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MTHFR AND ApoE genetic variants association with sudden sensorineural hearing loss

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ABSTRACT

Hypothesis: Although the pathogenesis of sudden sensorineural hearing loss (SSNHL) is not clear, however several causes including genetic factors seems to be implicated. We hypothesized that common genetic variants might be involved in SSNHL.
Background: SSNHL is known to be an idiopathic disease because the causative factors have not been identified. Several causes including genetic and viral infection besides immune system reaction, neurological disorders, medications, etc. have been previously reported. We examined the association between ApoE and MTHFR gene variants in SSNHL.
Methods: This study includes case-control scheme encompassing a total of 177 individuals, include patients inflicted with SSNHL and healthy subjects as control group. Genotyping of MTHFR and ApoE variants was conducted by PCR – RFLP method.
Result: Our study showed that MTHFR rs1801133 allele frequency is significantly different between cases and controls. Also genotype distribution of ApoE was significantly different between patients and healthy controls.
Conclusions: MTHFR C677T and ApoE gene variant may be associated with sudden sensorineural hearing loss in an Iranian population.

1. Introduction

Sudden hearing loss (SHL) is an emergency clinical condition with the symptom which is frightening the individuals and causing urgent visit to a physician [1]. Sudden sensorineural hearing loss (SSNHL) which is presenting as sensorineural deafness and mostly unilateral with more than or equal to 30 dB decline in hearing in at least three frequencies consecutively is mostly accompanied by tinnitus and aural fullness [2–4]. Several etiologies have been considered as pathogenic mechanism in development of idiopathic sudden sensorineural deafness but the causative factor is yet to be explored. SSNHL has been frequently observed with underlying diseases including genetically involved vascular diseases, viral infection, the response to antigen antibodies in the inner ear and neurological disorders [3,4]. Various mechanisms have been proposed as immune response, blood pressure and vascular obstruction [5]. The average prevalence of SSNHL has been approximately estimated as 5–20 patient in 100,000 population [4]. The most acknowledged hypothesized pathologies of SSNHL is damaged inner ear perfusion and ischemic vascular impairment of the cochlea. Moreover a number of hereditary vascular risk factors such as genetic variations in the folate-dependent homocysteine metabolism have been recognized. 5-methyltetrahydrofolate, a cofactor needed for methylation of homocysteine to methionine is produced by MTHFR from 5,10-methylenetetrahydrofolate in a reduction process. On one hand there is MTHFR as a key enzyme in folate metabolism, on the other hand its association with increased levels of plasma homocysteine as a risk factor for thrombosis and atherosclerosis declare probable MTHFR role in SSNHL development. There are studies which assess this connection with controversial results due to ethnic differences, various sample size, publication bias, low statistical power and uncorrected multiple hypothesis testing [6,7].

Thus the necessity of more well-designed investigations in dissimilar populations is clarified. The most investigated polymorphism of this 11-exon gene on chromosome1p36.3 is a substitution from alanine to valine at codon 222 (A222V) leading to a transition of C to T in exon 4 at position 677 (C677T, rs1801133) considering as enzyme activity

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reducer to the 30% and 65% of wild type for TT and CT genotypes respectively [6–8].

Apolipoprotein E is an important protein for lipid transformation which plays a key role in metabolism of cholesterol and triglyceride in the brain [9]. It is shown that it sustains and refurbishes neuronal cell membranes and act as a strongest genetic risk factor of age-related disorders such as Alzheimer's disease, generalized atherosclerosis and macular degeneration [10].

This gene has 3 common alleles including ε2, ε3 and ε4. It is suggested that ApoE function in a number of mechanism to develop SSNHL comprised of same whereas unknown mechanism of ε4 allele predisposing to Alzheimer's disease, predisposing to auditory neuropathy by ε4 allele and causing atherosclerotic vascular disease (ASVD) and so predisposing to ischemic injury to the cochlea [9]. Contrary to these proposals there is a report that suggests a less common APOE e4 allele in hearing loss populations which shows a protective role of this allele in healing loss patients [11]. Although ApoE as a risk factor for SSNHL might be a plausible hypothesis there are not clear view about the role of its alleles in association with SSNHL which is sought in this study.

