Iran J Public Health, Vol. 54, No.3, Mar 2025, pp.567-577

Review Article

Association of the RET Intronic Variant rs2435357 on Hirschsprung's Disease Susceptibility: A Systematic Review and Meta-Analysis

Fatemeh Bahreini [1,2], ***Ali Mahdavinezhad*** [2], ****Maryam Eghbali*** [3]

1. *Department of Medical Genetics, Hamadan University of Medical Sciences, Hamadan, Iran*
2. *Research Center for Molecular Medicine, Institute of Cancer, Hamadan University of Medical Sciences, Hamadan, Iran*
3. *Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran*

***Corresponding Author:** Email: eghbalim1385@gmail.com

(Received 19 Aug 2024; accepted 27 Nov 2024)

Abstract

Background: Hirschsprung disease (HSCR) is a congenital life-threatening intestinal disorder characterized by the absence of nerves in the myenteric and submucosal plexuses in the distal bowel. There are several studies on the association of rs2435357 polymorphism in the proto-oncogene *RET* gene and HSCR susceptibility. However, some of the results remain controversial. Therefore, we conducted this updated meta-analysis to estimate the association of this polymorphism and HSCR risk.

Methods: We searched PubMed, Scopus, Web of Science and Google Scholar according to PRISMA guidelines to assess the association of *RET* rs2435357 with HSCR up to Jan 2024. We included case-control/cohort studies to perform meta-analysis conducted using genotype models. Odd ratios (ORs) with 95%CI were utilized to determine the susceptibility to HSCR. Q-test and I^2 were used to evaluate heterogeneity, and Egger's/ Begg's tests were used to assess publication bias.

Results: Overall, 89 eligible studies meeting the inclusion criteria were retrieved with 2690 cases and 5408 controls from online databases. Finally, 17 studies were used for meta-analysis. *RET* rs2435357 showed a statistically significant association with HSCR under allelic model (OR = 4.50, 95%CI: 3.78-5.36, $P<0.05$), additive model (OR=2.02, 95%CI: 1.54-2.63, $P<0.05$), recessive model (OR=4.39, 95%CI: 3.33-5.78, $P<0.05$) and dominant model (OR=8.66, 95%CI: 6.96-10.76, $P<0.05$).

Conclusion: The polymorphism rs2435357 in *RET* gene provides substantial susceptibility in all inheritance models and to HSCR. However, more research is needed to clarify its specific role in prognosis and the interaction with other genetic and environmental factors affecting HSCR.

Keywords: Hirschsprung disease (HSCR); Polymorphism; Rs2435357; Meta-analysis

Introduction

Hirschsprung disease (HSCR) is a congenital life-threatening intestinal disorder with a global incidence of approximately 1 in 5000 live births, that characterized by the absence of nerves in the myenteric and submucosal plexuses in the distal

DOI: 10.18502/ijph.v54i3.18249

bowel (1). In some patients, the aganglionosis begins at the internal sphincter and extends to the complete colon but in most, aganglionosis is less severe and defined as short segment disorder (2). According to the extent of the aganglionic segment, HSCR is classified into three subtypes, known as short segment (80% of cases, extends to the sigmoid region), long segment (20% of cases, extends proximal to the sigmoid colon) and total colonic aganglionosis (5% of cases, extends to ileocecal valve) (3). Around 70% of HSCRs occur as an isolated case and the remaining is syndromic. HSCR is a complex disease and follows a multifactorial inheritance, although in familial cases, 10%-20% of patients show autosomal dominant inheritance (4). It is typically manifested in the newborn period with abdominal distention, constipation, vomiting and neonatal enterocolitis nevertheless in adulthood usually diagnosed with chronic abdominal distention and severe constipation (5). Several pieces of evidence have indicated that genetic factors play a major role in HSCR pathogenesis (6, 7).

Numerous molecular genetic studies have revealed rare, coding, high-penetrance variants in more than 14 genes and common, low-penetrance, noncoding variants close to *RET*, *NRG1*, and the *SEMA3* genes (2). Among these, the *RET* gene is the main susceptible gene is involved in the development of HSCR (8). The *RET* proto-oncogene is located on chromosome 10q11.2 and encodes a receptor tyrosine kinase. *RET* activation is involved in the proliferation, differentiation and migration of the enteric nervous system (9). There is a variety of evidence on the association between *RET* polymorphisms and Hirschsprung predisposition, however, the results are controversial (10). Among the variations of the *RET* gene, rs2435357 (T > C), the causal locus located in intron 1, has been wildly investigated in HSCR and it is necessary to update the meta-analysis data for rs2435357.

Therefore, we performed a current meta-analysis to understand the link between this polymorphism and susceptibility to HSCR.

