Research Article

miR-618 rs2682818 C>A polymorphism decreases Hirschsprung disease risk in Chinese children

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MicroRNAs (miRNAs) are endogenous non-coding small RNAs that play an important role in the development of many malignant tumors. In addition, recent studies have reported that single nucleotide polymorphisms (SNPs) located in the miRNA functional region was inextricably linked to tumor susceptibility. In the present study, we investigated the susceptibility between miR-618 rs2682818 C>A and Hirschsprung disease (HSCR) in the Southern Chinese population (1470 patients and 1473 controls). Odds ratios (ORs) and 95% confidence intervals (CIs) were used for estimating the strength of interrelation between them. We found that the CA/AA genotypes of miR-618 rs2682818 were associated with a decreased risk of HSCR when compared with the CC genotype (OR = 0.84, 95% CI = 0.72–0.99, P = 0.032). Based on the stratified analysis of HSCR subtypes, the rs2682818 CA/AA genotypes were able to significantly lessen the risk of HSCR compared with CC genotype in patients with long-segment HSCR (adjusted OR = 0.70, 95% CI = 0.52–0.93, P = 0.013). In conclusion, our results indicated that the miR-618 rs2682818 C>A polymorphism was associated with a reduced risk of HSCR in Chinese children, especially in patients with long-segment HSCR (L-HSCR) subtype.

Introduction

Hirschsprung disease (HSCR), also known as aganglionosis, is a common congenital digestive tract disease in pediatric surgery [1]. It is a significant racial difference in the incidence of HSCR [2]. The incidence in Asians is approximately 2.8/10000, while the incidence of Hispanics is approximately 1/10000 [3–5]. In addition, the incidence of HSCR is also significantly related to gender, with a male to female ratio of 4:1 [6]. Clinically, according to the length of the ganglion segment, HSCR can be divided into three types, including short-segment HSCR (S-HSCR), long-segment HSCR (L-HSCR) and total colon aganglionosis (TCA) [7,8]. The overwhelming majority of HSCRs exhibit sporadic traits, and approximately 20% of HSCR cases present a familial genetic pattern [7,9,10]. HSCR is characterized by siblings whose risk of recurrence is 3–17%, with different incidence rates being significantly associated with gender, ganglion segment length and familial [10,11]. In recent years, more than a dozen genes related to the pathogenesis of HSCR were confirmed, including RET [12], EDNRB [13], SOX10 [14], GDNF [15], EDN3 [16], PHOX2B [17,18], etc. However, these gene mutations associated with the onset of HSCR are only half of current HSCR cases [6]. Therefore, the genetic model of HSCR is a multifactorial genetic disease, and its specific pathogenesis remains to be further explored [19,20].

MicroRNAs (miRNAs) are single-stranded, non-coding RNAs containing 17–25 nucleotides that specifically bind to the 3’ untranslated region (UTR) region of mRNA and regulate the related gene expression [21,22]. It is estimated that approximately 30% of genes in the human genome regulated by miRNAs [23,24]. Single nucleotide polymorphisms (SNPs) are the most common, numerous and widely distributed variants of the human genome [25]. By interfering transcription, processing of pri-miRNA
and pre-miRNA, SNPs in the functional region can affect the function of miRNA, thereby regulating the expression of downstream genes [26].

Recent studies have shown that miR-618 deregulation is closely related to tumorigenesis, including hepatocellular carcinoma [27], thyroid cancer [28], breast cancer [29], bladder cancer [30] and Barrett’s esophageal cancer. The SNP rs2682818 located on the stem-loop sequence of the miR-618 precursor, so it can affect and/or alter the secondary structure of pre-miR-618, which in turn affects the miR-618 expression, thereby affecting the association of miR-618 with cancer risk [31,32]. In recent years, more and more reports have illustrated that SNP rs2682818 is closely related to the susceptibility of a variety of diseases, including follicular lymphoma [31], acute lymphocytic leukemia [33], colorectal cancer [34] and breast cancer [32]. However, the correlation between SNP rs2682818 and HSCR risk is still unclear. Therefore, we performed this case–control study with 1470 cases of HSCR and 1473 control subjects to assess the association between miR-618 rs2682818 C>A polymorphism and HSCR susceptibility in Chinese children.

