Hirschsprung disease (HSCR) is a congenital malformation of the hindgut produced by a disruption in the neural crest cells (NCC) migration during embryonic development. This disorder results in an absence of intramural ganglion cells in the submucosal and myenteric plexuses producing a functional intestinal obstruction [1]. HSCR is classified, according to the hindgut produced by a disruption in the neural crest cells (NCC) migration during embryonic development. This disorder results in an absence of intramural ganglion cells in the submucosal and myenteric plexuses producing a functional intestinal obstruction [1]. HSCR is classified, according to the}

Introduction

Hirschsprung disease (HSCR) is a congenital malformation of the hindgut produced by a disruption in the neural crest cells (NCC) migration during embryonic development. This disorder results in an absence of intramural ganglion cells in the submucosal and myenteric plexuses producing a functional intestinal obstruction [1]. HSCR is classified, according to the extent of aganglionosis, into long-segment (L-HSCR, 20% of affected individuals) and short-segment (S-HSCR, 80%) forms, each with distinct genetic characteristics [2]. The incidence of this disease is generally 1 per 5000 of live births, with males about 3.5–7.8 times more likely to be affected than females [3] and it usually presents in infancy, although some patients present with persistent, severe constipation later in life. There are also differences among races, with a higher incidence in Asians at 2.8 per 10,000 of live births [4]. Besides, HSCR can be either familial or sporadic.
absence of enteric ganglia, a feature which is reminiscent of HSCR [7][9].

RET locates in 10q11.2 and is composed of 21 exons. Three common SNPs in the coding region of RET, c135G>A (rs1800858, A45A), c1296X>G (rs1800860, A432A) and c2307T>G (rs1000061, I769L) lie in exon2, exon7 and exon13 respectively [5,9,10].-1A>C (rs10900297) locates in the promoter region of RET [11]. R2435357 has been proven to lie in the enhancer-like sequence within intron 1 of the RET-protooncogene [3]. The PHOX2B maps to chromosome 4p12, encoding 314 homeodomain protein of amino acids. One common SNP, IVS2+100A>G (rs28647582) lies in intron 2 of PHOX2B [8]. Several case-control studies have investigated the association between these gene polymorphisms and Hirschsprung’s disease risk, but the result is still not clear due to the inconsistency among these studies. The cause of this result may be due to sparseness of data, ethnic difference, different designs and publication bias.

Meta-analysis has the advantage of reducing the risk of random error and obtaining a precise estimation for the major effect by combining data from all eligible studies [12]. Besides, to our knowledge, there were no quantitative reviews of the literature on combining data from all eligible studies [12]. We conducted a meta-analysis of all available published case-control studies to verify the precise associations.

**Materials and Methods**

**Identification and eligibility of relevant Studies**

We conducted a comprehensive literature search in Pubmed, Elsevier Science Direct, China National Knowledge Infrastructure database, Chinese Biomedical database and Google scholar from January 2003 to December 2012 using the following search terms: hirschsprung disease, hirschsprung’s disease; polymorphism, genetic; RET; and PHOX2B. Moreover, the references of the selected papers were also checked by hand-search for other potential articles that possibly have been missed in the initial search. Only papers in Chinese and English were included.

**Inclusion and exclusion criteria**

The inclusion and exclusion criteria were drew up on the basis of the discussion studies. Studies eligible for this meta-analysis had to fulfill (1) the design type of study was a case-control study; (2) the study had examined the associations between the RET, PHOX2B gene polymorphisms and HSCR; (3) the frequencies of genotypes in case and control groups could be collected; (4) controls derived from a population within the same geographic area and ethnic background as HSCR cases. The exclusion criteria were as follows: (1) researches that did not meet the inclusion criteria; (2) the study reported useless or duplicated data.

**Data extraction**

All of the data were extracted independently by two reviewers (Chun-mei Liang and Dong-mei Ji) according to the prespecified selection criteria. Potential disagreements were resolved by consensus. The following characteristics were extracted: name of first author, year of publication, racial ancestry of the study participants, genotypes and sample size, the polymorphisms investigated in the studies, the genotyping method, type of study.

