Association of KRAS and NRAS gene polymorphisms with Wilms tumor risk: a four-center case-control study

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ABSTRACT

Wilms tumor is a type of pediatric solid tumor that arises partly due to somatic and germline mutations. Single-nucleotide polymorphisms (SNPs) in the RAS gene reportedly modify the risk for several types of human malignancies. We conducted a multicenter study to investigate whether RAS gene variants predispose individuals to Wilms tumor. Four SNPs in RAS were genotyped in 355 Wilms tumor cases and 1070 controls. The SNPs included rs12587 G>T, rs7973450 A>G and rs7312175 G>A in KRAS, and rs2273267 A>T in NRAS. Individuals harboring the rs12587 GT genotype were more likely to develop Wilms tumor than those carrying the GG genotype (adjusted odds ratio [OR]=1.30, 95% confidence interval [CI]=1.004-1.68, P=0.046). However, the other three SNPs seemed not to influence the risk for Wilms tumor. Compared to individuals without a risk genotype, those harboring one to three KRAS risk genotypes had an adjusted OR of 1.28 for developing Wilms tumor (95% CI=1.002-1.64, P=0.048). Stratification analysis revealed that rs12587 GT/TT was associated with Wilms tumor risk in children >18 months old (adjusted OR=1.39, 95% CI=1.02-1.89, P=0.037). Our findings indicate that the rs12587 G>T polymorphism in KRAS is associated with increased Wilms tumor susceptibility.

INTRODUCTION

Wilms tumor (nephroblastoma) is the most common pediatric renal malignancy [1]. It is normally derived from embryonal kidney precursor cells in which cell growth and/or differentiation are dysregulated during development [2, 3]. The incidence rate of Wilms tumor is about 1 in 10,000 children in Western countries [4].
The overall five-year survival rate exceeds 90% in developed countries [5-7]. Despite great achievements in the treatment of Wilms tumor, the outcomes for patients with high-risk disease (about 25%) remain disappointing [8]. Apart from this, high treatment costs and severe chronic health conditions that occur in nearly 25% of survivors are also challenging [9, 10].

There is strong evidence that genetic factors contribute to Wilms tumor risk. To date, five Wilms tumor susceptibility loci have been well characterized, including Wilms tumor gene 1 (WT1), Wilms tumor gene on the X chromosome (WTX), catenin beta 1 (CTNNB1), tumor protein 53 (TP53) and the imprinted 11p15 region [11-13]. Although additional genetic variants continue to be identified, the carcinogenesis of Wilms tumor remains to be fully explained [14-16]. Therefore, it is indispensable to identify other genes that increase Wilms tumor susceptibility.

The RAS oncogene family has three members: KRAS, NRAS and HRAS. These genes encode a family of highly homologous GTPases that are involved in various cellular activities, such as growth, proliferation and differentiation [17, 18]. RAS mutations have been detected in about 20% of human malignancies [19]. KRAS mutations are the most common, accounting for approximately 85% of all RAS mutations [20, 21], followed by NRAS mutations (15%). HRAS mutations are very rare, constituting less than 1% of all RAS mutations [22].

The impact of RAS gene variants on the risk of cancer has been widely investigated, including in studies of colorectal cancer [23], lung cancer [24, 25], breast cancer [26] and melanoma [27]. Clark et al. demonstrated that coordinated activation of RAS and β-catenin accelerated the growth and metastatic progression of Wilms tumor in a murine model [28]. They later reported that activating KRAS mutations were found in human Wilms tumor samples [29]. Recently, another team verified the importance of RAS mutations in the development and progression of Wilms tumor [30].

Despite these findings, the link between RAS gene polymorphisms and Wilms tumor risk remains obscure. To clarify the association of RAS with Wilms tumor risk, we selected single-nucleotide polymorphisms (SNPs) in the two most common diseased-related RAS genes, KRAS and NRAS, for analysis in a four-center hospital-based case-control study.

