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Bimodal strategy for excellent audiological rehabilitation in a subject with a novel nonsense mutation of the *SLC26A4* gene: A case report

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ABSTRACT

Sensorineural hearing loss is a heterogeneous disease caused by mutations in many genes. However, in the presence of enlarged vestibular aqueduct, it is frequently associated with mutations in the solute carrier family 26 member 4 (*SLC26A4*), a gene causative of a syndromic form (Pendred) as well as a non-syndromic form of hearing loss (DFNB4). We describe a clinical case presenting bilateral sensorineural hearing loss and enlarged vestibular aqueduct in which a novel homozygous *SLC26A4* mutation was identified. Despite a late diagnosis of hearing loss, a peculiar rehabilitation therapy strategy was identified that provided excellent results.

1. Introduction

Sensorineural hearing loss (SNHL) is the most common form of inherited deafness in the general population presenting a high genetic heterogeneity. Mutations in the *SLC26A4* gene (OMIM 605646), located on the chromosome 7q22.3, play an important role (1–5) in sensorineural hearing loss onset. Mutations can occur in an isolated non-syndromic form (DFNB4, OMIM#600791), or in Pendred syndrome (PDS, OMIM#274600). Enlarged vestibular aqueduct (EVA), a congenital malformation of the inner ear, is frequently associated with hearing loss both in the syndromic forms and in the isolated forms. In some patients affected by EVA undergoing cochlear implantation, there may be an intraoperative risk of a perilymphatic gusher, but the audiological performances obtained are still good as reported in literature [6–9]. The aim of this work was a description of a clinical case presenting sensorineural hearing loss and EVA in which a new *SLC26A4* mutation, in homozygous form, was found and which had never before been described in the literature. In this case report, we also outlined the new audiological strategy used to obtain a good audiological recovery with an improvement in low-frequency resolution in the patient.

2. Case presentation

The subject of our study is a six-year-old girl (Fig. 1, Panel A) affected by asymmetric bilateral sensorineural hearing loss. The diagnosis was made at the age of 27 months in the Audiology Unit (University of Federico II, Naples) where the child had been brought by her parents because she had a language delay. A complete audiological evaluation (Distortion Product Otoacoustic Emissions at frequencies 0.8–4 KHz; Tympanometry; Pure Tone Audiometry (PTA); Electrophysiological evaluation in Auditory Evoked potentials) was performed according to standard protocols [10] (Fig. 2, Panels A–C). The degree of her hearing loss was defined as severe-profound according to International Bureau for Audio phonology audiometric classification of hearing loss. Her parents were found to be unaffected. There were no pathologic signs at the vestibular examination; in fact, an absence of spontaneous and positional nystagmus, bilateral hyporeflexia of vestibulo-ocular reflex and regular eye movements were displayed.

The patient was subjected to computed tomography (CT) for the study of the middle and inner ear with a scan of petrous bone (temporal bone) and magnetic resonance imaging (MRI) for the study of brain tissues, brainstem structures, and internal acoustic meatuses. Using these radiological investigations, we found the vestibular aqueduct to

