Original Article
RET gene polymorphism is associated with hirschsprung disease: a meta-analysis

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Abstract: The association of RET rs1800858 polymorphism and Hirschsprung Disease (HSCR) risk was investigated by some epidemiological studies, however, the study results were contradictory. To derive a more precise estimate of the association, we conducted a meta-analysis. ORs with 95% CI were used to assess the strength of association between RET rs1800858 polymorphism and HSCR risk in fixed or random effect model. A total of 9 studies with 3825 subjects were identified. RET rs1800858 polymorphism was associated with a significantly increased risk of HSCR (OR = 5.24; 95% CI, 4.49-6.11; P<0.00001). In the race subgroup analysis, both Asians (OR = 4.82; 95% CI, 4.24-5.48; P<0.00001) and Caucasians (OR = 6.56; 95% CI, 5.26-8.18; P<0.00001) with RET rs1800858 polymorphism had increased HSCR risk. Sensitivity analysis suggested that the result of this meta-analysis was statistically stable. The shape of funnel plots and Egger’s test revealed that there was no statistical significance for evaluation of publication bias. In conclusion, this meta-analysis suggested that RET rs1800858 polymorphism might be associated with the risk of HSCR.

Keywords: Hirschsprung disease, RET, meta-analysis, polymorphism

Introduction
Hirschsprung Disease (HSCR) is a congenital neuropathy, characterised by the absence of enteric ganglion (aganglionosis) and impaired peristaltic movement along variable lengths of distal intestine. HSCR patients develop constipation, diarrhoea, vomiting and sometimes life-threatening colon complications such as enterocolitis. Therefore, further investigation into the molecular pathogenesis of HSCR and the consequential development of novel therapeutics are needed.

The RET (Rearranged during Transfection) is a gene consisting of 21 exons and located on chromosome 10q11.2. The RET gene encodes a transmembrane tyrosine kinase receptor. It plays an important role in the development, proliferation and differentiation of neuroendocrine cells. Inactivating germline RET mutations are found in about 15-20% of sporadic HSCR cases [1].

Published data has shown inconsistent findings about the association of RET rs1800858 polymorphism with the risk of HSCR [2-9]. This meta-analysis quantitatively assesses the results from published studies to provide a more precise estimate of the association between RET rs1800858 polymorphism as a possible predictor of the risk of HSCR.

Methods

Publication search

We performed a search of the literature to identify all studies that evaluated the association between RET variants and the risk of HSCR, using the following electronic databases: PubMed, Excerpta Medica Database (EMBASE), Wanfang and Chinese National Knowledge Infrastructure (CNKI). The search terms we used were as follows: (“Hirschsprung Disease” or HSCR) and genetic and RET. No language restriction was applied. The references of all studies included in the search were also checked to yield further eligible studies. If more than one study reported on a particular population, only the latest or the most complete study
was included. Additionally, when a study reported different subpopulation results, we identified each subpopulation as a separate study.

**Inclusion and exclusion criteria**

Studies were included if they met the following criteria: (1) case-control study; (2) about the associations between RETS rs1800858 polymorphism and HSCR risk; and (3) had available genotype frequencies of cases and controls or could be calculated from the paper. Accordingly, the exclusion criteria were (1) duplicate data, (2) only for HSCR samples, (3) for other disease compared with controls, and (4) number of the cases less than 30.

**Data extraction**

The following data were recorded from each article: first author, years of publication, country, ethnicity, gender, numbers of subjects, and

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**Table 1. Characteristics of the included studies**

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Gender</th>
<th>Total number (n)</th>
<th>Hardy-Weinberg equilibrium</th>
<th>Newcastle-Ottawa scale</th>
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</thead>
<tbody>
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<td>Fitze</td>
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<td>German</td>
<td>Caucasian</td>
<td>Mixed</td>
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<td>Yes</td>
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<tr>
<td>Burzynski</td>
<td>2004</td>
<td>Netherlander</td>
<td>Caucasian</td>
<td>Mixed</td>
<td>231</td>
<td>Yes</td>
<td>6</td>
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<tr>
<td>Garcia-Barcelo</td>
<td>2005</td>
<td>China</td>
<td>Asian</td>
<td>Mixed</td>
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<tr>
<td>Liu</td>
<td>2010</td>
<td>China</td>
<td>Asian</td>
<td>Mixed</td>
<td>373</td>
<td>Yes</td>
<td>8</td>
</tr>
<tr>
<td>Tou</td>
<td>2011</td>
<td>China</td>
<td>Asian</td>
<td>Mixed</td>
<td>291</td>
<td>Yes</td>
<td>6</td>
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<tr>
<td>Ngo 1</td>
<td>2012</td>
<td>Vietnam</td>
<td>Asian</td>
<td>Mixed</td>
<td>471</td>
<td>Yes</td>
<td>8</td>
</tr>
<tr>
<td>Ngo 2</td>
<td>2012</td>
<td>China</td>
<td>Asian</td>
<td>Mixed</td>
<td>971</td>
<td>Yes</td>
<td>8</td>
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<tr>
<td>Kim</td>
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<td>Korea</td>
<td>Asian</td>
<td>Mixed</td>
<td>555</td>
<td>Yes</td>
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</tr>
<tr>
<td>Vaclavikova</td>
<td>2014</td>
<td>Czech</td>
<td>Caucasian</td>
<td>Mixed</td>
<td>367</td>
<td>Yes</td>
<td>9</td>
</tr>
</tbody>
</table>
RET and hirschsprung disease

