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Gap Junction Beta 2 Gene Mutation In Indonesian Patients With Non Syndromic Congenital Hearing Loss

Devira Zahara[1], Jenny Bashiruddin[2], Gino Tann[1], Delfitri Munir[1], Nyilo Purnami[3], Eka Savitri[4], Rosita Juwita Sembiring[1], Harmein Nasution[5]

[1]Dept. of ENT – Head and Neck Surgery, Univ. of Sumatera Utara/Adam Malik General Hospital, Medan– Indonesia.
[2]Dept. of ENT - Head and Neck Surgery, Univ. of Indonesia /Cipto Mangunkusumo General Hospital, Jakarta– Indonesia.
[3]Dept. of ENT – Head and Neck Surgery. Univ. of Airlangga, Surabaya– Indonesia.
[4]Dept. of ENT – Head and Neck Surgery. Univ. of Hasanuddin , Makasar– Indonesia.
[5]Head of the Research Institute, Univ. of Sumatera Utara, Medan – Indonesia.

Abstract: Congenital hearing loss is caused by factors during pregnancy and delivery. Congenital deafness may affect speech development, social, cognitive, and academic abilities. More problems may arise if no early detection nor intervention are performed.[2-4] Hearing loss in infant and children sometimes are followed by mental underdevelopment, emotional problems, and aphasia. Congenital deafness is commonly detected by family members as delayed speech.

In current study, polymorphism of S86T was found in all cases (100%). This polymorphism was also found in all cases of Iranian patients including control patients (Hamid et al). According to Lee et al (1992) from uniprot data, amino acid difference in S86T position was called sequence conflict.

Etiological diagnosis of hearing impairment is of great importance to ensure early and adequate management. Even after thorough history taking, clinical and audiometric evaluation, the cause of hearing loss remains unclear in a majority of patients.

Keywords: Gap Junction Beta 2 Gene Mutation, Indonesian Patients, Non Syndromic Congenital Hearing Loss, Hearing Loss.

Introduction

Hearing is an important factor for speech ability and verbal communication. Hearing learning in infants and children is very complex due to the involvement of growing, embryological, anatomical, physiological, neurological, and auditory developments.[1-2]

Congenital hearing loss is caused by factors during pregnancy and delivery. Congenital deafness may affect speech development, social, cognitive, and academic abilities. More problems may arise if no early detection nor intervention are performed.[2-4] Hearing loss in infant and children sometimes are followed by mental underdevelopment, emotional problems, and aphasia. Congenital deafness is commonly detected by family members as delayed speech.[1]

Prevalence of congenital deafness in the world is about 1-3 cases in 1000 births.[2,5-6] A survey in 7 Indonesian provinces in 1994-1996 showed congenital deafness occured in 0.1% of 19,375 sample.[7] Indonesian 2005 health profile predicts 214.100 congenital deafness in 214.100.000 Indonesian citizens, and this number increases each year due to high birth rate at 0.22%.[3]

Problems in detecting deafness in Indonesia and other developing countries include lack of knowledge, information and awareness in the society.[8] Early detection is critical, it leads to early rehabilitation and development of communication skill.[9-10] For this reason, simple screening may be performed in the first day after birth. Without early screening, deafness might be unknown to the parents, teachers, or physicians until the child has speech problem in the age of 2 or 3 years old. Bashiruddin (2009) performed hearing screening in infants in six hospitals in Jakarta and found deafness in 297 of 12,757 (23%) infants.[7]

Two thirds of congenital deafness is genetic, the rest is caused by environmental factors and unidentified genetic factors.[11] There are two forms of deafness: syndromic and non-syndromic deafness.[12] Seventy percent of genetic deafness is non-syndromic, the rest 30% is syndromic. Syndromic deafness means that the patient also has other organ abnormality (e.g. the heart, kidney, eye, thyroid or other organs). Knowledge of genetic factor in a patient may help detecting other organ abnormalities.[6,13-4] Deklerck et al (2014) showed that genetic is the most common cause of congenital deafness (65.4%).[15] Eighty percent of deafness is inherited as autosomal recessive, 20% as autosomal dominant, 1-2% as x-linked recessive and mitochondrial mutation.[6,11]

Many molecular processes are impaired in genetic deafness, including transcription function, potassium and chloride channels, connexin, and stereocilia. Genetic testing may predict prognosis and may direct treatment. For example, GJB2 mutation means deafness is not neurological problem, and the patient is a good candidate for cochlear implantation.[16] Zhu et (2014) al showed that GJB2 knockout in mice in the 5th day resulted in progressive hearing loss at high frequencies followed by mid and low frequencies. Endocochlear potential decreased but not progressively. This showed that GJB2 mutation caused amplification damage in cochlea.[17]