Materials and Methods

The current research is a systematic review and meta-analysis that reviewed the studies related to the *RET* gene variant rs2435357 until Jan 2024.

Search strategy

To determine relevant studies on the association of *RET* rs2435357 with HSCR, a comprehensive literature search was performed using electronic databases: PubMed, Scopus, Web of Science, and Google Scholar. The search was limited to human studies and language in English. The search terms were as follows: ("Hirschsprung disease" OR "HSCR" OR "HD" OR "Congenital Megacolon") AND ("RET" OR "RET Proto-oncogene" OR "RET gene") AND ("polymorphism" OR "SNP" OR "variation" OR "mutation" OR "variant" OR "SNPs") AND (rs2435357).

Inclusion and exclusion criteria

Criteria for the screening process were established to ensure the stability of the included data. Inclusion criteria: 1) analysis of the association between rs2435357 and HSCR susceptibility, 2) cohort or case-control study, and 3) the collection of the genotype data in case and control groups. Exclusion criteria: 1) review articles or meta-analysis, 2) animal or in vitro experiments, 3) studies with duplicate data 4) linkage studies and family studiesm 5) studies with insufficient data on genotype frequency, 6) abstracts, case reports, commentaries, editorials, and conference papers.

Reviewing process

Studies were independently reviewed by two authors (ME and FB). Two authors (ME and FB) retrieved and extracted data from eligible studies by reading full text. Any disagreements were resolved through discussing the topic with AM.

Data extraction & quality assessment

An Excel sheet along with an Endnote file was prepared to record the results and scientometric characteristics of the entered studies. Parameters

extracted from studies included last name of author(s), place of publication (country), year of publication, HWE (Hardy–Weinberg) status, country of origin, ethnicity (Caucasian, Asian, African, mixed populations), total patients in there were two groups of case and control, age, gender, allele frequencies in wild and mutant states, genotype frequencies in wild homozygotes, heterozygotes and mutant homozygotes states in the case and control groups. In this study, people examined and diagnosed with Hirschsprung by surgical methods, pathology of rectal biopsies or other medical records were considered as the outcome. The Newcastle-Ottawa scale (NOS) was used to assess the methodological quality of the searched studies. Based on this scale, a maximum of 9 points were assigned to each study. Studies that received less than 4 points were classified as poor quality, studies with a score of 4 to 6 were classified as medium quality, and studies with a score of 7 or more were classified as high quality.

Heterogeneity and publication bias

Q-test was used to evaluate heterogeneity between studies. If all studies have the same true effect size, the expected value of Q will be equal to the degree of freedom (number of studies minus 1). Moreover, according to the I^2 value, heterogeneity was classified as low (less than 50%), moderate (50-74%) or high (75% or more). Publication bias was checked with Egger's and Begg's tests.

Statistical analysis

The effect measure selected in this study was the odds ratio (OR) with 95% confidence intervals (CIs). This criterion was chosen because all the final selected studies were case-control designs. Studies were weighted by sample size to reflect their value of evidence. Due to the heterogeneity of the studies, random effects model was used to estimate pooled OR; otherwise, the fixed effect model is used to estimate pooled OR. Data were analyzed at a significance level of <0.05 using Stata version 11 software (StataCorp, College Station, TX, USA).

Results

The search for sources was done systematically and manually. According to our search strategy, 89 articles were retrieved from online databases. As shown in Fig. 1, 36 duplicate studies were removed, resulting in 53 studies. Finally, after the evaluation and removal of irrelevant studies (for example, different genes, sample size in the study, unclear details of the working method, etc.), a total of 12 studies were selected. In the manual review, after screening 27 studies, 5 studies were added to the 12 studies found systematically, and a total of 17 studies were used for this systematic review and meta-analysis.