**RESULTS**

**Correlation of RAS gene polymorphisms with Wilms tumor risk**

We successfully genotyped 1070 controls and 351 cases for KRAS polymorphisms, along with 1070 controls and 355 cases for NRAS polymorphism. The demographic characteristics of the subjects are presented in Supplemental Table 1. All the SNP genotype frequencies were in Hardy-Weinberg equilibrium in controls (P>0.05). Our results indicated that the rs12587 GT genotype is a risk variant for Wilms tumor (Table 1), as individuals with this genotype had a 1.30-fold greater risk for developing Wilms tumor (95% confidence interval [CI]=1.004-1.68, P=0.046) than those with the GG genotype. The individual rs7973450 A>G, rs7312175 G>A and rs2273267 A>T variants did not predispose individuals to Wilms tumor.

We further examined the combined effects of the risk genotypes for KRAS on Wilms tumor risk. Compared to individuals without a risk genotype, those harboring one to three of these genotypes were at 1.28-fold greater risk for Wilms tumor (95% CI=1.002-1.64, P=0.048).

**Stratification analysis**

Tables 2 and 3 summarize the analysis of KRAS and NRAS polymorphisms and Wilms tumor risk after stratification by age, gender and clinical stage. A significant association between rs12587 GT/TT and Wilms tumor risk was only found in children >18 months old among the analyzed strata (adjusted odds ratio [OR]=1.39, 95% CI=1.02-1.89, P=0.037).

**False-positive report probability (FPRP) analysis**

In FPRP analysis (Table 4), only at a prior probability level of 0.25 and an FPRP threshold of 0.2 did the increased Wilms tumor risk remain noteworthy in carriers of rs12587 GT (FPRP=0.141), children >18 months old with rs12587 GT/TT (FPRP=0.131) and those with one to three risk genotypes (FPRP=0.139).