Materials and methods

Study subjects
In the present study, 1470 HSCR cases were collected from 2010 to 2015 and 1473 controls. These patients were diagnosed as HSCR in the pediatric outpatient clinic of Guangzhou Women and Children Children's Hospital (Guangzhou, China). The description of specific diagnostic criteria for HSCR could be found in our previous studies [35]. Participants without a history of HSCR and neurological disease were used as controls. According to the segment length of the ganglion, the case groups were divided into three subgroups, including S-HSCR, L-HSCR and TCA. The present study was approved by the Guangzhou Women and Children Children's Hospital Institutional Review Board. Written informed consent was obtained from the guardian of all participants. The ethics certificate number is: 201943800.

SNP genotyping
For the selection of potential functional polymorphisms, we have described in detail in our previous study [36]. Genomic DNA was extracted from venous blood and paraffins samples using the TIANamp Blood Genomic DNA Kit and TIANquick FFPE DNA Kit (TIANGEN Biotech Co. Ltd., Beijing, China) [37]. Subsequently, we amplified the genomic DNA samples by ABI-7900 real-time quantitative PCR instrument (Applied Biosystem, Foster City, CA) and performed TaqMan genotyping on the selected polymorphisms [38–40]. We randomly selected 10% of the DNA samples for the second genotyping. The consistency of all replicate samples was 100% to ensure the accuracy of the data [41].

Statistical analysis
Statistical analysis in the present study was performed by SAS 9.4 software (SAS Institute Inc, Cary, NC, U.S.A.). A two-sided chi-square test was used to verify the distribution of samples characteristics between all cases and controls group. The goodness-of-fit chi-squared test was used to examine that the genotype frequencies in the controls were consistent with the Hardy–Weinberg equilibrium (HWE). By multivariate logistic regression analysis, the adjusted odds ratio (OR) and 95% confidence interval (CI) were calculated and then used for assessing the association between miR-618 rs2682818 C>A polymorphism and HSCR risk. Only when P<0.05 could be considered statistically significant.

Results

Association between miR-618 rs2682818 polymorphism and HSCR susceptibility
In the present study, 1330 cases and 1453 controls were successfully genotyped. The frequency distribution of rs2682818 C>A genotype in the control groups was consistent with HWE (P=0.793). The case group and the control genotype frequencies in the present study were shown in Table 1. We found that the miR-618 rs2682818 C>A polymorphism was associated with a reduced risk of HSCR (OR = 0.84, 95% CI = 0.72–0.99, P=0.032). In addition, miR-618 rs2682818 C>A polymorphism showed significant correlations with HSCR risk in the additive (adjusted OR = 0.88, 95% CI = 0.77–0.99, P=0.038) and dominant models (adjusted OR = 0.85, 95% CI = 0.72–0.99, P=0.036).
**Table 1** Association between miR-618 rs2682818 C>A polymorphism and HSCR susceptibility

| Genotype | Cases (n=1330) | Controls (n=1453) | Crude OR (95% CI) | P1 | Adjusted OR (95% CI) | P2 |
|----------|----------------|-------------------|-------------------|----|----------------------|----|
| rs2682818C>A (HWE = 0.793) | | | | | | |
| CC | 788 (59.25) | 798 (54.29) | 1.00 | 1.00 | | |
| CA | 461 (34.66) | 555 (38.20) | 0.84 (0.72–0.99) | 0.032 | 0.85 (0.72–1.01) | 0.058 |
| AA | 81 (6.09) | 100 (6.88) | 0.82 (0.60–1.12) | 0.209 | 0.81 (0.58–1.11) | 0.184 |
| Additive | | | 0.87 (0.77–0.99) | 0.029 | 0.88 (0.77–0.99) | 0.038 |
| Dominant | 542 (40.75) | 655 (45.08) | 0.84 (0.72–0.97) | 0.021 | 0.85 (0.72–0.99) | 0.036 |
| Recessive | 1249 (93.91) | 1353 (93.12) | 0.88 (0.65–1.19) | 0.397 | 0.86 (0.63–1.17) | 0.332 |

1 χ2 test for genotype distributions between HSCR patients and controls.
2 Adjusted for age and gender.
For values in bold, P < 0.05.

