Original Article

Association between *IKBKAP* polymorphisms and Hirschsprung's disease susceptibility in Chinese children

Ning Wang[1#^], Jiaojiao Xi[1#], Chaoting Lan[2#], Yuxin Wu[2], Yun Zhu[1], Xiaoyu Zuo[1], Yan Zhang[1]

[1]Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China; [2]Department of Pediatrics, The First Affiliated Hospital of Jinan University, Guangzhou, China

Contributions: (I) Conception and design: All authors; (II) Administrative support: Y Zhang; (III) Provision of study materials or patients: Y Zhang; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: X Zuo; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Xiaoyu Zuo; Yan Zhang. Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou 510623, China. Email: zuoxiaoyu@foxmail.com; yannizy@gmail.com.

Background: Hirschsprung's disease (HSCR) is a rare congenital disease in which enteric nervous system (ENS) in the distal intestine is absent. HSCR is a disease involving genetic factors and environmental factors. Despite a series of genes have been revealed to contribute to HSCR, many HSCR associated genes were yet not identified. Previous studies had identified that a potential susceptibility gene of HSCR was an inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (*IKBKAP*). The study aimed to explore the association of genetic variants in *IKBKAP* and HSCR susceptibility in southern Chinese children.

Methods: Single nucleotide polymorphism (SNPs) were genotyped by the Mass ARRAY iPLEX Gold system (Sequenom, San Diego, CA, USA) on all samples, which included 1,470 HSCR children (cases) and 1,473 healthy children (controls). The associations between SNPs and HSCR or clinical subtypes were assessed by comparing their allele frequencies in corresponding case and control samples. Different genetic models, including additive, recessive, and dominant models, were tested using PLINK 1.9 software.

Results: Further subgroup analysis revealed rs2275630 as a total colonic aganglionosis (TCA)-specific susceptibility locus. The present study is the first to indicate that *IKBKAP* rs2275630 were associated with HSCR susceptibility, especially in TCA patients.

Conclusions: We conclude that *IKBKAP* rs2275630 is a susceptibility gene of HSCR.

Keywords: Hirschsprung's disease (HSCR); single nucleotide polymorphism (SNP); B-cells, kinase complex-associated protein (*IKBKAP*); genetic susceptibility

Submitted Nov 23, 2021. Accepted for publication Apr 22, 2022.
doi: 10.21037/tp-21-550
View this article at: https://dx.doi.org/10.21037/tp-21-550

Introduction

Hirschsprung's disease (HSCR), also called congenital ganglioside disease, is a rare congenital intestinal disease. Clinical manifestations of constipation, intestinal obstruction, and enterocolitis symptom seriously affect the life and growth of children, even life-threatened. HSCR varies widely among races. The occurrence rate of HSCR is highest in the Asian population (2.8 in 10,000 infants), with a male/female ratio 2:1 to 4:1. According to the absent length of the ganglion in the intestine, there are usually three types: short-segment

^ ORCID: 0000-0001-7349-9699.

HSCR (S-HSCR), long-segment HSCR (L-HSCR), and total colonic aganglionosis (TCA) (1).

HSCR is characterized by absent ganglion cells in the intestine (2). The abnormal migration, proliferation and differentiation of the intestinal neural crest cells in the development process lead to the development and growth of the intestinal nervous system. So far, more than 24 HSCR-associated genes were reported, for example ret proto-oncogene (*RET*), endothelin receptor type B (*EDNRB*), glial cell-line derived neurotrophic factor (*GDNF*), and SRY-box transcription factor 10 (*SOX10*) (3-5). Most of these genes are essential for the development of intestinal ganglia. For example, *RET* variants (rs2506030 and rs2435357) were associated with HSCR through the GDNF-RET pathway (5,6). However, the genetic architecture of HSCR has not been fully explained, calling for further researches on detecting disease-contributed genes.