**DISCUSSION**

Thus far, only a small portion of genetic loci have been found to increase the risk of Wilms tumor. This underscores the need to reveal more genetic loci that could predispose individuals to this disease. Herein, we evaluated the impact of KRAS and NRAS gene SNPs on
| Genotype | Cases (N=355) | Controls (N=1070) | $P^a$ | Crude OR (95% CI) | $P$ | Adjusted OR (95% CI) $^b$ | $P^b$ |
|----------|---------------|-------------------|------|------------------|-----|---------------------------|------|
| **KRA S rs12587 G>T (HWE=0.287)** | | | | | | | |
| GG       | 206 (58.69)   | 688 (64.30)       | 1.00 | 1.00             |     | 0.049                     | 1.30 (1.004-1.68) | 0.046 |
| GT       | 129 (36.75)   | 333 (31.12)       | 1.29 (1.002-1.67) | 0.049 | 1.30 (1.004-1.68) | 0.046 |
| TT       | 16 (4.56)     | 49 (4.58)         | 1.09 (0.61-1.96) | 0.772 | 1.08 (0.60-1.94) | 0.806 |
| Additive | | | 0.142 | 1.18 (0.96-1.44) | 0.117 | 1.18 (0.96-1.44) | 0.120 |
| Dominant | | | | | | | |
| Recessive | | | | | | | |
| G        | 541 (77.07)   | 1709 (79.86)      | 1.00 | 1.00             |     | 0.114                     | 1.18 (0.96-1.45) | 0.117 |
| T        | 161 (22.93)   | 431 (20.14)       | 0.114 | 1.18 (0.96-1.45) | 0.114 | 1.18 (0.96-1.45) | 0.117 |
| **KRA S rs7973450 A>G (HWE=0.080)** | | | | | | | |
| AA       | 282 (80.34)   | 881 (82.34)       | 1.00 | 1.00             |     | 0.040                     | 0.402 |
| AG       | 68 (19.37)    | 185 (17.29)       | 1.15 (0.84-1.56) | 0.380 | 1.14 (0.84-1.56) | 0.402 |
| GG       | 1 (0.28)      | 4 (0.37)          | 0.78 (0.09-7.02) | 0.825 | 0.83 (0.09-7.50) | 0.870 |
| Additive | | | 0.660 | 1.13 (0.84-1.52) | 0.436 | 1.12 (0.83-1.51) | 0.448 |
| Dominant | | | | | | | |
| Recessive | | | | | | | |
| A        | 632 (90.03)   | 1947 (90.98)      | 1.00 | 1.00             |     | 0.111                     | 0.84 (0.74-1.49) | 0.462 |
| G        | 70 (9.97)     | 193 (9.02)        | 0.450 | 1.12 (0.84-1.49) | 0.450 | 1.11 (0.84-1.49) | 0.462 |
| **KRA S rs7312175 G>A (HWE=0.130)** | | | | | | | |
| GG       | 270 (76.92)   | 851 (79.53)       | 1.00 | 1.00             |     | 0.040                     | 0.404 |
| GA       | 72 (20.51)    | 201 (18.79)       | 1.13 (0.84-1.53) | 0.431 | 1.14 (0.84-1.54) | 0.404 |
| AA       | 9 (2.56)      | 18 (1.68)         | 1.58 (0.70-3.55) | 0.272 | 1.54 (0.68-3.48) | 0.298 |
| Additive | | | 0.423 | 1.17 (0.91-1.51) | 0.222 | 1.17 (0.91-1.51) | 0.218 |
| Dominant | | | | | | | |
| Recessive | | | | | | | |
| G        | 612 (87.18)   | 1903 (88.93)      | 1.00 | 1.00             |     | 0.189                     | 0.91 (0.91-1.53) | 0.205 |
| A        | 90 (12.82)    | 237 (11.07)       | 0.208 | 1.18 (0.91-1.53) | 0.209 | 1.18 (0.91-1.53) | 0.205 |
| **NRA S rs2273267 A>T (HWE=0.723)** | | | | | | | |
| AA       | 183 (51.55)   | 541 (50.56)       | 1.00 | 1.00             |     | 0.111                     | 0.774 |
| AT       | 142 (40.00)   | 443 (41.40)       | 0.95 (0.74-1.22) | 0.676 | 0.95 (0.74-1.23) | 0.714 |
| TT       | 30 (8.45)     | 86 (8.04)         | 1.03 (0.66-1.61) | 0.893 | 1.02 (0.65-1.61) | 0.917 |
| Additive | | | 0.889 | 0.99 (0.82-1.19) | 0.883 | 0.99 (0.82-1.19) | 0.890 |
| Dominant | | | | | | | |
| Recessive | | | | | | | |
| A        | 508 (71.55)   | 1525 (71.26)      | 1.00 | 1.00             |     | 0.109                     | 0.84 (0.82-1.19) | 0.891 |
| T        | 202 (28.45)   | 615 (28.74)       | 0.883 | 0.99 (0.82-1.19) | 0.883 | 0.99 (0.82-1.19) | 0.891 |
| Combined effect of risk genotypes for KRA S $^c$ | | | | | | | |
| 0        | 200 (56.98)   | 673 (62.90)       | 1.00 | 1.00             |     | 0.102                     | 0.84 (0.82-1.19) | 0.891 |
| 1        | 13 (3.70)     | 28 (2.62)         | 1.56 (0.80-3.07) | 0.196 | 1.57 (0.80-3.10) | 0.192 |
| 2        | 132 (37.61)   | 345 (32.24)       | 1.29 (1.00-1.66) | 0.052 | 1.29 (1.00-1.66) | 0.052 |
| 3        | 6 (1.71)      | 24 (2.24)         | 0.84 (0.34-2.09) | 0.709 | 0.84 (0.34-2.09) | 0.709 |
| Trend    | | | 0.157 | 1.11 (0.98-1.25) | 0.094 | 1.11 (0.98-1.25) | 0.093 |
| 0        | 200 (56.98)   | 673 (62.90)       | 1.00 | 1.00             |     | 0.102                     | 0.84 (0.82-1.19) | 0.891 |
| 1-3      | 151 (43.02)   | 397 (37.10)       | 0.048 | 1.28 (1.002-1.64) | 0.048 | 1.28 (1.002-1.64) | 0.048 |

OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

$^a$ $\chi^2$ test for genotype distributions between Wilms tumor patients and cancer-free controls.

$^b$ Adjusted for age and gender.

