

# Sudden hearing loss and MTHFR 677C>T/1298A>C gene polymorphisms

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**Purpose:** Sudden hearing loss (SHL) can be caused by vascular disorders favoring impaired cochlear perfusion. A number of inherited prothrombotic risk factors have been considered in the pathogenesis of vascular impairment and the possible role of genetic alterations has recently been suggested. We aimed to investigate the relationship between SHL and MTHFR 677 and 1298 gene polymorphisms. **Methods:** DNA genotyping was performed on peripheral blood leukocytes in 45 SHL patients and 135 controls. **Results:** Wild-type MTHFR (677CC/1298AA) was significantly more frequent in the controls ( $P = 0.01$ ), and gene polymorphisms (677CT, 677TT, 1298AC, 1298CC, compound 677CT/1298AC) were significantly more frequent in the patients ( $P = 0.005$ ;  $P_{trend} = 0.001$ ).

**Conclusion:** These data suggest that MTHFR gene polymorphisms may be considered as risk factors for SHL and participate on vascular impairment related to this disorder. Further studies, based on large series of patients, are needed to definitely assess the role of this prothrombotic factor in the etiopathogenesis of SHL. **Genet Med 2005; 7(3):206–208.**

**Key Words:** MTHFR gene polymorphisms, vascular impairment, sudden hearing loss.

Sudden hearing loss is a frequent otological disease, with an incidence of approximately 1–2/10000 per year; it is defined as a sensorineural hearing loss of 30 dB or more over, at least, three contiguous audiometric frequencies that develops over a period of a few hours to three days, and whose etiology can be found only in 10–15% of patients.<sup>1</sup> Various etiological causes have been hypothesized, including vascular disorders, viral infections of the labyrinth or the cochlear nerve, or autoimmune causes.<sup>2</sup> Whatever the cause of the hearing loss, impaired cochlear perfusion seems to be the most important event and, as the cochlea is highly sensitive to minimal changes in localized blood flow and anoxia, cochlear ischemia is a likely cause.<sup>3</sup>

Over recent years, a number of inherited prothrombotic risk factors and their related genetic alterations, such as factor V Leiden, prothrombin 20210G>A transition, and MTHFR 677C>T and 1298A>C polymorphisms, have been studied and correlated with vascular disorders.<sup>4</sup> MTHFR 677C>T and 1298A>C polymorphisms have been identified in healthy subjects,<sup>5</sup> with a high frequency in Northern Italy,<sup>6,7</sup> and also in patients with vascular diseases known to have thrombosis as an etiology such as, venous and arterial thromboembolism,<sup>8,9</sup> coronary artery disease,<sup>10,11,12</sup> and spontaneous abortion.<sup>13,14</sup>

To the best of our knowledge, among prothrombotic risk factors, only the prothrombin 20210G>A gene mutation<sup>2</sup> and platelet GPIIb/IIIa 807C>T polymorphism<sup>15</sup> have been recently considered as risk factors for hearing loss in young patients. On the basis of these findings, genetic studies provide a possible new approach to investigating the pathogenesis of sudden hearing loss.

A number of general population studies have shown that slight increases in plasma homocysteine are associated with an increased risk of atherosclerosis and thrombosis. Homocysteine is an amino-acid produced as a result of methionine metabolism and related to folate through the active form of folic acid, 5-methyltetrahydrofolate. Its concentration depends on the three enzymes methylenetetrahydrofolate reductase (MTHFR), methionine synthase and cystathionine-B synthase; a deficiency of any of these, causes an accumulation of unmetabolised intracellular homocysteine that is exported from the cell into the plasma. This favors the functional impairment of endothelial and vascular smooth cells, and the extracellular matrix, as well as increased coagulation and impaired fibrinolysis. The endothelial damage may favor the formation of atheromatous plaques of cholesterol, which is known to have an additive negative effect on endothelial function.

We, therefore investigated the possible relationship between MTHFR 677C>T and 1298A>C polymorphisms that lead to reduced enzyme activity and sudden hearing loss.

## MATERIALS AND METHODS

The study included 45 consecutive patients affected by sudden hearing loss (24 females; mean age:  $48.5 \pm 14.9$  years) and

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DOI: 10.1097/01.GIM.0000157817.92509.45

135 healthy blood donors; each case was individually matched with three controls in terms of gender and age.

All of the patients underwent a general physical examination, audiological tests, MRI, and microbiologic, immunological and hematological examinations.