**Statistical analyses**

Allele frequencies of the RET and PHOX2B SNPs at genetic polymorphisms from the respective studies were determined by the allele counting method. All the statistical analyses were performed by Stata version 11.0 (Stata-Corp, College Station, TX). We assessed Hardy–Weinberg equilibrium (HWE) for the controls in each study by the Chi-square test. The odds ratio (OR) and its 95% confidence interval (95% CI) were estimated for each study by fixed or random effect model. Heterogeneity among studies was measured using the Chi-square based Q statistic [13]. We also quantified the effect of heterogeneity using I² statistic which measures the severe degree of heterogeneity. I² value ranges from 0 to 100% (I² = 0–25%, no heterogeneity; I² = 25–50%, moderate heterogeneity; I² = 50–75%, large heterogeneity; I² = 75–100%, extreme heterogeneity) [14]. If there was a statistical difference in terms of heterogeneity, a random effect model was selected to combine the data. Otherwise, a fixed effect model was used. Visual inspection of asymmetry in funnel plots was conducted. Beggger’s rank correlation method was used to statistically assess the publication bias [P<0.05 was considered to be representative of statistically significant publication bias].

**Main results of meta-analysis**

The process of selecting studies was showed in Figure 1. For RET gene, a total of 96 papers were identified after an initial search,11 of which were published in Chinese. Based on the exclusion and inclusion criteria in original manuscript, after reading the titles or abstracts, 11 papers were excluded for not relevant to these gene polymorphisms with HSCR risks; 5 were reviews; 46 were not about polymorphisms. After reading full texts of the remaining 34 papers, 5 did not contain a control group; 5 were excluded for not relevant to the loci2 had a duplication of data. Thus, 22 articles were left for data extraction. Of these, 6 studies were excluded owing to the absence of sufficient genotype frequencies. Finally, 16 separate studies including 1,527 cases and 1,963 controls were considered in the current meta-analysis. Among these studies, there were five SNPs discussed in this meta-analysis (rs1800858, rs1800860, rs1800861, rs10900297, rs2435357). The controls of studies [15–17] for rs1800858 and the controls of studies [15] for rs1800861 were not conformed to HWE (Table 1). The characteristics of the studies that investigated the association of RET gene polymorphisms with HSCR were showed in Table 1. For PHOX2B gene, a total of 16 papers were identified after an initial search, 3 were published in Chinese. Based on the exclusion and inclusion criteria mentioned above, 3 papers were excluded for the irrelevance of the gene polymorphisms with HSCR risks; 1 was review; 5 were not about polymorphisms, 1 had a duplication of data. Then, 6 articles were left for data extraction. Of these, 2 studies were excluded owing to the absence of sufficient genotype frequencies. Finally, 4 separate studies including 372 cases and 511 controls were considered in this meta-analysis. The characteristics of the four studies were showed in Table 2. The controls of studies [8] for rs28647582 was not conformed to HWE (Table 2).

**rs 28647582 polymorphism**

Totally, there were 4 studies [7,8,18,19] including 372 cases and 511 controls inspecting the correlation between rs 28647582 and HSCR risk. The comparisons of all genotypes did not detect any statistical association. The results were showed in Table 3 and supplement S2.

**rs1800858 polymorphism**

In total, we identified nine studies [3,15–17,20–24] including 863 cases and 1,118 controls examining the relation between rs1800858 and HSCR risk. We found a prominent association of rs1800858 gene polymorphism with HSCR. The results were presented in Table 3 and supplement S2.
On the basis of three studies [20,21,25] involving 328 cases and 396 controls, an association was not observed only in the GG/GA versus AA genotype. The detailed results were listed in Table 3 and supplement S2.

Six studies [3,15,21,24–26] including 571 cases and 730 controls researched the role of rs1800861 polymorphism in the occurrence of HSCR. The comparisons of all genotypes were detected for

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**Figure 1. Process of selecting studies about RET and PHOX2B gene.**

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**rs1800860 polymorphism**

On the basis of three studies [20,21,25] involving 328 cases and 396 controls, an association was not observed only in the GG/GA versus AA genotype. The detailed results were listed in Table 3 and supplement S2.

**rs1800861 polymorphism**

Six studies [3,15,21,24–26] including 571 cases and 730 controls researched the role of rs1800861 polymorphism in the occurrence of HSCR. The comparisons of all genotypes were detected for
The results were listed in Table 3 and supplement S2.

**rs10900297 polymorphism**

Totally, five studies [21,22,24,27,28] which contained 742 cases and 954 controls investigated the possible effect of rs10900297 polymorphism on the development of HSCR. We also successfully obtained a significant association of rs10900297 gene polymorphism with HSCR. The results were showed in Table 2 and supplement S2.