$^c$ Risk genotypes were carriers with rs12587 GT/TT, rs7973450 AG/GG and rs7312175 GA/AA genotypes.
Table 2. Stratification analysis for association between KRAS genotypes and Wilms tumor susceptibility.

| Variables          | rs12587 (case/control) | AOR (95% CI) | P | rs7973450 (case/control) | AOR (95% CI) | P | rs7312175 (case/control) | AOR (95% CI) | P | Combine genotypes (case/control) | AOR (95% CI) | P |
|--------------------|-------------------------|--------------|---|--------------------------|--------------|---|--------------------------|--------------|---|-------------------------------|--------------|---|
| Age, month         |                         |              |   |                          |              |   |                          |              |   |                               |              |   |
| ≤18                | 77/272                  | 46/153       | 1.06 (0.70-1.61) | 0.771 | 97/341                  | 26/84        | 1.09 (0.67-1.79) | 0.726 | 99/345 | 24/80 | 1.04 (0.63-1.74) | 0.870 | 74/269 | 49/156 | 1.14 (0.76-1.73) | 0.522 |
| >18                | 129/416                 | 99/229       | **1.39 (1.02-1.89)** | **0.037** | 185/540                 | 43/105       | 1.20 (0.81-1.77) | 0.373 | 171/506 | 57/139 | 1.21 (0.85-1.73) | 0.286 | 126/404 | 102/241 | 1.35 (0.99-1.83) | 0.056 |
| Gender             |                         |              |   |                          |              |   |                          |              |   |                               |              |   |
| Female             | 97/283                  | 66/165       | 1.17 (0.81-1.68) | 0.412 | 128/369                 | 35/79        | 1.28 (0.82-1.99) | 0.285 | 129/354 | 34/94 | 0.99 (0.64-1.54) | 0.973 | 93/277 | 70/171 | 1.22 (0.85-1.75) | 0.287 |
| Male               | 109/405                 | 79/217       | 1.37 (0.98-1.92) | 0.064 | 154/512                 | 34/110       | 1.03 (0.67-1.57) | 0.897 | 141/497 | 47/125 | 1.34 (0.91-1.97) | 0.135 | 107/396 | 81/226 | 1.35 (0.96-1.88) | 0.081 |
| Clinical stages    |                         |              |   |                          |              |   |                          |              |   |                               |              |   |
| I                  | 71/688                  | 48/382       | 1.23 (0.84-1.82) | 0.293 | 101/881                 | 18/189       | 0.82 (0.48-1.38) | 0.446 | 90/851 | 29/219 | 1.28 (0.82-2.00) | 0.276 | 69/673 | 50/397 | 1.25 (0.85-1.84) | 0.261 |
| II                 | 51/688                  | 39/382       | 1.37 (0.89-2.13) | 0.154 | 68/881                  | 22/189       | 1.48 (0.89-2.45) | 0.134 | 71/851 | 19/219 | 1.06 (0.62-1.80) | 0.836 | 50/673 | 40/397 | 1.36 (0.88-2.09) | 0.172 |
| III                | 47/688                  | 32/382       | 1.21 (0.76-1.93) | 0.425 | 67/881                  | 12/189       | 0.84 (0.44-1.58) | 0.587 | 57/851 | 22/219 | 1.48 (0.88-2.47) | 0.138 | 46/673 | 33/397 | 1.20 (0.75-1.91) | 0.443 |
| IV                 | 28/688                  | 17/382       | 1.08 (0.59-2.01) | 0.797 | 34/881                  | 11/189       | 1.51 (0.75-3.04) | 0.246 | 39/851 | 6/219 | 0.59 (0.25-1.42) | 0.241 | 27/673 | 18/397 | 1.12 (0.61-2.06) | 0.714 |
| I+II               | 122/688                 | 87/382       | 1.29 (0.96-1.75) | 0.096 | 169/881                 | 40/189       | 1.08 (0.74-1.58) | 0.698 | 161/851 | 48/219 | 1.19 (0.83-1.69) | 0.350 | 119/673 | 90/397 | 1.30 (0.96-1.75) | 0.093 |
| III+IV             | 75/688                  | 49/382       | 1.16 (0.79-1.71) | 0.439 | 101/881                 | 23/189       | 1.06 (0.66-1.72) | 0.800 | 96/851 | 28/219 | 1.12 (0.72-1.75) | 0.617 | 73/673 | 51/397 | 1.17 (0.80-1.71) | 0.413 |

AOR, adjusted odds ratio; CI, confidence interval.

* Adjusted for age and gender, omitting the corresponding stratify factor.
the risk of Wilms tumor in 355 Wilms tumor patients and 1070 healthy control subjects. To the best of our knowledge, we are the first to report the association of RAS gene polymorphisms with Wilms tumor risk in Chinese children.