**Table 2** Stratification analysis for the association between miR-618 rs2682818 C>A and HSCR susceptibility (by subtype)

| Variables | rs2682818 (cases/controls) | Crude OR (95% CI) | P1 | Adjusted OR1 (95% CI) | P1 |
|-----------|-----------------------------|-------------------|----|----------------------|----|
| CC | | 0.91 (0.77–1.08) | 0.274 | 0.93 (0.78–1.10) | 0.385 |
| CA/AA | 162/798 | 0.68 (0.51–0.89) | 0.006 | 0.70 (0.52–0.93) | 0.013 |
| S-HSCR | 545/798 | 0.70 (0.42–1.16) | 0.166 | 0.72 (0.42–1.21) | 0.212 |
| L-HSCR | 90/655 | | | |
| TCA | 42/798 | | | |

1 Adjusted for age and gender with omitting the corresponding stratification factor.
For values in bold, P < 0.05.

**Subtype analysis of the relationship between rs2682818 polymorphism and HSCR risk**

The different subclinical features of HSCR are classified according to the length of the ganglion. Compared with the rs2682818 CC genotype, the CA/AA genotypes were able to remarkably reduce the risk of HSCR in patients with L-HSCR (adjusted OR = 0.70, 95% CI = 0.52–0.93, P=0.013). However, no significant correlation was found in patients with S-HSCR and TCA (Table 2).

**Discussion**

miR-618 is a small molecule consisting of 98 nucleotides located on chromosome 12. In recent years, more and more reports have reported that miR-618 is closely related to various malignant tumors. Song et al. [42] found that miR-618 was down-regulated in metastatic androgen-independent prostate cancer, and patients with low miR-618 had a poor prognosis. It was worth mentioning that overexpression of miR-618 could induce MET (mesenchymal-to-epithelial transition) by binding to FOXP2 (Forkhead box p2), and inhibiting the migration and invasion of prostate cells. A study from Shi et al. [43] reported that miR-618 was down-regulated in gastric cancer tissue, whereas up-regulation of miR-618 expression inhibited migration and invasion of gastric cancer cells. These studies suggest that the low expression of miR-618 may be negatively correlated with the ability of malignant tumors to metastasize.

The SNP rs2682818 is a part of the miR-618 precursor stem loop sequence, so the rs2682818 polymorphism can alter the secondary stem-loop structure of miR-618, thereby affecting pre-miR-618 processing and expression levels [31,44]. It is worth mentioning that Zhu et al. have reported that miR-146a rs2910164 homozygous GG expression level is higher than CC, which indicates that C-to-G in rs2910164 may change the precursor of miR-146a, thereby improving mature miR-146a expression level in HSCR [45]. They suspected that the amount of mature miRNA changed by somatic mutation could affect HSCR risk through by inhibiting TRAF6 and IRAK1, key adapter molecules downstream of the Toll-like and cytokine receptors, which were frequently rearranged with RET proto-oncogene [46]. This is very similar to the expression pattern of miR-618, and it is worth further researching the role of rs2682818 in HSCR. Fu et al. [31] reported that the rs2682818 variant A allele was associated with decreased expression of mature miR-618 and increased susceptibility to follicular lymphoma. Interestingly, the rs2682818 C>A polymorphism presented a protective effect in chronic lymphocytic leukemia [47]. Chen et al. found that people with AA or AC genotype had a lower risk of colorectal cancer than CC genotype [34]. In addition, a recent meta-analysis study by

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Feng et al. [47] showed that rs2682818 of miR-618 was associated with breast cancer and miR-618 could be a potential biomarker. These studies indicate that rs2682818 variant A allele can influence the miR-618 expression, thereby affecting the risk of susceptibility to some malignancies or other diseases. The RET gene was the first gene discovered to be recognized as a major player in the pathogenesis of HSCR [48]. A meta-analysis study from Liang et al. reported that the SNPs of (rs1800858, rs1800860, rs10900297 and rs2435357) RET polymorphisms was significantly associated with an increased risk of HSCR [49]. Subsequently, Zhang et al. [35] found that the risk of RET SNPs in S-HSCR patients was significantly higher than in patients with L-HSCR and TCA in the sporadic cases. However, we found that the rs2682818 polymorphism significantly reduced HSCR susceptibility in the L-HSCR population in the stratified analysis based on ganglion length. In addition, early research found that patients with long segments were characterized by autosomal dominant inheritance, and patients with short segments were characterized by recessive or multifactorial inheritance [50]. Our results demonstrated that miR-618 rs2682818 C>A significantly reduced the risk of HSCR susceptibility in both dominant and additional models. This suggests that miR-618 rs2682818 C>A may be a dominant inheritance of non-RET gene variants.