A written informed consent has been obtained from all the patients.

The procedures followed were in accordance with the ethical standards of the Helsinki Declaration.

Genomic DNA was isolated from peripheral blood leukocytes, and MTHFR 677 and 1298 was genotyped in all of the subjects using a LightCycler DNA analyser and PCR.

Descriptive statistics have been calculated for continuous variables (mean and standard deviation) and for qualitative ones (absolute and percent frequencies). Comparisons have been carried out by means of Student's *t*-test<sup>7</sup> for unpaired data and by  $\chi^2$  test for qualitative variables, together the  $\chi^2$  test for trend in the case of ordinal variables. Statistical significance is obtained for  $P < 0.05$ ; statistical analyses have been performed with SAS<sup>®</sup> version 6.12.

## RESULTS

MTHFR 677C>T was of the wild type (CC) in ten patients (22.2%) and fifty-three controls (39.3%), heterozygous (CT) in twenty-three (51.1%) and sixty-two (45.9%), and homozygous (TT) in twelve (26.7%) and twenty (14.8%) ( $P = 0.05$ ;  $P_{trend} = 0.018$ ). The MTHFR 1298A>C polymorphism (AC, CC) was more frequent in the patients, but not significantly so.

With regard to the association of 677/1298 gene polymorphisms, one patient (2.2%) and nineteen controls (14.1%) were of the wild type (CC/AA) ( $P = 0.01$ ). Twelve patients (26.7%) and sixty controls (44.4%) were single heterozygous (CT/AA or CC/AC); seventeen patients (37.8%) and thirty controls (22.2%) were carriers of compound heterozygotes of both 677 and 1298 polymorphisms (CT/AC); and fifteen patients (33.3%) and twenty-six controls (19.3%) had homozygous polymorphisms (677TT or 1298CC) ( $P = 0.005$ ;  $P_{trend} = 0.001$ ). A double variant (homozygosity 677TT or 1298CC, compound heterozygosity for both polymorphisms 677CT/1298AC) was therefore present in thirty-two patients (71.2%) and fifty-six controls (41.5%) ( $P = 0.03$ ). None of the patients or controls had more than two variant alleles (Tab. 1).

Mean folate levels were  $8.4 \pm 4.3$  ng/mL in the patients and  $10.8 \pm 4.1$  ng/mL in controls ( $P < 0.0001$ ). Although normal ( $<15$   $\mu\text{mol/L}$ ), mean homocysteine levels were higher in the patients than in controls ( $13.5 \pm 2.9$  vs.  $11.3 \pm 3.1$   $\mu\text{mol/L}$ ) ( $P < 0.0001$ ), as were mean cholesterol levels ( $214 \pm 29.9$  vs.  $182.9 \pm 19.1$  mg/dL) ( $P < 0.0001$ ) and mean erythrocyte MCV ( $93.8 \pm 5.2$  vs.  $82.9 \pm 6.9$  fl) ( $P < 0.0001$ ). Mean homocysteine levels were higher and mean folate levels were lower in the groups with MTHFR polymorphisms: homocysteinemia showed an increasing trend and folatemia a decreasing trend in the groups with double polymorphisms (compound heterozygosity and homozygosity) with respect to the wild type group (Tab. 1 and Tab. 2).

**Table 1**  
MTHFR 677 and 1298 polymorphisms in cases and controls

MTHFR polymorphisms	Patients (45)	Controls (135)	Homocysteinemia	Folatemia
	n (%)	n (%)	Mean (DS)	Mean (DS)
<b>677C&gt;T</b>				
CC	10 (22.2)	53 (39.3)	10.2 (2.9)	12.2 (3.8)
CT	23 (51.1)	62 (45.9)	12.1 (3)	9.9 (4.1)
TT	12 (26.7)	20 (14.8)	14.2 (2.1)	7 (3.1)
<b>1298A&gt;C</b>				
AA	19 (42.2)	71 (52.6)	11.1 (3.2)	10.6 (4.3)
AC	23 (51.1)	58 (43)	12.4 (2.9)	9.9 (4.1)
CC	3 (6.7)	6 (4.4)	14.2 (2.4)	8.5 (3.7)
<b>677/1298</b>				
CC/AA	1 (2.2)	19 (14.1)		
CC/AC	6 (13.3)	28 (20.7)		
CT/AA	6 (13.3)	32 (23.7)		
CT/AC	17 (37.8)	30 (22.2)		
CC/CC	3 (6.7)	6 (4.4)		
TT/AA	12 (26.7)	20 (14.8)		
CT/CC	0	0		
TT/AC	0	0		
TT/CC	0	0		

677CC = wild-type, 677CT = heterozygous, 677TT = homozygous; 1298AA = wild-type, 1298AC = heterozygous, 1298CC = homozygous

One patient had the prothrombin gene mutation; none of the patients or controls had a factor V Leiden mutation. The hemochrome, coagulation, microbiologic and LLAC test results were normal in all of the cases and controls. The results of physical examination and MRI were negative in all of the patients.