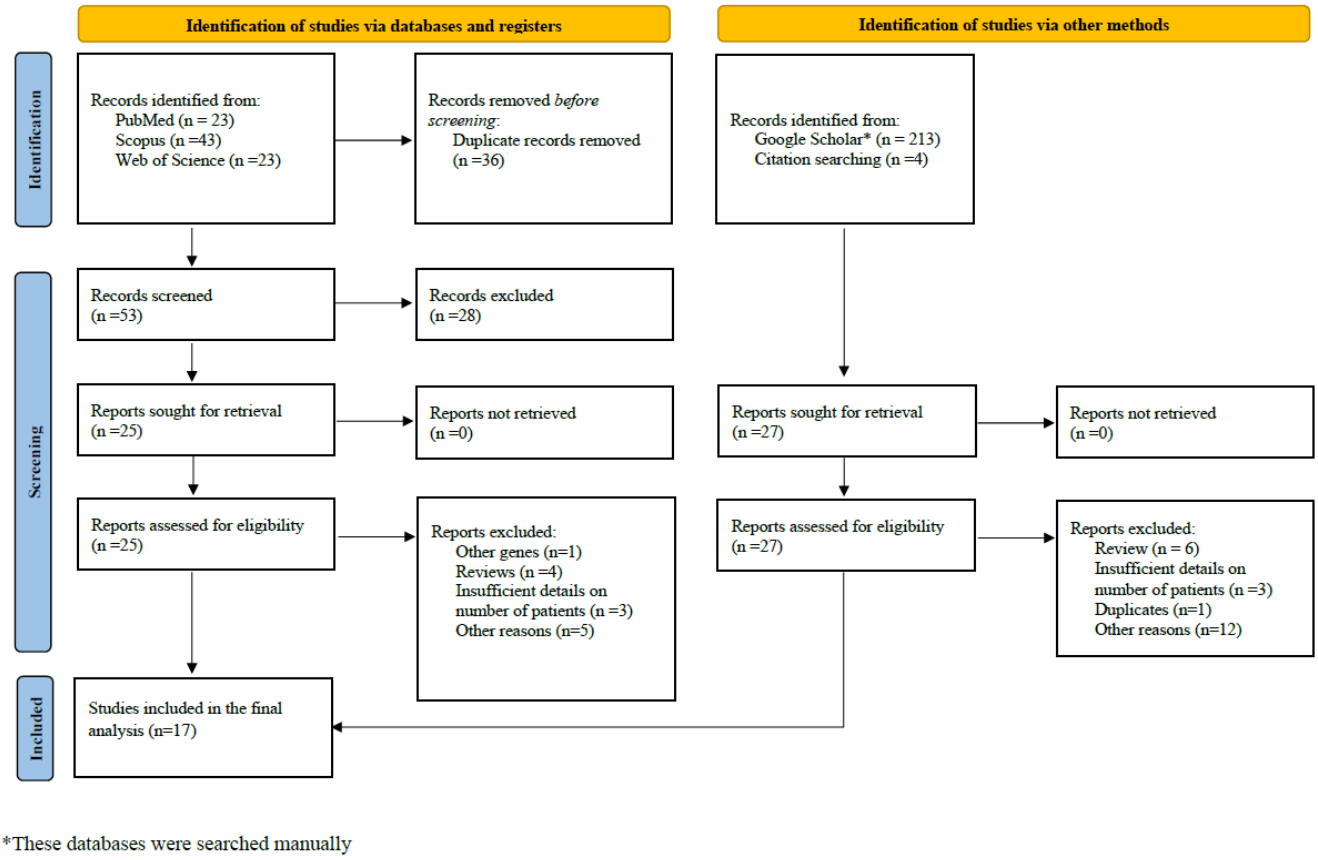

Fig. 1: Flowchart of the search path and selection of information in this systematic review and meta-analysis study

In this systematic review and meta-analysis study, most of the evaluated studies were case-control and samples from both men and women participated. In the study, CC was considered as the wild, TC as the heterozygous and TT as the minor (mutant) genotype. The frequency of cases and controls in these studies was 2690 and 5408 respectively. Among the total reviewed studies, 14 studies conducted on Asian race (70% focused on the Asian race). These studies were from different countries: China (3, 11-16), Thailand (17, 18), Indonesia (19-21). The general characteristics of these studies are shown in Table 1.

According to the NOS criteria, out of 21 studies included for meta-analysis, 15 studies had high quality (score 7 or more) and 6 studies had medium quality (score 5-6). Therefore, none of them were excluded in the meta-analysis stage (Table 2). In this study, heterogeneity was investigated according to Q and I^2 indices. Where the value of I^2 is less than 50% and the significance level of the Q test is less than 0.1, it means the homogeneity of the selected studies. In other words, if at least one of these two indicators destroys the assumption of heterogeneity, studies are considered heterogeneous. As the results of Table 2 show, there was heterogeneity in all considered allelic and genotypic models (additive, dominant and recessive). Therefore, odds ratio estimation from random effect model was used.