To the best of our knowledge, this was the first study to explore the association of rs2682818 polymorphism in miR-618 with HSCR susceptibility. In the present study, the proportion of males in the case group was 83.67%, and that in the control group was 34.35%. After adjusting for age and gender, the polymorphism of rs2682818 CA genotype was no significant association related to HSCR risk comparing with CC genotype. However, the P-value is bounded by a critical value and has a certain statistical significance. This indicates that participants with different genders of CA genotype have a close relationship with susceptibility to HSCR. On the other hand, this also implies that the small sample sizes, all of them from the southern Han population, which will bring a certain deviation to judge the association of rs2682818 genotype and HSCR susceptibility risk.

In addition, some limitations need to be mentioned in this experiment. First, we have not yet analyzed the association of HSCR with environmental factors, including the effects of diet, geographic location, etc. Second, there is still a lack of in-depth exploration of miR-618 rs2682818 and HSCR sensitivity mechanisms, which has potential implications for HSCR therapy.

In summary, in the present study, we found that the miR-618 rs2682818 C>A polymorphism could reduce the risk of HSCR susceptibility, especially in patients with L-HSCR. Next, multiregional and multicenter collaboration is needed to expand the sample sizes and verify this susceptibility, thus providing a new direction for clinical research.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution
All authors contributed significantly to this work. Y.Z., T.L., X.X., L.L., and Q.H. performed the research study and collected the data. Y.Z. and T.L. analyzed the data. T.L. and W.Z. designed the research study. Y.Z., T.L. and W.Z. wrote the paper. T.L. prepared all the tables. All authors reviewed the manuscript. In addition, all authors have read and approved the manuscript.

Abbreviations
CI, confidence interval; HSCR, Hirschspring disease; HWE, Hardy–Weinberg equilibrium; L-HSCR, long-segment HSCR; miRNA, microRNA; OR, odds ratio; S-HSCR, short-segment HSCR; SNP, single nucleotide polymorphism; TCA, total colon aganglionosis; UTR, untranslated region.

References
1 Burkardt, D.D., Graham, Jr, J.M., Short, S.S. and Frykman, P.K. (2014) Advances in Hirschsprung disease genetics and treatment strategies: an update for the primary care pediatrician. *Clin. Pediatr. (Phila)* 53, 71–81, https://doi.org/10.1177/0009922713500846
2 Tang, C.S., Cheng, G., So, M.T., Yip, B.H., Miao, X.P., Wong, E.H. et al. (2012) Genome-wide copy number analysis uncovers a new HSCR gene: NRG3. *PLoS Genet.* 8, e1002687, https://doi.org/10.1371/journal.pgen.1002687
3 Russell, M.B., Russell, C.A. and Niebuhr, E. (1994) An epidemiological study of Hirschsprung’s disease and additional anomalies. *Acta Paediatr.* 83, 68–71, https://doi.org/10.1111/j.1651-2227.1994.tb12955.x
4 Zhao, J., Xie, X., Yao, Y., He, Q., Zhang, R., Xia, H. et al. (2018) Association of VAMP5 and MCC genetic polymorphisms with increased risk of Hirschsprung disease susceptibility in Southern Chinese children. *Aging (Albany N.Y.)* 10, 689–700, https://doi.org/10.18632/aging.101423
5 Garcia-Barcelo, M.M., Sham, M.H., Lui, V.C., Chen, B.L., Song, Y.Q., Lee, W.S. et al. (2003) Chinese patients with sporadic Hirschsprung’s disease are predominantly represented by a single RET haplotype. J. Med. Genet. 40, e122, https://doi.org/10.1136/jmg.40.11.e122

6 Butter Tjaden, N.E. and Trainer, P.A. (2013) The developmental etiology and pathogenesis of Hirschsprung disease. Transl. Res. 162, 1–15, https://doi.org/10.1016/j.trsl.2013.03.001

