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Associations between common genetic variants in microRNAs and Hirschsprung disease susceptibility in Southern Chinese children

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Abstract

Introduction: Hirschsprung disease (HSCR), characterized by the defective migration of enteric neural crest cells, is a severe congenital tract disease in infants. Its etiology is not clear at present, although a genetic component plays an important role in its etiology. Many studies focused on the polymorphisms of microRNA (miRNA) in several disease progressions have been reported, including HSCR. However, the findings remain inconclusive. The present study aimed to explore the association of genetic variants in miRNAs and HSCR susceptibility in Southern Chinese children.

Methods: Five single nucleotide polymorphisms (SNPs) (*miR-146A* rs2910164, *miR-4318* rs8096901, *miR-3142* rs2431697, *miR-3142* rs2431097 and *miR-3142* rs5705329) were included to be genotyped in the stratified analysis through the Mass ARRAY iPLEX Gold system (Sequenom, San Diego, CA, USA) conducted on all the samples, comprising 1470 cases and 1473 controls. After quality control, the minor allele frequency was compared in cases and controls to analyze the association between SNPs and HSCR using PLINK 1.9 (https://www.cog-genomics.org/plink) and multiple heritability models were tested (additive, recessive and dominant models).

Results: Our results indicated that miR-4318 rs8096901 polymorphisms were associated with HSCR susceptibility in Southern Chinese children, especially in short-segment HSCR (S-HSCR) patients after stratified analysis.

Conclusions: In summary, we report that miR-4318 rs8096901 was associated with HSCR, especially in SHSCR patients.

KEYWORDS
association, genetic variants, Hirschsprung's disease, microRNAs, Southern Chinese Children

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1 | INTRODUCTION

Hirschsprung disease (HSCR), which is characterized by enteric ganglia absence, is a common disorder of the enteric nervous system (ENS) of infants.[1] HSCR occurs in approximately one case per 5000 live births, and male infants have four-fold greater likelihood that is likely to be infected compared to female infants.[2] Although Hirschsprung disease can affect all races, it is more common in Asians. There are three main types of this disease: short-segment HSCR (S-HSCR), long-segment HSCR (L-HSCR) and total colonic aganglionosis (TCA). This is defined by the length of the intestine that lacks nerve cells.[3]

Hirschsprung disease has very complex components, which are not very clear at present, although a genetic component plays an essential part in its etiology.[4] Over recent years, many studies have been conducted aiming to determine the relationship between genes and Hirschsprung disease, and *RET* was found as the dominant gene that associates with HSCR.[5] Other genes, such as *NRG1*, *EDNRB*, *GDNF*, *SOX10* and *ZFHX1B*, were also reported to link to HSCR.[4,6–10] However, variants within these genes could only account for half of the HSCR cases,[2] emphasizing the need to further uncover the pathogenesis of HSCR.

MicroRNAs (miRNAs) are endogenous small non-coding single-stranded RNAs with sizes ranging from 19 to 25 nucleotides.[11] They can bind to the 3'-UTR of mRNAs to regulate their target gene expression through the induction of the degradation or translation inhibition of the corresponding mRNA.[11,12] To date, more than 800 miRNAs have been identified, and many of these have been implicated in important biological processes, including cell proliferation, migration, metabolism and apoptosis.[13–16]

Recent studies have reported that miRNAs are closely related to HSCR.[17] It was revealed that polymorphism rs2910164 in pre-miR-146a has a vital role in the etiology of HSCR in Han Chinese. To explore the function of the genetic component in HSCR, Tang[18] performed a genome-wide association analysis in patients with HSCR, identifying thousands of single nucleotide polymorphisms (SNPs) that were associated with HSCR. However, the cases and controls in their study were all collected from the Hospital of Hong Kong. However, the relationship of rs8096901 with HSCR has not been reported in the Southern Chinese. Hence, we also chose this SNP to replicate in Southern Chinese children.

Moreover, microRNAs also contribute to autoimmune diseases, such as polymorphisms rs2431697, rs2431097 and rs57095329, which are all on the microRNAs. Xiao et al.[19] reported that rs2431697 and rs57095329 have closed relations with rheumatoid arthritis. SNP rs2431097 was associated with systemic lupus erythematosus in the study by Imgenberg-Kreuz et al.[20] However, the correlation between rs2431697, rs2431097, rs57095329 and HSCR remains unclear. Therefore, we also included these SNPs in this case–control study with 1470 HSCR cases and 1473 controls of Southern Chinese children to assess the associations among rs2910164, rs8096901, rs2431697, rs2431097, rs57095329 and HSCR susceptibility.