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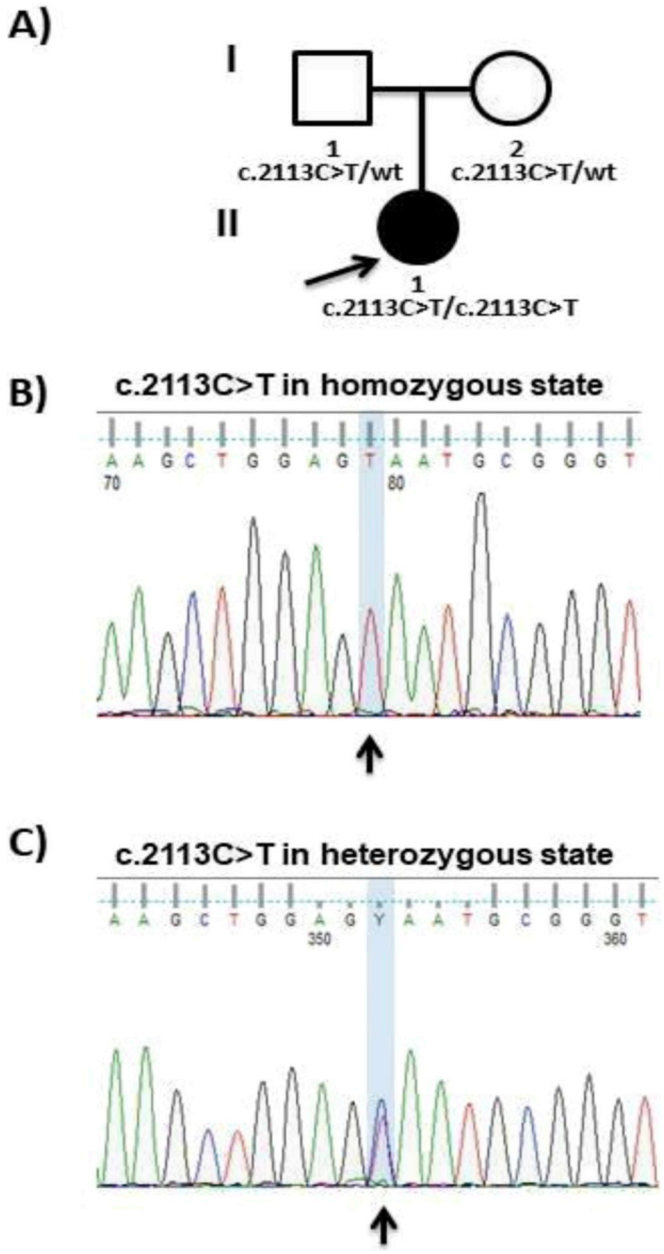

Fig. 1. Molecular analysis. Panel A) Family pedigree: unblackened and blackened symbols denote unaffected and affected subjects, respectively. Panel B) Electropherograms shows the mutation c.2113C > T (p.Q705*) in homozygosity in the proband and in heterozygosity in the parents (panel C).

be dilatated bilaterally (EVA). The other structures were normal with no further morphological anomalies.

The study of antibodies and thyroid hormones, associated with thyroid ultrasound, were normal. Up until now, no anatomical or functional alterations of the thyroid and kidney have been found.

