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REVIEW ARTICLE

The p.P240L variant of CDH23 and the risk of nonsyndromic hearing loss: a meta-analysis

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Abstract

Purpose We conducted a meta-analysis assessing the association between the p.P240L (c.C719T) variant and the risk of nonsyndromic hearing loss (NSHL).

Methods Literatures that reported prevalence rates were identified using PubMed, EMBASE, OVID, Cochrane library, Web of Science, Chinese National Knowledge Infrastructure, Wanfang Databases for the period from inception to August 2017. Random and fixed effects models were used to generate pooled ORs and I^2 values. The heterogeneity assumption decided the effect model.

Results A total of four relevant studies were included in the meta-analysis. The results of meta-analysis indicated that the p.P240L variant was correlated with the risk of NHSL in Asian populations (OR = 10.17, 95% CI = 2.74–37.82, $P = 0.001$). The T allele of p.P240L was associated with a 12-fold higher risk of NSHL than the C allele (OR = 11.68; 95% CI = 3.16–43.24, $P < 0.001$). Specifically, p.P240L heterozygotes (OR = 8.49; 95% CI = 2.28–31.59, $P = 0.001$), had a significantly higher risk of NSHL. Publication bias of our meta-analysis was attributed to the limited availability of relevant results and the number of studies included in our meta-analysis was relatively small.

Conclusions The p.P240L variant increased the risk of NHSL in Asian populations, suggesting a remarkable ethnic specificity linked with susceptibility to this mutation.

Keywords CDH23 · Hearing loss · Asian · Mutation

Introduction

There are 466 million persons in the world with disabling hearing loss (6.1% of the world's population) [1]. Hearing impairment can lead to inevitable problems, such as social isolations, depression, and a reduction in professional capabilities. Until now, there are hundreds of genes in which mutations cause hearing loss either as the sole clinical feature or in combination with extra-auditory manifestations as part of a syndrome [2]. In most cases, deafness is frequently caused by single gene mutations. Among them, CDH23 provides a striking example. As the DFNB12 locus-linked gene, CDH23 was identified which encoded cadherin 23. It was a non-conventional cadherin lacking the N-terminal tryptophan residue involved in canonical cadherin interactions. Additionally, mutations in the CDH23 gene was known to be responsible for Usher syndrome type 1D (USH1D). Affected individuals were manifested by severe to profound congenital hearing loss, vestibular dysfunction, and retinal degeneration beginning in childhood.

Deafness caused by CDH23 has been found in many populations worldwide, including African–American, Dutch, European, German, Pakistani, Turkish, and Japanese populations [3]. To date, a substantial amount of research has been devoted to elucidate the influence of this gene on the risk of NSHL, but conflicting results were obtained. Some studies reported that CDH23 mutations were related to prelingual-onset severe-to-profound hearing loss [4], while others suggested that the major form of CDH23-related hearing loss might be adult-onset progressive hearing loss [5]. Moreover, p.P240L (c.C719T) was likely to be the most common cause of CDH23-associated hearing loss in Asian populations. Astuto et al. reported that ~5% of recessive nonsyndromic hearing loss might be caused by mutation of CDH23 [3]. Thus, the frequency was suggested to be high. In previous

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research, such effects in the human genetic association were difficult to be detected due to small sample size of studies. To discuss the variable results, a further insight into the association between variants and hearing loss susceptibility was essential. In this study, we performed a meta-analysis from relevant studies published to determine whether CDH23 was an important causative gene for NSHL and then to comprehensively evaluate the role of p.P240L in the risk of NSHL.

Materials and methods

Search strategy

This study was conducted according to PRISMA guidelines (http://www.prisma-statement.org/). A structured literature search was performed consisting of relevant studies that were identified from the following electronic databases: PubMed, EMBASE, OVID, Cochrane library, Web of Science, Chinese National Knowledge Infrastructure (CNKI), Wanfang Databases up to August 2017 using various combinations of keywords including "CDH23", "cadherin 23", "DFNB12", "nonsyndromic hearing loss" and "case–control OR cohort studies". Only English language articles were included. The entire search was presented by two independent researchers. All the selected studies were retrieved, and their references were checked for other relevant publications. Review articles were also assessed to find additional eligible studies. As this was a study based on published articles instead of an original research, ethical approval was not needed.