2 | MATERIALS AND METHODS

2.1 | Study subjects

The sample involved in the present study was collected from Guangzhou Women and Children's Medical Center. All cases included in the present study were diagnosed with HSCR by barium enema and anorectal manometry evaluation, and these diagnoses were eventually confirmed by a clinicopathological test for aganglionosis after surgery. The study protocol was approved by the hospital's institutional review board. In the present study, there were 1470 patients and 1473 controls, with all patients being divided into three subgroups based on the length of the aganglionosis, including 1033 S-HSCR, 294 L-HSCR and 82 TCA. The controls in the present study were confirmed not to have HSCR or any other neurological-related disorders.

2.2 | SNP genotyping and quality control

Two previously examined SNPs *miR-146A* rs2910164 and *miR-4318* rs8096901 were chosen for replication in the present study. Moreover, three SNPs (*miR-3142* rs2431697, *miR-3142* rs2431097 and *miR-3142* rs5705329) associated with the immune system were selected to examine the correlation with HSCR in Southern Chinese children. These SNPs were included to be genotyped in the further analysis through the Mass ARRAY iPLEX Gold system (Sequenom, San Diego, CA, USA) conducted on all the samples. We carried out a Hardy–Weinberg equilibrium examination to exclude SNPs with $p < 0.05$. For quality control of the SNPs: (i) if the missing data of SNPs was more than 10%, the case/control was excluded from the final analysis and (ii) all subjects for whom 10% follow-up calls were missed were removed. After all of the quality control steps, the five SNPs were retained for further analysis, including 1470 patients and 1473 controls.

2.3 | Association analysis and stratified analysis

The investigators compared the minor allele frequency in cases and controls to analyze the association between SNPs and HSCR, as well as other measurements, using PLINK 1.9 (additive measurement of recessive and dominant model test) (https://www.cog-genomics.org/plink).[21,22] There are three main types of HSCR: S-HSCR, L-HSCR and TCA. Hence, by comparing the patients with a certain sub-phenotype with the control group, the association of sub-phenotype stratification was analyzed.

2.4 | Statistical analysis

The investigators used a chi-squared test to calculate the Hardy–Weinberg equilibrium for heterogeneity. The odds ratio (OR) was used to estimate the risk of children who suffered from HSCR, which was calculated by logistic regression. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Characteristics of the participants

HSCR is divided into three types: (i) short segments (S-HSCR), which only have the aganglionosis limited to the level of the rectosigmoid colon; (ii) long segments (L-HSCR), which could be classified as lack of ganglion cells reached to the part of descending colon, splenic flexure or transverse colon; and (ii) colonic aganglionosis (TCA), which has the entire gastrointestinal tract implicated.[3] The patients in the present study were also divided based on these types. There were 1470 cases and 1473 controls in the present research. The specific clinical information of these participants is summarized in the Supporting information (Table S1).

3.2 | Associations between the selected SNPs and Hirschsprung disease risk

In the present study, the investigators selected five SNPs that are located in or closed to miRNAs to investigate their associations with HSCR. Among these SNPs, three of these were in *miR-3142*, which included rs2431697, rs2431097 and rs57095329. As shown in Table 1, only *miR-4318* rs8096901 was significantly associated with HSCR [p = 0.041, odds ratio (OR) = 0.87, 95% confidence interval (CI) = 0.76–0.99 for the additive model; p = 0.038, OR = 0.76, 05% CI = 0.59–0.98 for the recessive model]. However, the association of *miR-146A* rs2910164 with HSCR reported by Zhu et al.[17] could not be replicated in the present study. In conclusion, it was found that rs8096901 is associated with HSCR in the present study.

3.3 | Associations between the selected SNPs and the three types of Hirschsprung disease risk

The investigators further explored the association of these selected SNPs with Hirschsprung disease through stratified analysis. The polymorphism rs8096901 was found to be significantly associated with S-HSCR (Table 2), under different genetic models (OR = 0.85, 95% CI = 0.73–0.99, p = 0.035 for the additive model; OR = 0.74, 95% CI = 0.55–0.98, p = 0.035 for the recessive model). However, none of the five polymorphisms were significantly associated with the other two types of HSCRs, L-HSCR and TCA.