After the audiological diagnosis, given the presence of EVA, to identify the etiology of the pathology in the girl, the subject, was analyzed, by direct sequencing, for *SLC26A4* mutations [11]. The molecular analysis showed the presence of the stop-gain variant c.2113C > T, p.Q705* (NM_000441) in homozygosity (Fig. 1, Panel B). This variant, never described in literature until now, introduces a termination codon causing or the formation of a truncated protein in the last cytoplasmic domain or a defective transcript that could be degraded by nonsense-mediated mRNA decay. The analysis of the inheritance pattern revealed the presence of the same mutation in heterozygous state in her parents (Fig. 1, Panel C). The variant was absent from the population databases 1000 Genomes Project, Exome Sequencing Project, and Exome Aggregation Consortium [12]. SLC26A4-Q705* mutation screening in a population of 116 healthy subjects was performed using restriction enzyme digest analysis, using the BsrDI enzyme. We did not identify any carriers of allele T. Using the online program Mutation Taster [13], the variant was predicted to be disease-causing with a score of 0.999 for Mutation Taster. Moreover, by using InterVar [14], the variant was predicted pathogenic with the following evidence scores: PVS1 (very strong), PM2 (moderate), PP3 (supporting). After the diagnosis of hearing loss, the young patient was rehabilitated with bilateral behind the ear (BTE) hearing aids (HA) and subjected to speech therapy with limited benefits. For these reasons, at the age four, it was changed therapeutic strategy by switching to a bimodal stimulation (Cochlear Implant [CI] on one side and HA on the other). This solution involved the use of a CI (Advanced Bionics Mid Scala electrode and Naida Q70 speech processor) in the worst ear (left) while in the contralateral ear (right) the patient wore an HA (Phonak Sky Q70 UP) fitted with classic pediatric formula DSL v.5. The electrode was placed through round window and a small gusher occurred during surgery. The coding strategy used was “Hires Optima-S″ with a directional microphone (“UltraZoom”). At the age of six years, as part of the clinical trial, a new HA specifically designed for use in bimodal system (Phonak Naida Link) was applied. At first, the fitting of the HA followed the National Acoustic Laboratories – NAL-NL2 - configuration (outcomes are shown in Fig. 3, Left side, Panels A, B, E, G) and later the new Phonak formula – Adaptive Phonak Digital Bimodal formula - (outcomes are shown in Fig. 4, Right side, Panels C, D, F, H) designed to optimally complement hearing with CI. With the new prescriptive formula, there was a substantial improvement in the performance of the patient, above all in the speech recognition, both in silence and with competitive noise. PTA in free field with HA and CI reaches 20 dB normal hearing level from 250 Hz to 4000 Hz (Fig. 3, Right side, Panel F). As regards speech audiometry with bisyllabic words, in a quiet environment, she reaches 30 dB in perception threshold and 50 dB in intellection threshold (Fig. 3, Right side, Panel H).

Linguo-specific tests of perceptual and cognitive-linguistic outcomes were administered to assess performance variations. For perceptual skills, the Common Assessment Protocol in Rehabilitative Audiology was used while for lexical skills, the TFL Protocol - tests of assessment of preschool lexical skills, in understanding and production [15].

In Fig. 4 A and 4 B we report the comparison of acoustic-perceptual and linguistic skills, with bilateral hearing aids (2 months before CI), bimodal system (CI - NAIDA Q 70 UP) 20 months after implantation and integrated bimodal system (CI - Naida Link) after 12 months.

With regard to the cognitive skills, working memory was assessed with the administration of metaphonological skills assessment tests. It was not possible to carry out such assessments with bilateral hearing aids as these skills develop in preschool, after the bimodal stimulation began. Fig. 4C shows the comparison of metaphonological abilities.

This study was approved by the University of Naples Federico II Ethical Committee. A signed informed-consent form was obtained from the parents.

