Association analysis of LDLR gene polymorphisms with susceptibility to sudden deafness

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Abstract

In order to explore the association of 3 SNPs of LDLR gene with susceptibility to sudden deafness (SD), serum lipid parameters were compared in 139 SD patients and 139 healthy individuals. Genotyping of the 3 SNPs, allele and genotype frequencies were performed and compared. The association between allele frequency of 3 SNPs and susceptibility to SD in different models were analysed. Serum lipid parameters were compared in patients with different genotypes. The comparison of serum lipid levels showed higher triglycerides, total cholesterol, and low-density lipoprotein cholesterol levels in patients compared to the controls. The frequencies of rs5929 T allele, rs2738464 G allele and rs1433099 T allele in SD group were higher than control group. We observed a statistically significant association of LDLR gene rs5929 T allele under dominant genetic model, rs2738464 G allele under additive and dominant genetic models, rs1433099 T allele under dominant genetic model with susceptibility to SD. There was no statistical difference in serum lipid levels of SD patients with different genotypes. Total cholesterol, triglycerides, and low-density lipoprotein cholesterol may be risk factors in the pathogenesis of SD. rs5929 T allele, rs2738464 G allele and rs1433099 T allele may be risk factors for SD.

Introduction

Sudden sensorineural hearing loss (SD) is defined as a hearing loss in at least three contiguous test frequencies of 30dB occurring within 3 days [1] and is one of the most common acute diseases of the ENT. Even with positive and timely treatment, many patients remain affected with permanent hearing loss, tinnitus, and other sequelae that cause great suffering. While the etiology and pathogenesis remain to be elucidated, current research has subsequently focused on exploring polymorphisms in genes that are important for normal microcirculation. Indeed, genetic susceptibility to SD occurs in individuals with mutations in genes critical for microcirculation. This subsequently affects the inner ear microcirculation which is related to the incidence of SD. Mutations in genes encoding for methyltetrahydrofolate reductase, prothrombin, and platelet glycoprotein are found in patients with microcirculation disorder and thus are genes of interest in understanding the basis of SD [2].

Several pieces of evidence suggest that abnormal lipid metabolism increases the risk of sudden sensorineural hearing loss [3,4]. Polymorphisms localized in the exons of low-density lipoprotein receptor (LDLR) gene may have a significant impact on lipid metabolism by changing in the expression or stability of LDLR [5,6]. There has been no report on the association of LDLR gene polymorphisms with susceptibility to SD. Thus, in our study we compared 3 SNPs gene polymorphisms of LDLR gene of 139 cases of patients of SD with 139 normal hearing volunteers.

The incidence of SD is estimated at 5-20/10,000 individuals per year[4], so it is necessary to unravel the origins of SD. Exploring the relationship between LDLR polymorphisms and susceptibility to SD may help identify SD susceptible populations and provide new methods for the prevention and diagnosis of SD.

Methods

Statement

All methods in this paper were performed in accordance with the relevant guidelines and regulations of the Second Affiliated Hospital of Xi’an Jiaotong University.

Informed consent has been obtained from a parent and/or legal guardian, for research involving human participants under the age of 18 years (including donors of tissue samples).

Informed consent has been obtained from adults themselves for research involving human participants (including donors of tissue samples).

Participants

SD group: 139 patients with SD visited the Department of Otolaryngology of the Second Affiliated Hospital of Xi’an Jiaotong University, from March 2013 to May 2014, included 70 males and 69 females, aged 17 to 72 years, mean 45.1 ± 14.2 years. All cases in this study were taken within the first 7 days of SD onset and provided initial treatment. Patients were selected by their relevant medical history and clinical examination to exclude hypertension, diabetes, coronary heart disease, and other diseases. All subjects had normal hearing volunteers.