For better understanding the mechanism of SSNHL we performed a case-control survey which analyses the association between MTHFR and ApoE gene polymorphism In an Iranian population.

2. Materials and methods

77 unrelated Iranian patients with SSNHL (mean age:43.5 ± 14.7, 58.4% male and 41.6% female) and 100 healthy controls without a family history of SSNHL were recruited from Amir Alam Hospital which is affiliated by Tehran University of Medical Sciences (TUMS), Tehran, Iran. The characteristics of patients and control are as described by detail previously [12]. All participants gave an informed consent before entry to the research project. TUMS ethics committee approved this study based on Helsinki declaration. Whole blood samples collected in ethylenediamine tetraacetic acid-coated (EDTA) tube was used for DNA extraction from lymphocyte using a phenol-based method. NanoDrop 2000c spectrophotometer, Thermo Fisher Scientific, was used for determination of DNA concentration. DNA samples were stored up in −20 °C until subsequent use for PCR amplification.

2.1. Genotyping of the MTHFR C677T polymorphism

In order to detect MTHFR A223V (C677T) (rs1801133) polymorphism forward primer: 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ and reverse primer: 5′-AGG ACG GTG CGG TGA GAG TG-3′ were used for amplification of a 198 bp DNA segment. The total volume of PCR reaction was 20 μl consisting of 1.6 μl H2O, 0.7 μl of each primer, 6.5 μl Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark) and 0.5 μl DNA sample. PCR protocol includes: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s and extension at 72 °C for 30 s ended by a final extension at 72 °C for 5 min in a MJ Mini Gradient Thermal Cycler produced by Bio-Rad. PCR product was electrophoresed on a 2% agarose gel and documented in UV gel documentation system transilluminator. Digestion was carried out using 5.2 μl H2O, 0.1 μl restriction enzyme *Hin*fI, 2000 U (Thermo Fisher Scientific), 1.7 μl 10× buffer R and 10 μl PCR product and incubated at 37 °C overnight. The digested product was visualized on a 3% agarose gel and genotype was determined. Wild types (C677C) produced a single band at 198 bp. Heterozygotes (C677T) produced 198, 175 and 23 bp fragments. Homozygotes (T677 T) produced 175 and 23 bp fragments (13).

2.2. Genotyping of apoE

For apoE genotyping 0.5 μl genomic DNA was amplified with 1 μl of each forward 5'-TCCAA GGAGC TGCAG GCGGC GCA-3′ and reverse 5'GCCCC GGCCT GGTAC ACTGC CA-3′ primers, 6.5 μl Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark) and 11 μl H2O in a total volume of 20 μl. PCR parameters were 35 cycle of denaturation at 95 °C for 5 min, annealing at 68.2 °C for 30 s and extension at 72 °C for 30 s which is started with a initial denaturation for 5 min at 95 °C and final extension for 5 min at 72 °C. The 7 μl of 218 bp PCR product was digested with 0.2 μl *Afl*III 250 units and 0.6 μl *Hae*II 2000 units (New England Biolabs), 1.8 H20, 1.2 of each enzyme buffer followed by incubation at 37 °C overnight. Digested product was electrophoresed on a 4% agarose gel and documented in UV gel documentation system transilluminator for genotype determination. Genotypes E2/E2, E3/E3, E4/E4, E2/E3, E2/E4 and E3/E4 produce: 50 and 168 bp; 23, 50 and 145 bp; 23 and 195 bp; 23, 50, 145 and 168 bp; 23, 50, 168 and 195 bp; 23, 50, 145 and 195 bp fragments respectively [14].

