Association between \textit{PHOX2B} gene rs28647582 T>C polymorphism and Wilms tumor susceptibility

Ao Lin\textsuperscript{1,*}, Wen Fu\textsuperscript{1,*}, Wenwen Wang\textsuperscript{2,*}, Jinhong Zhu\textsuperscript{3}, Jiabin Liu\textsuperscript{1}, Huimin Xia\textsuperscript{1}, Guochang Liu\textsuperscript{1} and Jing He\textsuperscript{1}

\textsuperscript{1}Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangzhou Women and Children’s Medical Center, Guangzhou Medical University, Guangzhou 510623, Guangdong, China; \textsuperscript{2}Department of Oncology, The Affiliated Wuxi No.2 People’s Hospital of Nanjing Medical University, Wuxi 214000, Jiangsu, China; \textsuperscript{3}Department of Clinical Laboratory, Biobank, Harbin Medical University Cancer Hospital, Harbin 150040, Heilongjiang, China

Correspondence: Jing He (hejing198374@gmail.com) or Guochang Liu (starbless2003@126.com)

Wilms tumor is one of the most common pediatric solid tumors. The pair-like homeobox 2b (\textit{PHOX2B}) gene is an important transcription factor that regulates cellular proliferation and differentiation in early life. The association between \textit{PHOX2B} single nucleotide polymorphisms (SNPs) and Wilms tumor risk has not been investigated. Therefore, we conducted a case-control study involving 145 Wilms tumor patients and 531 controls to explore the association between the \textit{PHOX2B} rs28647582 T>C polymorphism and Wilms tumor susceptibility. The association between the \textit{PHOX2B} rs28647582 T>C polymorphism and Wilms tumor susceptibility was assessed by odds ratios (ORs) and 95% confidence intervals (CIs). Our results indicated that \textit{PHOX2B} rs28647582 T>C polymorphism did not significantly alter Wilms tumor susceptibility. However, in the stratified analysis, we found that TC/CC genotypes significantly increased Wilms tumor risk among children older than 18 months (adjusted OR = 1.77, 95% CI = 1.07–2.95, \textit{P}=0.027) and those with clinical stages III+IV (adjusted OR = 1.75, 95% CI = 1.09–2.82, \textit{P}=0.022), when compared with those with TT genotype. Our study suggested that \textit{PHOX2B} rs28647582 T>C was weakly associated with Wilms tumor susceptibility. Our conclusions need further validation with a larger sample size.

\textbf{Introduction}

Wilms tumor, also known as nephroblastoma, is one of the most common pediatric malignant tumors, accounting for 7–8% of tumors in childhood [1,2]. The prevalence of Wilms tumor in Chinese children is approximately 3.3/million [3]. According to the report, 75% of patients are younger than 5-years old and incidence peak is 3-year old [1,4]. Survival rate of Wilms tumor once was less than 30%. Benefitting by the combined utilization of surgery, chemotherapy, radiotherapy and other treatment methods, 90% of Wilms tumors patients can be cured nowadays [4]. But, approximately 25% of Wilms tumor patients still have a poor survival rate of less than 70%, which results from unfavorable histopathological types [2]. Even though these patients survive, they also have a high recurrence rate and suffer from chronic health problems. There are some theories that try to explain the origin of nephroblastoma. The more frequently accepted theory is that posterior renal blastocyst fail to differentiate into glomeruli and renal tubules [5]. However, the mechanism of unsuccessful differentiation of posterior renal blastocyst is unknown. Therefore, it is necessary to explore genetic etiology of Wilms tumor to provide theoretical basis for its prediction and treatment.

With the development of genome-wide association studies (GWASs), more and more Wilms tumor susceptibility genes have been discovered. For example, single nucleotide polymorphisms (SNPs) in the \textit{FWT1} [6], \textit{FWT2} [7], \textit{BARD1} [8], \textit{CTR9} [9], \textit{HACE1} [10] and \textit{LMO1} genes [11] have been observed to modify Wilms tumor susceptibility. There are plenty of gene polymorphisms worth further exploring.