KRAS and NRAS have been mapped to chromosomes 12p12.1 and 1p13.2, respectively. Many studies have investigated the mechanisms by which RAS gene polymorphisms impact cancer risk. In particular, rs61764370 and rs712, two KRAS polymorphisms in miRNA-binding sites, have been intensively studied. These two SNPs are located in the 3’ untranslated region (UTR) of KRAS, where they disrupt a let-7 miRNA binding site, thus increasing KRAS expression and enhancing tumor growth [31]. Chin et al. studied 46 populations worldwide, and identified the rs61764370 SNP in the 3’ UTR of the KRAS gene (KRAS-LCS6). This SNP was associated with increased expression of KRAS, reduced expression of let-7 and increased risk of lung cancer [31]. Furthermore, this allele was demonstrated to elevate the risk of epithelial ovarian

| Variables          | rs2273267 (cases/controls) | Crude OR (95% CI) | P  | Adjusted OR a (95% CI) | P a |
|--------------------|---------------------------|-------------------|----|------------------------|-----|
| Age, month         |                           |                   |    |                        |     |
| ≤18                | 58/199                    | 1.02 (0.68-1.52)  | 0.934 | 1.01 (0.67-1.50)       | 0.975 |
| >18                | 125/342                   | 0.95 (0.70-1.28)  | 0.730 | 0.96 (0.71-1.30)       | 0.799 |
| Gender             |                           |                   |    |                        |     |
| Females            | 91/234                    | 0.87 (0.60-1.24)  | 0.431 | 0.87 (0.60-1.24)       | 0.432 |
| Males              | 92/307                    | 1.06 (0.77-1.46)  | 0.727 | 1.05 (0.76-1.45)       | 0.764 |
| Clinical stages    |                           |                   |    |                        |     |
| I                  | 67/541                    | 0.79 (0.54-1.16)  | 0.235 | 0.80 (0.55-1.17)       | 0.252 |
| II                 | 41/541                    | 1.27 (0.83-1.95)  | 0.271 | 1.27 (0.83-1.96)       | 0.269 |
| III                | 41/541                    | 0.95 (0.60-1.50)  | 0.819 | 0.95 (0.60-1.51)       | 0.832 |
| IV                 | 28/541                    | 0.69 (0.38-1.26)  | 0.229 | 0.70 (0.38-1.26)       | 0.233 |
| I+II               | 108/541                   | 0.98 (0.73-1.31)  | 0.868 | 0.98 (0.73-1.32)       | 0.888 |
| III+IV             | 69/541                    | 0.85 (0.58-1.22)  | 0.373 | 0.85 (0.59-1.23)       | 0.392 |

OR, odds ratio; CI, confidence interval.

a Adjusted for age and gender, omitting the corresponding stratify factor.

Table 3. Stratification analysis for the association between NRAS rs2273267 A>T polymorphism and Wilms tumor risk.

| Genotype         | Crude OR (95% CI) | P a  | Statistical power b | Prior probability |
|------------------|------------------|------|---------------------|-------------------|
| rs12587 G>T      |                  |      |                     |                   |
| GT vs. GG        | 1.29 (1.002-1.67)| 0.049| 0.886               | 0.141             |
| GT/TT vs. GG     |                  |      |                     |                   |
| >18 months Risk  | 1.39 (1.02-1.89) | 0.037| 0.682               | 0.131             |
| genotypes 1-3 vs.| 1.28 (1.002-1.64)| 0.048| 0.903               | 0.139             |

OR, odds ratio; CI, confidence interval.

a χ² test was used to calculate the genotype frequency distributions.

b Statistical power was calculated using the number of observations in the subgroup and the OR and P values in this table.
cancer [32] and triple-negative breast cancer [33]. In a population-based case-control study conducted in the US by Christensen et al., the KRAS-LCS6 variant genotype (rs61764370) was not associated with the overall risk of head and neck squamous cell carcinoma, but was associated with a significantly reduced survival time [34].