7 Amiel, J., Sproat-Eisman, E., Garcia-Barcelo, M., Lantieri, F., Burzynski, G., Borrego, S. et al. (2008) Hirschsprung disease, associated syndromes and genetics: a review. J. Med. Genet. 45, 1–14, https://doi.org/10.1136/jmg.2007.053959

8 Moore, S.W. (2012) Total colonic aganglionosis in Hirschsprung disease. Semin. Pediatr. Surg. 21, 302–309, https://doi.org/10.1053/j.sempedsurg.2012.07.004

9 Luzon-Toro, B., Gui, H., Ruiz-Ferrer, M., Sze-Man Tang, C., Fernandez, R.M., Sham, P.C. et al. (2015) Exome sequencing reveals a high genetic heterogeneity on familial Hirschsprung disease. Sci. Rep. 5, 16473, https://doi.org/10.1038/srep16473

10 Tilghman, J.M., Ling, A.Y., Turner, T.N., Soisa, M.X., Krumm, N., Chattarjee, S. et al. (2019) Molecular genetic anatomy and risk profile of Hirschsprung's disease. N. Engl. J. Med. 380, 1421–1432, https://doi.org/10.1056/NEJMoa1706594

11 Passarge, E. (2002) Dissecting Hirschsprung disease. Nat. Genet. 31, 11–12, https://doi.org/10.1038/ng878

12 Romeo, G., Ronchette, P., Luo, Y., Barone, V., Seri, M., Ceccherini, I. et al. (1994) Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung’s disease. Nature 367, 377–378, https://doi.org/10.1038/367377a0

13 Carrasquillo, M.M., McCailllon, A.S., Paffenbeger, E.G., Kashuk, C.S., Nouri, N. and Chakravarti, A. (2002) Genome-wide association study and mouse model identify interaction between RET and EDNIRB pathways in Hirschsprung disease. Nat. Genet. 32, 237–244, https://doi.org/10.1038/ng998

14 Sham, M.H., Lui, V.C., Fu, M., Chen, B. and Tam, P.K. (2001) SOX10 is abnormaly expressed in aganglionic bowel of Hirschsprung’s disease infants. Gut 49, 220–226, https://doi.org/10.1136/gut.49.2.220

15 Shen, L., Pichel, J.G., Mayelli, T., Sarola, H., Lu, B. and Westphal, H. (2002) Gdfn haploinsufficiency causes Hirschsprung-like intestinal obstruction and early-onset lethality in mice. Am. J. Hum. Genet. 70, 435–447, https://doi.org/10.1086/338712

16 Duan, X.L., Zhang, X.S. and Li, H.W. (2003) Clinical relationship between EDN-3 gene, EDNRB gene and Hirschsprung’s disease. World J. Gastroenterol. 9, 2839–2842, https://doi.org/10.3748/wjg.v9.i2.2839

17 Garcia-Barcelo, M., Sham, M.H., Lui, V.C., Chen, B.L., Ott, J. and Tam, P.K. (2003) Association study of PHOX2B as a candidate gene for Hirschsprung’s disease. Gut 52, 563–567, https://doi.org/10.1136/gut.52.4.563

18 Zhao, J., Zhu, Y., Xie, X., Yao, Y., Zhang, J., Zhang, R. et al. (2019) Pleiotropic effect of common PHOX2B variants in Hirschsprung disease and neuroblastoma. Aging (Albany N.Y.) 11, 1252–1261, https://doi.org/10.18632/aging.101834

19 Emison, E.S., Garcia-Barcelo, M., Grice, E.A., Lantieri, F., Amiel, J., Burzynski, G. et al. (2010) Differential contributions of rare and common, coding and noncoding Ret mutations to multifactorial Hirschsprung disease liability. Am. J. Hum. Genet. 87, 60–74, https://doi.org/10.1016/j.ajhg.2010.06.007

20 Jannott, A.S., Amiel, J., Polet, A., Lantieri, F., Fernandez, R.M., Verheij, J.B. et al. (2012) Male and female differential reproductive rate could explain parental transmission asymmetry of mutation origin in Hirschsprung disease. Eur. J. Hum. Genet. 20, 917–920, https://doi.org/10.1038/ejhg.2012.35