The homeobox genes are transcription factors that play an important part in embryonic development, including cellular differentiation, migration, apoptosis, signal transduction and angiogenesis [12]. The
pair-like homeobox 2b (PHOX2B) gene belongs to the homebox gene family which locates in 4p13. This gene accounts for 4.8 k bases approximately, contains three exons and two introns, encodes a peptide containing 314 amino acids [13]. PHOX2B is expressed during neural development in central autonomic circuits and peripheral neural crest derivatives, particularly in retrotrapezoid nucleus, noradrenergic centers and hindbrain. PHOX2B is an indispensable transcription factor in the proliferation and differentiation of neural crest tissue [14–17]. It can also regulate the expression of cancer-related genes such as TH [18], PHOX2 itself [19], RET [20], TLX-2 [21], ALK [22], SOX10 [23] and MSX1 [24].

In the past, research about PHOX2B polymorphisms mostly focused on exons. With the continuous development of biotechnology, many introns have been found to assume the regulation roles in genetic expression. Previous investigations have shown that the PHOX2B rs28647582 T>C polymorphism significantly alters Hirschsprung disease susceptibility [25–27]. In addition, Perotti et al. [28] and Zin et al. [29] have widely found the heterozygous loss of 4p13 in sporadic Wilms tumor. To our knowledge, no study has investigated the association between PHOX2B rs28647582 T>C polymorphism and Wilms tumor susceptibility. Considering this polymorphism may affect the differentiation of posterior renal blastocyst, we conducted this experiment about PHOX2B rs28647582 T>C polymorphism and Wilms tumor susceptibility.

Materials and methods

Study population

It was a case-control study with 145 patients and 531 controls (Supplementary Table 1). The patients were collected from the Guangzhou Women and Children's Medical Center during March 2001 to June 2016 [30–32]. Wilms tumor patients were histopathologically confirmed and distinguished depending on the NWTS-5 criteria. At the same time, a total of 531 tumor-free controls were randomly selected from the same center and matched patients with age, gender and ethnicity. In accordance with the relevant laws and regulations, participants or their guardians were requested to sign informed consent. The present study was approved by the Institutional Review Board of Guangzhou Women and Children's Medical Center (ethic approve number: 2018022102).

Genotyping

DNA was extracted from 2 ml venous blood samples using TIANamp Blood DNA Kit (TianGen Biotech Co. Ltd., Beijing, China). The PHOX2B rs28647582 T>C polymorphism was genotyped by TaqMan real-time PCR [33–35]. The information of samples was kept secret from staffs for a reliably experimental results. Moreover, we randomly selected 10% of the samples for repetitive test. The results showed that repeatability is 100%.

Statistical analysis

T-test was employed to check the differences in age. Frequency distribution of gender and genotype between patients and the controls were evaluated by χ² test. A goodness-of-fit test was used to estimate Hardy–Weinberg equilibrium (HWE) in controls. After adjusting for age and gender, we performed an unconditional multiple logistic regression model to assess the association between the PHOX2B rs28647582 T>C polymorphism and Wilms tumor susceptibility by odds ratios (ORs) and 95% confidence intervals (CIs). Finally, stratified analysis was conducted by age, gender and clinical stages. All data were analyzed by SAS statistical software, using a two-sided test. The results were considered statistically significant when P<0.05.

Results

PHOX2B rs28647582 T>C polymorphism and Wilms tumor susceptibility

The genotype distribution of PHOX2B rs28647582 T>C polymorphism in Wilms tumor patients and controls is shown in Table 1. The genotype distribution of PHOX2B rs28647582 T>C polymorphism obeyed the HWE genetic balance in controls (P=0.505). Unfortunately, we could not observe significant association between PHOX2B rs28647582 T>C polymorphism and Wilms tumor susceptibility (TC vs. TT: adjusted OR = 1.42, 95% CI = 0.95–2.12, P=0.090; CC vs. TT: adjusted OR = 0.78, 95% CI = 0.22–2.76, P=0.699; TC/CC vs. TT: adjusted OR = 1.35, 95% CI = 0.91–2.00, P=0.133; CC vs. TC/TT: adjusted OR = 0.71, 95% CI = 0.20–2.48, P=0.587; and C vs. T: adjusted OR = 1.22, 95% CI = 0.87–1.72, P=0.252).

