Original Paper

Audiology & Neurotology

Audiol Neurotol 2011;16:185–190
DOI: 10.1159/000319310

Received: December 22, 2009
Accepted after revision: July 5, 2010
Published online: August 25, 2010

Role of Genetic and Acquired Prothrombotic Risk Factors in Genesis of Sudden Sensorineural Hearing Loss

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Key Words
Sudden sensorineural hearing loss · Risk factors · MTHFR gene

Abstract
The methylenetetrahydrofolate reductase C677T mutation, factor V G1691A (factor V Leiden) mutation, prothrombin G20210A mutation and 8 other laboratory values associated with increased thrombotic risk were analyzed in 40 patients with sudden sensorineural hearing loss (SSHL). The results were compared with those obtained from 120 controls not affected by SSHL. We found a statistically significant higher frequency of hyperhomocysteinemia in the SSHL group compared with controls, and that this was also associated with the presence of homozygosity for the MTHFR C677T mutation. The study results suggest that SSHL might be caused, among other factors, by a combination of these 2 variables. We suggest that this analysis of the MTHFR C677T mutation should be further investigated to establish the etiology of SSHL, and that the same analysis should be taken into account in those patients with high levels of homocysteine.

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Introduction

Sudden sensorineural hearing loss (SSHL) is defined as a hearing loss >30 dB in 3 contiguous frequencies that occurs in a period of time ranging from a few hours to 3 days.

It is usually unilateral, but bilateral forms have been described arising simultaneously or in succession [Xenellis et al., 2007]. The incidence of SSHL is estimated as between 5 and 20 new cases every 100,000 people per year [Byl et al., 1984; Rudack et al., 2006]. Viral infection and ischemia are widely recognized etiological hypotheses. Some cases of SSHL have been also attributed to rupture of the cochlear membrane [Goodhill and Harris, 1979; Gussen, 1981] or inner ear immune-mediated disorders [Toubi et al., 2004].

The possibility of inner ear ischemia due to vascular thrombosis is currently arousing particular interest: the cochlea is an organ with terminal type arterial circulation supplied by the labyrinthine artery and therefore particularly sensitive to these kinds of injuries [Capaccio et al., 2007; Mom et al., 2008].

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1420–3030/11/0163–0185$38.00/0

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Many risk factors have been investigated, particularly cardiovascular ones such as diabetes, smoking, increased blood viscosity, elevated fibrinogen, triglycerides, LDL, total cholesterol and several mutations including the glycoprotein la [Ohinata et al., 1994; Suckfüll et al., 2002; Ullrich et al., 2004].

Recently, it has been suggested that congenital and/or acquired thrombophilia may have a pathogenic role in SSHL. Congenital thrombophilias are represented by widely known gene polymorphisms, such as factor V Leiden which is characterized by a G1691A mutation leading to production of a protein resistant to proteolytic action of activated protein C (phenotype known as activated protein C resistance). Other known polymorphisms that have already been studied are a mutated prothrombin characterized by G20210A substitution in the promoter region of the gene and deficiencies of physiological coagulation inhibitors such as protein C, antithrombin and protein S.

The most common acquired thrombophilia is the presence of lupus anticoagulant or antiphospholipid antibodies. Hyperhomocysteinemia is another condition predisposing to the development of arteriovenous thrombosis which can have genetic (MTHFR C677T homozygosity, homocystinuria) or acquired causes (eating disorders, thyroid disorders, kidney diseases) [Frosst et al., 1995].

The MTHFR enzyme is responsible for the reduction of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, required for the transformation of homocysteine into methionine [Lucock, 2000].

The genetic mutation, named MTFHR C677T, leads to a cytosine (C)→thymine (T) substitution in nucleotide 677 of the MTHFR gene (coding therefore for an alanine instead of a valine residue), and is responsible for the formation of a thermolabile enzyme.

The MTHFR C677T mutation and another mutation in the nucleotide 1298 A→C (MTHFR A1928C) have been analyzed: the mutation 1298 A→C has no influence on the activity of the enzyme, whereas the mutation C677T generates an enzyme that rapidly decreases its activity [Yamada et al., 2001].