Inclusion and exclusion criteria

The criteria of included studies were as follows: (1) studies were under case–control or cohort designs; (2) papers that investigated the association between CDH23 mutations and NSHL; (3) the study that reported genotypes or allelic data to estimate OR and 95% CI; (4) the control groups were people with normal hearing.

The exclusion criteria of studies were: (1) irrelevant to p.P240L variant; (2) irrelevant to our topic; (3) review; (4) duplicate data. All collected papers were scored and categorized according to the Newcastle–Ottawa Scale (NOS) for assessing the quality of studies [6].

Data extraction

Data extraction was independently presented with the standardized form by two authors. Disagreement was resolved by discussion between the two authors. The following information was extracted from each study: the first author, year of publication, ethnicity and number of genotypes in case and control subjects. The review process is showed in the flow chart (Fig. 1).

Data analysis

The combined OR which was reported under an allele model and 95% CI were used to estimate the strength of association between p.P240L mutant and NSHL. Cochrane χ^2 and I^2 values were used to evaluate the heterogeneity between studies. A P of heterogeneity (Ph) > 0.05 of a study indicated a lack of heterogeneity, and this study was analyzed using a fixed effects model; a Ph < 0.05 led to the use of a random effects model [7, 8]. Hardy–Weinberg equilibrium (HWE) in the control groups were assessed by Chi-square interval. It is not necessary to assess the potential publication bias when there are fewer than ten studies because the low number implies inherent weaknesses in the review. So we did not emphasize publication bias. Sensitivity analysis was performed by sequentially removing an individual study each time to check whether any single study could bias the overall estimate. Statistical analyses were performed using Stata version 13.0 (Stata Corporation, College Station, TX, USA).

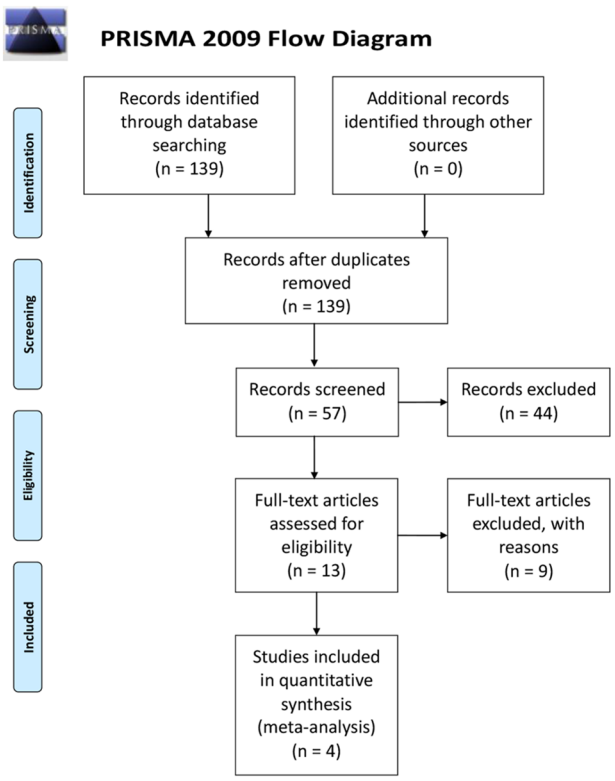

Fig. 1 PRISMA 2009 Flow Diagram PRISMA flow diagram with reasons for filtering articles

Table 1 NOS scale checklists

Relevant study	Selection	Comparability	Exposure
Wagatsuma et al. [9]	★★★	★	★★
Miyagawa et al. [10]	★★★	★	★★
Mutai et al. [11]	★★	★	★★
Kim et al. [12]	★★★★	★★	★★

Results

Study characteristics

There were 139 papers relevant to the keywords through searching the potential studies (Fig. 1). After examining abstracts, 126 papers were excluded. Of these, 13 papers were preliminarily selected for further identification of full review. Those articles were excluded for the following reasons: not cohort or case–control studies, duplicated reports, and not related to CDH23 mutations. Consequently, a total of four studies comprising 1624 cases with NSHL and 2793 controls were subjected to our meta-analysis [9–12]. Each study was evaluated with a high score (not less than 5) and the result was shown in Table 1. All the included studies were consistent with the Hardy–Weinberg Equilibrium test, indicating a good representation of control population.