Stratification analysis

We further conducted a stratified analysis by age, gender and clinical stages (Table 2). Stratified analysis revealed that
Table 1 PHOX2B rs28647582 T>C polymorphism and Wilms tumor susceptibility

| Genotype | Cases (N = 145) | Controls (N = 531) | Crude OR (95% CI) | P | Adjusted OR (95% CI) | P† |
|----------|----------------|-------------------|------------------|---|-------------------|---|
| rs28647582 (HWE = 0.505) | | | | | | |
| TT | 95 (65.52) | 380 (71.56) | 1.00 | 0.100 | 1.00 | 0.100 |
| TC | 47 (32.41) | 136 (25.61) | 1.38 (0.93–2.06) | 0.113 | 1.42 (0.95–2.12) | 0.090 |
| CC | 3 (2.07) | 15 (2.82) | 0.80 (0.23–2.82) | 0.729 | 0.78 (0.22–2.76) | 0.699 |
| Additive | | | 0.249 | 0.280 | 1.22 (0.87–1.72) | 0.254 |
| Dominant | 50 (34.48) | 151 (28.44) | 1.33 (0.90–1.96) | 0.159 | 1.35 (0.91–2.00) | 0.133 |
| Recessive | 142 (97.93) | 516 (97.18) | 0.73 (0.21–2.55) | 0.618 | 0.71 (0.20–2.48) | 0.587 |
| T | 237 (81.72) | 896 (84.37) | 1.00 | 0.279 | 1.22 (0.86–1.70) | 0.252 |
| C | 53 (18.28) | 166 (15.63) | 0.279 | 0.158 | 1.77 (1.07–2.95) | 0.027 |

*χ² test for genotype distributions between Wilms tumor patients and controls.
†Adjusted for age and gender.

Table 2 Stratification analysis for the association between PHOX2B rs28647582 T>C polymorphism and Wilms tumor susceptibility

| Variables | Phox2b rs28647582 (cases/controls) | Crude OR (95% CI) | P | Adjusted OR* (95% CI) | P† |
|-----------|---------------------------------|------------------|---|-------------------|---|
| Age, month | | | | | |
| ≤18 | 51/174 | 15/59 | 0.87 (0.45–1.66) | 0.667 | 0.88 (0.45–1.65) | 0.651 |
| >18 | 44/206 | 35/92 | 1.78 (1.07–2.96) | 0.026 | 1.77 (1.07–2.95) | 0.027 |
| Gender | | | | | |
| Females | 45/184 | 19/69 | 1.00 (0.55–1.84) | 0.991 | 1.02 (0.56–1.88) | 0.945 |
| Males | 50/216 | 31/82 | 1.63 (0.98–2.73) | 0.062 | 1.65 (0.98–2.76) | 0.059 |
| Clinical stage | | | | | |
| I+II | 37/380 | 16/151 | 1.09 (0.59–2.02) | 0.788 | 1.14 (0.61–2.12) | 0.685 |
| III+IV | 49/380 | 34/151 | 1.75 (1.08–2.81) | 0.022 | 1.75 (1.09–2.82) | 0.022 |

*Adjusted for age and gender, omitting the corresponding factor.
Results in bold indicate P <0.05.

TC/CC genotypes carriers had higher risk to develop Wilms tumor than those with TT genotype in the groups of older than 18 months (adjusted OR = 1.77, 95% CI = 1.07–2.95, P=0.027) and clinical stages III+IV (adjusted OR = 1.75, 95% CI = 1.09–2.82, P=0.022). No significant correlation was found between PHOX2B rs28647582 T>C polymorphism and Wilms tumor susceptibility in other groups.