Homozygosity for the MTHFR C677T mutation is the most common genetic cause of hyperhomocysteinemia (from 5 to 14% of the general population is homozygous for this mutation) [Kaul et al., 2006].

The purpose of this study was to identify the incidence of thrombophilia and cardiovascular risk factors in 40 patients with SSHL.

Patients and Methods

Patients

Forty patients with sudden hearing loss came under our observation between January 2007 and February 2009 and were enrolled in the study (17 males and 23 females, mean age 52.28 ± 15 years). The following conditions were used as exclusion criteria: previous conductive or sensorineural hearing loss, diabetes, hypertension, previous myocardial infarction or stroke, deep vein thrombosis, and intake of anticoagulants/anti-aggregators or oral contraceptives. Healthy controls (n = 120) with a negative personal history of thrombotic events were selected using the same exclusion criteria as the SSHL group.

Evaluation

All patients underwent an objective ENT examination and anamnesis collection, tonal audiometry, brain MRI with contrast and the following hematologic examinations: complete blood count, fibrinogen, international normalized ratio, partial thromboplastin time, presence of lupus anticoagulant (LAC) tested by diluted Russel viper venom, antithrombin, protein C and protein S activity levels, and homocysteine plasma levels. Blood samples were analyzed to identify mutation of factor V G1691A (factor V Leiden) and prothrombin G20210A and MTHFR C677T mutations.

Genomic DNA was isolated from leucocytes from peripheral venous blood of patients and controls, amplified by PCR and analyzed to determine the genotype of the 3 mutations. To assess the presence of the MTHFR C677T gene mutation, we used the AA901 MTHFR (C677T) real time FRET kit by NLM Diagnostic. The test is based on the real-time FRET PCR technique which allows real-time monitoring of sample amplification. The progression of PCR analysis is evaluated by quantification of fluorescence emitted by molecules associated with hybridization probes. Hybridization probes allow identification of mutations of the target sequence by analyzing the dissociation curve (melting curve), which is obtained by subjecting the amplified sequences to a scale of increasing temperatures. The melting curve analysis helps to distinguish the 3 genotypes (homozygous, heterozygous and wild-type) according to the position of the peaks determined by the different dissociation temperatures.

Alleles MTHFR 677CC, prothrombin 20210GG and factor V Leiden 1691GG indicate a homozygous wild genotype (no mutation), while MTHFR 677CT, prothrombin 20210GA and factor V Leiden 1691GA are heterozygous mutated alleles and, finally, homozygous variants have been named MTHFR 677TT, prothrombin 2021AA and factor V Leiden 1691AA.

Statistical Analysis

Statistical analysis was conducted by calculating all the qualitative and quantitative variables, and transforming the latter into qualitative variables to facilitate analysis. The different blood levels of homocysteine, proteins C and S and antithrombin were transformed into qualitative dichotomous variables (0 and 1) using a cutoff (≥15 and <15 μmol/l for homocysteine, <70 and ≥70% for proteins C and S and antithrombin). Qualitative variables of cases and controls were then compared using the χ^2 test. During the analysis of the MTHFR C677T mutation, the association of heterozygous and wild-type genotypes against homozygosity were also compared. The association between hyperhomo-

Table 1. Frequencies of mutations and prothrombotic risk factors in SSHL patients and controls

	Controls (n = 120)	SSHL patients (n = 40)	p
Factor V Leiden (MTHFR G1691A)			
1691GG (wild-type)	111 (92.5)	38 (95)	0.8568
1691GA (heterozygous)	9 (7.5)	2 (5)	
MTHFR C677T			
677CC (wild-type)	32 (26.6)	9 (22.5)	0.0327, p trend = 0.0599
677CT (heterozygous)	57 (47.5)	12 (30)	
677TT (homozygous)	31 (25.8)	19 (47.5)	
PTH G2021A			
2021GG (wild-type)	111 (92.5)	35 (87.5)	0.5182
2021GA (heterozygous)	9 (7.55)	5 (12.5)	
LAC			
Absent	118 (98.2)	40 (100)	0.9436
Present	2 (1.8)	0	
Antithrombin			
≥70%	120 (100)	40 (100)	n/a
<70%	0	0	
Protein S			
≥70%	120 (100)	39 (97.5)	0.0578
<70%	0	1 (2.5)	
Protein C			
≥70%	116 (96.6)	40 (100)	0.5716
<70%	4 (3.4)	0	
Homocysteinemia[1]			
<15 μmol/l	107 (88.9)	24 (60)	0.0001, p trend = 0.0001
≥15≤24 μmol/l	13 (11.1)	11 (27.5) } 16 (40)	
≥25 μmol/l	0	5 (12.5) }	

Figures in parentheses are percentages.