21 He, L. and Hannon, G.J. (2004) MicroRNAs: small RNAs with a big role in gene regulation. Nat. Rev. Genet. 5, 522–531, https://doi.org/10.1038/nrg1379

22 Tang, F., Hajkova, P., Barton, S.C., Lao, K. and Surani, M.A. (2006) MicroRNA expression profiling of single whole embryonic stem cells. Nucleic Acids Res. 34, e9, https://doi.org/10.1093/nar/gnj009

23 Lewis, B.P., Burge, C.B. and Bartel, D.P. (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120, 15–20, https://doi.org/10.1016/j.cell.2004.12.035

24 Erson, A.E. and Petty, E.M. (2008) MicroRNAs in development and disease. Clin. Genet. 74, 296–306, https://doi.org/10.1111/j.1399-0004.2008.01076.x

25 Theuerb, J., Olubosun, A. and Vihinen, M. (2011) Performance of mutation pathogenicity prediction methods on missense variants. Hum. Mutat. 32, 358–368, https://doi.org/10.1002/humu.21445

26 Ryan, B.M., Rundles, A.J. and Harris, C.C. (2010) Genetic variation in microRNA networks: the implications for cancer research. Nat. Rev. Cancer 10, 389–402, https://doi.org/10.1038/nrc2867

27 Abdalla, M.A. and Haj-Ahmad, Y. (2012) Promising urinary protein biomarkers for the early detection of hepatocellular carcinoma among high-risk hepatitis C virus Egyptian patients. J. Cancer 3, 390–403, https://doi.org/10.7150/jca.4280

28 Cheng, Q., Zhang, X., Xu, X. and Lu, X. (2014) miR-618 inhibits anaplastic thyroid cancer by repressing XIAP in one ATC cell line. Ann. Endocrinol. (Paris) 75, 187–193, https://doi.org/10.1016/j.anенд.2014.01.002

29 Fassan, M., Baffa, R., Palazzo, J.P., Lloyd, J., Crosario, M., Liu, C.G. et al. (2009) MicroRNA expression profiling of male breast cancer. Breast Cancer Res. 11, R58, https://doi.org/10.1186/bcr2348

30 Fassan, M., Volinia, S., Patalini, J., Pizzi, M., Baffa, R., De Bernard, M. et al. (2011) MicroRNA expression profiling in human Barrett’s carcinogenesis. Int. J. Cancer 129, 1661–1670, https://doi.org/10.1002/ijc.25823

31 Fu, A., Hoffman, A.E., Liu, R., Jacobs, D.I., Zheng, T. and Zhu, Y. (2014) Targetome profiling and functional genetics implicate miR-618 in lymphomagenesis. Epigenetics 9, 730–737, https://doi.org/10.4161/epi.27996

32 Morales, S., Gulphi, F., Gonzalez-Hormazabal, P., Fernandez-Ramires, R., Bravo, T., Reyes, J.M. et al. (2016) Association of single nucleotide polymorphisms in pre-miR-27a, pre-miR-196a2, pre-miR-423, miR-608 and pre-miR-618 with breast cancer susceptibility in a South American population. BMC Genet. 17, 109, https://doi.org/10.1186/s12863-016-0415-0

33 Dzikiewicz-Krawczyk, A. (2015) MicroRNA polymorphisms as markers of prognosis and treatment response in hematologic malignancies. Crit. Rev. Oncol. Hematol. 93, 1–17, https://doi.org/10.1016/j.critrevonc.2014.08.006

34 Chen, Y., Du, M., Chen, W., Zhu, L., Wu, C., Zhang, Z. et al. (2018) Polymorphism rs2682818 in miR-618 is associated with colorectal cancer susceptibility in a Han Chinese population. Cancer Med. 7, 1194–1200, https://doi.org/10.1002/cam4.1409
35. Zhang, Y., He, Q., Zhang, R., Zhang, H., Zhong, W. and Xia, H. (2017) Large-scale replication study identified multiple independent SNPs in RET synergistically associated with Hirschsprung disease in Southern Chinese population. Aging (Albany N.Y.) 9, 1996–2009, https://doi.org/10.18632/aging.101294

