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Glutathione peroxidase and catalase enzyme gene polymorphisms in profound congenital hearing loss*

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Abstract

Objective: The aim of this study was to search the codon 200 polymorphism on the glutathione peroxidase 1 gene (GPX1) and A/T changes on the promoter region of the catalase gene (CAT) in cochlear implant patients with congenital profound hearing loss.

Methods: Sixty-five cochlear implant patients with congenital hearing loss and 100 age- and gender-matched healthy volunteers were evaluated between 2011 and 2013. Genomic DNA was extracted from peripheral blood samples by using the salting out procedure. The T/A polymorphism in the promoter region of the CAT gene (rs7943316) and GPX1 gene codon 200 proline to leucine substitution (rs1050450) were determined by polymerase chain reaction and restriction fragment length polymorphisms.

Results: No statistically significant difference was found in CC and CT genotypes in codon 200 on GPX1 (CC, p=0.10; CT, p=0.48) However, there was a statistically significant difference in the TT genotype (p=0.04). In the CAT promoter region, there was no statistically significant difference between the patients and control groups (AA, p=0.41; TA, p=0.16; TT, p=0.08).

Conclusion: As a conclusion, the TT genotype on the GPX1 codon 200 may have a relationship with congenital profound sensorineural hearing loss.

Keywords: Catalase, cochlear implant, congenital sensorineural hearing loss, glutathione peroxidase, polymorphism.

Özet: Ağır konjenital işitme kaybında glutatyon peroksidaz ve katalaz enzim gen polymorfizmleri

Amaç: Bu çalışmanın amacı konjenital ağır işitme kaybı olan koklear implantlı hastalarda glutatyon peroksidaz 1 geni (GPX1) üzerindeki kodon 200 polimorfizmini ve katalaz geni (CAT) üzerindeki A/T değişimlerini araştırmak idi.

Yöntem: Doğuştan işitme kaybı olan 65 koklear implant hastası ve yaş ve cinsiyet eşleşmeli 100 gönüllü 2011 ila 2013 yılları arasında değerlendirilmiştir. Tuzla çökeltme yöntemi kullanılarak periferik kan numunelerinden genomik DNA ekstrakte edilmiştir. Polimeraz zincir reaksiyonu ve restriksiyon fragment uzunluk polimorfizmleri kullanılarak CAT geninin promoter bölgesindeki T/A polimorfizmi (rs7943316) ve GPX1 gen kodon 200 prolinin lösine çevrimi (rs1050450) belirlenmiştir.

Bulgular: GPX1 üstündeki kodon 2000 içindeki CC ve CT genotipleri arasında istatistiksel açıdan anlamlı herhangi bir istatistiksel açıdan anlamlı farklılık bulunmamıştır (CC, p=0.10; CT, p=0.48). Ancak TT genotipi açısından istatistiksel açıdan anlamlı bir farklılık vardı (p=0.04). CAT promoter bölgesi açısından hastalarla kontrol grupları arasında istatistiksel açıdan anlamlı bir farklılık yoktu (AA, p=0.41; TA, p=0.16; TT, p=0.08).

Sonuç: Sonuç olarak GPX1 kodon 200 üzerindeki TT genotipi konjenital ağır sensorinöral işitme kaybıyla ilişkili olabilir.

Anahtar sözcükler: Glutatyon peroksidaz; katalaz; koklear implant; konjenital sensörinöral işitme kaybı; polimorfizm.

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Congenital hearing loss affects approximately 1–3 children of every 1000 births. Of these, 60% are prelingual deaf patients (hereditary), while 40% are environmental or iatrogenic.[1,2] Genetic hearing loss has been well studied in cochlear implant patients.[1] However, further studies are still needed for genetic hearing impairments. Free radicals are the molecules that carry one or more unshared electrons in their outer orbitals. They have strong reaction potential and can therefore damage cell membranes, lipids and proteins. The system that defends the formation of free radicals and helps with detoxification is called antioxidants.[3,4]

The aim of this study was to investigate any possible relationship between the polymorphisms of the promoter A/T (A= adenine, T= thymine) nucleotide in the catalase (CAT) gene and of the codon 200 proline to leucine (T/C) substitution (C= cytosine) in the glutathione peroxidase 1 (GPX1) gene and congenital sensorineural hearing loss.