[1] OR = 5.4872 (95% CI = 2.3329–12.9066) for patients having hyperhomocysteinemia (homocysteinemia ≥15 μmol/l) vs. controls.

cysteinemia and homozygosity for the MTHFR C677T mutation was verified by calculating the φ coefficient, which measures the association of 2 binary variables. The relationship between all variables recorded and SSHL was evaluated by multivariate backward logistic regression, removing the non-significant variables one by one and retaining only statistically significant variables in the final model.

All analyses were calculated using MedCalc release 10.2 for Windows (MedCalc Software, Mariakerke, Belgium).

Results

Table 1 summarizes data collected regarding the MTHFR C677T polymorphism, mutation of factor V Leiden, presence of LAC, prothrombin 20210A mutation, the values of antithrombin, protein C, protein S and hyperhomocysteinemia. There was no significant difference between the SSHL group and controls, taking into account mutation of factor V, prothrombin, presence of LAC and plasma values of antithrombin, proteins C and S ($p > 0.1$).

The differences between the SSHL group and controls in regard to hyperhomocysteinemia (homocysteinemia ≥15 μmol/l) and the MTHFR C677T mutation were statistically significant. The frequency of hyperhomocysteinemia was higher in the SSHL group than in controls ($\chi^2 = 15.288$ and $p = 0.0001$) with an odds ratio equal to 5.4872 (95% CI 2.3329–12.9066, $p = 0.0001$). Hyperhomocysteinemia was found to be associated with the presence of homozygosity for the MTHFR C677T mutation ($\chi^2 = 15.286$ and φ coefficient = 0.523, $p = 0.0001$). Five SSHL patients had homocysteine values >25 μmol/l (exact values were 26, 28.58, 40.49, 65 and 67 μmol/l) and they were all homozygous for the C677T mutation. The compari-

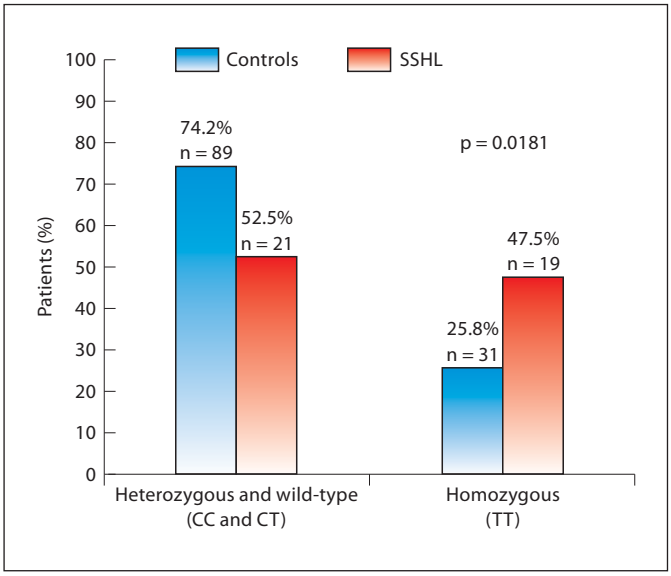

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Fig. 1. Genetic status of SSHL patients and controls for the MTHFR C677T mutation. OR = 2.5975 (95% CI 1.2355–5.4612) for patients having homozygosity for MTHFR C677T mutation vs. controls.

son between patients who had both copies of the gene for mutated MTHFR (i.e. homozygous) and patients who had 1 or 2 functional copies of the gene (i.e. heterozygous or wild-type) was statistically significant (χ^2 = 5.585, p = 0.0181) with an odds ratio of 2.5975 (95% CI = 1.2355–5.4612, p = 0.0118; fig. 1). During the logistic regression which considered the presence/absence of homozygosity for the MTHFR C677T mutation and the homocysteine values, the presence of the latter was retained as a statistically significant variable.