Many genetic mutations may cause syndromic and non-syndromic deafness. Clinical manifestations occur when genetic mutations produce different amino acids that in turn will build different proteins.[18] Deklerck, Acke, Janssens, De Leenheer (2015) analysed 191 patients, where 65,4% are genetic and GJB2 mutation was the most common cause.[15] Nishio and Usami (2015) found that from 1389 samples taken from 1120 non-syndromic deaf patients, there were 8376 variations of genetic mutation, the most common mutation is GJB2, followed by CDH23, SLC26A4, MYO15A, COL11A2, MYO7A, and OTOF.[19]

Gap junction beta 2 (GJB2) gene produces connexin 26 protein that plays a role in congenital deafness.[20-1] This gene is related to non-syndromic deafness in western population. Connexin 26 is a component of gap junction protein that acts as intercellular channel. It is believed to play a crucial role in regulating potassium ion flux during auditory transduction in the inner ear.[22]

About 50% of GJB2 mutation is inherited as autosomal recessive, carriers has prevalence of 1 in 33 patients.[6,23] More than 101 GJB2 mutations are found in deaf patients. The type of mutation and its prevalence are different in different ethnic groups, for example 35delG is common in Europeans, 167delT in Jewish Ashkenazi dan 235delC mutation in Japanese and Asian.[6,24-6] One in 40 white United States people is GJB2 mutation carrier.[16] Congenital deafness is frequently found in Indonesia, and most patients suffer non-syndromic deafness with normal-hearing parents.

Material and Methods

Subjects

This is cross-sectional study to observe gap junction beta 2 (GJB2) gene mutations in non-syndromic congenital deafness patients in Indonesia. Subjects were 74 non-syndromic congenital deafness patients with severe to profound sensorineural hearing loss who came from different parts of Indonesia (Medan, Jakarta, Jogjakarta, Surabaya and Makassar) with different ethnic background. Non probability consecutive sampling was performed in this study.

Clinical Protocol

After obtaining informed consent from all participants, 1 mL peripheral venous blood was taken from patients. We analysed GJB2 genes mutation by using DNA extracted and PCR technique. Detection of mutations within GJB2 gene was carried out by DNA sequencing. The primers were Cx148F2 (5-CCTGTGTTGTGTGYGCATTCGTC-3) and Cx929R3 (5-CTCATCCCTCTCTCATGCTGTC-3). For PCR amplification at 3 minutes, initial denaturation at 95^0C was followed by four steps including denaturation 95^oC for two minutes, annealing 59^oC for 45 seconds and elongation 72^oC for 2 minutes. Next step was 25 cycles consist of denaturation 95^0C for one minute, annealing 59^0C for 30 seconds and elongation 72^0C for one minute and the last elongation 72^0C for 5 minutes. PCR analysis by using 1 % agarose gel. DNA were sequenced by using EZ sequencing method on an Applied Biosystems 3730xl DNA analyzer (USA). The sequencing results were analysed by Chromas 2.13 software (Technelgsim, Queensland, Australia).

Results

We found 20 of the 74 patients (27.03%) with GJB2 gene mutation. Of these 20 cases, 12 (16.21%) patients were homozygotes and 8 (10.81%) patients were heterozygotes. The most common congenital deafness subjects were male (n=46; 62.16%), age group 2-4 years old (n=27; 36.49%). The youngest patient was 1.5 years old and the oldest was 14 years old, average age was 5.5 $\pm$ 3.4 years old. The most ethnic group was Javanese (n=37; 50%).

Table 1. Patients Characteristics

Patients Characteristics	Deaf Individual (n)	Persentase (%)
Sex		
Male	46	62.16
Female	28	37.84
Age		
< 2 Years	7	9.46
2-4 Years	27	36.49
>4-6 Years	23	31.09
>6-8 Years	9	12.16
>8-10 Years	4	5.40
>10 Years	4	5.40
Ethnics		
Jawa Tengah	37	50.00
Jawa Timur	7	9.47
Bugis	5	6.76
Aceh	4	5.41
Batak	4	5.41
Minang	2	2.70
Cina	2	2.70
Melayu	2	2.70
Sunda	1	1.35
Betawi	1	1.35
Flores	1	1.35
Subang	1	1.35
Nias	1	1.35
Mandar	1	1.35
Toraja	1	1.35
Flores	1	1.35
Kalimantan Timur	1	1.35
Bima	1	1.35
Papua	1	1.35

The most frequent mutation in patients group is missence 636 C>A (p.F146L) in 8 patients (10.81%). Other mutations consist of 2 variants of silent mutations: 439 C>T (p.L145L) in 6 patients (8.11%) and 501 G>A (p.E167E) in 1 patient (1.35%), 3 variants of missence mutations: 430 G>A (p.A78T) and 626 G>A (p.R143Q) in 3 patients (4.05%), missence 634 T>A (p.F146I) in 2 patients (2.70%), and 1 variant of nonsense mutation: 672 C>A (p.Y158X) in 1 patient (1.35%).