**rs2435357 polymorphism**

In all, five studies [3,27,29–31] including 566 cases and 719 controls assessed the association between rs2435357 polymorphism and HSCR. Amongst them, two studies [3,29] were conducted in European population. We found a very significant

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### Table 1. Characteristics of studies included in this meta-analysis about RET gene.

| First author      | Year | Population | Race | Cases/controls | Genotype polymorphism | Genotype method | Type of study | HWE  |
|------------------|------|------------|------|----------------|-----------------------|----------------|--------------|------|
| Phusantisampan    | 2012 | Thai       | Asia | 68/120         | rs1800858(G>A)        | PCR–RFLP       | Hospital-based | 0.12 |
|                  |      |            |      |                | rs1800861(T>G)       | TaqMan         |              | 0.77 |
|                  |      |            |      |                | rs2435357(T>C)       | TaqMan         |              | 0.40 |
| Liu              | 2010 | Chinese    | Asia | 125/148        | rs1800858(G>A)       | PCR            | Hospital-based | 0.79 |
|                  |      |            |      |                | rs1800860(A>G)       | PCR            |              | 0.86 |
|                  |      |            |      |                | rs1800860(A>G)       | PCR            |              | 0.81 |
|                  |      |            |      |                | rs1800861(T>G)       | PCR            |              | 0.09 |
|                  |      |            |      |                | rs10900297(A>C)      | PCR            |              | 0.77 |
| Miao             | 2010 | Chinese    | Asia | 315/352        | rs10900297(A>C)      | PCR            | Hospital-based | 0.99 |
|                  |      |            |      |                | rs2435357(T>C)       | PCR            |              | 0.39 |
| Fitze            | 2003 | German     | Europe| 80/120        | rs1800858(G>A)       | NA             | Hospital-based | 0.90 |
|                  |      |            |      |                | rs10900297(A>C)      | PCR            |              | 0.97 |
| Burzynski        | 2004 | Netherlander| Europe| 105/126       | rs1800858(G>A)       | NA             | Hospital-based | 0.24 |
|                  |      |            |      |                | rs1800861(T>G)       | PCR-RFLP       |              | 0.06 |
| Sadeva           | 2008 | Indonesians| Asia | 34/46          | rs1800861(T>G)       | PCR-RFLP       |              | 0.54 |
|                  |      |            |      |                | rs1800861(T>G)       | PCR-RFLP       |              | 0.08 |
|                  |      |            |      |                | rs10900297(A>C)      | PCR            |              | 0.36 |
| Li               | 2011 | Chinese    | Asia | 80/80          | rs1800860(A>G)       | PCR            | Hospital-based | 0.29 |
|                  |      |            |      |                | rs1800861(T>G)       | PCR            |              | 0.12 |
| Du               | 2006 | Chinese    | Asia | 94/122         | rs1800858(G>A)       | PCR            | Hospital-based | <0.01 |
|                  |      |            |      |                | rs1800861(T>G)       | PCR            |              | <0.01 |
| Zhang            | 2005 | Chinese    | Asia | 16/40          | rs1800858(G>A)       | PCR            | Hospital-based | <0.05 |
| Zhao             | 2012 | Chinese    | Asia | 80/80          | rs1800858(G>A)       | PCR-HRM        | Hospital-based | <0.01 |
| Wang             | 2006 | Chinese    | Asia | 52/120         | rs10900297(A>C)      | PCR            | Hospital-based | 0.41 |
| Arnold           | 2008 | Caucasian  | Europe| 62/30         | rs2435357(T>C)       | TaqMan         | Hospital-based | 0.66 |
| Pini Prato       | 2009 | Italian    | Europe| 22/85         | rs2435357(T>C)       | PCR            | Hospital-based | 0.44 |
| Zhang            | 2007 | Chinese    | Asia | 99/132         | rs2435357(T>C)       | PCR            | Hospital-based | 0.54 |

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### Table 2. Characteristics of studies included in this meta-analysis about PHOX2B gene.

| First author      | Year | Population | Race | Cases/controls | Genotype polymorphism | Genotype method | Type of study | HWE  |
|------------------|------|------------|------|----------------|-----------------------|----------------|--------------|------|
| Garcia-Barcelo    | 2003 | Chinese    | Asia | 91/71          | rs28647582(A>G)       | PCR            | Hospital-based | 0.07 |
| Liu              | 2009 | Chinese    | Asia | 100/96         | rs28647582(A>G)       | PCR            | Hospital-based | <0.05 |
| Dou              | 2007 | Chinese    | Asia | 123/194        | rs28647582(A>G)       | PCR            | Hospital-based | 0.09 |
| Xiao             | 2009 | Chinese    | Asia | 58/150         | rs28647582(A>G)       | PCR            | Hospital-based | 0.54 |

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association of rs2435357 gene polymorphism with HSCR. The detailed results were showed in Table 3 and supplement S2.