IKBKAP is also known as elongator complex protein 1 (*ELP1*) (7). It has been reported that *IKBKAP* was the most common cause of familial autonomic dysfunction (FD) (8,9), and about 60% of FD patients had gastrointestinal dysfunction (10). It is of interest that the number of neurons in FD patients decreases with time after birth, which is to some extent similar to that observed in HSCR. In fact, the simultaneous occurrence of FD and HSCR has been reported in the literature (11), raising a question of partially shared etiology between HSCR and FD such as shared susceptible genes. It was shown that the expressions of several genes essential in the development of enteric nervous system (ENS), such as *phox2bb*, a homologous gene of *PHOX2B* (12), and *ret* were decreased in *ikbkap*-knocked out zebrafish (7). Previous genome-wide association study revealed association between six single nucleotide polymorphisms (SNPs) within or nearby *IKBKAP* and HSCR susceptibility in southern China population, including rs10979596, rs10979597, rs2230793, rs2275630, rs10979607, and rs4369056 (13). These associations, however, required further replication on a large-scale independent population with similar geographical regions and genetic backgrounds. In this study, two *IKBKAP* SNPs (rs2230793 and rs2275630) were genotyped in a large-scale cohort representing the southern Chinese population to validate the relationship between the *IKBKAP* gene and HSCR. We present the following article in accordance with the MDAR reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-21-550/rc).

Methods

Study subjects

A total of 2,943 samples (1,470 disease groups and 1,473 healthy groups without a history of nervous system diseases) were recruited from Guangzhou Women and Children's Medical Center. All recruited patients were confirmed as HSCR by tissue biopsy and had received surgical treatments. The relevant clinical data such as age, gender, and clinical types were recorded. Based on the length of the aganglionic segment, 1,470 disease groups were further divided into short-segment type (S-HSCR, 1,033 patients), long-segment type (L-HSCR, 294 patients), total colon aganglionic type (TCA, 82 patients), total intestinal aganglionosis type (TIA, 3 patients) and unknown subtype (58 patients). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Ethics Review Board of Guangzhou Women and Children's Medical Center (No. 2016042036) and informed consent was taken from all the patients or their legal guardians.

SNP genotyping and quality control

The previously reported SNPs of *IKBKAP* included rs10979596, rs10979597, rs2230793, rs2275630, rs10979607, and rs4369056 (13). Among the six SNPs, rs10979596, rs10979597 and rs10979607 are highly correlated (i.e., high linkage disequilibrium). Hence, we randomly selected rs10979597, as well as rs2230793 and rs2275630, as tag-SNPs for *IKBKAP*. However, rs10979597 was found non-polymorphic in our cohort such that it was excluded from the subsequent analysis. SNPs were genotyped by the Mass ARRAY iPLEX Gold system (Sequenom, San Diego, CA, USA) on all samples. SNPs were excluded if they met any of the following quality control criteria: (I) genotyping call rate <90% in all samples; (II) significant departure from Hardy-Weinberg equilibrium expectation ($P<0.05$). Finally, all the two SNPs were kept for subsequent analyses using 1,470 disease groups and 1,473 healthy groups.

Association analysis and subgenotype analysis

The associations between SNPs and HSCR or its clinical subtypes were assessed by comparing the allele frequency in cases and the controls. Different genetic models, including additive, recessive, and dominant models, were tested using

Table 1 Sample characteristics of the study subjects

HSCR subphenotype	Cases (n=1,470)	Controls (n=1,473)	P
Gender, n (%)			
Female	240 (16.33)	458 (31.09)	<0.001
Male	1,230 (83.67)	1,015 (68.91)	<0.001
Age (months), mean ± SD	8.37±20.5	18.61±19.75	<0.001
Clinical manifestation, n (%)			
S-HSCR	1,033 (70.27)	N/A	–
L-HSCR	294 (20.00)	N/A	–
TCA	82 (5.58)	N/A	–
TIA	3 (0.20)	N/A	–
Unknown subtype	58 (0.70)	N/A	–

SD, standard deviation; S-HSCR, short-segment HSCR; L-HSCR, long-segment HSCR; HSCR, Hirschsprung's disease; TCA, total colonic aganglionosis; TIA, total intestinal aganglionosis.