Meta-analysis of overall data

A database, including first author, published year, background, sample size of case and control and genotyping method was established according to the extracted information (Table 2). Genotype, allele distributions, as well as HWE for each study are shown in Table 3.

Overall, there was a significant association between the p.P240L variant and increased risk of NSHL (Fig. 2). Specifically, the T allele of p.P240L was associated with a 12-fold higher risk of NSHL than the C allele (Fig. 2a, OR = 11.68; 95% CI = 3.16–43.24; *P*h = 0.088; $I^2 = 54.1\%$, $P < 0.001$). The pooled OR was calculated using fixed effects model, and forest plot on the association of mutation carriers with the risk of NSHL was fulfilled (Fig. 2b, OR = 10.17, 95% CI = 2.74–37.82; *P*h = 0.070; $I^2 = 57.5\%$, $P = 0.001$). Moreover, compared with individuals with wild type, p.P240L heterozygotes (Fig. 2c; OR = 8.49; 95% CI = 2.28–31.59; *P*h = 0.051; $I^2 = 61.3\%$, $P = 0.001$), had a significantly higher risk of NSHL.

Sensitivity analysis, after removing one study at a time, was performed to evaluate the stability of the results. For the p.P240L mutation, when successively excluded one study (Fig. 3), the estimated pooled odd ratio has changed because of the sample size. The pooled odd ratio became even larger. Thus, sensitivity analysis indicated that our results were reliable.

Discussion

The incidence of prelingual hearing loss is about 1 in 1000 newborns in Western Europe. At least half of these cases are genetically determined. The nonsyndromic forms of hearing loss account for 70% of these cases, of which about 85% are autosomal recessively inherited [13, 14]. Genetic heterogeneity and the small size of families with recessive hearing loss have hindered the unravelling of the genetic causes. Consequently, a quantitative synthesis to accumulate data from different studies may provide evidence on the association of genetic variants with susceptibility to NSHL.

Table 2 Characteristics of the studies included in the meta-analysis

References	Year	Country	Survey age	Case	Control	Genotyping
Wagatsuma et al. [9]	2007	Japan	All	64	146	PCR
Miyagawa et al. [10]	2012	Japan	All	1396	192	PCR
Mutai et al. [11]	2013	Japan	All	36	2183	NGS; PCR
Kim et al. [12]	2015	Korea	Children	128	272	PCR

Table 3 Distribution of the p.P240L variant genotype and allele among NSHL patients and controls

Relevant study	Case					Control					HWE	
	CC	C/T	TT	C	T	CC	C/T	TT	C	T	χ^2	*P*
Wagatsuma et al. [9]	60	4	0	124	4	145	1	0	291	1	0.0017	0.97
Miyagawa et al. [10]	1358	31	7	2747	45	191	1	0	383	1	0.0013	0.97
Mutai et al. [11]	33	3	0	69	3	2182	1	0	4365	1	0.0001	0.99
Kim et al. [12]	124	2	2	250	6	272	0	0	544	0	NA	NA