Subgroup analyses
Moreover, we performed subgroup analyses by the race. The detailed results were showed in Table 4. Seven studies [3,15–17,20,21,24] were conducted in Asia and Two [22,23] were in Europe for rs1800858 polymorphism. When stratified by race, the results remained statistical significant. For other SNPs, we were not able to stratify with insufficient information of subgroup.

Sensitivity Analysis
Although the distribution of genotypes in the controls in some studies did not follow HWE, the corresponding pooled OR and between-study heterogeneity were not significant altered without these studies for rs1800858. (AA vs. GG: OR = 8.56, 95% CI = 2.47–29.71, \( P_{\text{heterogeneity}} = 0.000 \); AA vs. AG/GG: OR = 5.91, 95% CI = 2.55–13.69, \( P_{\text{heterogeneity}} = 0.000 \); AA/AG vs. GG: OR = 3.38, 95% CI = 1.46–8.81, \( P_{\text{heterogeneity}} = 0.000 \); A vs. G: OR = 3.46, 95% CI = 1.73–6.95, \( P_{\text{heterogeneity}} = 0.000 \). However, sensitivity analysis showed that the studies by Du et al was the main origin of heterogeneity for rs1800858. The heterogeneity significantly decreased when this study was excluded (\( P_{\text{heterogeneity}} = 0.10 \) for GG vs. TT), while the value of pooled OR was not significantly altered without this studies (GG vs. TT:OR = 7.41, 95% CI = 4.89–11.23; (Supplement S4)

Publication bias
Both Begg’s funnel plot and Egger’s test were performed to assess the publication bias of the studies. The shape of funnel plots of all contrasts models was symmetrical, and \( P \) values of Egger’s tests were more than 0.05, providing statistical evidences of funnel plots’ symmetry. The results of Egger’s test suggested no publication bias (Table 3 and Supplement S3).

Discussion
Hirschsprung disease (HSCR), a congenital malformation characterized by intestinal obstruction and colonic distension in

Table 3. Main results of the meta-analysis.

| Gene polymorphism | Number of studies | Test of association | Test of heterogeneity | Publication bias |
|-------------------|-------------------|--------------------|-----------------------|-----------------|
|                   | Comparison        | OR (95%CI)         | \( P \) value | \( Q \)     | \( P \) value | \( I^2 \)% | \( P \) value (Begg’s) |
| rs28647582        | GG vs AA          | 2.05 (0.94–4.48)   | 0.07 | 3.63 | 0.305 | 17.3 | 0.497 |
|                   | GG vs GA+AA       | 2.14 (0.98–4.69)   | 0.06 | 3.12 | 0.373 | 4.0  | 1.000 |
|                   | GG+GA vs AA       | 0.76 (0.40–1.45)   | 0.41 | 12.69 | 0.005 | 76.4 | 0.174 |
|                   | G vs A            | 0.86 (0.46–1.66)   | 0.64 | 14.90 | 0.002 | 79.9 | 0.174 |
| rs1800858         | AA vs GG          | 8.36 (3.45–20.25)  | 0.000 | 68.06 | 0.000 | 88.2 | 0.835 |
|                   | AA+GA vs GG       | 3.59 (1.83–7.02)   | 0.000 | 55.29 | 0.000 | 85.5 | 0.835 |
|                   | AA vs GA+GG       | 6.60 (3.66–11.89)  | 0.000 | 53.05 | 0.000 | 84.9 | 0.835 |
|                   | A vs G            | 3.81 (2.28–6.35)   | 0.000 | 99.13 | 0.000 | 91.9 | 1.000 |
| rs1800860         | GG vs AA          | 4.56 (1.14–18.27)  | 0.032 | 0.89 | 0.347 | 0.0  | 0.317 |
|                   | GG vs GA+AA       | 2.38 (1.66–3.43)   | 0.000 | 0.64 | 0.728 | 0.0  | 0.602 |
|                   | GG+GA vs AA       | 3.77 (0.94–15.07)  | 0.061 | 0.87 | 0.351 | 0.0  | 0.317 |
| rs1800861         | G vs A            | 2.23 (1.60–3.11)   | 0.000 | 0.80 | 0.669 | 0.0  | 0.117 |
|                   | GG vs TT          | 5.38 (2.68–10.80)  | 0.000 | 16.29 | 0.006 | 69.3 | 0.851 |
|                   | GG+TG vs TT       | 3.07 (2.17–4.34)   | 0.000 | 5.23 | 0.388 | 4.5  | 0.348 |
|                   | GG vs TT+TG       | 4.14 (1.84–9.30)   | 0.001 | 50.09 | 0.000 | 90.0 | 0.348 |
|                   | G vs T            | 2.85 (1.84–4.47)   | 0.000 | 30.02 | 0.000 | 83.3 | 0.188 |
| rs10900297        | CC vs AA          | 9.73 (5.94–15.94)  | 0.000 | 1.45 | 0.835 | 0.0  | 1.000 |
|                   | CC+AC vs AA       | 5.31 (3.27–8.62)   | 0.000 | 1.43 | 0.839 | 0.0  | 0.624 |
|                   | CC vs AC+AA       | 7.06 (5.60–9.81)   | 0.000 | 4.84 | 0.304 | 17.3 | 1.000 |
|                   | C vs A            | 5.05 (4.16–6.13)   | 0.000 | 4.02 | 0.403 | 0.5  | 0.624 |
| rs2435357         | TT vs CC          | 11.44 (5.67–23.10) | 0.000 | 10.06 | 0.039 | 60.3 | 0.327 |
|                   | TT+TC vs CC       | 4.04 (2.92–5.57)   | 0.000 | 4.39 | 0.355 | 9.0  | 0.624 |
|                   | TT vs TC+CC       | 9.01 (5.25–15.46)  | 0.000 | 10.30 | 0.036 | 61.2 | 0.327 |
|                   | T vs C            | 4.53 (3.27–6.27)   | 0.000 | 9.73 | 0.045 | 58.9 | 1.000 |