PLINK 1.9 software (14,15). Univariate and multivariate logistic regression analyses were applied to estimate the unadjusted and adjusted effect size in terms of the odds ratio (OR). Age and sex were adjusted in the multivariate logistic regression. The Hardy-Weinberg disequilibrium was assessed by the chi-squared test. P<0.05 is viewed as statistically significant.

Bioinformatic analysis

At NCBI, rs2275630 showed the presence of known enhancement markers in the human fetal intestine (https://www.ncbi.nlm.nih.gov/geo/roadmap/epigenomics/). We used the web site (http://bioinfo.life.hust.edu.cn/HumanTFDB/) to check whether polymorphism would destroy the putative transcription factor motif. Associations of rs2275630 genotypes with *IKBKAP* expression in colon tissues based on data from the GTEx portal database (https://www.gtexportal.org/home/).

Statistical analysis

The Hardy-Weinberg equilibrium for heterogeneity was calculated by the researchers using the chi-square test. The researchers estimated the risk of developing HSCR in children using the OR, and logistic regression calculated the OR. A P value <0.05 was considered statistically significant.

Results

Characteristics of study subjects

The features of the subjects are summarized in *Table 1*. In our cohort of 1,470 patients with HSCR, the age of onset ranged from a few days after birth to a few years old, with an average age of 8.37±20.50 months. In total, 1,473 age-matched healthy controls from the study cohort were recruited from other pediatrics. HSCR was divided into: (I) S-HSCR: 1,033 (70.27%); (II) L-HSCR: 294 (20.00%); (III) total colonic aganglionosis (TCA): 82 (5.58%). There was statistical significance in age and gender distribution between disease groups and healthy groups (P<0.05). Therefore, in the next multivariate analysis, gender and age were adjusted.

Association of IKBKAP gene SNPs with HSCR

In this article, two SNPs (rs2230793 and rs2275630) of *IKBKAP* were selected to test the association with HSCR. Four groups, including additive, dominant, recessive, and genotypic models, were tested in 1,470 cases and 1,473 controls. We did not find significant association for the two SNPs in any model, with P values ranged from 0.48 to 1.00 (*Table 2*). Next, meta-analyses for rs2230793 and rs2275630 were performed to evaluate the association between *IKBKAP* SNPs and HSCR (*Table 3*). Compared with the published data of Tang *et al.* (13), considerable heterogeneity was observed in rs2230793 (Q=86.8%, P_{het}=0.006) and rs2275630 (Q=78.0%, P_{het}=0.033). A marginal significant meta-association was found for rs2230793 (P=0.08), but not for rs2275630 (P=0.16).

Associations between IKBKAP gene SNPs and the clinical subtypes of HSCR

Next, we further carried out subgroup analyses to assess the relationship between the two SNPs and clinical subtypes of HSCR. The results showed that rs2275630 was significantly associated with TCA (OR =1.81, 95% CI: 1.17–2.80, P=0.01) rather than that with S-HSCR (OR =1.01, 95% CI: 0.83–1.24, P=0.71) and L-HSCR (OR =1.03, 95% CI: 0.76–1.40, P=0.98), respectively (*Table 4*). No significant associations were found between the other SNP rs2230793 and any of the HSCR subtypes.

Bioinformatic analysis of rs2275630

For rs2275630, there are known enhancer marks like H3K4me1 in the human fetal gut present over the region

Table 2 Associations between selected polymorphism and Hirschsprung's disease risk in southern Chinese children