Wang et al. [7] reported that the rs712 polymorphism in the KRAS 3’ UTR was associated with a reduced risk for oral squamous cell carcinoma, while rs1137282 in KRAS exon 6 was not [35]. In contrast, in a study of 181 gastric cancer patients and 674 cancer-free controls, Li et al. found that the T allele of rs712 significantly enhanced the susceptibility to gastric cancer [36]. As different types of tissues and cells have different miRNA profiles, the effects of SNPs in specific 3’ UTRs may vary accordingly. Moreover, differences in the population sources, environmental exposures, sample sizes and selection criteria of subjects may also have influenced the contribution of RAS SNPs to cancer susceptibility of different types. Therefore, it is necessary to define the impact of RAS polymorphisms on the risk of a certain cancer type in a certain population.

Our findings indicated that carriers of the KRAS rs12587 GT genotype had a genetic predisposition to Wilms tumor risk. Unexpectedly, rs7973450 A>G, rs7312175 G>A and rs2273267 A>T were not significantly associated with Wilms tumor risk. The rs12587 G>T, rs7973450 A>G and rs2273267 A>T polymorphisms reside in different complementary miRNA sites. The different locations of these SNPs may be one reason for their different effects on cancer risk. Other plausible interpretations of the null association include the relatively small sample size and the low-penetrance susceptibility of single polymorphisms.

One limitation of this study was the relatively small sample size, which may have impaired the statistical power, especially for the stratification analysis. Another limitation was the restriction of the included population to a single ethnicity (Chinese Han), which may render the findings inapplicable to other populations. Further, though we analyzed four SNPs in the current study, additional SNPs should be considered in future studies. Lastly, the current study only focused on genetic factors, and gene-environment interaction analysis was not performed due to the lack of relevant information. Wilms tumor is a heterogeneous disease, and both genetic and environmental factors contribute to its tumorigenesis. Thus, more comprehensive studies are warranted.

In conclusion, this was the first multi-center evaluation of the association of KRAS and NRAS gene SNPs with Wilms tumor susceptibility. Our study has provided the first evidence that KRAS gene SNPs may increase Wilms tumor susceptibility. Ongoing epidemiological studies in other independent populations are warranted prior to extrapolation of the current conclusions.

**MATERIALS AND METHODS**

**Study subjects**

In total, 355 cases and 1070 healthy controls were included in this study (Supplemental Table 1). The subject selection criteria were described in detail in our previous study [37-43]. In brief, cases with newly diagnosed and histologically confirmed Wilms tumor were recruited from four centers in China (Guangzhou Women and Children’s Medical Center [37-43], The First Affiliated Hospital of Zhengzhou University, The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University, and The Second Affiliated Hospital of Xi’an Jiao Tong University). All the included cases were sporadic cases. The controls were healthy volunteers without a history of Wilms tumor, matched to the cases by age, gender and city of residency. All the subjects or their guardians provided written informed consent before participating. Approval of the study protocol was obtained from the Institutional Review Board of each center prior to the study.

**Polymorphism selection and genotyping**

We analyzed three potential functional SNPs in the KRAS gene and one potential functional SNP in the NRAS gene. SNPs were selected from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) and SNPinfo (http://snpinfo.niehs.nih.gov/snpfunc.htm). These four SNPs could capture an additional 89 SNPs with R^2 ≥ 0.8 (Supplemental Table 2). The selection criteria were set as previously described [42, 44]. Genomic DNA was extracted from venous blood with a TIANamp Blood DNA Kit (TianGen Biotech Co. Ltd., Beijing, China). SNP genotyping was performed with a TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). Negative controls with water and 10% replicates were also genotyped to ensure genotyping accuracy. No discordant genotypes were found in the replicates.

**Statistical analysis**

Statistical analysis was performed in SAS release 9.1 (SAS Institute, Cary, NC, USA). The genotype frequency distributions of the polymorphisms were first
evaluated among the controls, and Hardy-Weinberg equilibrium was assessed with the $\chi^2$ test. The distribution of subject characteristics between cases and controls was examined with a two-sided $\chi^2$ test. Unadjusted and adjusted (for age and gender) ORs and 95% CIs were generated for both single and combined SNPs. We then determined the association of the SNPs with Wilms tumor risk using the OR and 95% CI calculated from multivariable logistic regression analysis. FPRP analysis was performed as described previously [45]. All results were considered statistically significant if $P<0.05$.

CONFLICTS OF INTEREST

There are no competing interests to declare.