## DISCUSSION

In this study MTHFR gene polymorphisms showed a significant statistical association with SHL, suggesting that MTHFR genomic analysis should be considered in its etiological assessment.

It is well known that reduced MTHFR enzymatic activity leads to folate dysmetabolism and increased plasma homocysteine levels, both of which may play a role in microvascular diseases.<sup>16</sup> It has also been hypothesized that, although slight, the increase in homocysteine levels associated with altered folate metabolism may favor the impairment of endothelial function in small vessels,<sup>17</sup> such as the auditory artery and its branches. Furthermore, high blood cholesterol levels are known to be an important risk factor for endothelial dysfunction and vascular thrombosis.<sup>18</sup> The significantly higher levels of serum homocysteine and cholesterol, and the lower levels of

**Table 2**  
Relevant data with their statistical significance

	Patients (45)	Controls (135)	P
677/1298 MTHFR polymorphisms	n (%)	n (%)	
CC/AA (wild-type)	1 (2.2)	19 (14.1)	
CC/AC or CT/AA (single heterozygosis)	12 (26.7)	60 (44.4)	
CT/AC (double heterozygosis)	17 (37.8)	30 (22.2)	
CC/CC or TT/AA (homozygosis)	15 (33.3)	26 (19.3)	0.005
(Ptrend = 0.001)			
	Mean (SD)	Mean (SD)	
Homocysteinemia	13.5 (2.9)	11.3 (3.1)	<0.0001
CC/AA (wild-type)	10.6 (—)	8.5 (2.1)	
CC/AC or CT/AA (single heterozygosis)	12.2 (2.6)	9.7 (2.4)	
CT/AC (double heterozygosis)	16.2 (2.1)	13.9 (1.5)	
CC/CC or TT/AA (homozygosis)	14.9 (2.3)	13.7 (2.1)	
Folatemia	8.4 (4.3)	10.8 (4.1)	<0.0001
CC/AA (wild-type)	13.2 (—)	13 (3.2)	
CC/AC or CT/AA (single heterozygosis)	13 (3.7)	12.6 (3.4)	
CT/AC (double heterozygosis)	7.9 (2.8)	7.8 (2.6)	
CC/CC or TT/AA (homozygosis)	8.1 (3.6)	7.5 (2.9)	
Cholesterolemia	214 (29.9)	182.9 (19.1)	<0.0001

677CC = wild-type, 677CT = heterozygous, 677TT = homozygous; 1298AA = wild-type, 1298AC = heterozygous, 1298CC = homozygous

serum folate, observed in the patients versus controls might support the relationship between MTHFR polymorphisms and microvascular impairment in SHL (Tab. 2).

Although the homocysteine and folate levels in our patients and controls were statistically different, they were both within the normal range (at the upper and lower limit, respectively). We suppose that although not sufficient to cause major cardiovascular disease slight modifications in these substances may favor microvascular impairment, especially in such terminal vascular systems as the cochlear.

Moreover, it has been recently described the potential influence of low folate levels on hearing impairment in SHL patients possibly related to the effects on homocysteine metabolism and to the diminution of folate antioxidant capacity.<sup>19</sup>

Though folate is the strongest nutritional and pharmacological determinant of plasma homocysteine concentrations, recent evidence suggests that the administration of high doses of folic acid may have a direct positive effect on vascular endothelial function, regardless of homocysteine levels.<sup>11</sup> From this

point of view, the possible folate dysmetabolism associated with the MTHFR gene polymorphisms occurring in patients with SHL might suggest the use of folate supplementation in such patients as is currently the case in patients with other vascular disorders.

In conclusion, MTHFR gene polymorphisms may be considered as risk factors for SHL; however, further studies, based on larger series of patients, and including genomic analysis of multiple inherited prothrombotic factors, are needed to definitely assess their role in the etiopathogenesis of SHL.

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