2.3. Statistical analysis

Odds ratios, 95% confidence intervals and chi-squared (χ^2) Genetic testing analysis was done for examination the association of genotype and allele frequency differences between case and controls and SSNHL using STATA version 12. P value < 0.05 was described as probability value and significance cut off.

3. Results

3.1. Prevalence of the MTHFR 677 Genotypes in the cases and controls

Available data for genotyping of 77 cases and 100 controls showed difference in distribution of each genotype (CC,CT and TT) between case and control although this was not significant statistically ($p = 0.107$) (Table 1). Cross tabulation of genotype frequencies between case and controls when C is considered as risk allele (CC vs. CT + TT) illustrated a not significant difference, ($P = 0.07$; OR = 1.7, 95% confidence interval [CI]: 0.901–3.37). Cross tabulation of allele frequencies (C vs.T) between two groups depicted that allele C makes individuals susceptible to SSNHL (OR = 1.7, 95% CI: 1.04–3.03) So C is a risk allele for development of SSNHL in Iranian population ($P = 0.02$).

3.2. Prevalence of apoE genotypes in cases and controls

Statistical analysis on the relationship between ApoE polymorphism and SSNHL demonstrated that there is a significant difference in distribution of various genotype between cases and controls ($p = 0.008$). However there was no such statistical difference in allele distribution between two groups ($p = 0.11$). The analysis showed that E3 is a protective allele for SSNHL progress and presence of E3 make Iranian population 0.1 times less susceptible to SSNHL, ($p = 0.002$; OR = 0.17, 95% CI: 0.03–0.62). Table 2 summarizes the genotypes and allele frequency of ApoE in SSNHL patients and healthy controls.

Table 1
Genotype and allele frequency of case and control group.

Genotype/allele frequency of C667T polymorphism	SSNHL group $N = 77$ N (%)	Control group $N = 100$ N (%)	Total N (%)
Genotypes			
CC	51 (66.2)	53 (53)	103 (58.2)
CT	22 (28.6)	34 (34)	56 (31.6)
TT	4 (5.2)	13 (13)	17 (9.6)
Alleles			
C	124(80.5)*	140 (70)	164 (46.3)
T	30 (19.5)	60 (30)	90 (25.4)

* $P = 0.02$, OR = 1.7, 95% CI: 1.04–3.03.

Table 2
ApoE genotype and allele frequency in association with SSNHL.

ApoE genotypes and alleles frequency	SSNHL group $N = 75$ N (%)	Control group $N = 92$ N (%)	Total N (%)
Genotypes			21 (12.6)
E3E3	3 (4)	18 (19.6)	
E4E4	0 (0)	0 (0)	0 (0)
E2E2	6 (8)	0 (0)	6 (3.4)
E3E4	4 (5.3)	4 (4.3)	8 (4.8)
E3E2	61 (81.3)	66 (71.7)	127 (76)
E2E4	1 (1.3)	3 (3.3)	4 (2.4)
Alleles			
E3	71 (47.3*	106 (57.6)	177 (53)
E2	74 (49.3)	69 (37.5)	143 (42.8)
E4	5 (3.3)	7 (3.8)	
			12 (3.6)

* p = 0.002; OR = 0.17, 95% CI: 0.03–0.62.

4. Discussion

Sudden sensorineural hearing loss, one of the most common diseases of inner ear with unknown etiology is usually unilateral with a various range of recovery during 1 or 2 month after the onset of the symptoms is considered as one of the most important inner ear disease as well [15]. In the present case-control study the role MTHFR C677T polymorphism (rs1801133) and ApoE in susceptibility of SSNHL in Iranian population is investigated and results shows that allele C of MTHFR gene at position 677 is a risk factor for development of SSNHL in patients while ApoE allele E3 play a protective role when genotype frequency is significantly different between cases and controls. This is the first study that explores the association of ApoE and MTHFR polymorphism in patients with SSNHL in Iranian population to the best of our knowledge.