Table 1: Characteristics of the included studies in the present meta-analysis

Author	Country	HWE in control group	Frequency of Genotype TT/TC/CC *		Sex	Age: case/control	Ethnicity
			Case	Control			
Dehua Yang	China	Yes	209/126/27	329/802/317	F/M	NA	Asian
Karun Eadyow	Thai-land	Yes	75/30/15	67/130/45	F/M	0–15 /17-64	Asian
Yang Wang	China	Yes	320/146/25	100/252/157	F/M	1.34 ± 2.12/2.70 ± 3.13	Asian
Valtter B Vir-tanan	Finland	Yes	56/17/12	30/129/140	F/M	4-46	European
Theerawut Phusantisam-pan	Thai-land	Yes	47/14/7	31/64/25	F/M	1.2±2.1/34.5±10.3	Asian
Xian-Ning Zhang	China	No	57/28/14	29/62/41	F/M	2 days to 17	Asian
Gunadi	Indo-nesia	No	67/22/4	27/83/26	F/M	NA	Asian
Ashish Kapoor	USA	Yes	148/109/95	42/240/345	F/M	NA	European
Alessio Pini Prato	Italy	yes	11/6/5	3/32/50	F/M	6.9±5.3 (case)	European
Arnold	USA	yes	30/16/16	1/11/18	F/M	NA	European
Miao	China	yes	228/65/22	62/169/95	NA	NA	Asian
Zhang	China	yes	42/16/1	13/30/16	F/M	5 days to 10.5	Asian
Zhang	China	yes	59/15/2	13/30/16	F/M	5 days to 10.5	Asian
Qi Li	China	yes	69/27/3	19/58/37	F/M	NA	Asian
Qian Jiang	China	yes	88/30/2	100/252/157	NA	NA	Asian
Kristy Iskandar	Indo-nesia	yes	62/11/0	20/34/6	NA	<18	Asian
Gunadi	Indo-nesia	yes	42/14/4	26/68/24	F/M	NA	Asian
Tonia C Carter	USA	yes	2/8/51	2/24/222	NA	NA	African-American
Tonia C Carter	USA	yes	10/21/18	4/66/126	NA	NA	Hispanic
Tonia C Carter	USA	yes	14/6/2	20/36/35	NA	NA	Asian

* CC and TT are wild and mutant genotypes, respectively; NA is denoted Not Applicable

Table 2: Risk of bias assessment using the Newcastle-Ottawa scal for included studies

Study	Selection				Comparability	Exposure			Total score (0-9)*
	Is the Case Definition Adequate? (+)	Representativeness of the Cases (+)	Selection of Controls (+)	Definition of Controls (+)	Comparability of CASES and controls on the Basis of the Design or Analysis (++)	Ascertainment of Exposure (+)	Same Method of Ascertainment for Cases and Controls (+)	Non-Response Rate (+)	
Dehua Yang	+	+	+	+	++	+	+	+	9
Karun Eadyow	+	+	+	+	++	+	+	+	9
Yang Wang	+	+	+	+	- -	+	+	+	7
Valtter B Virtanan	+	+	+	+	- -	+	+	+	7
Theerawut Phusantisampan	+	+	-	+	- -	+	+	+	6
Xian-Ning Zhang	+	+	-	-	- -	+	+	+	5
Gunadi, 2016	+	+	-	-	- -	+	+	+	5
Ashish Kapoor	+	+	-	-	- -	+	+	+	5
Alessio Pini Prato	+	+	-	-	- -	+	+	+	5
Arnold	+	+	+	+	++	+	+	+	9
Miao	+	+	+	+	++	+	+	+	9
Zhang	+	+	-	-	++	+	+	+	7
Zhang	+	+	-	-	++	+	+	+	7
Qi Li	+	+	+	+	++	+	+	+	9
Qian Jiang	+	+	+	+	++	+	+	+	9
Kristy Iskandar	+	+	+	+	++	+	+	+	9
Gunadi, 2014	+	+	+	+	++	+	+	+	9
Tonia C Carter	+	+	+	+	++	+	+	+	9
Tonia C Carter	+	+	+	+	++	+	+	+	9
Tonia C Carter	+	+	+	+	++	+	+	+	9

*Total score < 4 denoted low quality, 4-6 denoted moderate quality and ≥7 denoted high quality.

Egger's and Begg's tests were used to detect publication bias. Only in the comparison of TT genotype compared to CT+CC (i.e., recessive model) the diffusion bias was observed. For this purpose, Trim and Fill analysis was used for this model (Table 3). Meta-trim results showed that the odds ratio for this model is equal to 1.48 (CI 95%: 1.204-1.754, P<0.001) (Fig. 2).

