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Original Article

Glutathione peroxidase 3 gene polymorphisms and the risk of sudden sensorineural hearing loss

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Abstract The glutathione peroxidase 3 gene (*GPX3*) is reported to be a risk factor for arterial ischaemic stroke and cerebral venous thrombosis. *GPX3* may be one of the aetiologies of sudden sensorineural hearing loss (SSNHL), which might be attributed to the genetic effect of *GPX3* by influence reactive oxygen species (ROS). Unbalanced ROS have been associated with susceptibility to SSNHL. Therefore, we conducted a case–control study with 416 SSNHL cases and 255 controls. Five single nucleotide polymorphisms (SNPs) were selected. The genotypes were determined using TaqMan genotyping assays. Each SNP was tested using the Hardy–Weinberg equilibrium (HWE), and the genetic effects were evaluated using three inheritance models. All five SNPs were in HWE. As the result, the AG genotype of rs3805435 had an adjusted odds ratio (OR) of 0.54 (95% confidence interval = 0.37–0.79, $p = 0.001$) compared with the AA genotype in the SSNHL cases. The GG and AG genotypes of the SNP

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rs3805435 were associated with SSNHL under the dominant model ($p = 0.002$, OR = 0.58). In conclusion, these results suggest that *GPX3* polymorphisms influence susceptibility to SSNHL in southern Taiwan.

Introduction

Sudden sensorineural hearing loss (SSNHL) is defined as a loss of at least 30 dB in three contiguous frequencies over 3 days or less [1]. The incidence rates per 100,000 population in Taiwan are 8.85 for men and 7.79 for women [2]. The aetiology and pathogenesis of SSNHL are unknown, but its causes may include viral infection, vascular disease, and autoimmunity [3]. SSNHL, a complex multifactorial disease, may also be associated with genetic factors including heat shock protein 70 (HSP70), phosphodiesterase 4D (*PDE4D*), factor V Leiden G1691A, prothrombin G20210A, and methylene-tetrahydrofolate reductase (MTHFR) C677T [3–6].

Glutathione peroxidase (GPX) is an oxygen radical-metabolising enzyme. Four major GPX isoenzymes (GPX1–4) are encoded by distinct genes, and the isoenzymes vary in tissue distribution and substrate specificity [7]. The decreased activity of these antioxidant enzymes may increase oxidative stress. GPX3 is a member of the selenocysteine-containing GPX family. GPX3 is the only GPX isoform found in the extracellular space.

Oxidative stress plays a key role in endothelial dysfunction and results in damage to the terminal microvascular system. Berjis et al. revealed an association between endothelial dysfunction and SSNHL by measuring flow-mediated dilation [8]. Capaccio et al. noted unbalanced reactive oxygen species (ROS) levels in SSNHL patients [9]. A significantly higher ROS level was observed in SSNHL patients than in a group of controls [9]. Antioxidant enzymes such as GPX function as the primary cellular defence mechanism against ROS by reducing oxidative stress.

GPX3 plays a pivotal role in arterial and venous thrombosis [10,11]. GPX3 maintains the bioavailability of nitric oxide (NO) in the vascular system, and GPX3 deficiency leads to the decreased vascular bioavailability of NO, which attenuates its effect on platelet function and subsequently results in a prothrombotic state. Decreased GPX3 levels may lead to the loss of antioxidant effects, promoting oxidative modification of fibrinogen, thereby facilitating fibrin thrombus formation [12]. Akhter et al. reported decreased plasma GPX3 levels in stroke patients compared with a group of controls [12].

Therefore, in the present study, we hypothesise that single nucleotide polymorphisms (SNPs) of *GPX3* are associated with the risk of SSNHL. *GPX3* polymorphisms may play a role in the pathogenesis of SSNHL. No study has investigated the relationship between *GPX3* polymorphisms and the risk of SSNHL. Thus, we conducted a case–control study to investigate whether *GPX3* SNPs are risk factors for SSNHL for people in southern Taiwan.

Material and methods

Study population

We recruited 416 SSNHL patients and 255 controls from our hospital between October 2010 and July 2014. The diagnostic criteria of SSNHL were sensorineural hearing loss of at least 30 dB in three contiguous frequencies detected using a pure tone audiogram, with an onset within 3 days [2]. Healthy volunteers without a history of hearing loss or any ear disorders were enrolled as controls. Demographic information was collected.

SSNHL patients underwent audiometric testing, including pure tone audiometry and auditory brainstem-evoked responses, and computed tomography or magnetic resonance imaging (to exclude acoustic neuroma).

The study protocol was approved by the Institutional Review Board of our hospital, and all subjects provided informed consent.

SNP selection and genotyping

We selected all tagging SNPs (tSNPs) of *GPX3* from the release 2.0 phase II data of the HapMap Project (www.hapmap.org) [13] by using the tagger pairwise method [14]. tSNPs were selected according to the following criteria: r2 of 0.8 or higher and a minor allele frequency of more than 10% in the Han Chinese population. Five tSNPs of *GPX3* met the aforementioned criteria and were selected for genotyping: rs3763013, rs8177412, rs3805435, rs3828599, and rs2070593.

Genomic DNA was extracted from peripheral blood through a standard method. Genotyping was performed using TaqMan technology (7500 Real-Time PCR System, Applied Biosystems, Foster City, CA, USA), and reactions were performed in 96-well microplates in ABI 9700 thermal cyclers (Applied Biosystems, Foster City, CA, USA). Fluorescence was measured using the ABI 7500 Real-Time PCR System and analysed using System SDS software version 1.2.3 (Applied Biosystems, Foster City, CA, USA). All SNPs were typed in each subject.

Statistical analysis

Continuous variables were analysed using independent *t* tests, the results of which are presented as means ± SD. The allele frequency was obtained through direct gene counting. The Hardy–Weinberg equilibrium (HWE) was examined in the controls by using the chi-squared test. The effect of the minor allele of each SNP was examined in

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