Discussion

Little is known about the pathogenesis of SSHL. To date it is uncertain whether SSHL is due to a single disease process or the result of different etiological factors such as infections, vascular diseases, embolism or autoimmune mechanisms [Cadoni et al., 2002]. Impaired perfusion of the inner ear is widely recognized as a possible pathogenic mechanism [Byl et al., 1984; Cadoni et al., 2004; Mattox and Leyles, 1989; Yildiz et al., 2008]. Recent studies have shown the involvement of genetic mutations (associated with thrombotic microangiopathic disorders) in the development of SSHL; unfortunately, the results of different studies are contradictory. The discrepancies observed may be due to the relatively small number of cases and controls analyzed or to the different criteria used in patient enrollment (age, diabetes and type of hearing loss).

In our study, we found a statistically significant difference in the incidence of homozygosity for the MTHFR C677T mutation between SSHL patients and controls.

The results of our study are in line with those of other authors. Capaccio et al. [2007] found in 2 separate studies a statistically significant association ($p < 0.012$ and $p < 0.001$) between the presence of mutations in the MTHFR gene (both in position 1298 and in position 677) and SSHL. In both these studies, there was a difference in the number of homozygous patients found in the SSHL group compared to controls. Yildiz et al. [2008] found a significantly higher frequency of heterozygosity for the MTHFR C677T mutation in patients with SSHL compared to controls (50.9 vs. 25%, p = 0.03). On the other hand, Gross et al. [2006] and Rudack et al. [2004] did not obtain significant results when studying the C677T mutation in patients with SSHL (p = 0.17 and 0.34).

Studies of ischemic heart disease showed similar discrepancies in the results; however, in a meta-analysis of 72 studies of MTHFR C677T mutation in 16,849 patients, Wald et al. [2002] found a statistically significant increase (OR = 1.21) in ischemic heart disease in homozygous patients. According to Klerk et al. [2002], the MTHFR C677T mutation becomes a risk factor for cardiovascular disease in those populations, such as the European one, where folate fortification of foods is not mandatory [Food and Drug Administration et al., 1996].

In our study, we also analyzed genetic mutations of prothrombin and factor V Leiden, but we found no statistically significant differences between the SSHL group and controls. These mutations also show conflicting results: Patscheke et al. [2001] found a statistically significant difference for the prothrombin mutation, whereas in the study of Yildiz et al. [2008] and Görür et al. [2005] the data did not reach significance. Görür et al. [2005] also showed a significant difference in factor V Leiden frequency in patients with SSHL, while Yildiz et al. [2008] did not find any significant results.

We believe this is due to the low frequency with which different mutations are found in the general population, and we think that a larger number of patients is therefore required.

The patients with SSHL analyzed in our study also had significantly higher levels of homocysteine compared to controls. In the literature, the association between MTHFR C677T mutation and hyperhomocysteinemia

has been confirmed by various authors [Almawi et al., 2009; Fujimaki et al., 2009; Husemoen et al., 2009; Oterino et al., 2010; Szamosi et al., 2009; Yamada et al., 2001] although in another study [Antoniades et al., 2009] the same mutation showed a stronger correlation with low folate levels. In our study we did not analyze folate levels; however, according to several studies, low levels of folate are inversely related to homocysteine levels [Cadoni et al., 2004; Quaranta et al., 2008; Schuknecht et al., 1973; Suckfüll et al., 2002].

The association between homozygosity for the MTHFR C677T mutation and hyperhomocysteinemia suggests that this combination of variables may be involved, along with other factors, in the onset of SSHL.

Conclusions

Hyperhomocysteinemia was statistically higher in the SSHL group compared with controls and was also associated with the presence of homozygosity for the MTHFR C677T mutation. The study therefore supports the hypothesis that SSHL might be caused, among other factors, by the combination of the 2 variables and confirms the hypothesis of arteriovenous thrombosis etiology for SSHL.

Genetic analysis of the MTFHR gene should be taken into account in the investigation of SSHL etiology, particularly in those patients who have high levels of homocysteine.

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