Table 4. The results of subgroup analyses.

| Gene polymorphism | Comparison | OR (95%CI) |
|-------------------|------------|------------|
|                   | Asia       | Europe     |
| rs1800858         | AA vs GG   | 5.92 (2.14–16.34) | 26.71 (13.92–51.24) |
|                   | AA vs AG+GG| 5.28 (2.66–10.46) | 14.78 (8.34–26.16) |
|                   | AA+AG vs GG| 2.89 (1.29–6.48) | 7.04 (4.41–11.24) |
|                   | A vs G     | 3.25 (1.75–6.04) | 6.52 (4.83–8.81) |

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newborns, and constipation in adults, that occurs in 1 in 5000 live births [3]. Mutations of the RET proto-oncogene have been detected in HSCR cases, nucleotide changes include microdeletions, insertions, variants affecting the correct RNA splicing, nonsense mutations, silent mutations, and missense mutations, with more than 100 different mutations described so far [32]. However, RET mutations have been detected in only up to 50% of familial patients and in 7%–35% of sporadic cases [33].

Recently, SNP, as the third generation of genetic markers, becomes the focus study. Studies showed that the SNPs of multiple introns and exons of RET expressed very highly or very lowly, which was associated with the phenotype of HSCR [16]. Besides, the SNPs of other genes such as PHOX2B also have been reported to relate to HSCR.

Although the association between polymorphisms of RET, PHOX2B and HSCR risk has been reported by a number of studies, the conclusions remained controversial due to the inconsistent findings. For rs1800858 polymorphism, Phussantisampan et al [3] suggested the A allele was protective against HSCR and G was risk allele, however, other studies [15–17,20–24] found that the carriers of the G allele showed significantly increased risk of HSCR. Liu et al [20] found that rs1800960 played a protective role in the pathogenesis of HSCR in Chinese population, which differed from the findings of Tou et al [21], Li et al [25] and Lanteri et al [26]. Sadowa et al [26] showed the (GG) genotype of rs180061 gene did not associate with the risk of HSCR occurrence, but some studies [3,21,24] suggested that the (GG) genotype was was significantly higher in HSCR patients compared to healthy controls. Liu et al [8] and Xiao et al [19] suggested that the (GG) genotype of rs20647592 was a risk genotype in HSCR patients, but Dou et al [10] showed that the (GG) genotype of rs20647592 gene did not associate with the risk of HSCR occurrence. Meta-analysis is a powerful method for quantitatively summarizing the results from different studies, so we conducted this study to obtain a more comprehensive and reliable conclusion.

In brief, our study included five gene polymorphisms of RET (rs1800858, rs1800960, rs1800661, rs1900297 and rs24395357) and one gene polymorphism (rs20647592) of PHOX2B. For all we know, this is the first meta-analysis investigating the association between these gene polymorphisms and HSCR.

Because the sample size was larger than previous ones, the meta-analysis reduced the probability that random error produced false-positive or false-negative association. We found a significant association between these gene polymorphisms (rs1800858, rs1800661, rs1900297 and rs24395357) and one gene polymorphism (rs20647592) of PHOX2B. For all we know, this is the first meta-analysis investigating the association between these gene polymorphisms and HSCR. Meta-analysis is a powerful method for quantitatively summarizing the results from different studies, so we conducted this study to obtain a more comprehensive and reliable conclusion.

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