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REFERENCES

1. Davidoff AM. Wilms’ tumor. Curr Opin Pediatr. 2009; 21:357–64. https://doi.org/10.1097/MOP.0b013e32832b323a
2. Beckwith JB, Kiviat NB, Bonadio JF. Nephrogenic rests, nephroblastosomatosis, and the pathogenesis of Wilms’ tumor. Pediatr Pathol. 1990; 10:1–36. https://doi.org/10.3109/15513819009067094
3. Hohenstein P, Pritchard-Jones K, Charlton J. The yin and yang of kidney development and Wilms’ tumors. Genes Dev. 2015; 29:467–82. https://doi.org/10.1101/gad.256396.114
4. Breslow N, Olshan A, Beckwith JB, Green DM. Epidemiology of Wilms tumor. Med Pediatr Oncol. 1993; 21:172–81. https://doi.org/10.1002/mpo.2950210305
5. Gadd S, Huff V, Walz AL, Ooms AH, Armstrong AE, Gerhard DS, Smith MA, Avul JM, Mezerman D, Chen QR, Hsu CH, Yan C, Nguyen C, et al. A Children’s Oncology Group and TARGET initiative exploring the genetic landscape of Wilms tumors. Nat Genet. 2017; 49:1487–94. https://doi.org/10.1038/ng.3940
6. Dome JS, Liu T, Krasin M, Lott L, Shearer P, Daw NC, Billups CA, Willimas JA, and Jude Children’s Research Hospital. Improved survival for patients with recurrent Wilms tumor: the experience at St. J Pediatr Hematol Oncol. 2002; 24:192–98. https://doi.org/10.1097/00043426-200203000-00007
7. Dome JS, Graf N, Geller JI, Fernandez CV, Mullen EA, Spreeafico F, Van den Heuvel-Eibrink M, Pritchard-Jones K. Advances in Wilms Tumor treatment and biology: progress through international collaboration. J Clin Oncol. 2015; 33:2999–3007. https://doi.org/10.16020/jico.2015.62.1888
8. Sonn G, Shortliffe LM. Management of Wilms tumor: current standard of care. Nat Clin Pract Urol. 2008; 5:551–60. https://doi.org/10.1038/ncpuro1218
9. Pritchard-Jones K, Moroz V, Vujanic G, Powis M, Walker J, Messahel B, Hobson R, Levitt G, Kelsey A, Mitchell C, and Children’s Cancer and Leukaemia Group (CCLG) Renal Tumours Group. Treatment and outcome of Wilms’ tumour patients: an analysis of all cases registered in the UKW3 trial. Ann Oncol. 2012; 23:2457–63. https://doi.org/10.1093/annonc/mds025
10. Malogolowkin M, Cotton CA, Green DM, Breslow NE, Perlman E, Miser J, Ritchey ML, Thomas PR, Grundy PE, D’Angio GJ, Beckwith JB, Shamberger RC, Haase GM, et al, and National Wilms Tumor Study Group. Treatment of Wilms tumor relapsing after initial treatment with vincristine, actinomycin D, and doxorubicin. A report from the National Wilms Tumor Study Group. Pediatr Blood Cancer. 2008; 50:236–41. https://doi.org/10.1002/pbc.21267
11. Little SE, Hanks SP, King-Underwood L, Jones C, Rapley EA, Rahman N, Pritchard-Jones K. Frequency and heritability of WT1 mutations in nonsyndromic Wilms’ tumor patients: a UK Children’s Cancer Study Group Study. J Clin Oncol. 2004; 22:4140–46. https://doi.org/10.1200/JCO.2004.02.136
12. Scott RH, Douglas J, Baskcomb L, Huxter N, Barker K, Hanks S, Craft A, Gerrard M, Kohler JA, Levitt GA, Picton S, Pizer B, Ronghe MD, et al, and Factors Associated with Childhood Tumours (FACT) Collaboration. Constitutional 11p15 abnormalities, including heritable imprinting center mutations, cause nonsyndromic Wilms tumors. Nat Genet. 2008; 40:1329–34. https://doi.org/10.1038/ng.243
13. Andrade RC, Cardoso LC, Ferman SE, Faria PS, Seuánez NH, Achatz MI, Vargas FR. Association of TP53 polymorphisms on the risk of Wilms tumor. Pediatr Blood Cancer. 2014; 61:436–41. https://doi.org/10.1002/pbc.24775
14. Rakheja D, Chen KS, Liu Y, Shukla AA, Schmid V, Chang TC, Khokhar S, Wickiser JE, Karandikar NJ, Malter JS, Mendell JT, Amatruda JF. Somatic
mutations in DROSHA and DICER1 impair microRNA biogenesis through distinct mechanisms in Wilms tumours. Nat Commun. 2014; 2:4802. https://doi.org/10.1038/ncomms5802