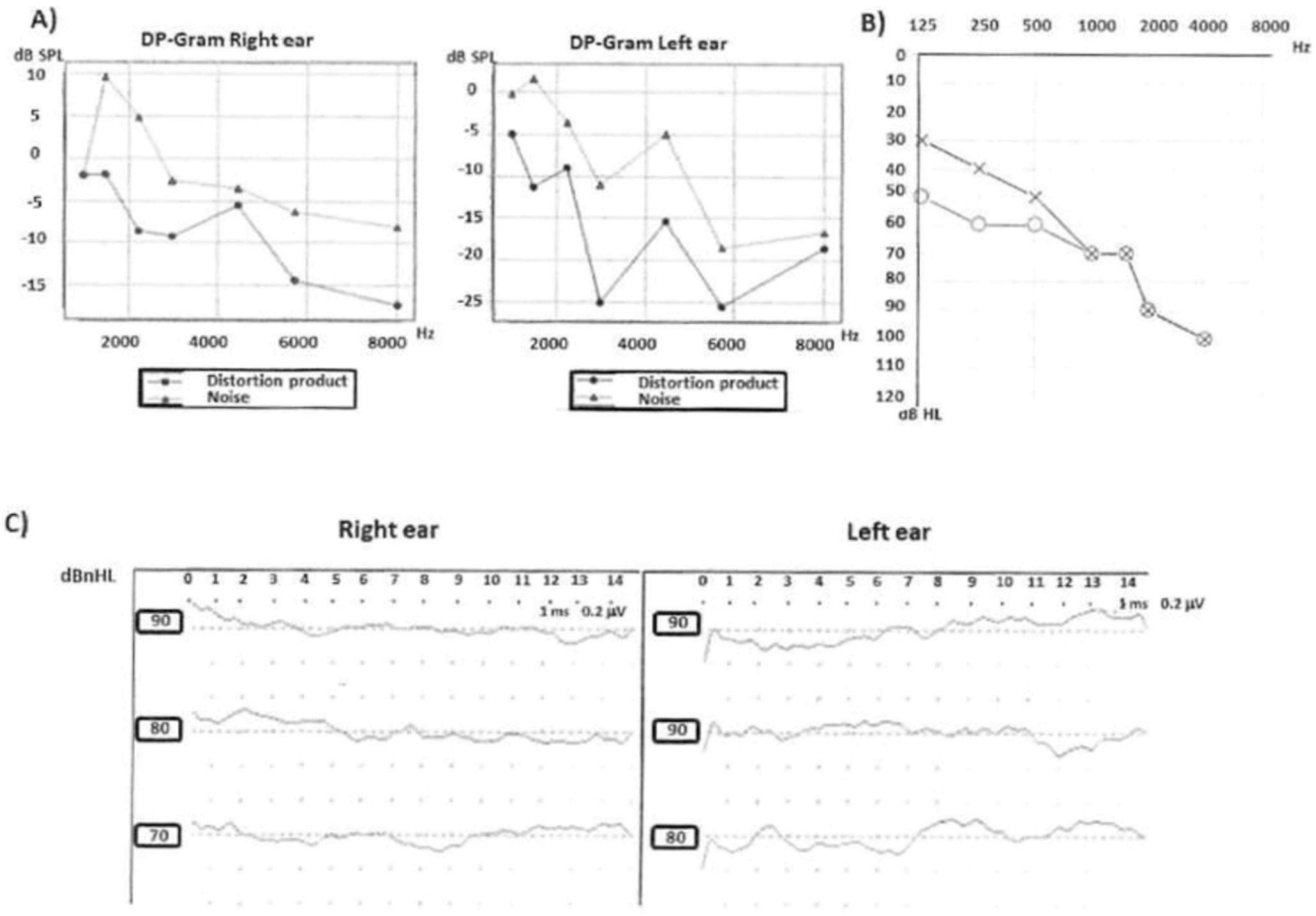

Fig. 2. Audiological analysis of the proband before rehabilitation. Panel A) Distortion product otoacoustic emissions of both ears. Panel B) PTA. Symbol o denotes right ear, symbol × denotes left ear. Panel C) Auditory evoked potentials.