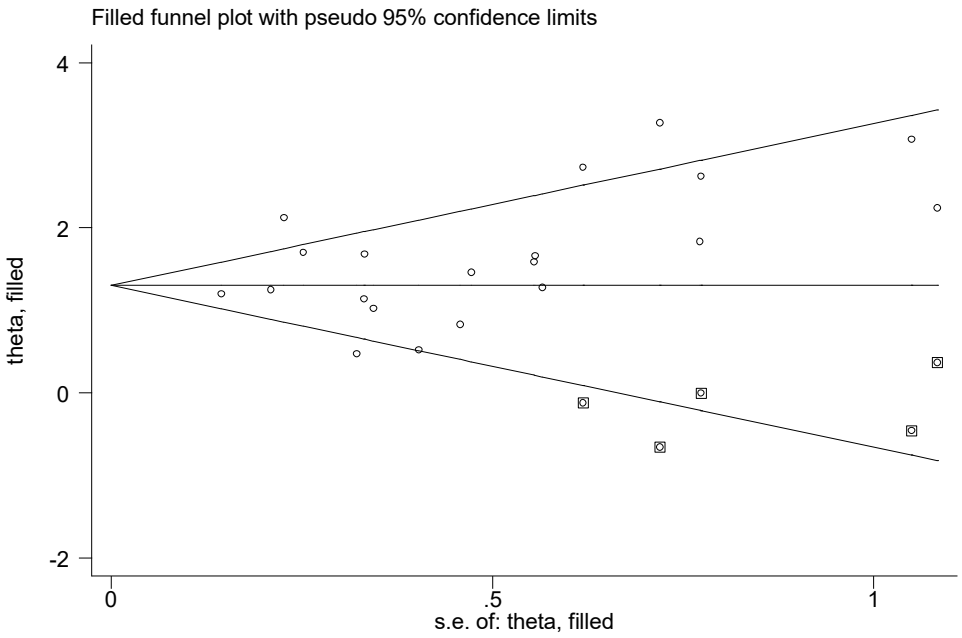

Fig. 2: Funnel plot based on Trim and Fill analysis. θ represents the odds ratio and s.e.(θ) represents the standard error of the odds ratio

Table 3: The results of the heterogeneity and skewness test of the selected studies along with the odds ratio (OR) values

Genetic Model	Type of Model	Heterogeneity			Odds Ratio (OR)			Publication Bias	
		I^2(%)	Q	P_Q	OR	95% CI	P	P_{Beggs}	P_{Eggers}
RET rs2435357									
T vs. C	Random	47.4	36.13	0.010	4.50	3.78-5.36	<0.001	0.974	0.254
TT vs. CT [a]	Random	50.0	38.03	0.006	2.02	1.54-2.63	<0.001	0.206	0.912
TT+CT vs. CC [b]	Random	62.8	51.13	<0.001	8.66	6.96-10.76	<0.001	0.871	0.059
TT vs. CT+CC [c]	Random	61.2	49.00	<0.001	4.39	3.33-5.78	<0.001	0.041	0.148

[a] additive model; [b] Dominant model; [c] Recessive model

The risk of Hirschsprung 's disease in people with T allele is 4.50 times higher than C allele (OR=4.50, CI 95%: 3.78-5.36, *P*<0.001) (Fig. 3A). Moreover, significant association was observed under dominant, recessive and additive models (Table 2) and the most significant association was observed in the dominant model (OR=8.66, CI 95%: 6.96-10.76, *P*<0.001).