Table 2. Variants of GJB2 gene mutation

Variants of GJB2 gene mutation	Protein	Deaf Individual no.	%
439 C>T	p.L145L	6	8.11
430 G>A	p. A78T	3	4.05
636 C>A	p.F146L	8	10.81
672 C>A	p. Y158X	1	1.35
626 G>A	p. R143Q	3	4.05
634 T>A	p.F146I	1	1.35
694 C>T	p.L232L	0	0
501 G>A	p.E167E	3	4.05
Polymorphism 455-456 G>C-C>G	p.S86T	74	100

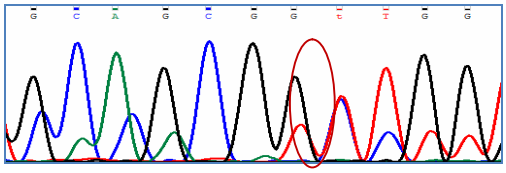

Figure 1. 439 C>T (p.L145L)

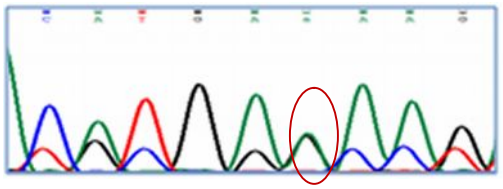

Figure 2. 430 G>A (p.A78T)

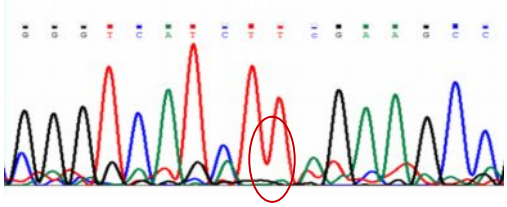

Figure 3. 636 C>A (p.F146L)

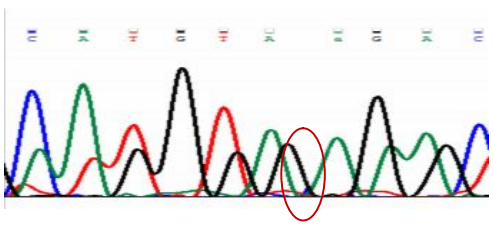

Figure 4. 672 C>A (p.Y158X)

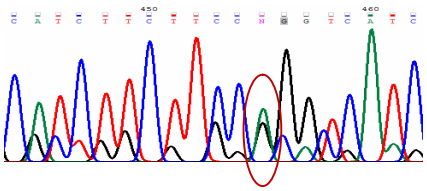

Figure 5. 626 G>A (p.R143Q)

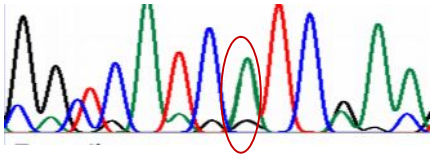

Figure 6. 634 T>A (p.F146I)

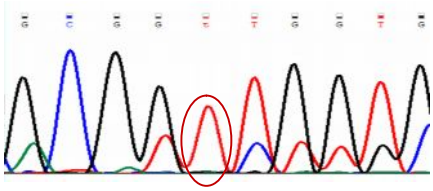

Figure 7. 694 C>T (p.L232L)

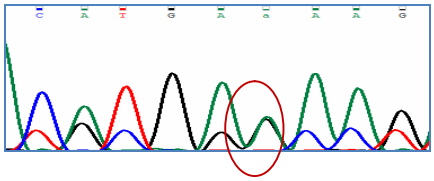

Figure 8. 501 G>A (p.E167E)

Discussion

Mutations in GJB2 gene mutation is the most common cause of non syndromic sensoryneural hearing loss in many populations with different ethnic background.[27-8] The present findings showed that the spectrum of mutations in GJB2 in Indonesian people is significantly different from that found in other ethnic groups in other countries. GJB2 gene encodes the protein connexin 26 (Cx26), a member of the connexin family of proteins.[22] Connexins are transmembrane proteins that oligomerize with five other connexin molecules to form a homomeric or a heteromeric connexon. Connexons in adjoining cells fuse through disulfide bonding to form gap junctions, which allow molecules to pass from cell to cell. Connexins 26 is highly expressed in epithelial supporting cells of the mammalian cochlea and are believed to play a key role in the cycling of potassium from the hair cells back to the endolymph.[29-30]

From 74 patients we found 20 patients (27.03%) with GJB2 gene mutations result and two of them had two affected family members (their sibling). About five patients had two kinds of GJB2 mutation.