Discussion

PHOX2B mutations were observed in congenital central hypoventilation syndrome, neuroblastoma and Hirschsprung disease [36,37]. Subsequent studies confirmed that polyalanine repeat expansion mutation (PARMs) in exon 3 increased the risk of congenital central hypoventilation syndrome [38,39]. As for exon 3 NPARMs (exon 3 nucleotide 673 G>T and 702_714dup13) and mutations in other locations (exon 2 nucleotide 299 G>T and nucleotide 421 C>G), they were more likely to modify the susceptibility to familial and sporadic neuroblastoma [40,41].

Introns are widely distributed in the genome of eukaryotic cells. They are much longer than exons and accumulate more mutations, accounting for 95–97% in human genome. Although introns do not code for proteins, an increasing number of studies found that introns not only played a regulatory role in transcription, but also had selective splicing and promoter-like functions during mRNA maturation and translation [42]. PHOX2B rs28647582 is located in intron 2. Existing studies had no idea how rs28647582 affected PHOX2B. In the past few years, some conflicting results about PHOX2B rs28647582 polymorphism and Hirschsprung's disease were drawn. Liu et al. [25] found that
rs28647582 C allele increased Hirschsprung’s disease risk. But, Garcia-Barcelo et al. [26] and Xiao et al. [27] indicated that rs28647582 CC/CT were protective genotypes to Hirschsprung’s disease. Interestingly, a meta-analysis showed that the rs28647582 T>C polymorphism failed to significantly change Hirschsprung’s disease risk [43]. These researches suggested that rs28647582 polymorphism may modify certain diseases susceptibility.

This is the first investigation about PHOX2B rs28647582 T>C polymorphism and Wilms tumor. We observed that PHOX2B rs28647582 TC/CC genotypes significantly increased Wilms tumor susceptibility in subgroups older than 18 months or clinical stages III+IV. Based on previous studies [26,41], rs28647582 T>C polymorphism would change PHOX2B structure and expression by abnormally splicing and regulating, which would weaken PHOX2B ability to promote the proliferation and differentiation of renal cells. In addition, PHOX2B rs28647582 T>C polymorphism may combine with tumor-related genes to modify Wilms tumor susceptibility.

Although we found the correlation between PHOX2B rs28647582 T>C polymorphism and Wilms tumor susceptibility in stratified analysis, some limitations existed in our study. First, our research had a relatively small sample size, containing 145 patients and 531 controls. It was difficult to discover the genuine association between PHOX2B rs28647582 T>C polymorphism and Wilms tumor. Second, we selected only one polymorphism in PHOX2B, which made genotype association analysis impossible. It may omit the combined effect of PHOX2B rs28647582 T>C polymorphism and other potential polymorphisms in Wilms tumor [44]. Third, as a hospital-based retrospective study, hospital admission bias and information bias inevitably existed in population selection, information collection and experimental operation. Finally, this paper merely revealed the connection between PHOX2B rs28647582 T>C polymorphism and Wilms tumor susceptibility. In order to achieve reliable results, we should expand the sample size, add genotype association analysis and perform a strict quality monitoring in follow-up studies.

In conclusion, we have exposed that PHOX2B rs28647582 T>C polymorphism is weakly associated with Wilms tumor susceptibility. Our result need further verified.

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

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

Funding
This work was supported by the National Natural Science Foundation of China [grant number: 81803320] the Pearl River S&T Nova Program of Guangzhou [grant number: 201710010086]; the Science and Technology Project of Guangzhou [grant number: 201804010037]; and the Wuxi Health Commission Foundation [grant number: Q201601]. The authors declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations
CI, confidence interval; GWAS, genome-wide association study; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; PHOX2B, pair-like homeobox 2b; SNP, single nucleotide polymorphism.