3. Discussion and conclusions

Sensorineural hearing loss is reported in syndromic and non-syndromic forms and, currently, more than 400 syndromic forms are known. About 70% of SNHL cases are non-syndromic and about 80% are recessive. Most of these recessive non-syndromic cases result from mutations of the *GJB2* and *GJB6* genes [16]. In addition to these, pathogenic variants in the solute carrier family 26 member 4 (*SLC26A4*) gene, which provides instructions to make an anion transporter protein called pendrin, can be considered another relevant cause of recessive hereditary hearing loss, accounting for about 14% of cases of hearing loss [1–5]. Mutations in this gene have been linked with both non-syndromic and syndromic forms (Pendred Syndrome) with thyroid dysfunction (goiter and a partial defect in iodide organification) associated to hearing loss. Moreover, in many cases, both for syndromic and not syndromic forms, EVA is associated to hearing loss. Variability both in hearing loss and thyroid disease is considerable, even within the same family; the hearing loss can fluctuate over time and some vestibular disorder may also be present. The onset of thyroid dysfunction may vary widely. Some studies suggest that the thyroid goiter is present only in 50% of individuals with PDS [17,18]. Goiter is usually displayed in late childhood or early puberty in approximately 40% of patients; in the remaining 60%, it begins in early adult life. In these patients, thyroid function was studied and revealed a slight increase in thyroid-stimulating hormone levels, while serum thyroxine levels were below average values. Thyroid microsomal antibodies were negative. Our study expands the mutation spectrum in the *SLC26A4* presenting the novel nonsense mutation c.2113C > T in homozygosity as the causative variant in an affected girl showing a sensorineural hearing loss and EVA. This private variant is located near to a previously described stop-gain variant, p.C706* (c.2118A > C), observed in a patient suffering from non-syndromic EVA [19]. The parents of the affected child are not consanguineous, yet, the presence of the new mutation in homozygosis could be explained considering that the analyzed family lives in a small village in the province of Salerno (Campania Region, South Italy) where it is known that there are several genetically isolated villages [10,20]. Sensorineural hearing loss is highly heterogeneous, but the presence of EVA in this subject has led us to choose to analyze the gene *SLC26A4*. This study, then, adds another case that fits into the known correlation between EVA and *SLC26A4* mutations as already described in the literature [11]. Actually, thyroid anomalies are not present in the girl, but considering her young age, it is not possible to exclude the development