Materials and Methods

Study design

The study was approved by University's Ethical Committee (date: 10/02/2014, number: January 10th, 2014/70). In this study, changes in promoter A/T in the catalase gene and polymorphism of codon 200 proline to leucine substitution (C/T) in glutathione peroxidase 1 gene were analyzed in pediatric cochlear implant patients with congenital profound hearing loss. In this study there were 65 consecutive children (32 males; mean age, 36 months old) who underwent cochlear implantation as a result of profound bilateral congenital sensorineural hearing loss. The preoperative evaluation included medical history, physical examination and a series of audiological tests. Imaging studies of the temporal bone (CT and MRI) were performed. All the children underwent cochlear implantation. The control group consisted of 100 participants of similar age and gender. 3 cc venous blood samples were obtained from both the patients and control cases.

DNA analysis

Genomic DNA was extracted from 2 ml peripheral blood samples by using the salting out procedure.[5] The T/A polymorphism in the promoter region of the CAT gene (rs7943316) and GPX1 gene codon 200 proline to leucine substitution (rs1050450) were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) as previously described.[6,7] To detect the CAT gene promotor polymorphism, the amplified products of 250 bp were digested with Hinf1 restriction enzyme (Thermo Fisher Scientific, Waltham, MA, USA) at 37ºC, producing fragments of 177 and 73 bp of the A allele or fragments of 250 bp of the T allele. The GPX1 gene polymorphic region was amplified using PCR. Amplified products of 338bp were digested with Apa1 restriction enzyme (Thermo Fisher Scientific, Waltham, MA, USA) at 37ºC, producing fragments of 257 and 81 bp of the C allele (proline) or fragments of 338 bp of the T allele (leucine). All PCR and digested products were separated on agarose gels and visualized under a UV transluminator.

Statistic analysis

Statistics were evaluated using SPSS version 11.0 (SPSS, Inc., Chicago, IL, USA). The gene polymorphisms of CAT (T/A) and GPX1 codon 200 were compared between the patients and healthy subjects using the chi-square test and Fisher exact test (Graphpad Instat, version 3). Statistical significance was considered when p value was <0.05. The Hardy-Weinberg equilibrium (HWE) was calculated using the De Finetti programme, and $p<0.05$ was accepted as statistically significant.[8]

Results

There were 32 boys and 33 girls aged 11 months to 108 months (mean, 36 months) at implantation. There was a positive family history of hearing loss in 17 (26%) of the 65 patients. Results were obtained from all patients with cochlear implant (CI) and 100 age- and gender-matched controls (43 girls). Distribution of the GPX1 gene codon 200 (T/C) polymorphism in the CI patients was as follows: CC genotype, 38% (25 patients); CT genotype, 45% (29 patients); and TT genotype, 17% (11 patients). Among the control group, the CC genotype was found in 50% (50 participants), while the CT genotype was found in 43% (43 participants) and the TT genotype in 7% (7 participants). In CI patients, the C allele was found in 69% (79 patients) and the T allele in 39% (51 patients); whereas in the control group, our findings were 71.5% (43) and 28.5% (57), respectively (Fig. 1). Having the TT genotype increased the risk of sensorineural hearing loss for 2.7 times (OR=2.706; 95% CI, 1.015–2.584), while having the T allele increased the risk for 1.6 times (OR=0.6174; 95% CI, 1.015–2.584). Having the C allele decreased the risk for 0.61 times (OR=0.6174; 95% CI, 0.3878–0.9852). The TT genotype and T allele were found to be related to the predisposition of disease. The C allele was found to be related to protection from the disease. Incidence of the T

Table 1. Allele distribution of GPX1 gene polymorphism among patients and control cases.

Gene	Genotype/Allele	Patients n* (%)	Control n† (%)	OR	95% CI	p
GPX1 codon 200	CC	25 (38%)	50 (50%)	0.6250	0.3311–1.180	0.10
	CT	29 (45%)	43 (43%)	1.068	0.5691–2.004	0.48
	TT	11 (17%)	7 (7%)	2.706	1.0220–7.397	0.04‡
	C allele	79 (61%)	143 (71.5%)	0.6174	0.3878–0.9852	0.03
	T allele	51 (39%)	57 (28.5%)	1.620	1.015–2.584	0.03
	HWE (p)	0.60	0.58			

*Number of patients was 65; †Number of controls was 100; ‡Fisher's exact test. **C:** cytosine; **CC:** cytosine/cytosine; **CT:** cytosine/thymine; **HWE:** Hardy-Weinberg equilibrium; **T:** thymine; **TT:** thymine/thymine.

allele was higher in the CI group than controls (0.390 and 0.285, respectively). There was a statistically significant difference between two groups, as shown in Table 1.