Genotype	Cases	Controls	Crude OR (95% CI)	P	Adjusted OR_{adj} (95% CI)	P_{adj}
rs2230793 T>G	n=1,442	n=1,455				
TT, n (%)	828 (57.42)	836 (57.46)	1.00		1.00	
TG, n (%)	538 (37.31)	546 (37.52)	1.00 (0.85–1.16)	0.95	0.96 (0.81–1.13)	0.60
GG, n (%)	76 (5.27)	73 (5.02)	1.05 (0.75–1.47)	0.76	1.10 (0.77–1.57)	0.59
Additive, G count/T count	690/2,194	692/2,218	1.01 (0.89–1.41)	0.89	1.00 (0.88–1.14)	1.00
Dominant, TG+GG/TT	614/828	619/836	1.00 (0.87–1.16)	0.98	0.98 (0.83–1.14)	0.76
Recessive, GG/TT+TG	76/1,366	73/1,382	1.06 (0.76–1.47)	0.75	1.14 (0.80–1.61)	0.48
rs2275630 A>G	n=1,454	n=1,461				
AA, n (%)	1,195 (82.19)	1,206 (82.55)	1.00		1.00	
AG, n (%)	246 (16.92)	238 (16.29)	1.05 (0.86–1.27)	0.65	1.03 (0.84–1.27)	0.76
GG, n (%)	13 (0.89)	17 (1.16)	0.77 (0.37–1.60)	0.49	0.87 (0.40–1.87)	0.72
Additive, G count/A count	272/2,636	272/2,650	1.01 (0.85–1.20)	0.93	1.01 (0.84–1.22)	0.90
Dominant, AG+GG/AA	259/1,195	255/1,206	1.03 (0.85–1.24)	0.78	1.02 (0.84–1.25)	0.82
Recessive, GG/AA+AG	13/1,441	17/1,444	0.77 (0.37–1.59)	0.48	0.87 (0.40–1.88)	0.72

Additive, dominant, and recessive indicate the association test following dominant, recessive, and additive models, respectively. The P value indicates the significance based on different genetic models. The calculation of the OR is also based on the risk allele of each SNP. CI, confidence interval; OR, odds ratio; P_{adj}, adjusted for age and gender; SNP, single nucleotide polymorphism; G, guanine; T, thymine; A, adenine.

Table 3 Meta-analysis results for SNPs reported in previous studies on Hirschsprung's disease

SNP	Gene	BP	Study	Minor allele	OR	P	P_{meta}	Q (%)	P_{het}
rs2230793	*IKBKAP*	108897203	Our data	G	1.03 (0.91–1.17)	0.900	0.08	86.8	0.006
		110699304	Tang *et al.*	G	1.58 (1.20–2.09)	0.024			
rs2275630	*IKBKAP*	108900127	Our data	G	1.04 (0.87–1.24)	0.950	0.16	78.0	0.033
		110702228	Tang *et al.*	G	1.68 (1.12–2.51)	0.021			

SNP, single nucleotide polymorphism; BP, base-pair where the SNP is located; G, guanine; OR, odds ratio; P_{meta}, P value for meta-analysis; Q, Q-test for heterogeneity; P_{het}, P value for heterogeneity.

Table 4 The association results of two independent SNPs with different subclinical features classified by aganglionosis length, including short-length (S-HSCR), long-length (L-HSCR) and TCA

CHR	SNP	BP	A1/A2	Gene	Test	Patient, A1 count/ A2 count	Control, A1 count/ A2 count	OR	P	P_{adj}
9	rs2230793	108897203	G/T	*IKBKAP*	S-HSCR	471/1,559	692/2,218	1.00 (0.87–1.15)	0.63	0.98
					L-HSCR	151/431	692/2,218	1.16 (0.94–1.42)	0.26	0.18
					TCA	47/117	692/2,218	1.33 (0.93–1.90)	0.15	0.12
9	rs2275630	108900127	G/A	*IKBKAP*	S-HSCR	184/1,862	272/2,650	1.01 (0.83–1.24)	0.71	0.91
					L-HSCR	54/528	272/2,650	1.03 (0.76–1.40)	0.98	0.84
					TCA	26/142	272/2,650	1.81 (1.17–2.80)	0.01	7.30E–03

The P value indicates the significance based on different genetic models. The calculation of the OR is also based on the risk allele of each SNP. A1/A2 indicate the risk allele and protective allele to disease, respectively. CHR, chromosome; SNP, single nucleotide polymorphism; BP, base-pair where the SNP is located; G, guanine; T, thymine; A, adenine; OR, odds ratio; P_{adj}, P value adjusted for age and gender; TCA, total colonic aganglionosis; S-HSCR, short-segment HSCR; L-HSCR, long-segment HSCR; HSCR, Hirschsprung's disease.