NA not available

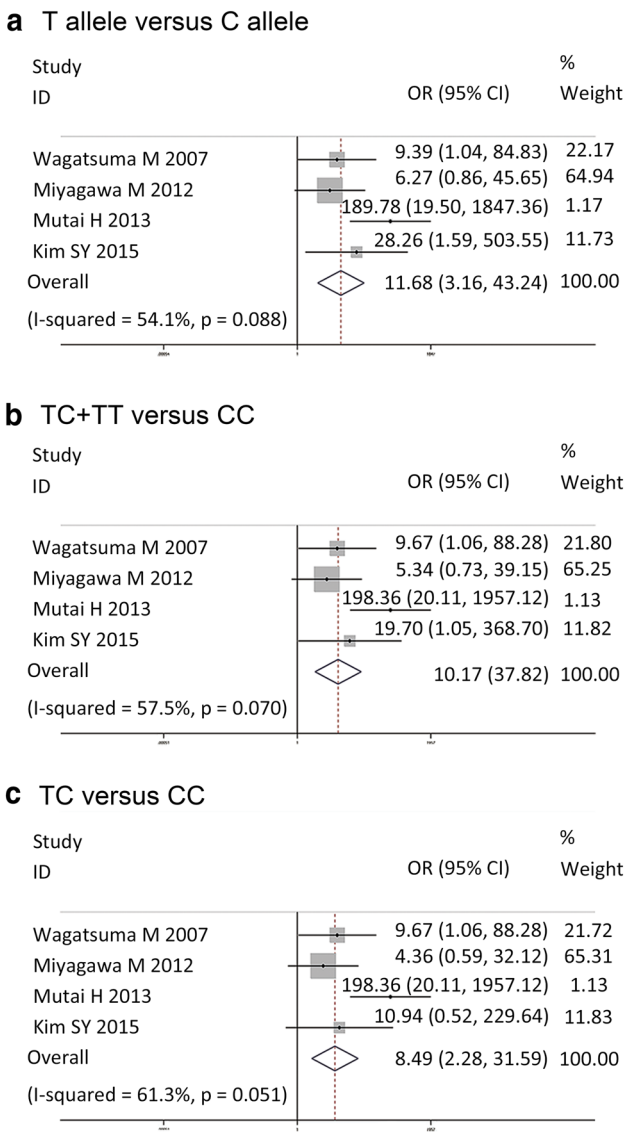

Fig. 2 Forest plots of the effects of p.P240L on NSHL risk. **a** Allelic model referred to T allele versus C allele. **b** Dominant model referred to TT + TC genotype versus CC genotype. **c** Codominant model referred to CT genotype versus CC genotype

CDH23 mutations have been associated with prelingual severe-to-profound sensorineural hearing loss (SNHL) in either syndromic or nonsyndromic pattern. The product of CDH23 has similarity to the well-characterized protein E-cadherin; the EC repeats of E-cadherin are responsible for homophilic cell–cell adhesion, which is established by the formation of parallel stable EC-domain dimers at the same cell surface and at two opposing cell surfaces. This stability is due to interdomain rigidification of the EC domains, achieved by the binding of three calcium ions to highly conserved peptide sequences (LDRE, DXNDN, and DXD) [15]. Thus, the importance of CDH23 as a deafness gene has increasingly been recognized. Several