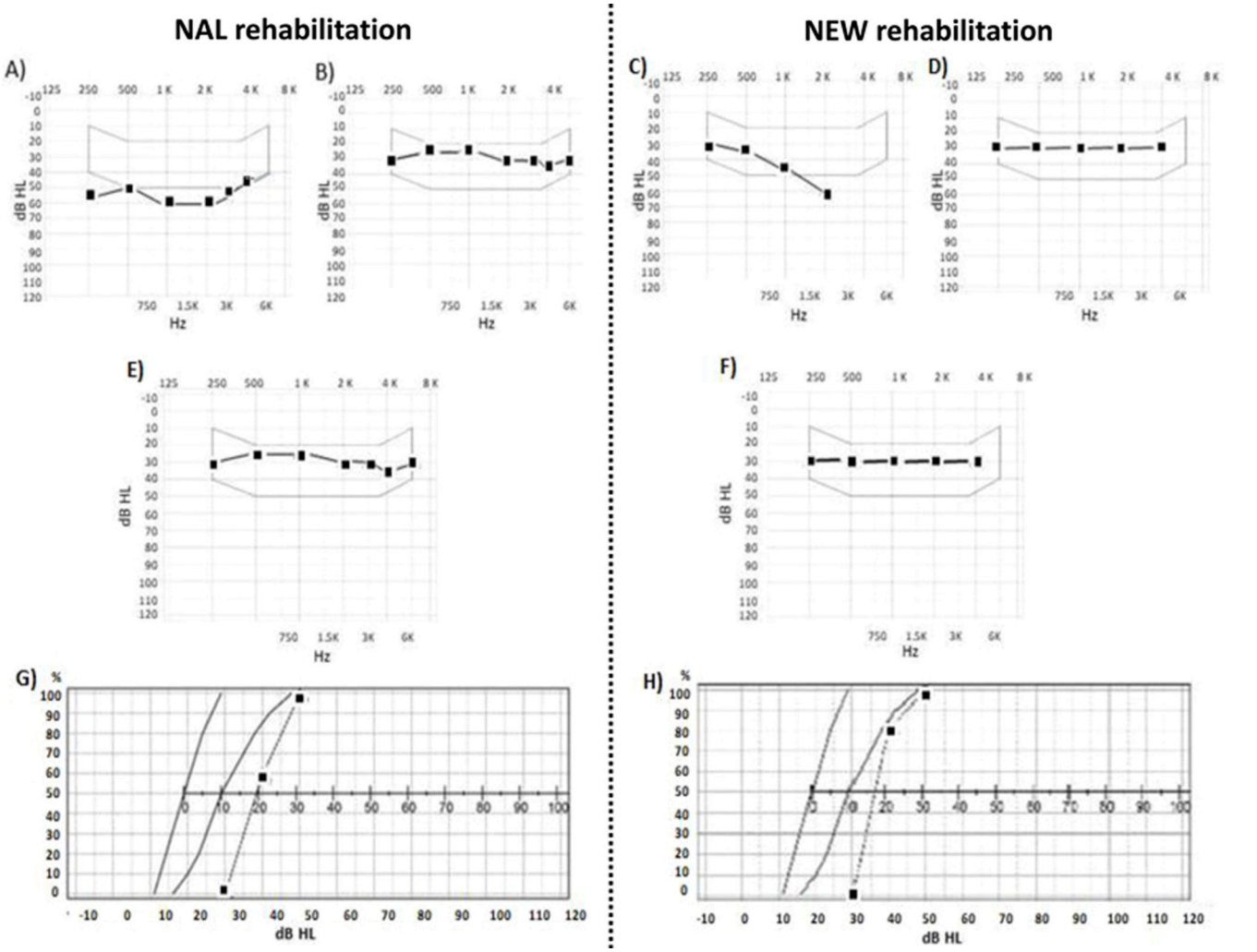

Fig. 3. Audiological outcomes with NAL-NL2 (left side) and APDB formula (right side): Pure tone liminar audiometry testing respectively right (panels A, C) and left (panels B, D) ear with HA; pure tone liminar audiometry testing both ears (HA plus CI) (panels E, F). Speech audiometry with HA (right ear, continuous line) and with CI (left ear, dashed line) (panels G, H).

of later symptoms. This study, moreover, reports remarkable audiological performance in this girl obtained using a bimodal strategy with a new prescriptive formula (APDB), as can be seen by comparing the outcomes shown in Fig. 3. The level of rehabilitation obtained was based on the hearing aids performance and the periodic (every 6 months) evaluations about relational, communicative and linguistic outcomes, as well as with respect to the truly remarkable academic results reached by the young girl. The use of the bimodal system in this patient has improved the development of both perceptual and linguistic skills, but also of cognitive skills, such as working memory, essential for the development of more complex learnings. These skills have shown a further improvement with the use of an integrated bimodal system (CI - Naida Link).

This study gives useful information for other similar cases to further improve results of audiological rehabilitation and confirms data reported in the literature [21] that for subjects presenting EVA, the CI can be used with excellent results. .

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Declaration of competing interest

None.