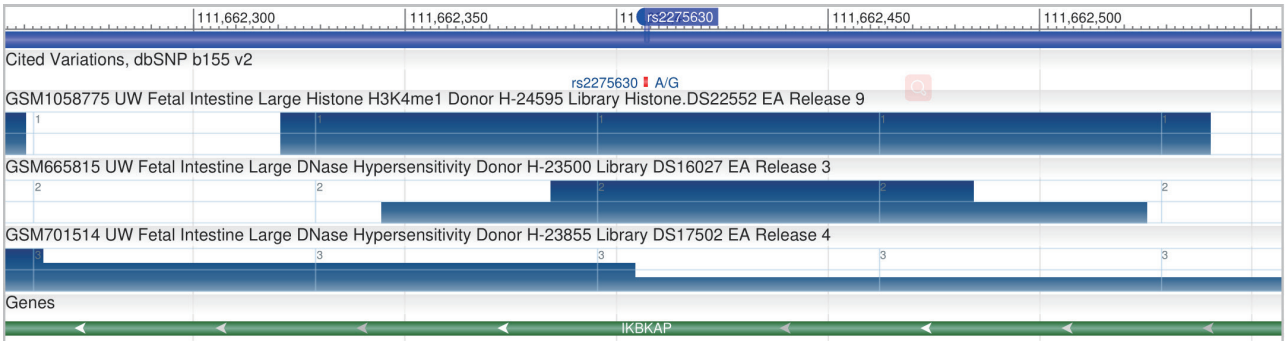

Figure 1 Analysis of enhancer markers of rs2275630 previously reported in human fetal intestine.

Table 5 Changes of TF before and after mutation with rs2275630

TF	Combined position	Predicted target sequence (direction)	Score	P	q
Pre-mutation prediction binding TF					
ZNF92	−14 to +1	TTTCTGTTAGCTTTAT (−)	11.2143	0.0000409	0.0138
SRF	−7 to +8	TAACAGAAAACTATCA (+)	11.1316	0.0000361	0.0115
MEIS1	−4 to +8	TGATAGTTTTCTG (−)	12.8333	0.000021	0.00761
BCL6	−13 to +2	GAAAACTATCAAGGAA (+)	12.6111	0.0000154	0.00509
Post-mutation prediction combined with TF					
BCL6	−13 to +2	GAGAACTATCAAGGAA (+)	12.3556	0.0000195	0.0065

ZNF92, zinc finger protein 92; SRF, serum response factor; MEIS1, myeloid ecotropic viral insertion site 1; BCL6, B-cell lymphoma 6; q, false discovery rate; TF, transcription factor.

in NCBI (*Figure 1*). The polymorphism disrupts putative transcription factor motif (*Table 5*). In HumanTFDB, the genes predicted to bind transcription factor before mutation are *ZNF92* (P=0.0000409), *SRF* (P=0.0000361), *MEIS1* (P=0.000021) and *BCL6* (P=0.0000154), and the genes predicted to bind transcription factor before mutation are *BCL6* (P=0.0000195), in which *SRF*, *MEIS1* and *BCL6* are related to nerves respectively. This point itself does not affect the expression on GTEx.

Discussion

As a disease of intestinal ganglion cell deficiency, HSCR can cause constipation and diarrhea (16,17), which may be life-threatening in severe cases. The most common therapeutics is surgery, which resected the diseased intestinal tract and reconnected the normal intestinal tract to the anus. However, the late recovery of this operation is not ideal, and many patients may have gastrointestinal diseases in their whole life (18). Therefore, it is essential to study the pathogenesis of HSCR, which can better diagnose and treat HSCR patients. So far, a series of genes have been revealed to contribute to the etiology of HSCR, including *RET*, *GDNF*, *EDNRB*, *EDN3*, *ECE1*, *SOX10*, *ZEB2*, *PHOX2B* (19-25). However, there is still much work to unravel the mysteries in the etiology of HSCR.

