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Polymorphisms in folate metabolism genes are associated with susceptibility to presbycusis

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ABSTRACT

Aim: Presbycusis or age related hearing loss is caused by several extrinsic and intrinsic factors that damage the auditory system. Gene polymorphisms in folate metabolism were found to play an important role in the etiology of presbycusis. The present study aimed to investigate the role of 5,10-methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*) and thymidylate synthase (*TYMS*) gene polymorphisms in the onset of presbycusis in a South Indian population.

Main methods: A total of 220 subjects confirmed with presbycusis along with 270 age and sex matched healthy controls visiting MAA ENT Hospitals, Hyderabad, India were enrolled for the study. Genotyping of *MTHFR* C677T (rs180133) and A1298C (rs1801131), *MTR* A2756G (rs1805087), *TSER* (rs1801136) and *TS*1494indel6 bp (rs16430) was carried out using PCR & PCR-RFLP methods.

Key findings: The 'TT' genotype of *MTHFR* C677T and '152 bp/152 bp' genotype of *TS*1494indel6 bp showed statistically significant risk for presbycusis while CC genotype of *MTHFR* A1298C, '2R/2R' genotype of *TSER at* 3′UTR and 6 bp ins/6 bp ins of *TYMS* at 5′UTR were found to be protective. The **T**-A-A haplotype combination of *MTHFR* C677T, *MTHFR* A1298C and *MTR* A2756G as well as 3R- 152 bp of *TYMS* at 5′UTR and 3′UTR were also found to contribute significant risk for the onset of presbycusis. Further, the combination of SNP loci *TSER*: *TS*1494indel6 bp exhibited moderate linkage in presbycusis.

Significance: The present pilot study identified the significant association of gene variants of *MTHFR* and *TYMS* with presbycusis. These findings aid in early diagnosis of hearing loss in the elderly population.

1. Introduction

Hearing loss is one of the major chronic health problems affecting 360 million people globally and the prevalence is higher in the aging population [1]. Approximately 24% of the subjects belonging to the age group of 65–74 years and about 40% of the subjects above 75 years are reported to be affected with hearing loss [2]. The loss of hair cells and other cellular elements in cochlea leads to auditory dysfunction in elderly causing presbycusis [3]. The dysfunction in cochlear transduction depends upon many extrinsic and intrinsic factors [4,5]. It is reported that deficiency of several metabolites and micronutrients like homocysteine, vitamin B12 and folate have an influence on the onset of hearing loss [6,7,8].

Folate, a vitamin of B group plays an important role in the synthesis and methylation of DNA and also contributes to sustain methionine levels in case of low methionine availability. Polymorphisms in genes encoding enzymes such as 5,10-methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*), methionine synthase reductase (*MTRR*) and thymidylate synthase (*TYMS*) are reported to regulate dNTP levels [9,10]. The most associated SNPs with disease pathogenicity are *MTHFR* C677T (rs1801133); *MTHFR* A1298C (rs18011310) and *MTR* A2756G (rs1805087) (Fig. 1). These polymorphisms interfere with the synthesis of methyltetrahydrofolate and remethylation of homocysteine. 5,10-Methylenetetrahydrofolate reductase (*MTHFR*) is a key enzyme in folate metabolism that plays a central role in DNA methylation through the methionine-homocysteine cycle [10,11]. Methionine synthase (*MTR*) with vitamin B12 as a cofactor uses the methyl group from 5-*MTHF* to remethylate homocysteine and produce methionine and tetra hydrofolate (THF) [12,13]. The methionine thus formed is used for the production of S-adenosylmethionine that is required for DNA methylation. Thymidylate synthase, also a crucial enzyme of the folate metabolism along with 5,10-methylenetetrahydrofolate (5,10-