There are some studies which have studied the association between ApoE and MTHFR polymorphisms and SSNHL among other populations and with the same study design in which different results reported making difficult to clarify the possible cause of SSNHL. In 2006 for the aim of investigating presence of congenital thrombophilic risk factors MTHFR C677T, the factor V Leiden G1691A and the prothrombin G20210A allele have been studied in 48 Italian SSNHL patients and 48 healthy controls. But they could not prove a significant association between SSNHL and MTHFR C677T alleles and genotypes frequencies between case and controls and also between this distribution and hearing outcomes [16]. This is in accordance with the result of study by Rudack et al. [17]. Another case control study in 2006 working on 81 patients and 264 healthy controls. In order to determine whether selected polymorphisms in folate dependent homocysteine metabolism genes can play a predisposing vascular risk in the development of SSNHL MTHFR C677T, MTHFR A1298C, and methionine synthase (MTR) A2756G SNPs were analyzed which showed higher prevalence of MTHFR 677TT/MTR 2675GG (double homozygous) in patients ($p = 0.030$) in addition to detection of homozygote MTHFR 677TT combined with heterozygote MTHFR 1298 AC genotype only in patients (6%) highlighting the inherited vascular risk factors in SSNHL progress [7]. This is similar results to report of study by Capaccio P et al. which had shown (677CT, 677TT, 1298 AC, 1298CC, compound 677CT/1298 AC) as more frequent in Sudden hearing loss (SHL) patients ($P = 0.005$; Ptrend = 0.001) [18]. Two years later the same author provided information on significant relationship between MTHFR C677T/A1298C polymorphisms, the prothrombin G20210A transition, and the platelet GlyIIIa$^{A1/A2}$ and V Leiden G1691A mutations and SSNHL in a case control study consisting of 100 patients and 200 controls reopening the idea that inherited variant of prothrombotic factors may be causative reason of SSNHL and useful for detection of individuals with potential risk of SSNHL [19]. Furthermore T allele association by itself with increased risk of SSNHL has been stated independent of blood folic acid and homocysteine in Japanese population which is a solitary determination of MTHFR effect on SSNHL risk until 2010. This indicate that MTHFR C677T polymorphism affect the circulating form of folic acid, 5-methyltetrahydrofolate (5-MTHF), which have impact on endothelial function and vascular superoxide production, but not homocysteine [20]. Pollak et al. showed a result in line with this result reporting significantly increased risk of severe/profound Hearing impairment (HI) among males carriers of rs1801133 TT genotype from Poland (odds ratio = 4.88, $p = 0.001$) [8]. A meta analysis working on 1271 extracted from six studies up to May 1, 2014 could prove no association between C677T polymorphism and risk of SSNHL under none of the genetic models in Asian population while this relationship was significantly present in European populations (T vs. C, OR = 1.542, 95% CI 1.008–2.359, $P = 0.046$; TT vs. CT + CC, OR = 1.856, 95% CI 1.245–2.767, $P = 0.002$) [6]. This is in line with result of another meta analysis as association of low level of serum folate, cardiovascular risk factors (smoking, increased alcohol consumption) with increased risk of SSNHL and higher frequency of Factor V Leiden and MTHFR gene polymorphisms as inherited prothrombophilic SNPs in patient which illustrate the function of acquired and inherited cardiovascular risk factors on SSNHL expansion [21].

Although inconsistent with previous results in terms of allele frequency of MTHFR C677T in case and control groups, we found that allele distribution is significantly different between SSNHL patients in comparison to healthy controls; more specifically allele C confer risk for development of SSNHL in Iranian population (*P value = 0.02)*. The mechanism by which C allele may cause higher risk of SSNHL is unknown yet. However, our result shed light on the probable influence of folate-dependent homocysteine metabolism genes on development of SSNHL.