The observed genotype counts did not significantly deviate from those expected according to the Hardy-Weinberg Equilibrium (HWE) for glutathione peroxidase 1 codon 200 T/C polymorphism (Table 1).

The catalase gene promoter region A/T polymorphism was analyzed using Hinf I enzyme. The CAT gene promoter region A/T polymorphism genotype was found to be distributed unevenly: AA genotype, 14% (9 patients); TA genotype, 39% (26 patients) and TT genotype, 47% (30 patients) among the patient group. Among the controls, it was 17% (17 participants), 49% (49 participants) and 34% (34 participants), respectively. In the patient group, the A allele was found in 34% (44 patients) and T allele in 66% (86 patients), whereas among controls, the results were 41.5% (83 participants) and 58.5% (17 participants), respectively (Fig. 2).

There was no statistically significant difference between the patients and controls for possession of the CAT gene promoter region A/T polymorphism (AA, p=0.41; TA, p=0.16; TT, p=0.08). Incidence of the T allele was higher in the patient group than in controls (0.660 and 0.585, respectively); however there was no statistical significance (p=0.10). Results are shown in Table 2.

The observed genotype counts did not significantly deviate from those expected according to the Hardy-Weinberg Equilibrium (HWE) for the CAT gene promoter region A/T polymorphism.

Discussion

Congenital sensorineural hearing loss is one of the most common congenital diseases, affecting approximately 1–3 of every 1000 newborns. Genetic factors account for approximately 60% of congenital hearing loss.[1] It is reported that 50% of profound hearing loss is the result of a single gene mutation. There are more than 120 genes in the etiology of profound hearing loss.[2]

The cell produces energy from aerobic and anaerobic metabolism. In aerobic metabolism or oxidative metabolism, the oxygen molecule is reduced to water and a small

Table 2. CAT gene promoter region A/T polymorphism allele distribution.

Gene	Genotype/Allele	Patients n* (%)	Control n† (%)	OR	95% CI	p
CAT A/T	AA	9 (14%)	17 (17%)	0.7847	0.3266–1.885	0.41‡
	TA	26 (39%)	49 (49%)	0.6939	0.3686–1.306	0.16
	TT	30 (47%)	34 (34%)	1.664	0.8776–3.154	0.08
	A allele	44 (34%)	83 (41.5%)	0.7212	0.4555–1.142	0.10
	T allele	86 (66%)	117 (58.5%)	1.387	0.8757–2.195	0.10
	HWE (p)	0.72	0.93			

*Number of patients was 65; †Number of controls was 100; ‡Fisher's exact test. **A:** adenine; **AA:** adenine/adenine; **HWE:** Hardy-Weinberg equilibrium; **T:** thymine; **TA:** thymine/adenine; **TT:** thymine/thymine.

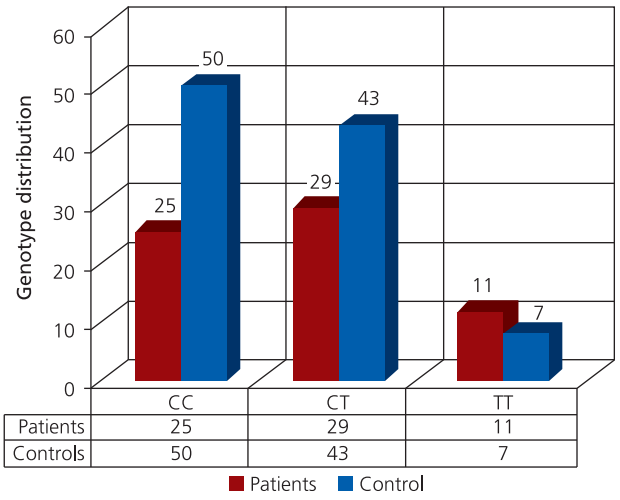

Fig. 1. Distribution of GPX1 gene codon 200 T/C polymorphism among patients and controls. **CC:** cytosine/cytosine, **CT:** cytosine/thymine, **TT:** thymine/thymine.