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Table 2. The results of association of LDLR gene polymorphisms with susceptibility to sudden deafness.

| Number | SNP | Group     | Genotype (n, %) | Allele (n, %) | P       | P*     | P**     | OR, 95%CI |
|--------|-----|-----------|----------------|--------------|---------|--------|---------|----------|
| 1      | rs5929 | Control   | TT            | 19(3.7)      | 0.346   | 0.081  | 0.022*  | 1.529    |
|        |      |           | CG            | 60(43.2)     | 98(35.3) | 180(64.7) |
|        |      |           | CC            | 60(43.2)     | 73(26.3) | 205(73.7) |
| 2      | rs2738464 | Control  | TT            | 15(10.8)     | 0.276   | 0.007**| 0.016*  | 1.572    |
|        |      |           | CG            | 58(42.4)     | 95(34.2) | 183(65.8) |
|        |      |           | CC            | 58(42.4)     | 69(24.8) | 209(75.2) |
| 3      | rs1433099 | Control  | TT            | 17(12.2)     | 0.381   | 0.059  | 0.015*  | 1.578    |
|        |      |           | CT            | 60(43.2)     | 94(33.8) | 184(66.2) |
|        |      |           | CC            | 60(43.2)     | 68(24.5) | 210(75.5) |

Table 3 shows the results of 3 SNPs associated with SD between SD group and control group in three gene models. LDLR gene rs5929 T allele in a dominant genetic model was associated with susceptibility to SD (P = 0.031 <0.05), rs2738464 G allele in an additive genetic model was associated with susceptibility to SD (P = 0.007 <0.05), rs1433099 T allele in a dominant model was associated with susceptibility to SD. (P=0.023 <0.05).
The comparison of serum lipid parameters in patients with different genotypes

Tables 4-6 shows serum lipid parameters were compared in 139 patients with different genotypes of rs2738464, rs1433099 and rs5929. We found there was no statistical difference in serum lipid levels of SD patients with different genotypes.

Statistical analyses

Serum lipid parameters were analysed using independent t tests, and the results are presented as means ± SD (Standard Deviation). GENEPOP v4.0 software was used to genotype Hardy-Weinberg test for SD group and control group; The software SPSS18.0 computed allele and genotype frequency for the two groups. Pearson’s chi-square test indicated different alleles and genotype frequency distribution between the two groups as well as the association of alleles with susceptibility to SD among different gene models. Nonlinear logistic regression analysis was used to calculate the OR (odds ratios) and 95% CI (95% confidence intervals); All data with P<0.05 was considered statistically significant.

Discussion and conclusion

SD features have similarities to acute ischemic diseases especially in treatment and principles. This is because features in both cases are usually sudden onset and have rapid progression. Moreover, better prognosis of treatment in the acute phase and healing of SD also resembles ischemic disease. Some scholars believe that lipid metabolism disorders are risk factors for SD and coronary heart disease [7]. The pathogenesis of vascular risk factors was also similar: for example, coronary artery embolization is the main artery embolism affecting myocardial blood supply, and SD is microvascular embolism, affecting the inner ear blood supply [7]. Tagaya reported that patients with SD were similar with acute ischemic events in their magnetic resonance imaging, such as myocardial infarction and cerebral vascular accident [8]. Chien found that phosphodiesterase 4D gene polymorphism was not only associated with ischemic stroke, but also increased women’s susceptibility to SD [9]. Numerous studies confirmed that polymorphisms within IL-6, methylenetetrahydrofolate reductase, prothrombin, and Factor V Leiden genes are related to coronary heart disease and SD. To a certain extent, these gene polymorphisms may also determine the susceptibility to SD.

Table 3. The results of association of LDLR gene SNPs with SSHL under three gene models. Pd represented the difference of allele gene frequency in dominant model, Pe represented the difference of allele gene frequency in additive model, Pf represented the difference of allele gene frequency in recessive model, using Bonferroni-Holm correction, *P<0.05, **P<0.01 represented the differences were statistically significant.