IKBKAP, with HGNC symbol *ELP1*, encodes the protein *IkappaB* kinase complex associated protein, also called *ELP1*. It is a scaffold protein that forms the elongator complex with ELP2, 3, 4, 5, and 6 (7). *IKBKAP* may be the best candidate to explain the risk of inflammatory bowel disease (IBD) in susceptibility loci 9q31.2 (26). *IKBKAP* is widely expressed in the central nervous system and in the critical nuclei of the brain and brainstem that can regulate the autonomic nervous system (27). *IKBKAP* may be required in the developing and adult mouse central nervous system (27). Mutations in *IKBKAP* have been reported to cause familial dysautonomia (FD) (8,9) a neuronal abnormally developmental and progressively degenerative disease (28). Over 60% of FD

patients also suffered from gastrointestinal dysfunction (10). Notably, decreased ganglion and neuron density in the ENS was found in FD patients (29), indicating that HSCR and FD share partial pathogenic mechanisms. Indeed, the concurrence of HSCR and FD had been discussed in works of literature (11). Knockout of *IKBKAP* in human neuroblastoma cells *in vitro* down-regulated *RET* expression, a well-known HSCR-pathogenic gene (30), suggesting the potential association of *IKBKAP* in developing the HSCR.

Previous zebrafish experiments (7) and fine-mapping of the 9q31 susceptible locus (13) had revealed the involvement of *IKBKAP* in HSCR. Tang *et al.* conducted a genome-wide association study in 173 Chinese HSCR patients [31 of them with *RET* no coding sequence (CDS) mutation] and 436 controls (13). They found that the *IKBKAP* SNPs rs2230793 and rs2275630 were associated with HSCR. Furthermore, after stratifying patients using the *RET* CDS mutation status, rs2275630 tended to increase the risk of HSCR along with the *RET* CDS mutations. Besides, Cheng *et al.*'s zebrafish experiment found that *ikbkap*-knockout zebrafish presented down-regulated *RET* expressions (7). However, in our study, we only measured rs2230793 and rs2275630, not RET coding variables, which will be considered later. Tang et.al conducted the research based on the background of RET coding variants. We do not have RET coding variants, so our results are not completely consistent. In this study, two previously reported *IKBKAP* SNPs, rs2230793 and rs2275630, were randomly selected to replicate in a large-scale cohort of the southern Chinese population, including 1,470 disease groups and 1,473 healthy groups. Unluckily, we failed to find significant associations for both SNPs in the four models (additive model, dominant model, recessive model, and genotype model), with P values varying between 0.48 and 0.98. However, further subgroup analysis identified a significant association between rs2275630 and TCA (OR =1.81, 95% CI: 1.17–2.80, P=0.01), indicating that *IKBKAP* acted as a TCA-specific susceptibility loci.

In summary, this study replicated *IKBKAP* as a susceptible gene for HSCR. More specifically, rs2275630 of *IKBKAP* was a TCA-specific susceptibility variant. For rs2275630, the polymorphism disrupts putative transcription factor motif (*Table 5*). Like *SRF* (31,32), it mediates developmental neuronal migration, *MEIS1* (33) is one of the decisive factors involved in differentiation during striatal development, *BCL6* (34) is an adverse biological risk factor for lymphoma. And there are known enhancer marks like H3K4me1 in the human fetal gut present over the region in NCBI (*Figure 1*). Despite the large sample size setting, some limitations of this study should be noted. First, the sample size of TCA was limited, making our result at a risk of accidental finding. Hence, the explanation of the association between rs2275630 and TCA need to be cautious. A TCA-specific case-control study that utilize large TCA sample size would be helpful to validate our findings. Second, only two SNPs were selected to tag *IKBKAP* in this study, leaving the effect of other *IKBKAP* SNPs unexploited. In particular, the functional variant accounting for the gene effect is still hidden in the shadow. Third, our results can only be responsible for the southern Chinese population. Further studies incorporating multi-ethnic populations are essential to comprehensively investigate the genotype-phenotype relationship.