This study showed a high prevalence of two of these mutations, F146L (10.81%) and L141L (8.11%). Among them, F146L was the most frequent and was found in eight of the 74 patients. GJB2 is located in 13q11-q12 locus. GJB2 mutation may cause either recessive autosomal deafness (DFNB1) or dominant autosomal (DFNA3).[16,28,31-2] More than 50% of congenital deafness is hereditary, the majority is non-syndromic, and more than half of recessive autosomal non-syndromic deafness is caused by GJB2 mutation.[33-4]

There were three patients (4.05%) with 626 G>A (p.R143Q) heterozygotes mutation. In Uniport data, one publication showed that p.R143Q mutation caused autosomal dominant deafness. A few autosomal dominant deafness causing mutations in Cx26 have also been described, classified clinically as DFNA3.

Huang et al (2014) in China found nine very severe to profound deaf patients with dominant autosomal caused by mutations of R75W,G130V, R143Q and p.R184Q.[32]

The type and rate of mutations is strongly influenced by ethnic groups of a population.[30] A study by Abe et al (2000) in Japan found three types of missense mutations: R143W (427C>T), G45E (134G>A), and V37I (109G>A), one type of nonsense mutation Y136X (408C>A) and three types of frameshift deletion mutation: 235delC, 176-191del16 and 299-300delAT. Frameshift 235 del C is the most common mutation.[26,35] Hamid et al in Iran (1999) found 10 types of GJB2 mutations: 35delG, R127H, V27I+E114G, Y155X, M163V, R143W, R32H, R165W, 333–334 delAA, 355–357delGAG. They found that 35 del G is the most common mutation.[28] Mutation of 35 del G is also the majority in Caucasian (1-5% of population) and United States populations but rarely found in Asia and Africa populations.[30] Hall et al found that carriers of GJB C35delG mutation had worse hearing especially in high frequency than those who are not carrier.[36-7] De Castro et al (2013) in their study in non-syndromic deaf subjects in Brazil found that V27I missense mutation is the most frequent mutation (26%) followed by frameshift 35delG mutation, missense M34T, V95M dan V27I.[6] These findings showed that each population has its own unique mutation.[38]

GJB2 gene abnormality is the molecular cause of 10-50% of non-syndromic deafness, and there are more than 150 known mutations, polymorphisms, and unclassified variants of this gene. GJB2 gene should be the first gene to analyse in patients with hearing loss because most gene mutations in congenital deafness especially in very severe to profound deafness is GJB2 mutation.[31,39,40-1]

Several different connexin (Cx) subunits are reported to be expressed in the mammalian inner ear, including Cx26, Cx30, Cx31, and Cx43. Gap junctions mediate ionic intercellular communication in both epithelial and connective tissues within the spiral limbus, organ of Corti, and stria vascularis. Individual gap junctions appear to harbor different combinations of Cx26 and Cx30 heteromers rather than just one of the isoforms.[42] Cohen-Salmon et al demonstrated that targeted ablation of Cx26 in the epithelial cell network of the cochlea resulted in normal cochlear development, but following the onset of hearing, cell death of the supporting cells ensued. The timing of this cell death coincided with decrease in potassium concentration driving the endolymphatic potential. They hypothesized that the loss of Cx26 prevented recycling of K+ ions after sound stimulation and that elevated K+ in the extracellular perilymph inhibited uptake of the neurotransmitter glutamate, which ultimately resulted in cell death within the hair cell population. This K+ recycling theory' is the leading hypothesis for Cx26 function in the cochlea.[16,43]

In current study, polymorphism of S86T was found in all cases (100%). This polymorphism was also found in all cases of Iranian patients including control patients (Hamid et al). According to Lee et al (1992) from uniprot data, amino acid difference in S86T position was called sequence conflict.[44]

Conclusion

Etiological diagnosis of hearing impairment is of great importance to ensure early and adequate management. Even after thorough history taking, clinical and audiometric evaluation, the cause of hearing loss remains unclear in a majority of patients. Further examinations can imply imaging, ophthalmologic investigations, laboratory tests, electrocardiography and genetic testing. Carriers of GJB2 may also have hearing problems. The combination of genetic and audiological screening may play an important role in deafness detections of infants.

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