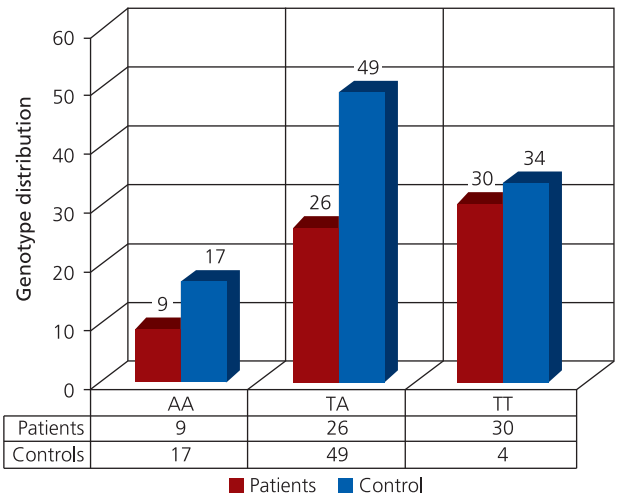

Fig. 2. CAT gene promoter region A/T polymorphism genotype distribution. **AA:** adenine/adenine, **TA:** thymine/adenine, **TT:** thymine/thymine.

amount of free radicals. Free radicals are the molecules that carry one or more unshared electrons in their outer orbits. Therefore, they have a high reaction potential and a short half-life. They can be harmful to cell membranes, lipids and proteins and cause DNA damage. These effects are more obvious among cells with the most active metabolisms.[3] The defense systems that prevent the production of free radicals and the effects of these substances are called antioxidants. Antioxidant molecules are endogenous enzymes such as superoxide dysmutase, glutathione peroxidase, catalase and so on. Endogenous substances that are not in enzyme form (transferrin, albumin, bilirubin, etc.) or exogenous substances (vitamins, food or medications) disable the effects of oxidant molecules.[3,4] Because cochlea has a very active metabolism, the normal pathway of the reduction of oxygen results in formation of reactive oxygen species (ROS), which are harmful for cochlear hairy cells. The corti contains a large amount of catalase.[9] Recently, the studies of gene polymorphisms of antioxidant and detoxifying enzymes support the idea of oxidative damage. Catalase enzyme gene polymorphisms were studied in many diseases and found to be significant in hypertension, diabetes mellitus and vitiligo.[7,10] However, in this study, we did not find a statistically significant difference between patients with sensorineural hearing loss and a normal control group for catalase gene polymorphism.

Yamane et al. found that the cochleas of animals that were exposed to noise resulted in high amounts of superoxide anions and hydroxyl radicals.[11] Labbe et al. suggested that apoptotic cell death along with oxidative stress and related cell damage might cause hearing loss in patients with Meniere's disease.[12] In Meniere's disease, the patient's plasma and lymphocyte oxide glutathione amounts were found to be higher than the reduced glutathione amount.[13]

In the GPX1 codon 200 C/T polymorphism, the balance between oxidative stress and antioxidants failed, and this was thought to be the reason for many diseases, such as cancer, Alzheimer's etc.[14,15]

In noise-induced hearing loss and idiopathic sudden hearing loss, investigating the effect of oxidative stress included studying various enzyme polymorphisms, and some relationships between the gene polymorphisms and the diseases were found.[16,17] In this study of CAT gene promoter region A/T polymorphism genotypes, we did not find a statistically significant difference between those with sensorineural hearing loss and a normal control group.

Among those with the GPX1 codon 200 C/T polymorphism, the TT genotype and the T allele were found to be related to predisposition to disease. The C allele was found to be related to protection from the disease. Because the cochlea has a very active metabolism, the normal pathway of reduction of oxygen results in formation of ROSs which are harmful to cochlear hairy cells. The GPX1

codon 200 C/T polymorphism TT genotype resulted in a decrease in the activity of antioxidants and detoxifying enzymes, supporting the idea of oxidative damage.

Limitations of our study are relatively small size of our series. In addition, some details of history and factors that may influence the outcome may not be completely documented. Due to these restrictions, associations should be interpreted with caution.

Conclusion

The TT genotype on the GPX1 codon 200 may have a relationship with congenital profound sensorineural hearing loss. Further studies with larger populations and a simultaneous evaluation of enzyme activity are suggested.

Conflict of Interest: No conflicts declared.

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