TABLE 1 Associations between selected polymorphism and Hirschsprung disease risk in Southern Chinese children

CHR	SNP	Gene	BP	A1/A2	F_A	F_U	Model	OR	p
5	rs2431697	MIR3142	160,452,971	C/T	0.12	0.13	ALLELIC	0.93 (0.80–1.08)	0.35
							ADD	0.90 (0.66–1.22)	0.51
							REC	0.82 (0.45–1.52)	0.54
							DOMDEV	1.04 (0.74–1.46)	0.83
5	rs2431097	MIR3142	160,463,878	T/C	0.24	0.24	ALLELIC	1.03 (0.91–1.17)	0.60
							ADD	1.04 (0.89–1.22)	0.62
							REC	1.08 (0.79–1.47)	0.65
							DOMDEV	0.98 (0.80–1.20)	0.87
5	rs57095329	PTTG1, MIR3142	160,467,840	G/A	0.21	0.21	ALLELIC	0.98 (0.86–1.11)	0.77
							ADD	0.99 (0.82–1.18)	0.89
							REC	0.98 (0.69–1.40)	0.92
							DOMDEV	0.99 (0.79–1.23)	0.92
5	rs2910164	MIR146A	160,485,411	G/C	0.38	0.37	ALLELIC	1.01 (0.91–1.13)	0.82
							ADD	0.99 (0.88–1.11)	0.84
							REC	0.93 (0.75–1.16)	0.53
							DOMDEV	1.10 (0.94–1.29)	0.25
18	rs8096901	MIR4318, LINC00669	38,065,879	T/C	0.30	0.32	ALLELIC	0.91 (0.82–1.02)	0.12
							ADD	0.87 (0.76–0.99)	**0.041**
							REC	0.76 (0.59–0.98)	**0.038**
							DOMDEV	1.13 (0.95–1.35)	0.16

CHR, chromosome; SNP, single nucleotide polymorphism; BP, base pair where the SNP is located; A1/A2 indicates the minor allele and major allele to disease; F_A/F_U, minor allele frequency of the SNP in cases or controls; ALLELIC, ADD, REC and DOMDEV indicate the association test following allelic, additive, recessive, dominant models; OR, odds ratio. The p value indicates the significance based on allelic each association test model, respectively, adjusted for age and genders. The use of bold in p-value means the result is meaningful statistically.

TABLE 2 The association results of selected SNPs with different subclinical features classified by aganglionosis length, including short-length (S-HSCR), long-length (L-HSCR) and total colonic aganglionosis (TCA)