References
1. Hohenstein, P., Pritchard-Jones, K. and Charlton, J. (2015) The Yin and Yang of Kidney Development and Wilms’ Tumors. Genes Dev 29, 467–482, https://doi.org/10.1101/gad.256396.114
2. Dome, J.S., Fernandez, C.V., Mullen, E.A., Kalapurakal, J.A., Geller, J.I. et al. (2013) Children’s Oncology Group’s 2013 blueprint for research: renal tumors. Pediatr Blood Cancer 60, 994–1000, https://doi.org/10.1002/pbc.24419
3. Bao, P.P., Li, K., Wu, C.X., Huang, Z.Z., Wang, C.F. et al. (2013) Recent incidences and trends of childhood malignant solid tumors in Shanghai, 2002-2010. Zhonghua Er Ke Za Zhi 51, 288–294
4. Davidoff, A.M. (2009) Wilms’ tumor. Curr. Opin. Pediatr. 21, 357–364, https://doi.org/10.1097/MOP.0b013e32832b323a
5. Charlton, J., Williams, R.D., Sebire, N.J., Popov, S., Vujanic, G. et al. (2015) Comparative methylation analysis identifies new tumour subtypes and biomarkers for transformation of nephrogenic rests into Wilms tumour. Genome Med. 7, 11, https://doi.org/10.1186/s13073-015-0136-4
6. Rahman, N., Arbour, L., Tonin, P., Renshaw, J., Pelletier, J. et al. (1996) Evidence for a familial Wilms’ tumor gene (FWT1) on chromosome 17q12-q21. Nat. Genet. 13, 461–463, https://doi.org/10.1038/ng0896-461
7. Rapley, E.A., Barfoot, R., Bonati-Pellie, C., Chompret, A., Fouilkes, W. et al. (2000) Evidence for susceptibility genes to familial Wilms tumour in addition to WT1, FWT1 and FWT2. Br. J. Cancer 83, 177–183, https://doi.org/10.1054/bjoc.2000.1283
8 Fu, W., Zhu, J., Xiong, S.W., Jia, W., Zhao, Z. et al. (2017) BARD1 gene polymorphisms confer nephroblastoma susceptibility. EBioMedicine 16, 101–105, https://doi.org/10.1016/j.ebiom.2017.01.038

9 Hanks, S., Perdeaux, E.R., Seal, S., Ruark, E., Mahamadali, S.S. et al. (2014) Germline mutations in the PAF1 complex gene CTR9 predispose to Wilms' tumour. Nat. Commun. 5, 4398, https://doi.org/10.1038/ncomms5398

10 Jia, W., Deng, Z., Zhu, J., Fu, W., Zhu, S. et al. (2017) Association between HACE1 gene polymorphisms and Wilms' tumor risk in a Chinese population. Cancer Invest. 35, 633–638, https://doi.org/10.1080/07357907.2017.1405016

11 Liu, G.C., Zhuo, Z.J., Zhu, S.B., Zhu, J., Jia, W. et al. (2017) Associations between LMO1 gene polymorphisms and Wilms' tumor susceptibility. Oncotarget 8, 50665–50672

12 Holland, P.W., Booth, H.A. and Bruford, E.A. (2007) Classification and nomenclature of all human homeobox genes. BMC Biol. 5, 47, https://doi.org/10.1186/1471-2142-5-47

13 Yokoyama, M., Watanabe, H. and Nakamura, M. (1999) Genomic structure and functional characterization of NBPhox (PMX2B), a homeodomain protein specific to catecholaminergic cells that is involved in second messenger-mediated transcriptional activation. Genomics 59, 40–50, https://doi.org/10.1006/geno.1999.5845

14 Dubreuil, V., Hirsch, M.R., Jouve, C., Brunet, J.F. and Goridis, C. (2002) The role of Phox2b in synchronizing pan-neuronal and type-specific aspects of neurogenesis. Development 129, 5241–5253

15 Pattyn, A., Morin, X., Cremer, H., Goridis, C. and Brunet, J.F. (1999) The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature 399, 366–370, https://doi.org/10.1038/20700

16 Stornetta, R.L., Moreira, T.S., Takakura, A.C., Kang, B.J., Chang, D.A. et al. (2006) Expression of Phox2b by brainstem neurons involved in chemosensory integration in the adult rat. J. Neurosci. 26, 10305–10314, https://doi.org/10.1523/JNEUROSCI.2917-06.2006