**Table 2. Meta-analysis results and subgroup analyses**

<table>
<thead>
<tr>
<th></th>
<th>( P_{\text{heterogeneity}} )</th>
<th>Model</th>
<th>( OR ) (95% CI)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.08</td>
<td>R</td>
<td>5.24 (4.49-6.11)</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Asian</td>
<td>0.13</td>
<td>F</td>
<td>4.82 (4.24-5.48)</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Caucasian</td>
<td>0.95</td>
<td>F</td>
<td>6.56 (5.26-8.18)</td>
<td>&lt;0.000001</td>
</tr>
</tbody>
</table>

R, random effects model; F, fixed effects model.

**Quality assessment**

The quality of selected studies was independently evaluated on basis of Newcastle-Ottawa scale (NOS). Studies with six or more stars were considered as high quality.

**Statistical analysis**

The strength of association between RET rs1800858 polymorphism and HSCR risk was estimated by OR with corresponding 95% CI. HWE was assessed by using \( \chi^2 \) test to compare expected and actual genotype frequencies among controls of each study. Q-statistic was applied to investigate heterogeneity among studies. \( P \)-value greater than 0.1 for Q test suggested a lack of statistically significant heterogeneity, and the fixed-effect model (Mantel-Haenszel method) was used to calculate pooled ORs. Otherwise, heterogeneity was present and the random-effect model (DerSimonian-Laird method) was more appropriate. In addition, the \( I^2 \)-test was employed to accurately measure the degree of heterogeneity. Furthermore, the \( I^2 \)-value less than 25% was equivalent to mild heterogeneity, and values between 25% and 50% was equivalent to moderate heterogeneity, whereas values greater than 50% was equivalent to large heterogeneity among studies. Potential publication bias was estimated by symmetry of funnel plot of OR versus the standard error of log (OR) and the visual symmetrical plot indicated that there was no publication bias among studies. Sensitivity analyses were conducted to assess the robustness of the results by eliminating each study in turn to show whether the individual data set influenced the pooled OR. Stratified analyses were conducted in terms of ethnicity. All statistical tests in this meta-analysis were two-tailed and \( P \)-value \( \leq 0.05 \) was considered statistically significant unless otherwise noted. All statistical analyses
were performed with Stata 11 software (Stata Corporation, College Station, Texas).

Results

Study characteristics

In this study, we searched 147 related references. When removing the duplicates and other unrelated references, 8 references met our inclusion criteria and were recruited in the meta-analysis (Figure 1). The information of the included studies was listed in Table 1. There were 3825 subjects. All studies received a score of more than or equal to 6, indicating good quality.

Results of meta-analysis

The results of the association between RET rs1800858 polymorphism and HSCR risk are summarized in Table 2. RET rs1800858 polymorphism was associated with a significantly increased risk of HSCRs (OR = 5.24; 95% CI, 4.49-6.11; P<0.00001; Figure 2). In the race subgroup analysis, both Asians (OR = 4.82; 95% CI, 4.24-5.48; P<0.00001) and Caucasians (OR = 6.56; 95% CI, 5.26-8.18; P<0.00001) with RET rs1800858 polymorphism had increased HSCR risk. Sensitivity analyses were conducted to assess the robustness of the results by eliminating each study in turn and all the results were not essentially altered, suggesting that the results of our meta-analysis were statistically stable (Figure 3). The shape of funnel plots did not indicate any evidence of funnel plot asymmetry (Figure 4). Egger’s test revealed that there was no statistical significance for evaluation of publication bias (P = 0.754).

Discussion

The current study used a comprehensive meta-analysis to reveal an association of RET rs1800858 polymorphism and HSCR susceptibility. We found that individuals with RET rs1800858 polymorphism showed an increased risk of HSCR in the overall population. In the stratified analysis by ethnicity, the significant association was found in Asians and Caucasians.

RET has been extensively studied in HSCR patients and over 100 mutations have been identified along the gene. However, mutations in the RET coding sequence (CDS) account for only up to 50% or 7-20% of familial and sporadic cases, respectively [1]. Ohgami et al. suggested that impaired phosphorylation of c-Ret at tyrosine 1062 causes HSCR-linked syndromic congenital deafness in c-Ret knockin (KI) mice [10]. Carniti et al. found that the Ret (C620R) allele is responsible for HSCR and affects the development of kidneys and the enteric nervous system [11].

The meta-analysis had several limitations that should be taken into account. First, there was heterogeneity for the outcome of the association between RET rs1800858 polymorphism and HSCR susceptibility. Although we reduced the degree of heterogeneity by stratified analyses based on ethnicity, other sources of heterogeneity were not verified, such as different genotyping methods. Second, the sample size was relatively small. Therefore, there was insufficient statistical power to demonstrate the association. Third, the meta-analysis was based on unadjusted risk estimates for confounding factors not provided by all of the studies. Thus, the existence of effect modifiers may have produced the large heterogeneity between studies, leading to bias.

In conclusion, this meta-analysis suggested that RET rs1800858 polymorphism might be associated with the risk of HSCR.
Disclosure of conflict of interest

None.

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References