Almost rare studies have been investigated the relationship of apolipoprotein E gene polymorphism and different aspect of hearing loss including the age, severity, hearing thresholds and risk of hearing impairment. Kurniawan et al. studied 435, 85-year-old patients and revealed that APOE-ε4 is associated with a 2.0-fold higher risk of hearing impairment (95% CI: 1.0–4.0) which related this allele to age-related hearing loss. They also demonstrated that individuals who have APOE- ε4/ε4 genotype have the most level of hearing loss while those with APOE- ε3/ε4 or ε2/ε4 genotype are intermediately impaired and lack of APOE-ε4 protect aged persons from hearing loss so they have the lowest level of this trait which highlight the dose-dependent effect of APOE-ε4 [10]. Grady et al. grouped 89 subjects with sensorineural hearing loss (SNHL) based on severity of hearing loss and severity of impairment of word recognition and recognized allele APOE-ε4 less prevalence in patients than normal population. This study reported no relationship between ε4 allele and severity of hearing loss or severity of impairment of word recognition which is claimed that it is related to recruitment bias of their participant such selecting younger adults with SNHL [9]. Furthermore APOE-ε4 allele is significantly associated with the better hearing thresholds since it is shown that one ε4 carriers have better thresholds at 4.0 kHz ($\beta = -2.72$ dB, $p = 0.013$) and 8.0 kHz ($\beta = -3.05$ kHz, $p = 0.006$) and two ε4 allele carriers have better hearing thresholds at 1.0 kHz ($\beta = -8.56$ dB, $p = 0.021$). This contrary result is a probable modest contribution of APOE-ε4 allele to better hearing thresholds might be because of high proportion of black participant who carry only one copy of ε4 and have lower odds of hearing loss, and selecting well-functioning community participants [22]. Finally a survey by Dawes could not identify a significant association between ApoE polymorphism and age-related hearing loss [11].

Various action of ApoE comprised of 1) working as aunique apolipoprotein in nervous system by mobilizing and redistributing lipids (re enervation process), 2) strongly related to age related diseases due to its contribution to stroke, dementia, macular degeneration generalized and atherosclerosis, and 3) antioxidant and anti-inflammatory effect, are

connected with hearing loss as protection from hearing loss by improvement of innervations process, reduction of atherosclerosis burden and oxidative stress prevention which all are totally weak in APOE-ε4 carrier and very strong in patients who carry APOE- ε2 [10]. Accordingly our results may suggest the intermediary protective impact of APOE- ε3 against hearing loss.

It can be assumed that a critical combination of variants alleles may be responsible for rising the disease susceptibility. Study new combination of loci and allele distribution of specific genes in Iranian population can be of great importance to declare more cause of SSNHL. Hence confirm data extracted from our study and other population based investigations.

Previous reports showed significant difference in genotype frequency of MTHFR C677T polymorphism in Iranian population in association with various conditions. It is suggested that C allele is a risk factor for development of neuropathy in patients with type 2 diabetes with genotype CC or CT; ($p = 0.03$; OR = 2.84, 95%CI: 1.03–7.83) [13]. Other reports include: increased risk of Alzheimer's disease (AD) in carriers of T allele ($P = 0.001$, OR = 1.97, 95% CI: 1.3–2.09) [23], lower susceptibility to gastric cancer ($p = 0.02$) in heterozygouts (CT genotype) when T allele has a protective role with of patients [24], lower susceptibility to retinoblastoma in individuals with MTHFR 677CT and 677TT genotypes (p values, 0.012 and 0.034, respectively) as lower T allele carriers among patients than controls ($p = 0.003$) [25], Higher risk of Multiple sclerosis (MS) in Iranian patients with CT and TT genotypes due to effect of 677 T allele (OR for CT = 2.9, 95% CI = 1.88–4.49; OR for TT = 6.23, 95% CI = 3.08–12.59) [26] and so on. Same significant associations have been described between ApoE polymorphism and different medical conditions among Iranian population such as report of lower frequency of Apo ε4 allele in patients with Diabetic nephropathy ($P < 0.05$) [27], Increased risk of Coronary artery disease (CAD) among patients with allele APOE-epsilon4 and -epsilon2 ($P < 0.001$) [28], higher susceptibility to Alzheimer disease (AD) in Iranian carriers of APOE-varepsilon4 allele due to low levels of apoA1 and HDL-C [29] and lastly based on the result of a systematic review on association of genetic variant and diabetes or diabetic complications in Iranian population, 677 T allele when pooling CT + TT genotypes is significantly connected to macroalbuminuria ($P < 0.001$) and ApoE4 is associated significantly with lipid profile among diabetic patients ($P < 0.05$) whereas it is not significantly associated with T2DM [30]. This study is the first exploration with aim of study the possible role of genetic polymorphism of MTHFR and ApoE in risk of SSNHL among Iranian population so far.