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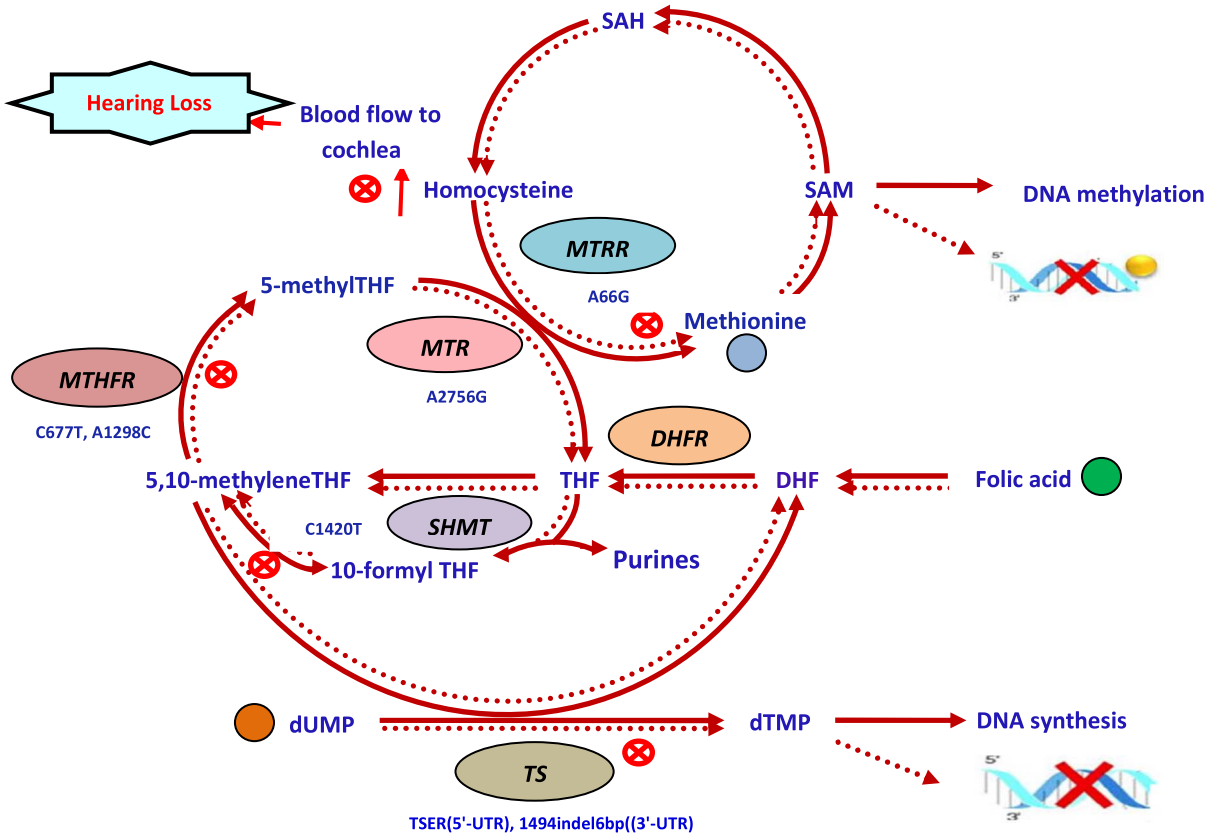

Fig. 1. Genes involved in folate metabolism.
(Source: Skibola et al., Blood 2004; 104: 2155–2162 (modified))

MTHF) produces thymidylate that plays a significant role in DNA synthesis and repair [14,15].

The *MTHFR* and *MTR* genes are located on chromosome 1p36.3 and *TYMS* gene is located on Chr 18p11.32. The two common *MTHFR* polymorphisms C677T in exon 4 and A1298C in exon 7 present in the catalytic domain of the protein result in decreased enzyme activity that has led to the onset of several diseases [12]. *MTR* A2756G polymorphism that results in substitution of aspartic acid for glycine decreases methionine synthase activity increases the cellular homocysteine level and causes hypomethylation of DNA [14]. The variations in *TYMS* gene promoter enhancer region (*TSER*: rs1801136) commonly constituted by a double repeat of 28 bp (2R, *TSER**2) and triple repeat (3R, *TSER**3) in 5′-untranslated enhancer region (5′-UTR) are involved in modulating *TYMS* mRNA expression and translational efficiency [16,17]. In addition, 3R allele with substitution of G > C (*TSER**3 G > C, rs2853542) at the 12th nucleotide in the E-box consensus element is implicated in decreased *TYMS* transcription [17]. Further, a 6 bp insertion/deletion in the 3′-UTR at 1494 position of *TYMS* (*TYMS*1494indel6bp: rs16430) has an influence on mRNA stability and protein expression [9,18].

Recent studies carried out in different populations have reported that the gene polymorphisms in folate metabolism played a key role in the etiopathogenesis of hearing loss [19]. In prebycusis, Uchida et al., (2011) reported an association of *MTHFR* and *MTR* gene polymorphisms in Japanese population and Durga et al., (2006) reported *MTHFR* 677 'TT' genotype to be associated in Netherlands population [20,21]. However, no studies have been reported on functional polymorphism of *MTHFR*, *MTR and TYMS* genes in relation with presbycusis in Indian population. Therefore, the present pilot study has been undertaken to evaluate the role of gene polymorphisms of folate metabolism pathway in the onset of presbycusis in a South Indian population.

2. Subjects and methods

2.1. Subjects

In the present case–control study, 220 cases confirmed with presbycusis at MAA ENT HOSPITALS, Hyderabad, Telangana State, over a period of three years from 2011 to 2014 and 270 age and sex matched healthy controls were recruited for the study. All patients underwent a detailed medical otoscopic examination that included tympanometry and pure tone audiometric test analysis for evaluating hearing loss at 0.5, 1, 2, 4 and 8 kHz frequencies. The patients whose age was equal or > 40 years and with no vestibular or any history of otological surgery, sensorineural form of hearing loss (bilateral as well as symmetrical) and co-morbidities such as diabetes, hypertension and hypothyroidism were included in the study. Subjects suffering from outer & middle ear diseases and noise induced hearing loss were excluded from the study. Informed written consent was taken from all participants, and the study was carried out with the Institutional Ethics committee (Institute of Genetics and Hospital for Genetic Diseases) approval. Blood samples were collected from all the study subjects in EDTA vials. Genomic DNA was isolated from whole blood samples by salting out method [22] and stored at −80 °C for molecular analysis.

2.2. Genotyping

The *MTHFR* C677T (rs1801133), A1298C (rs1801131), *MTR* A2756G (rs1805087), and *TYMS*1494indel6 bp (rs16430) polymorphisms were determined using the PCR-RFLP and *TSER* (rs1801136) by using amplified fragment length polymorphism (AFLP) assays. The details of the genes under study along with their respective primers, PCR reaction conditions, and restriction enzymes are presented in Table 1.