CHR	SNP	BP	A1/A2	F_A	F_U	Model	OR	*p*
S-HSCR								
5	rs2431697	160,452,971	C/T	0.13	0.13	ALLELIC	0.95 (0.80–1.13)	0.55
						ADD	1.01 (0.84–1.20)	0.96
						REC	1.01 (0.71–1.43)	0.97
						DOMDEV	1.01 (0.81–1.26)	0.95
5	rs2431097	160,463,878	T/C	0.24	0.24	ALLELIC	1.01 (0.88–1.15)	0.90
						ADD	0.96 (0.69–1.33)	0.79
						REC	0.93 (0.48–1.79)	0.82
						DOMDEV	0.99 (0.68–1.43)	0.96
5	rs57095329	160,467,840	G/A	0.20	0.21	ALLELIC	0.97 (0.84–1.11)	0.62
						ADD	1.02 (0.90–1.16)	0.75
						REC	0.99 (0.78–1.26)	0.94
						DOMDEV	1.07 (0.90–1.28)	0.42
5	rs2910164	160,485,411	G/C	0.38	0.37	ALLELIC	1.04 (0.92–1.17)	0.53
						ADD	0.93 (0.73–1.18)	0.55
						REC	0.90 (0.56–1.44)	0.65
						DOMDEV	0.95 (0.72–1.26)	0.73
18	rs8096901	38,065,879	T/C	0.30	0.32	ALLELIC	0.90 (0.79–1.02)	0.096
						ADD	0.85 (0.73–0.99)	**0.035**
						REC	0.74 (0.55–0.98)	**0.035**
						DOMDEV	1.14 (0.94–1.39)	0.18
L-HSCR								
5	rs2431697	160,452,971	C/T	0.13	0.13	ALLELIC	0.95 (0.72–1.24)	0.70
						ADD	1.13 (0.87–1.47)	0.35
						REC	1.28 (0.77–2.13)	0.34
						DOMDEV	0.88 (0.63–1.23)	0.46
5	rs2431097	160,463,878	T/C	0.25	0.24	ALLELIC	1.06 (0.86–1.31)	0.56
						ADD	0.66 (0.32–1.36)	0.26
						REC	0.43 (0.10–1.84)	0.26
						DOMDEV	1.55 (0.72–3.36)	0.26
5	rs57095329	160,467,840	G/A	0.22	0.21	ALLELIC	1.04 (0.83–1.29)	0.74
						ADD	0.95 (0.77–1.17)	0.65
						REC	0.89 (0.60–1.31)	0.56
						DOMDEV	1.09 (0.82–1.44)	0.55
5	rs2910164	160,485,411	G/C	0.37	0.37	ALLELIC	0.98 (0.81–1.18)	0.80
						ADD	0.86 (0.57–1.29)	0.46
						REC	0.76 (0.34–1.69)	0.50
						DOMDEV	1.05 (0.66–1.69)	0.83
18	rs8096901	38,065,879	T/C	0.33	0.32	ALLELIC	1.04 (0.85–1.26)	0.72
						ADD	0.99 (0.79–1.24)	0.94
						REC	0.93 (0.60–1.43)	0.73
						DOMDEV	1.13 (0.84–1.53)	0.41

(Continues)

TABLE 2 (Continued)

CHR	SNP	BP	A1/A2	F_A	F_U	Model	OR	*p*
TCA								
5	rs2431697	160,452,971	C/T	0.1	0.13	ALLELIC	0.75 (0.45–1.25)	0.26
						ADD	0.83 (0.30–2.29)	0.73
						REC	0.74 (0.10–5.58)	0.77
						DOMDEV	0.87 (0.28–2.69)	0.81
5	rs2431097	160,463,878	T/C	0.27	0.24	ALLELIC	1.17 (0.82–1.67)	0.39
						ADD	1.27 (0.84–1.92)	0.27
						REC	1.57 (0.70–3.51)	0.28
						DOMDEV	0.84 (0.48–1.46)	0.53
5	rs57095329	160,467,840	G/A	0.19	0.21	ALLELIC	0.90 (0.60–1.33)	0.59
						ADD	0.89 (0.49–1.61)	0.69
						REC	0.82 (0.25–2.65)	0.73
						DOMDEV	1.02 (0.50–2.07)	0.97
5	rs2910164	160,485,411	G/C	0.34	0.37	ALLELIC	0.88 (0.63–1.23)	0.46
						ADD	0.79 (0.52–1.21)	0.28
						REC	0.61 (0.28–1.35)	0.22
						DOMDEV	1.33 (0.79–2.24)	0.29
18	rs8096901	38,065,879	T/C	0.26	0.32	ALLELIC	0.76 (0.53–1.09)	0.14
						ADD	0.78 (0.50–1.20)	0.26
						REC	0.69 (0.29–1.61)	0.39
						DOMDEV	0.96 (0.54–1.70)	0.89

CHR, chromosome; SNP, single nucleotide polymorphism; BP, base pair where the SNP is located; A1/A2 indicates the minor allele and major allele to disease; F_A/F_U, minor allele frequency of the SNP in cases or controls; ALLELIC, ADD, REC and DOMDEV indicate the association test following allelic, additive, recessive, dominant models; OR, odds ratio. The *p* value indicates the significance based on allelic each association test model, respectively, adjusted for age and genders. The use of bold in *p*-value means the result is meaningful statistically.

3.4 | Systematic analysis of HSCR susceptibility of the selected SNPs

A meta-analysis was conducted to better understand the relationships among these SNPs and HSCR. After careful literature searching, only *miR-146A* rs2910164 and *miR-4318* rs8096901 were found in HSCR-related studies (see Supporting information, Table S2). Among them, a great amount of heterogeneity was found for *miR-146A* rs2910164 between our data and the data reported by Zhu et al.[17] (I^2 = 79.8%, p_{het} = 0.026), resulting in a non-significant association (p = 0.606). Unfortunately, a meta-analysis of *miR-4318* rs8096901 was unavailable because of the lack of effect size information in the original study.