| Number | SNP   | Group | Genotype (n, %) | Pd   | Pe   | Pf   |
|--------|-------|-------|----------------|------|------|------|
| 1      | rs5929|       |                |      |      |      |
|        |       |       | TT             | 0.031*| 0.081| 0.182|
|        |       |       | CT             |       |      |      |
|        |       |       | SSHL 19(13.7)  | 60(43.2) | 60(43.2) |      |
|        |       |       | Control 12(8.6)| 49(35.3) | 78(56.1) |      |
| 2      | rs2738464| |                  |      |      |      |
|        |       |       | GG             | 0.003*| 0.007*| 0.844|
|        |       |       | CT             |       |      |      |
|        |       |       | SSHL 15(10.8)  | 65(46.8) | 59(42.4) |      |
|        |       |       | Control 14(10.1)| 41(29.5) | 84(60.4) |      |
| 3      | rs1433099| |                  |      |      |      |
|        |       |       | TT             | 0.023*| 0.059| 0.156|
|        |       |       | CT             |       |      |      |
|        |       |       | SSHL 17(12.2)  | 60(43.2) | 62(44.6) |      |
|        |       |       | Control 10(7.2)| 48(34.5) | 81(58.3) |      |

Table 4. The comparison of serum lipid parameters in patients with different genotype of rs5929. P <0.05 was considered statistically significant.

| CC   | n     | CT   | n     | TG    |
|------|-------|------|-------|-------|
|      |       |      |       |       |
| TG   | 1.499±0.903| 60  | 1.438±0.933| 60  |
| TC   | 5.026±1.152| 60  | 4.908±1.150| 60  |
| HDL-C| 1.27(1.03-1.513)| 60| 1.306(1.038-1.51)| 60  |
| LDL-C| 3.202±0.933| 60  | 2.989±0.806| 60  |
| APOA1| 1.399±0.277| 60  | 1.43±0.269| 60  |
| APOB | 0.705(0.55-0.84)| 60| 0.66(0.61-0.8)| 60  |

Table 5. The comparison of serum lipid parameters in patients with different genotypes of rs2738464. P <0.05 was considered statistically significant.

| CC   | n     | CC   | n     | TG    |
|------|-------|------|-------|-------|
|      |       |      |       |       |
| TG   | 1.495±0.893| 59  | 1.249±0.935| 65  |
| TC   | 4.887±1.064| 59  | 4.835±1.005| 65  |
| HDL-C| 1.301±0.325| 59  | 1.405±0.315| 65  |
| LDL-C| 3.064±0.880| 65  | 2.871±0.759| 65  |
| APOA1| 1.404±0.244| 59  | 1.56±0.322| 65  |
| APOB | 0.67(0.6-0.85)| 59| 0.68(0.58-0.8)| 65  |

Table 6. The comparison of serum lipid parameters in patients with different genotypes of rs1433099. P <0.05 was considered statistically significant.
LDLR as a candidate gene for coronary heart disease has been studied extensively. As a result, in this study we choose LDLR as a candidate gene for research of susceptibility to SD. LDLR gene is located on the short arm of human chromosome 19 terminal (ie 19p13.3) and is composed of 18 exons and 17 introns, a total length of 45 Kb. LDLR polymorphisms are thought to affect the activity of the LDLR protein by altering its structure and subsequent function [10]. LDLR plays an important role in lipid metabolism and can be identified and combined with cholesterol specifically via apolipoprotein E and apolipoprotein B_{ox}, which decompose into bile acids, steroid, and vitamin D3 after being swallowed. 2/3 of normal human plasma low-density lipoprotein (LDL) is degraded in the LDL-R pathway, which can clear 65% -70% of LDL. Under long-term high cholesterol, macrophages are induced to swallow excess LDL and absorb the LDL cholesterol, causing cholesterol to be deposited in the phagocytes and become "foam" cells [11]. Low density lipoprotein of oxidative modification (OX-LDL) and cholesterol will affect the structure and function of artery intima. Some studies have shown that LDLR polymorphisms play a key role in regulating body's cholesterol and triglycerides (TG) concentrations [12]. Through many articles and reviews, Ashavaid found that LDLR gene polymorphism is closely related to lipid metabolism which has a strong correlation to early atherosclerosis plaque formation [13]. The blood supply of the inner ear is from the anterior inferior cerebellar artery, which is a terminal artery and has no collateral circulation. If some changes in blood lipid factors occur in the inner ear, dysfunction in microcirculation of cochlea and vestibular can easily occur. Blood lipid metabolism is closely linked to blood rheology and vascular disease. Lipids attached to the surface of the red blood cells and platelets, can reduce the ability of red blood cells to carry charges, enhancing the viscosity between cells and increase blood viscosity consequently slowing blood flow. An increase in cholesterol in the blood cell membrane components promotes cell sclerosis, weakens its ability to carry oxygen, and causes deformation, leading to hypoxia injury of capillary endothelial cell in inner ear. Triglyceride metabolites can damage the vascular endothelial cells directly, promote microthrombosis, reduce blood supply in the inner ear, and result in the onset of SD [14]. Chang et al. [15], found that SD of subjects with high cholesterol was 1.62 times than normal lipid objects, Cox proportional risk-adjusted return ratio of 1.6 (95% CI = 1.39 -1.85) by a retrospective cohort study. As a result, high cholesterol was considered a risk factor for the onset of SD and focus on hearing changes of objects with high cholesterol in clinical practice. X. Zhang found that there were 20.4% SD patients with hypertension, coronary artery disease, or diabetes, and 49.6% patients with hyperlipidemia [16]. When Chinchillas were fed a high cholesterol diet, visible lipid deposits in the edge layer of cochlear stria and outer hair cell histology, suggesting that high cholesterol can reduce cochlear blood vessels and cause hearing loss [17]. It was shown that lipid fluidity and sturdiness of the outer hair cells in the cochlea membrane are associated with cochlear potential amplification [18]. High cholesterol not only affects the blood supply to the inner ear, but also destroys the structure of outer hair cell, as result of affecting its activity.