17 Kang, B.J., Chang, D.A., Mackay, D.D., West, G.H., Moreira, T.S. et al. (2007) Central nervous system distribution of the transcription factor Phox2b in the adult rat. J. Comp. Neurol. 503, 627–641, https://doi.org/10.1002/cne.21409

18 Lo, L., Morin, X., Brunet, J.F. and Anderson, J.D. (1999) Specification of neurotransmitter identity by Phox2 proteins in neural crest stem cells. Neuron 22, 693–705, https://doi.org/10.1016/S0896-6273(00)00729-1

19 Cargin, F., Flora, A., Di Lascio, S., Battaglioli, E., Longhi, R. et al. (2005) PHOX2B regulates its own expression by a transcriptional auto-regulatory mechanism. J. Biol. Chem. 280, 37439–37448, https://doi.org/10.1074/jbc.M508368200

20 Di Zanni, E., Adamo, A., Belligni, E., Leron, M., Martucciello, G. et al. (2017) Common PHOX2B poly-alanine contractions impair RET gene transcription, predisposing to Hirschsprung disease. Biochim. Biophys. Acta Mol. Basis Dis. 1863, 1770–1777, https://doi.org/10.1016/j.bbadis.2017.04.017

21 Borghini, S., Bachetti, T., Fava, M., Di Duca, M., Cargin, F. et al. (2006) The TLX2 homeobox gene is a transcriptional target of PHOX2B in neural-crest-derived cells. Biochim. J. 395, 355–361, https://doi.org/10.1016/B20051396

22 Bachetti, T., Di Paolo, D., Di Lascio, S., Mirisola, V., Brignole, C. et al. (2010) PHOX2B-mediated regulation of ALK expression: in vitro identification of a functional relationship between two genes involved in nephroblastoma. PLoS ONE 5, e13108, https://doi.org/10.1371/journal.pone.0013108

23 Nagashimada, M., Ohta, H., Li, C., Nakao, K., Uesaka, T. et al. (2012) Autonomic neurocristopathy-associated mutations in PHOX2B dysregulate Sox10 expression. J. Clin. Invest. 122, 3145–3158, https://doi.org/10.1172/JCI63401

24 Revet, I., Huizenga, G., Chan, A., Koster, J., Volckmann, R. et al. (2008) The MSX1 homeobox transcription factor is a downstream target of PHOX2B and activates the Delta-Notch pathway in neuroblastoma. Exp. Cell Res. 314, 707–719, https://doi.org/10.1016/j.yexcr.2007.12.008

25 Liu, C.P., Li, X.G., Lou, J.T., Xue, Y., Luo, C.F. et al. (2009) Association analysis of the PHOX2B gene with Hirschsprung disease in the Han Chinese population. J. Pediatr. Surg. 44, 1805–1811, https://doi.org/10.1016/j.jpedsurg.2008.12.009

26 Garcia-Barcelo, M., Sham, M.H., Lui, V.C., Chen, B.L., Ott, J. et al. (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

27 Xiao, D., Liu, L., Mao, X.P. and Mao, J.X. (2009) Association between genetic polymorphisms of PHOX2B and susceptibility of Hirschsprung's disease in Shenzhen region. J. Shansi Med. Univ. 40, 1083–1084

28 Perotti, D., Spreafico, F., Torri, F., Gamba, B., D'Adamo, P. et al. (2012) Genomic profiling by whole-genome single nucleotide polymorphism array in Wilms tumor and association with relapse. Genes Chromosomes Cancer 51, 644–653, https://doi.org/10.1002/gcc.21951

29 Zin, R., Pham, K., Ashleigh, M., Ravine, D., Waring, P. et al. (2012) SNP-based arrays complement classic cytogenetics in the detection of chromosomal aberrations in Wilms' tumour. Cancer Genet. 205, 80–93, https://doi.org/10.1016/j.cancergen.2011.12.003