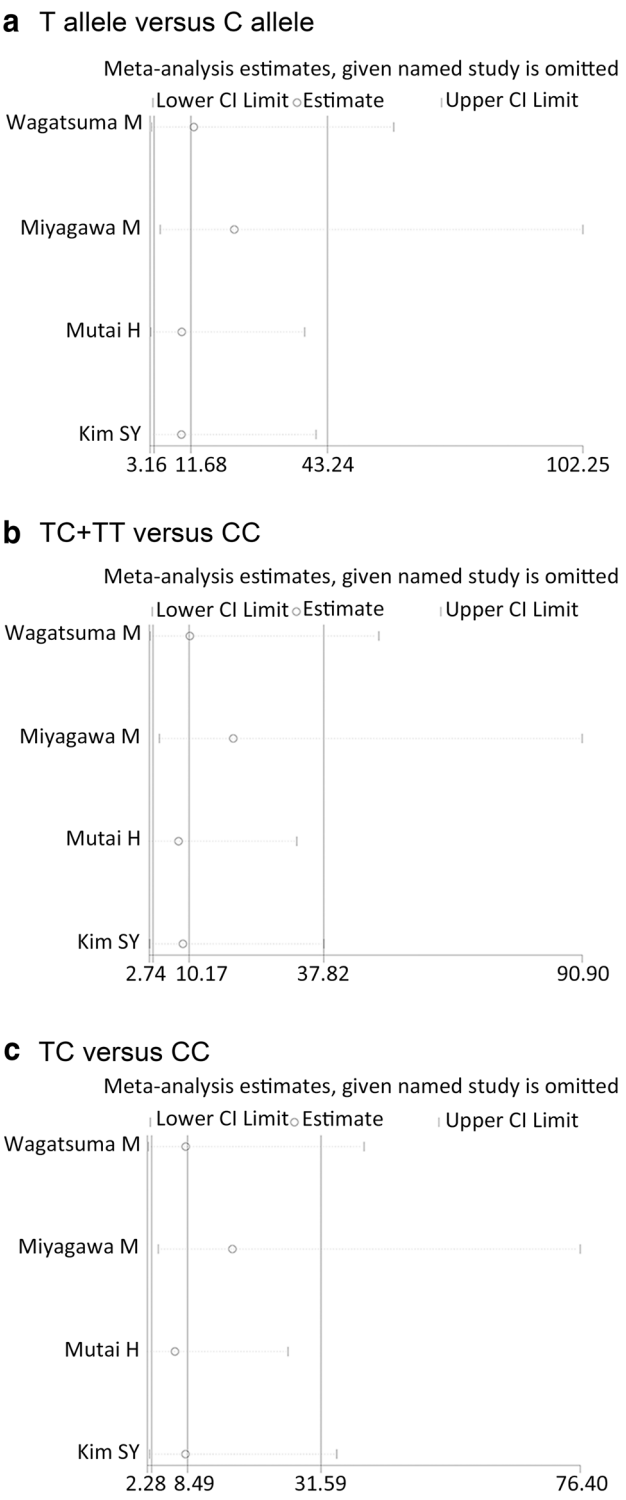

Fig. 3 Sensitivity analyses of the effects of p.P240L on NSHL risk. **a** Allelic model referred to T allele versus *C* allele. **b** Dominant model referred to TT + TC genotype versus CC genotype. **c** Codominant model referred to CT genotype versus CC genotype

CDH23 mutations have previously been reported in different populations, respectively [16–29]. In the Japanese study, CDH23 mutations were reported to be frequent after GJB2 and SLC26A4 in children and adults with hearing impairment [10]. Recently, CDH23 mutations have been reported in the Korean deaf population [28, 29] and genetic loads of CDH23 and its implications in the Korean pediatric population have also recently been reported [12]. Accordingly, it is an interesting question whether p.P240L is frequent because of mutational hot spot in Asian.

We performed a meta-analysis to analyze the association between NSHL risk factor and the p.P240L variant in the CDH23 gene. We combined 4 studies of 1624 patients and 2793 controls, and observed that the T allele carriers of p.P240L was generally associated with a 12-fold higher risk of NSHL than the C allele (Fig. 2a; OR = 11.68; 95% CI = 3.16–43.24; *P*h = 0.088; $I^2 = 54.1\%$). The results indicated that the mutant was significantly associated with NSHL risk. The assessment of the quality of the studies using NOS indicated all articles were of high quality. Moderate or even low heterogeneity performed across the studies indicated that the association assessed in this meta-analysis is statistically reliable.

Concerning these findings, the frequency of p.P240L mutant in Japanese and Korean may be representative of those in Eastern Asian populations. In addition, some other certain mutations of CDH23 occurred frequently in patients with Usher Syndrome. For example, P1502X and the IVS4 + 1G → A should be the first two mutations screened from a Swedish background [3]. It has also been known that prevalent GJB2 mutations are highly ethnic-specific (see The connexin-deafness homepage; http://davinci.crg.es/deafness/): c.35delG is common in the Caucasoid population, c.167delT is reported as prevalent in Ashkenazi Jews, p.R143W in a restricted village in Africa, and c.235delC in East Asian populations. Thus, p.P240L is elucidated to perform a found effect by a series of studies. Finally, future research will prove whether detection of this variant can facilitate the molecular diagnosis of NSHL.

By increasing the sample size, we intended to improve statistical power and obtain more compelling results. However, some inevitable limitations were still noticed. First, a lack of further adjustments for other risk factors, such as environmental agent, co-variables, and potential gene-environment interactions might bias the present results. Second, the p.P240L homozygotes were reported to cause prelingual severe-to-profound SNHL in a majority of cases [4, 10, 12]. However, audiological phenotypes of compound heterozygotes that carried one p.P240L allele seemed to be highly variable [10]. Third, because of the availability of publication, the number of studies included was relatively small. Additionally, more studies will be expected to evaluate the risk in different ethnic groups. Based on these, we might confirm that CDH23 could be an important cause for NSHL and should be borne in mind next to GJB2 or SLC26A4 screening.

In conclusion, we conducted a meta-analysis of the association of NSHL risk with genetic variants in CDH23. The results based on current evidence from all relevant studies indicated that the p.P240L variant increased the risk of NSHL in East Asian populations. Further replication studies in distinct populations are required to confirm whether p.P240L has a found effect in other origins. In addition, more research on the genetic mechanism of the NSHL susceptibility related will be carried out in our future work.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. There was no evaluation or vote of an Ethical Committee necessary because this study was designed as a meta-analysis.

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