Acknowledgments

We thank Yanlu Tong and Hezhen Wang for their assistance in DNA extraction and the collection of medical histories.
Funding: This study was supported by grants from National Natural Science Foundation of China (No. 81970450) and the Science and Technology Project of Guangzhou (No. 201903010074) to Yan Zhang.

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-21-550/rc

Data Sharing Statement: Available at https://tp.amegroups.com/article/view/10.21037/tp-21-550/dss

Peer Review File: Available at https://tp.amegroups.com/article/view/10.21037/tp-21-550/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tp.amegroups.com/article/view/10.21037/tp-21-550/coif). YZ reports the support by the grant of National Natural Science Foundation of China (Grant No. 81970450) and the Science and Technology Project of Guangzhou (No. 201903010074). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related

to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Ethics Review Board of Guangzhou Women and Children's Medical Center (No. 2016042036) and informed consent was taken from all the patients or their legal guardians.

References

1. Amiel J, Sproat-Emison E, Garcia-Barcelo M, et al. Hirschsprung disease, associated syndromes and genetics: a review. J Med Genet 2008;45:1-14.
2. Butler Tjaden NE, Trainor PA. The developmental etiology and pathogenesis of Hirschsprung disease. Transl Res 2013;162:1-15.
3. Chatterjee S, Chakravarti A. A gene regulatory network explains RET-EDNRB epistasis in Hirschsprung disease. Hum Mol Genet 2019;28:3137-47.
4. Gath R, Goessling A, Keller KM, et al. Analysis of the RET, GDNF, EDN3, and EDNRB genes in patients with intestinal neuronal dysplasia and Hirschsprung disease. Gut 2001;48:671-5.
5. Chatterjee S, Kapoor A, Akiyama JA, et al. Enhancer Variants Synergistically Drive Dysfunction of a Gene Regulatory Network In Hirschsprung Disease. Cell 2016;167:355-368.e10.
6. Jiang Q, Arnold S, Heanue T, et al. Functional loss of semaphorin 3C and/or semaphorin 3D and their epistatic interaction with ret are critical to Hirschsprung disease liability. Am J Hum Genet 2015;96:581-96.
7. Cheng WW, Tang CS, Gui HS, et al. Depletion of the IKBKAP ortholog in zebrafish leads to hirschsprung disease-like phenotype. World J Gastroenterol 2015;21:2040-6.
8. Anderson SL, Coli R, Daly IW, et al. Familial dysautonomia is caused by mutations of the IKAP gene. Am J Hum Genet 2001;68:753-8.
9. Slaugenhaupt SA, Blumenfeld A, Gill SP, et al. Tissue-specific expression of a splicing mutation in the IKBKAP gene causes familial dysautonomia. Am J Hum Genet 2001;68:598-605.
10. Axelrod FB. Familial dysautonomia. Muscle Nerve 2004;29:352-63.
11. Azizi E, Berlowitz I, Vinograd I, et al. Congenital megacolon associated with familial dysautonomia. Eur J Pediatr 1984;142:68-9.
12. Pattyn A, Morin X, Cremer H, et al. The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature 1999;399:366-70.
13. Tang CS, Sribudiani Y, Miao XP, et al. Fine mapping of the 9q31 Hirschsprung's disease locus. Hum Genet 2010;127:675-83.
14. Marchini J, Howie B, Myers S, et al. A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet 2007;39:906-13.
15. Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.
16. Kapur RP. Practical pathology and genetics of Hirschsprung's disease. Semin Pediatr Surg 2009;18:212-23.
17. Gao ZG, Chen QJ, Shao M, et al. Preliminary identification of key miRNAs, signaling pathways, and genes associated with Hirschsprung's disease by analysis of tissue microRNA expression profiles. World J Pediatr 2017;13:489-95.
18. Leenders E, Sieber WK, Kiesewetter WB. Hirschsprung's disease. Surg Clin North Am 1970;50:907-18.
19. Chatterjee S, Karasaki KM, Fries LE, et al. A multi-enhancer RET regulatory code is disrupted in Hirschsprung disease. Genome Res 2021. [Epub ahead of print].
20. Di Zanni E, Adamo A, Belligni E, et al. Common PHOX2B poly-alanine contractions impair RET gene transcription, predisposing to Hirschsprung disease. Biochim Biophys Acta Mol Basis Dis 2017;1863:1770-7.
21. Porokuokka LL, Virtanen HT, Lindén J, et al. Gfra1 Underexpression Causes Hirschsprung's Disease and Associated Enterocolitis in Mice. Cell Mol Gastroenterol Hepatol 2019;7:655-78.
22. Soret R, Schneider S, Bernas G, et al. Glial Cell-Derived Neurotrophic Factor Induces Enteric Neurogenesis and Improves Colon Structure and Function in Mouse Models of Hirschsprung Disease. Gastroenterology 2020;159:1824-1838.e17.

