posukh OL, Teryutin FM, Solovyev AV et al (2016) Spectrum and frequency of the *GJB2* gene pathogenic variants in a large cohort of patients with hearing impairment living in a subarctic region of Russia (the Sakha Republic). PLoS One 11(5):e0156300. doi:10.1371/journal.pone.0156300

Boudreault P, Baldwin EE, Fox M, Dutton L, Tullis LE et al (2010) Deaf adults' reasons for genetic testing depend on cultural affiliation: results from a prospective, longitudinal genetic counseling and testing study. J Deaf Stud Deaf Educ 15(3):209–227. doi:10.1093/deafed/enq012

Brunger JW, Murray GS, O'Riordan M, Matthews AL, Smith RJH, Robin NH (2000) Parental attitudes toward genetic testing for pediatric deafness. Am J Hum Genet 67(6):1621–1625. doi:10.1086/316901

Burton SK, Withrow K, Arnos KS, Kalfoglou AL, Pandya A (2006) A focus group study of consumer attitudes toward genetic testing and newborn screening for deafness. Genet Med 8(12):779–783

Derekoy FS (2000) Etiology of deafness in Afyon School for the Deaf in Turkey. Int J Pediatr Otorhinolaryngol 55(2):125–131

Dzhemileva LU (2011) Molekyliarno-geneticheskii analiz nasledstvennoi nesindromalnoi sensonevralinoi glyhoti, Dissertation, Moscow. (in Russian)

Gray RF (1989) Causes of deafness in schools for the deaf in Madras. Int J Pediatr Otorhinolaryngol 18(2):97–106

Kelley PM, Harris DJ, Comer BC, Askew W, Fowler T et al (1998) Novel mutations in the connexin 26 gene (*GJB2*) that cause autosomal recessive (DFNB1) hearing loss. Am J Hum Genet 62(4):792–799. doi:10.1086/301807

Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN et al (1997) Connexin 26 mutations in hereditary non-syndromic sensorineural deafness. Nature 387(6628):80–83. doi:10.1038/387080a0

Li Y, Steinberg AG, Bain L, Yaeger D, Bieler A et al (2007) Assessing parental attitudes toward genetic testing for childhood hearing loss: before and after genetic consultation. Am J Med Genet A 143A(14):1546–1553. doi:10.1002/ajmg.a.31730

Marazita ML, Ploughman LM, Rawlings B, Remington E, Amos KS, Nance WE (1993) Genetic epidemiological studies of early-onset deafness in the U.S. school-age population. Am J Med Genet 46(5):486–491. doi:10.1002/ajmg.1320460504

Middleton A, Hewison J, Mueller RF (1998) Attitudes of deaf adults toward genetic testing for hereditary deafness. Am J Hum Genet 63(4):1175–1180. doi:10.1086/302060

Mitchel RE, Karchmer MA (2004) When parents are deaf versus hard of hearing: patterns of sign use and school placement of deaf and hard-of-hearing children. J Deaf Stud Deaf Educ 9(2):133–152. doi:10.1093/deafed/enh017

Morton C, Walter E, Nance MD (2006) Newborn hearing screening—a silent revolution. N Engl J Med 354(20):2151–2164. doi:10.1056/NEJMra050700

Nahar R, Puri RD, Saxena R, Verma IC (2013) Do parental perceptions and motivations towards genetic testing and prenatal diagnosis for deafness vary in different cultures? Am J Med Genet A 161A(1):76–81. doi:10.1002/ajmg.a.35692

Palmer CG, Martinez A, Fox M, Zhou J, Shapiro N et al (2009) A prospective, longitudinal study of the impact of *GJB2*/*GJB6* genetic testing on the beliefs and attitudes of parents of deaf and hard-of-hearing infants. Am J Med Genet A 149A(6):1169–1182. doi:10.1002/ajmg.a.32853

Rodrigues F, Paneque M, Reis C, Venâncio M, Sequeiros J, Saraiva J (2013) Non-syndromic sensorineural prelingual deafness: the importance of genetic counseling in demystifying parents' beliefs about the cause of their children's deafness. J Genet Couns 22(4):448–454. doi:10.1007/s10897-012-9565-z

Schein JD, Delk MT (1974) The deaf population of the United States. National Association of the deaf:1–336

Sirmaci A, Akcayoz-duman D, Tekin M (2006) The c. IVS1+1G>A mutation in the *GJB2* gene is prevalent and large deletions involving the *GJB6* gene are not present in the Turkish population. J Genet 85(3):213–216

Steinberg AG, Kaimal GS, Bain L, Krantz I (2007) Parental narratives on genetic testing for children with hearing loss: a qualitative inquiry. Am J Med Genet A 143A(14):1533–1545. doi:10.1002/ajmg.a.31731

Zelante L, Gasparini P, Estivill X, Melchionda S, Dy. Agruma L et al (1997) Connexin 26 mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in Mediterraneans. Hum Mol Genet 6(9):1605–1609