30 Fu, W., Zhou, Z.J., Jia, W., Zhu, J., Zhu, S.B. et al. (2017) Association between TP53 gene Arg72Pro polymorphism and Wilms' tumour risk in a Chinese population. Onco. Targets Ther. 10, 1149–1154, https://doi.org/10.2147/OTT.S131014

31 Zhu, J., Fu, W., Jia, W., Xia, H., Liu, G.C. et al. (2018) Association between NER pathway gene polymorphisms and Wilms tumor risk. Mol. Ther. Nucleic Acids 12, 854–860, https://doi.org/10.1038/s41596-018-0002

32 Zhu, J., Jia, W., Wu, C., Fu, W., Xia, H. et al. (2018) Base excision repair gene polymorphisms and Wilms tumor susceptibility. EBioMedicine 33, 88–93, https://doi.org/10.1016/j.ebiomed.2018.06.018

33 He, J., Qiu, L.X., Wang, M.Y., Hua, R.X., Zhang, R.X. et al. (2012) Polymorphisms in the XPG gene and risk of gastric cancer in Chinese populations. Hum. Genet. 131, 1235–1249, https://doi.org/10.1007/s00439-012-1152-8

34 Chang, J., Zhong, R., Tian, J., Li, J., Zhai, K. et al. (2018) Exome-wide analyses identify low-frequency variant in CYP26B1 and additional coding variants associated with esophageal squamous cell carcinoma. Nat. Genet. 50, 338–343, https://doi.org/10.1038/s41588-018-0045-S

35 Chen, X., Wang, Y., Cheng, K., Li, J., Lou, J. et al. (2016) Genetic variants in the regulatory region of SLC10A1 are not associated with the risk of hepatitis B virus infection and clearance. Infect. Genet. Evol. 44, 495–500, https://doi.org/10.1016/j.meegid.2016.07.043

36 Amiel, J., Laudier, B., Attie-Bitach, T., Trang, H., de Pontual, L. et al. (2003) Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. Nat. Genet. 33, 459–461, https://doi.org/10.1038/ng1130

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37 Trochet, D., Bourdeaut, F., Janoueix-Lerosey, I., Deville, A., de Pontual, L. et al. (2004) Germline mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma. *Am. J. Hum. Genet.* **74**, 761–764, https://doi.org/10.1086/383253

38 Sasaki, A., Kanai, M., Kijima, K., Akaba, K., Hashimoto, M. et al. (2003) Molecular analysis of congenital central hypoventilation syndrome. *Hum. Genet.* **114**, 22–26, https://doi.org/10.1007/s00439-003-1036-z

39 Weese-Mayer, D.E., Berry-Kravis, E.M., Zhou, L., Maher, B.S., Silvestri, J.M. et al. (2003) Idiopathic congenital central hypoventilation syndrome: analysis of genes pertinent to early autonomic nervous system embryologic development and identification of mutations in PHOX2b. *Am. J. Med. Genet. A* **123a**, 267–278, https://doi.org/10.1002/ajmg.a.20527

40 Mosse, Y.P., Laudenslager, M., Khazi, D., Carlisle, A.J., Winter, C.L. et al. (2004) Germline PHOX2B mutation in hereditary neuroblastoma. *Am. J. Hum. Genet.* **75**, 727–730, https://doi.org/10.1086/424530

41 Raabe, E.H., Laudenslager, M., Winter, C., Wasserman, N., Cole, K. et al. (2008) Prevalence and functional consequence of PHOX2B mutations in neuroblastoma. *Oncogene* **27**, 469–476, https://doi.org/10.1038/sj.onc.1210659

42 Blencowe, B.J. (2006) Alternative splicing: new insights from global analyses. *Cell* **126**, 37–47, https://doi.org/10.1016/j.cell.2006.06.023

43 Liang, C.M., Ji, D.M., Yuan, X., Ren, L.L., Shen, J. et al. (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

44 Tolbert, V.P., Coggins, G.E. and Maris, J.M. (2017) Genetic susceptibility to neuroblastoma. *Curr. Opin. Genet. Dev.* **42**, 81–90, https://doi.org/10.1016/j.gde.2017.03.008