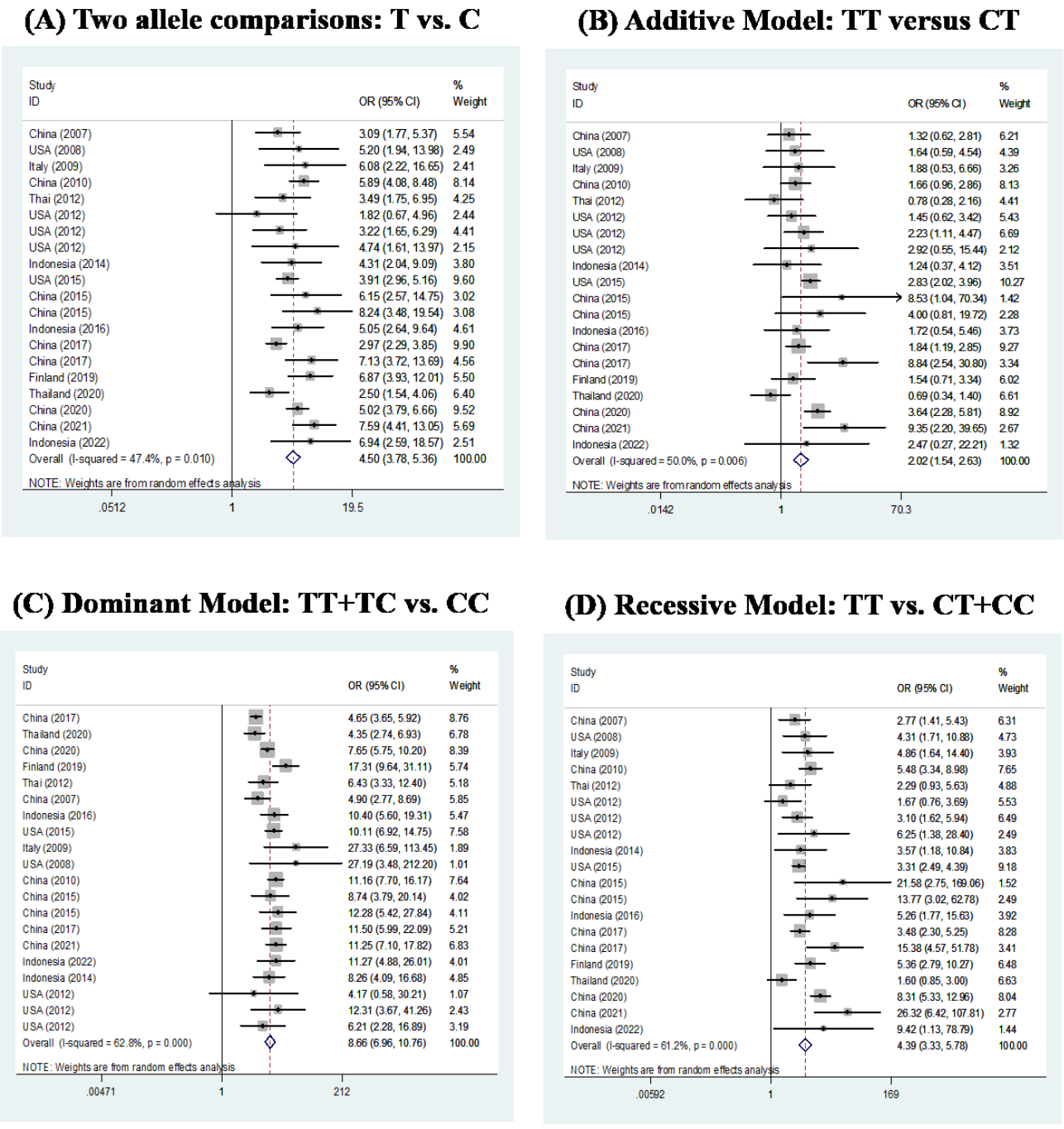

Fig. 3: In models showed forest plots for the connection of *RET* rs2435357 polymorphism and genotype with Hirschsprung disease. A) Allelic model, B) Additive model TT vs CT, C) Dominant model TT+CT vs CC, D) Recessive model TT vs CT+CC. Where horizontal lines denote the 95% CIs, square boxes indicate the odds ratios, and the size of the box is proportional to the weight of the study. The vertical line represents the null value (OR = 1.0). Solid diamonds represent the point estimate of each study and the overall summary estimate is derived from random-effects (RE)

Discussion

HSCR is a multifactorial genetic disorder that appears as a sporadic (85%) or a familial disorder (15%) (7). *RET* is the major gene for HSCR with >80% of all known loss-of-function mutations. It is responsible for the development, proliferation, and differentiation of neuroendocrine cells and is expressed in neural crest-derived cells (8, 22, 23). Surprisingly, the polymorphic noncoding risk variant (rs2435357) at *RET*, in ~80% of HSCR cases, causes disease risk with both European and Asian descents. rs2435357 is located in intron 1 of *RET* and has a high (~24%) allele frequency (24).

In this study, 17 articles searching the association of the rs2435357 in *RET* with HSCR were covered 2690 cases and 5408 controls. In the previous two meta-analyses on the association of rs2435357 with HSCR, Mu et al and Liang et al, applied a total of 12 studies and 5 studies, respectively. The study by Mu et al used 1939 cases and 3613 controls and also the study by Liang et al done with 566 cases and 719 controls. To obtain precise and effective information about the potential association our meta-analysis study was performed by more articles. In our meta-analysis, more five articles were analyzed; these studies' outcomes were in line with two previous meta-analysis articles that were mentioned (25, 26). Among them, one study carried out with a population of USA (27) estimates the genetic effect of the rs2435357 on HSCR risk as significant with an OR of 3.9 ($P=4.3 \times 10^{-44}$), three studies observed for rs2435357 association with HSCR risk in Finland, Indonesia, and Thailand (17, 20, 28) and the last study included in our meta-analysis having at least one copy of the minor T-allele has been reported to be associated with HSCR in Hispanic, non-Hispanic, and Asian ancestries (29). All studies collected in this study, consistent with two previous meta-analysis articles, were from Europe and Asia, with no studies from America or Africa which fulfilled the inclusion criteria to assess the relationship between *RET* and HSCR susceptibility. However, in Tonia C Carter'sstudy, *RET* SNPs including rs2435357 were not associated with HSCR in African-Americans for the number of people who have minor alleles homozygous very low (29).