This study is the first large-sample case-control association analysis study about the relationship between LDLR gene polymorphism with SD. SNP genotyping methods chose fluorescent multiplex ligation reaction (iMLDR) technology to ensure high accuracy and detection efficiency. It established dominance, additive, and recessive genetic models to analyze the association between the LDLR gene polymorphisms and SD, thus reducing false positive rates in case and control groups of genotype and allele frequencies results. By evaluating the relationship of 3 SNPs loci of LDLR gene and SD, the study found the frequencies of LDLR gene rs5929 T allele, rs2738464 locus G allele, rs1433099 T allele of SD group were significantly higher in the control group, and non-conditional logistic regression analysis showed that LDLR gene rs5929 T allele, rs2738464 locus G allele, rs1433099 T allele may be risk factor for onset of SD. Scholars have found that the rs1433099 polymorphism associated with cerebral infarction and T allele of this locus may increase the risk of cerebral infarction [19]. Our study is the first to discover the correlation between this site and SD and reflect the similar pathogenesis of SD and acute ischemic disease; rs5929 located in 13th exon of LDLR gene. By consideration of the relation of LDLR gene structure and function, the 7-14th exon is the second protein domain of encoding LDLR, EGF precursor homology domain, and it plays an important role in the fixed membrane protein, receptor binding ligand, the solution receptor of ligands in the lysosome, receptor synthesis and recycling. This region of SNP polymorphisms may affect LDLR activity or structure and will not only result in the reduced cholesterol and LDLR binding and intracellular swallow decomposition, but also increases the level of serum total cholesterol, one risk factor of SD [20]. The rs2738464 located in the 3’UTR region of LDLR gene. It belongs to transcriptional regulation which affects the stability of LDLR expression and protein synthesis structure, thereby affecting LDL via LDLR degradation pathway. Excessive plasma LDL was modified by oxidation to form OX-LDL. OX-LDL could cause sudden hearing loss by affecting the structure and function of the arterial intima and forming thrombosis in the microcirculation [21]. These positive SNPs loci are different from the existing positive SNPs loci of the LDLR gene which are associated with coronary heart disease, due to different pathogenesis of two diseases and the selection of the target population, or other factors. Therefore, individuals carrying these three alleles may be high-risk groups of SD. In this study, no association is found between serum lipid levels and different genotypes in rs5929, rs2738464 and rs1433099 loci for SD patients. It may be caused by the limited sample number and the sample imbalance on gender and age. In the future research, we need to increase the sample number and then explore the correlation between LDLR genotype and blood lipid level.

In this study, all subjects were from Han nationality, Shaanxi population, only representative of Han nationality of the northern population, indicating a need to extend the study to different regions, different ethnic groups, and the choice of a larger sample size for verification.

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