According to literature association of Lys198Asn (G/T) polymorphism (rs5370) of the endothelin-1 gene (EDN1) [31], MCP-1(CCL2) 2518 A/G, *E*-selectin Ser128Arg, and interleukin (IL)-62174G/C gene polymorphisms [32], interleukin 1A (IL1A) (889C/T; rs1800587) and interleukin 1B (IL1B) (511C/T; rs16944) [15], glutathione peroxidase 3 gene (GPX3) SNPs including rs3763013, rs8177412, rs3805435, rs3828599, and rs2070593 [33] and 16 SNP in 13 genes related to the oxidative stress cascade or steroid hormone receptors [34] with SSNHL have been investigated that showed increased susceptibility to SSNHL in individuals with recessive homozygous genotype of Lys198Asn (G/T) polymorphism ($p = 0.054$) with milder SSNHL than homozygous for wild allele [31], no association of genotype distribution of MCP-1, *E*-sel polymorphisms between case and control contrary to −174G/G polymorphism of IL-6 gene that is significantly related to risk of SSNHL [32], −889 T allele of *IL1A* with significantly higher prevalence in SSNHL patients in contrast with no effect of *IL1B*-511C/T genotype on risk of SSNHL [15], the only significance different distribution of allele and genotype frequency of SNP rs3805435 between SSNHL patients and healthy control as protective role of G allele (GG + AG) against SSNHL with dominant effect in Taiwanese population [33] and finally a significant correlation of polymorphisms rs2251780 and rs3779647 of GSR gene and NOS3 gene (rs1799983) with treatment outcome and prognosis in patients with SSNHL [34] that all depict that study of genetic factor may provide applicable information for prediction of SSNHL.

Limitation of our study is restricted number of patients with SSNHL and control. Especially for ApoE inefficient digestion method with probably not completely active enzyme due to improper delivery process from factory led to less available presented data than total number of investigations (92 out of 100 controls and 75 out of 77 cases). Low number of homozygous genotypes, E2/E2, $N = 6$; E3/E3, $N = 3$ and E4/E4, $N = 0$ among cases and E2/E2, N = 0; E4/E4, N = 0 among controls in our study shows lack of power for example if the researcher wants to limit the analysis for E4. Although there was low number of homozygous E3/E3 among patients we could propose a significant effect of E3 in SSNHL protection in comparison to other genotypes.

In conclusion genetic heterogeneity of SSNHL and variants that have been studied to influence on its higher risk of development in addition to its unknown etiology necessitate the investigation of different genes related to SSNHL and their variants in different populations to provide valid data for clear genetic mechanism underlying this disease progress which contribute to better schematic of this study and more possibility for successful prediction and prevention approaches. This study is the first study that surveyed the association between genetic polymorphism of MTHFR C677T and ApoE, and SSNHL in Iranian population that disclosed MTHFR 677C allele carriers are more susceptible to SSNHL whereas ApoE3 protect them from SSNHL improvement.

Compliance with ethical standards

Funding

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Conflict of interest

None to declare.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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