The meta-analysis concluded that people with the T allele are at a greater risk of Hirschsprung's disease than those with the C allele (OR=4.50, $P<0.001$). It was consistent with the two mentioned meta-analyses above shown T-allele is a susceptible allele to HSCR. And as well, the causal molecular basis of this association at *RET* explored in functional research has proven that rs2435357, the intronic enhancer, at the *RET* locus disrupts SOX10 binding site that compromises *RET* transactivation and decreases *RET* expression (24). In addition, this functional study with 690 European and 192 Chinese descent participants demonstrated that T-allele raises HSCR risk by 4-fold ($P=3.9\times10^{-43}$ European ancestry and $P=1.1\times10^{-21}$) and also indicated the T-allele increases penetrance in cases of rare *RET* coding mutations. In another functional study in 2016 (30), based on the observations in human patients, mouse models, and cellular assays, has been shown that *RET* reduced expression or *RET* loss-of-function is necessary for clinical appearance of HSCR. Every HSCR patient who transfers noncoding and common *RET* alleles such as T-allele rs2435357 transmitted along with rare inherited *RET* mutations arises the risk of HSCR by reducing of *RET* expression. This loss-of-function of *RET* occurs through the reduction of the risk variant enhancer activity and then disruption of binding of its particular transcription factor *SOX10* for T-allele in rs2435357, therefore, T-allele increases HSCR risk.

Some limitations need to be known. First, all study data came from Europe and Asia and we cannot distribute them to whole ethnicities especially Africans and Americans. Second, the lack of age data in all studies meant that we couldn't evaluate the relationship between age and the polymorphism rs2435357 and HSCR risk. Finally, the HSCR is a multifactorial disease with a combination of environmental and genetic factors that cannot be thoroughly investigated in a meta-analysis due to no sufficient data.

Conclusion

rs2435357 of the *RET* gene is strongly linked to the risk of HSCR. To confirm these findings, larger samples from different ethnicities are needed in further studies to investigate the association between this SNP and HSCR. In addition, more studies are recommended to focus on gene-gene and gene-environment interaction to further clarify this association.

Ethics approval

Ethics approval was taken from the Hamadan University of Medical Sciences, Hamadan, Iran, with code: IR.UMSHA.REC.1401.520**.**

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

With thanks for vice-chancellor of research and technology of Hamadan University of Medical Sciences, (number: 140106155056), which supported this study.

Funding

This study was supported and funded by the Hamadan University of Medical Sciences in Iran (IR.UMSHA.REC.1401.520). The funders did not play any role in the design of the study, analysis, and interpretation of data, or in writing of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interests.

References

1. Montalva L, Cheng LS, Kapur R, et al (2023). Hirschsprung disease. *Nat Rev Dis Primers,* 9 (1):54.
2. Huizer V, Wijekoon N, Roorda D, et al (2022). Generic and disease-specific health-related quality of life in patients with Hirschsprung disease: A systematic review and meta-analysis. *World J Gastroenterol,* 28 (13):1362-1376.
3. Jiang M, Li C, Cao G, et al (2017). Effects of NRG1 Polymorphisms on Hirschsprung's Disease Susceptibility: A Meta-analysis. *Sci Rep,* 7 (1):9913.
4. Karim A, Tang CS, Tam PK (2021). The Emerging Genetic Landscape of Hirschsprung Disease and Its Potential Clinical Applications. *Front Pediatr,* 9:638093.
5. Doodnath R, Puri P (2010). A systematic review and meta-analysis of Hirschsprung's disease presenting after childhood. *Pediatr Surg Int,* 26 (11):1107-10.
6. Borrego S, Ruiz-Ferrer M, Fernández RM, Antiñolo G (2013). Hirschsprung's disease as a model of complex genetic etiology. *Histol Histopathol,* 28 (9):1117-36.
7. Amiel J, Lyonnet S (2001). Hirschsprung disease, associated syndromes, and genetics: a review. *J Med Genet,* 38 (11):729-39.
8. Alves MM, Sribudiani Y, Brouwer RW, et al (2013). Contribution of rare and common variants determine complex diseases-Hirschsprung disease as a model. *Dev Biol,* 382 (1):320-9.
9. Bhattarai C, Poudel PP, Ghosh A, Kalthur SG (2022). The RET gene encodes RET protein, which triggers intracellular signaling pathways for enteric neurogenesis, and RET mutation results in Hirschsprung's disease. *AIMS Neurosci,* 9 (1):128-149.
10. Amooee A, Lookzadeh MH, Mirjalili SR, et al (2019). Association of rs2435357 and rs1800858 polymorphisms in Ret Proto-Oncogene with hirschsprung disease: systematic review and meta-analysis. *Arq Bras Cir Dig,* 32 (3):e1448.
11. Wang Y, Jiang Q, Cai H, et al (2020). Genetic variants in RET, ARHGEF3 and CTNNAL1, and relevant interaction networks, contribute to the risk of Hirschsprung disease. *Aging (Albany NY),* 12 (5):4379-4393.