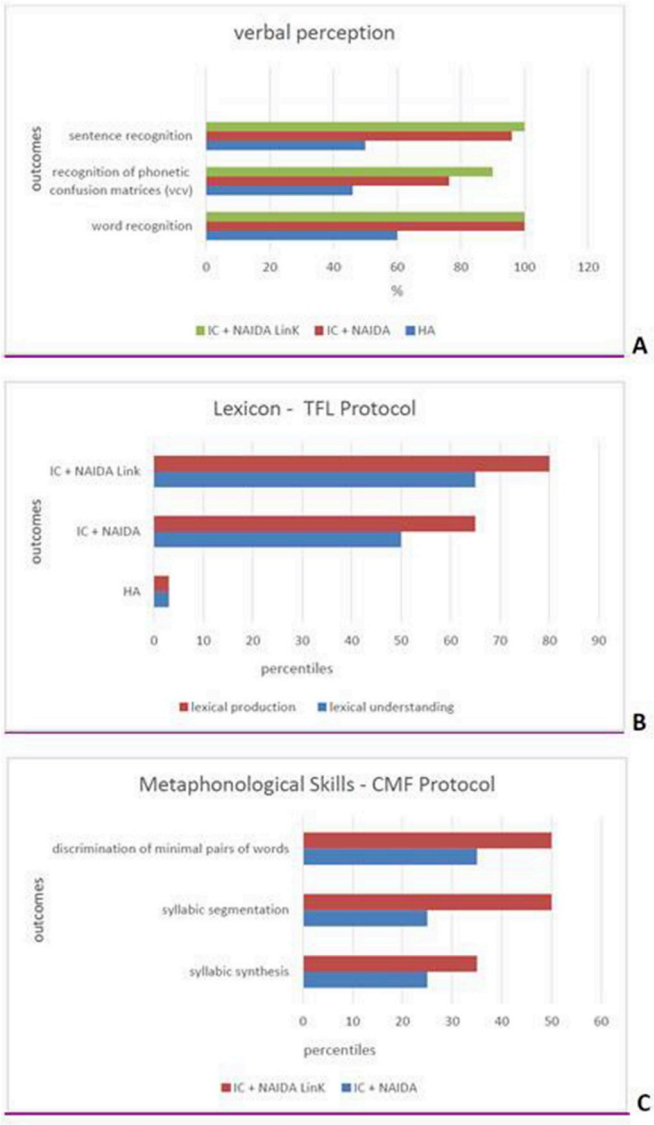

Fig. 4. Comparison of acoustic-perceptual (panel A) and linguistic skills (panel B) with bilateral hearing aids, bimodal system and integrated bimodal system; comparison of metaphonological abilities (panel C) with bimodal and integrated bimodal system.

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References

[1] V.C. De Moraes, N.Z. Dos Santos, P.Z. Ramos, M.C. Svidnicki, A.M. Castilho, E.L. Sartorato, Molecular analysis of SLC26A4 gene in patients with nonsyndromic hearing loss and EVA: identification of two novel mutations in Brazilian patients, Int. J. Pediatr. Otorhinolaryngol. 77 (2013) 410–413 https://doi:10.1016/j.ijporl.2012.11.042.

[2] Y. Chai, X. Pang, D. Chen, L. Li, Y. Chen, L. Sun, X. Wang, H. Wu, T. Yang, Molecular etiology of non-dominant, hearing impairment in Chinese Hans, Am. J. Med. Genet. A. 164A (2014) 3115–3119 https://doi:10.1002/ajmg.a.36785.

[3] M.C. Svidnicki, S.M. Silva-Costa, P.Z. Ramos, N.Z. dos Santos, F.T. Martins, A.M. Castilho, E.L. Sartorato, Screening of genetic alterations related to non-syndromic hearing loss using MassARRAY iPLEX(R) technology, BMC Med. Genet. 16 (2015) 85 https://doi:10.1186/s12881-015-0232-8.

[4] V.C.S. de Moraes, E. Bernardinelli, N. Zocal, J.A. Fernandez, C. Nofziger, A.M. Castilho, E.L. Sartorato, M. Paulmichl, S. Dossena, Reduction of cellular expression levels is a common feature of functionally affected pendrin (SLC26A4) protein variants, Mol. Med. 22 (2016) 41–53 https://doi:10.2119/molmed.2015.00226.

[5] S. Naz, A. Imtiaz, G. Mujtaba, A. Maqsood, R. Bashir, I. Bukhari, M.R. Khan, M. Ramzan, A. Fatima, A.U. Rehman, et al., Genetic causes of moderate to severe hearing loss point to modifiers, Clin. Genet. 91 (2017) 589–598 https://doi:10.1111/cge.12856.

[6] K. Mey, M. Bille, P. Cayé-Thomasen, Cochlear implantation in Pendred syndrome and non-syndromic enlarged vestibular aqueduct - clinical challenges, surgical results, and complications, Acta Otolaryngol. 136 (2016) 1064–1068 https://doi:10.1080/00016489.2016.1185538.

[7] P. Clarós, J.V. Fokouo, A. Clarós, Cochlear implantation in patients with enlarged vestibular aqueduct. A case series with literature review, Cochlear Implants Int. 18 (2017) 125–129 https://doi:10.1080/14670100.2016.1268754.