4 | DISCUSSION

Comprising the most common congenital digestive disease, HSCR is characterized by chronic constipation and distention of the proximal bowel.[23,24] Surgery is the most common solution for HSCR, which removes the affected segments and reconnects the healthy gut to the anus. However, the long-term effect of this surgery is not satisfactory. Many patients may suffer from gastrointestinal problems during their life, such as enterocolitis.[25] Therefore, it is necessary to identify the exact pathogenesis underlying HSCR. It would be helpful to better understand the early diagnosis and therapy of HSCR. Over recent decades, many studies have determined the relationship between the genetic component and HSCR. miRNAs can affect cell proliferation, differentiation, metabolism, apoptosis and carcinogenesis by regulating the gene expression at the post-transcriptional level.[26] Therefore, miRNA expression changes are frequent in many diseases and act as critical parts in the development of these diseases, including HSCR. Five SNPs located in or close to miRNAs were included in the present study to determine their relationship with HSCR. Among these SNPs, we found that only *miR-4318* rs8096901 was associated with HSCR. In the three types of HSCR, only *miR-4318* rs8096901 was associated with SHSCR.

According to Tang,[18] rs8096901 was associated with HSCR in the Hong Kong Chinese population. After replicating this result in Southern Chinese children, the association between rs8096901 and HSCR was successfully identified. Furthermore, all patients were divided into three types, including S-HSCR, L-HSCR, and TCA. The relationship between rs8096901 and S-HSCR, L-HSCR, and TCA was further investigated individually; unfortunately, rs8096901 was only found to

be associated with S-HSCR. Rs8096901 was reported to be close to *BRUNOL4* with a distance of 50 Mb in the study by Tang.[18] In the present study, we found that rs8096901 was close to another gene, *MIR4318*. Belonging to the family of microRNAs, MIR4318 has more than 4000 predicted targets according to the prediction of microRNA using TargetScan (http://www.targetscan.org). *RET*, one of these predicted targets, was reported to be the dominant gene contributing to the susceptibility of HSCR.[26] Hence, *MIR4318* was inferred that influences the expression of *RET* by some unknown mechanism, then associates with HSCR. Unfortunately, there was no experiment in this study to confirm the exact mechanism of how *MIR4318* influences the expression of *RET*, and more studies are needed to verify this hypothesis.

To determine the relationship between the different types of HSCR and the selected SNP, we used stratified analysis. It was found that the only rs8096901 was associated with S-HSCR and, for the other two types of HSCR, there was no SNP association with these. This may be explained by the sample size, for which the sample size in the present study was still moderate, especially regarding the number of TCAs. Another possible reason may be that S-HSCR is more common than L-HSCR and TCA in HSCR, making it easier to collect the sample of S-HSCR. As a result, it was easier to identify the relationship between SNPs and S-HSCR. Indeed, further studies with a large sample size are needed to explore the potential association between SNP in miRNA and these types of HSCR.

There are several limitations to the present study. First, the sample size of L-HSCR and TCA was less than S-HSCR. Hence, for L-HSCR and TCA, it was more challenging to determine their association with SNPs. For further studies, a larger sample size of these two types is needed to explore their association with SNPs. Second, we just chose five polymorphisms which were in the miRNA. To fully illuminate the contribution of SNPs in miRNAs, more polymorphisms should be involved in future studies.

To date, the functional role of *miR-4318* rs8096901 in HSCR has not been investigated. In the present study, we replicated the potential genetic association of this variant with the susceptibility to HSCR in Southern Chinese Children and demonstrated the correlation of *miR-4318* rs8096901 with S-HSCR, which is relevant in HSCR with respect to pathology. More studies with larger case sizes and including multicenter and functional evaluations are needed to explore the role of miRNA in HSCR.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

YZ and WZ designed experiment. QW, JZ, YZ, XX, QH, YZ, NW, LH, LL, TH, JZ and HX collected samples and conducted the study. YZ and WZ analyzed the data. QW, JZ, YZ, YZ and WZ wrote the paper. All authors read and approved the final manuscript submitted for publication.

DATA AVAILABILITY STATEMENT

The datasets used during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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