12. Yang D, Yang J, Li S, et al (2017). Effects of RET, NRG1 and NRG3 Polymorphisms in a Chinese Population with Hirschsprung Disease. *Sci Rep,* 7:43222.
13. Zhang XN, Zhou MN, Qiu YQ, et al (2007). Genetic analysis of RET, EDNRB, and EDN3 genes and three SNPs in MCS + 9.7 in Chinese Patients with isolated Hirschsprung disease. *Biochem Genet,* 45 (7-8):523-7.
14. Miao X, Leon TY, Ngan ES, et al (2010). Reduced RET expression in gut tissue of individuals carrying risk alleles of Hirschsprung's disease. *Hum Mol Genet,* 19 (8):1461-7.
15. Zhang Z, Jiang Q, Li Q, et al (2015). Genotyping analysis of 3 RET polymorphisms demonstrates low somatic mutation rate in Chinese Hirschsprung disease patients. *Int J Clin Exp Pathol,* 8 (5):5528-34.
16. Li Q, Zhang Z, Diao M, et al (2017). Cumulative Risk Impact of RET, SEMA3, and NRG1 Polymorphisms Associated With Hirschsprung Disease in Han Chinese. *J Pediatr Gastroenterol Nutr,* 64 (3):385-390.
17. Eadyow K, Phusantisampan T, Maneechay W, Sangkhathat SJSJST (2020). Genetic polymorphisms of the SOX10 gene in Thai patients with sporadic Hirschsprung disease. *Songklanakarin J Sci Technol,* 42 (3):596-601.
18. Phusantisampan T, Sangkhathat S, Phongdara A, et al (2012). Association of genetic polymorphisms in the RET-protooncogene and NRG1 with Hirschsprung disease in Thai patients. *J Hum Genet,* 57 (5):286-293.
19. Gunadi, Dwihantoro A, Iskandar K, Makhmudi A, Rochadi (2016). Accuracy of polymerase chain reaction-restriction fragment length polymorphism for RET rs2435357 genotyping as Hirschsprung risk. *J Surg Res,* 203 (1):91-4.
20. Gunadi, Kapoor A, Ling AY, et al (2014). Effects of RET and NRG1 polymorphisms in Indonesian patients with Hirschsprung disease. *J Pediatr Surg,* 49 (11):1614-8.
21. Iskandar K, Simanjaya S, Indrawan T, et al (2022). Is There Any Mosaicism in REarranged During Transfection Variant in Hirschsprung Disease's Patients? *Front Pediatr,* 10:842820.
22. Iwashita T, Kurokawa K, Qiao S, et al (2001). Functional analysis of RET with Hirschsprung mutations affecting its kinase domain. *Gastroenterology,* 121 (1):24-33.
23. Burzynski GM, Nolte IM, Bronda A, et al (2005). Identifying candidate Hirschsprung disease-associated RET variants. *Am J Hum Genet,* 76 (5):850-8.
24. Emison ES, Garcia-Barcelo M, Grice EA, et al (2010). Differential contributions of rare and common, coding and noncoding Ret mutations to multifactorial Hirschsprung disease liability. *Am J Hum Genet,* 87 (1):60-74.
25. Liang CM, Ji DM, Yuan X, et al (2014). RET and PHOX2B genetic polymorphisms and Hirschsprung's disease susceptibility: a meta-analysis. *PLoS One,* 9 (3):e90091.
26. Mu J, Zhang Y, Liao G, et al (2022). Association of rs2435357 and rs2506030 polymorphisms in RET with susceptibility to hirschsprung disease: A systematic review and meta-analysis. *Front Pediatr,* 10:1030933.
27. Kapoor A, Jiang Q, Chatterjee S, et al (2015). Population variation in total genetic risk of Hirschsprung disease from common RET, SEMA3 and NRG1 susceptibility polymorphisms. *Hum Mol Genet,* 24 (10):2997-3003.
28. Virtanen VB, Salo PP, Cao J, et al (2019). Noncoding RET variants explain the strong association with Hirschsprung disease in patients without rare coding sequence variant. *Eur J Med Genet,* 62 (4):229-234.
29. Carter TC, Kay DM, Browne ML, et al (2012). Hirschsprung's disease and variants in genes that regulate enteric neural crest cell proliferation, migration and differentiation. *J Hum Genet,* 57 (8):485-93.
30. Chatterjee S, Kapoor A, Akiyama JA, et al (2016). Enhancer Variants Synergistically Drive Dysfunction of a Gene Regulatory Network In Hirschsprung Disease. *Cell,* 167 (2):355-368.e10.