23. Hong M, Li X, Li Y, et al. Hirschsprung's disease: key microRNAs and target genes. Pediatr Res 2021. [Epub ahead of print]. doi:10.1038/s41390-021-01872-1.
24. Rogers JM. Search for the missing lncs: gene regulatory networks in neural crest development and long non-coding RNA biomarkers of Hirschsprung's disease. Neurogastroenterol Motil 2016;28:161-6.
25. Karim A, Tang CS, Tam PK. The Emerging Genetic Landscape of Hirschsprung Disease and Its Potential Clinical Applications. Front Pediatr 2021;9:638093.
26. Bonfiglio F, Zheng T, Garcia-Etxebarria K, et al. Female-Specific Association Between Variants on Chromosome 9 and Self-Reported Diagnosis of Irritable Bowel Syndrome. Gastroenterology 2018;155:168-79.
27. Chaverra M, George L, Mergy M, et al. The familial dysautonomia disease gene IKBKAP is required in the developing and adult mouse central nervous system. Dis Model Mech 2017;10:605-18.
28. Naumanen T, Johansen LD, Coffey ET, et al. Loss-of-function of IKAP/ELP1: could neuronal migration defect underlie familial dysautonomia? Cell Adh Migr 2008;2:236-9.
29. Bar-Shai A, Maayan C, Vromen A, et al. Decreased density of ganglia and neurons in the myenteric plexus of familial dysautonomia patients. J Neurol Sci 2004;220:89-94.
30. Cohen-Kupiec R, Pasmanik-Chor M, Oron-Karni V, et al. Effects of IKAP/hELP1 deficiency on gene expression in differentiating neuroblastoma cells: implications for familial dysautonomia. PLoS One 2011;6:e19147.
31. Knöll B, Nordheim A. Functional versatility of transcription factors in the nervous system: the SRF paradigm. Trends Neurosci 2009;32:432-42.
32. Kalita K, Kuzniewska B, Kaczmarek L. MKLs: co-factors of serum response factor (SRF) in neuronal responses. Int J Biochem Cell Biol 2012;44:1444-7.
33. Rataj-Baniowska M, Niewiadomska-Cimicka A, Paschaki M, et al. Retinoic Acid Receptor β Controls Development of Striatonigral Projection Neurons through FGF-Dependent and Meis1-Dependent Mechanisms. J Neurosci 2015;35:14467-75.
34. Qualls D, Abramson JS. Advances in risk assessment and prophylaxis for central nervous system relapse in diffuse large B-cell lymphoma. Haematologica 2019;104:25-34.

Cite this article as: Wang N, Xi J, Lan C, Wu Y, Zhu Y, Zuo X, Zhang Y. Association between *IKBKAP* polymorphisms and Hirschsprung's disease susceptibility in Chinese children. Transl Pediatr 2022;11(6):789-796. doi: 10.21037/tp-21-550