[8] C.M. Wu, H.C. Ko, Y.T. Tsou, Y.H. Lin, J.L. Lin, C.K. Chen, P.L. Chen, C.C. Wu, Long-term cochlear implant outcomes in children with GJB2 and SLC26A4 mutations, PloS One 10 (2015) e0138575 https://doi:10.1371/journal.pone.0138575.

[9] S. Wang, W. Ding, C. Chen, B. Xu, X. Liu, P. Bian, Y. Guo, Analysis between phenotypes and genotypes of inner ear malformation, Acta Otolaryngol. 139 (2019) 223–232 https://doi:10.1080/00016489.2018.1554262.

[10] V. Chinetti, S. Iossa, G. Auletta, V. Corvino, M. De Luca, F. De Falco, P. Giannini, G. Lilli, R. Malesci, P. Riccardi, et al., Mutational analysis for GJB2, GJB6, and GJB3 genes in Campania within a universal neonatal hearing screening programme, Int. J. Audiol. 20 (2011) 866–870 https://doi:10.3109/14992027.2011.603757.

[11] A. Franzè, G. Esposito, C. Di Domenico, S. Iossa, G. Sauchelli, T. Fioretti, M. Cavaliere, G. Auletta, V. Corvino, C. Laria, et al., SLC26A4 genotypes associated with enlarged vestibular aqueduct malformation in south Italian children with sensorineural hearing loss, Clin. Chem. Lab. Med. 54 (2016) e259–e263 https://doi:10.1515/cclm-2015-1216.

[12] IGSR, The international genome sample resource homepage, available at https://www.internationalgenome.org/home/ accessed on July 24, 2019.

[13] Mutation t@sting homepage available at https://www.mutationtaster.org/ accessed on July 24, 2019.

[14] InterVar Homepage available at https://wintervar.wglab.org/search.php/ accessed on July 24, 2019.

[15] S. Vicari, C. Marotta, A. Luci, S. Banal, Erickson (Eds.), Test Fono-Lessicale, 2007.

[16] H. Kremer, Hereditary hearing loss; about the known and the unknown, Hear. Res. 376 (2019) 58–68, https://doi.org/10.1016/j.heares.2019.01.003.

[17] W. Reardon, R. Coffey, T. Chowdhury, A. Grossman, H. Jan, K. Britton, P. Kendall-Taylor, R. Trembath, Prevalence, age of onset, and natural history of thyroid disease in Pendred syndrome, J. Med. Genet. 36 (1999) 595–598.

[18] J.L. Wémeau, P. Kopp, Pendred syndrome, Best Pract. Res. Clin. Endocrinol. Metabol. 31 (2017) 213–224, https://doi.org/10.1016/j.beem.2017.04.011.

[19] F. Zhang, X. Bai, Y. Xiao, X. Zhang, G. Zhang, J. Li, L. Xu, H. Wang, Identification of a novel mutation in SLC26A4 gene in a Chinese family with enlarged vestibular aqueduct syndrome, Int. J. Pediatr. Otorhinolaryngol. 85 (2016) 75–79 https://doi:10.1016/j.ijporl.2016.03.020.

[20] E. Bedin, A. Franzè, C. Zadro, M.G. Persico, M. Ciullo, U. Hladnik, D. Dolcetta, D.L. Grasso, P. Riccardi, T. Nutile, et al., Age-related hearing loss in four Italian genetic isolates: an epidemiological study, Int. J. Audiol. 48 (2009) 465–472 https://doi:10.1080/14992020902822039.

[21] Y.C. Rah, A.R. Kim, J.W. Koo, J.H. Lee, S.H. Oh, B.Y. Choi, Audiologic presentation of enlargement of the vestibular aqueduct according to the SLC26A4 genotypes, Laryngoscope 125 (2015) E216–E222 https://doi:10.1002/lary.25079.