36. He, J., Qiu, L.X., Wang, M.Y., Hua, R.X., Zhang, R.X., Yu, H.P. et al. (2012) Polymorphisms in the XPG gene and risk of gastric cancer in Chinese populations. Hum. Genet. 131, 1235–1244, https://doi.org/10.1007/s00439-012-1152-8

37. Lu, T., Li, L., Zhu, J., Liu, J., Lin, A., Fu, W. et al. (2019) AURKA rs8173 G>C polymorphism decreases Wilms tumor risk in Chinese children. J. Oncol. 2019, 9074908

38. He, J., Wang, F., Zhu, J., Zhang, R., Yang, T., Zou, Y. et al. (2016) Association of potentially functional variants in the XPG gene with neuroblastoma risk in a Chinese population. J. Cell. Mol. Med. 20, 1481–1490, https://doi.org/10.1111/jcmm.12836

39. He, J., Yang, T., Zhang, R., Zhu, J., Wang, F., Zou, Y. et al. (2016) Potentially functional polymorphisms in the LIN28B gene contribute to neuroblastoma susceptibility in Chinese children. J. Cell. Mol. Med. 20, 1534–1541, https://doi.org/10.1111/jcmm.12846

40. He, J., Zhang, X., Zhang, J., Zhang, R., Yang, T., Zhu, J. et al. (2018) LM01 super-enhancer polymorphism rs2168101 G>T correlates with decreased neuroblastoma risk in Chinese children. J. Cancer 9, 1592–1597

41. Liu, J., Jia, W., Hua, R.X., Zhu, J., Zhang, J., Yang, T. et al. (2019) APEX1 polymorphisms and neuroblastoma risk in Chinese children: a three-center case-control study. Oxid. Med. Cell Longev. 2019, 5736175, https://doi.org/10.1155/2019/5736175

42. Song, X.L., Tang, Y., Lei, X.H., Zhao, S.C. and Wu, Z.Q. (2017) miR-618 inhibits prostate cancer migration and invasion by targeting FOXP2. J. Cancer 8, 2501–2510, https://doi.org/10.7150/jca.17407

43. Shi, J., Gong, L., Chen, L., Luo, J., Song, G., Xu, J. et al. (2019) miR-618 suppresses metastasis in gastric cancer by downregulating the expression of TGF-beta2. Anat. Rec. (Hoboken) 302, 931–940, https://doi.org/10.1002/ar.24083

44. Zhang, Z., Xu, G., Cai, B., Zhang, H., Zhu, W. and Liu, X. (2017) Genetic variants in microRNAs predict recurrence of ischemic stroke. Mol. Neurobiol. 54, 2776–2780, https://doi.org/10.1007/s12035-016-9865-7

45. Zhu, H., Cai, P., Zhu, D., Xu, C., Li, H., Tang, J. et al. (2014) A common polymorphism in pre-miR-146a underlies Hirschsprung disease risk in Han Chinese. Exp. Mol. Pathol. 97, 511–514, https://doi.org/10.1016/j.yexmp.2014.11.004

46. Jazdzewski, K., Murray, E.L., Franssila, K., Jarzab, B., Schoenberg, D.R. and de la Chapelle, A. (2008) Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc. Natl. Acad. Sci. U.S.A. 105, 7269–7274, https://doi.org/10.1073/pnas.0802682105

47. Feng, X., Ji, D., Liang, C. and Fan, S. (2019) Does miR-618 rs2682818 variant affect cancer susceptibility? Evidence from 10 case-control studies. Biosci. Rep. 39, BSR20190741, https://doi.org/10.1042/BSR20190741

48. Edery, P., Lyonnet, S., Mulligan, L.M., Pelet, A., Dew, E., Abel, L. et al. (1994) Mutations of the RET proto-oncogene in Hirschsprung’s disease. Nature 367, 378–380, https://doi.org/10.1038/367378a0

49. Liang, C.M., Ji, D.M., Yuan, X., Ren, L.L., Shen, J. and Zhang, H.Y. (2014) RET and PHOX2B genetic polymorphisms and Hirschsprung’s disease susceptibility: a meta-analysis. PLoS ONE 9, e90091, https://doi.org/10.1371/journal.pone.0090091

50. Badner, J.A., Sieber, W.K., Garver, K.L. and Chakravarti, A. (1999) A genetic study of Hirschsprung disease. Am. J. Hum. Genet. 46, 568–580