15. Torrezan GT, Ferreira EN, Nakahata AM, Barros BD, Castro MT, Correa BR, Krepsihi AC, Olivieri EH, Cunha IW, Tabori U, Grundy PE, Costa CM, de Camargo B, et al. Recurrent somatic mutation in DROSHA induces microRNA profile changes in Wilms tumour. Nat Commun. 2014; 5:4039. https://doi.org/10.1038/ncomms5039

16. Walz AL, Ooms A, Gadd S, Gerhard DS, Smith MA, Guidry Auvil JM, Meerzaman D, Chen QR, Hu Y, Browby R, et al. Recurrent DGCR8, DROSHA, and SIX homeodomain mutations in favorable histology Wilms tumors. Cancer Cell. 2015; 27:286–97. https://doi.org/10.1016/j.ccell.2015.01.003

17. Bos JL. ras oncogenes in human cancer: a review. Cancer Res. 1989; 49:4682–89.

18. Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nat Rev Cancer. 2003; 3:459–65. https://doi.org/10.1038/nrc1097

19. Hobbs GA, Der CJ, Rossman KL. RAS isoforms and mutations in cancer at a glance. J Cell Sci. 2016; 129:1287–92. https://doi.org/10.1242/jcs.182873

20. Bos JL, Fearon ER, Hamilton SR, Verlaan-de Vries M, van Boom JH, van der Eb Aj, Vogelstein B. Prevalence of ras gene mutations in human colorectal cancers. Nature. 1987; 327:293–97. https://doi.org/10.1038/327293a0

21. Kranenburg O. The KRAS oncogene: past, present, and future. Biochim Biophys Acta. 2005; 1756:81–82. https://doi.org/10.1016/j.bbcan.2005.10.001

22. Irahara N, Baba Y, Nosho K, Shima K, Yan L, Dias-Santagata D, Iafrate AJ, Fuchs CS, Haigis KM, Ogino S. NRAS mutations are rare in colorectal cancer. Diagn Mol Pathol. 2010; 19:157–63. https://doi.org/10.1097/PDM.0b013e3181c93fd1

23. Yang M, Xiao X, Xing X, Li X, Xia T, Long H. KRAS and VEGF gene 3′-UTR single nucleotide polymorphisms predicted susceptibility in colorectal cancer. PLoS One. 2012; 12:e0174140. https://doi.org/10.1371/journal.pone.0174140

24. Nelson HH, Christensen BC, Plaza SL, Wiencke JK, Marsit CJ, Kelsey KT. KRAS mutation, KRAS-LCS6 polymorphism, and non-small cell lung cancer. Lung Cancer. 2010; 69:51–53. https://doi.org/10.1016/j.lungcan.2009.09.008

25. Kim M, Chen X, Chin LJ, Paranjape T, Speed WC, Kidd KK, Zhao H, Weidhaas JB, Slack FJ. Extensive sequence variation in the 3′ untranslated region of the KRAS gene in lung and ovarian cancer cases. Cell Cycle. 2014; 13:1030–40. https://doi.org/10.4161/cc.27941

26. Uvirova M, Simova J, Kubova B, Dvorackova N, Tomaskova H, Sedivcova M, Dite P. Comparison of the prevalence of KRAS-LCS6 polymorphism (rs61764370) within different tumour types (colorectal, breast, non-small cell lung cancer and brain tumours). A study of the Czech population. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2015; 159:466–71. https://doi.org/10.5507/bp.2015.029

27. Tomei S, Adams S, Uccellini L, Bedognetti D, De Giorgi V, Erdenebileg N, Ascierto ML, Reinboth J, Liu Q, Bevilacqua G, Wang E, Mazzanti C, Marincola F. Association between HRAS rs12628 and rs112587690 polymorphisms with the risk of melanoma in the North American population. Med Oncol. 2012; 29:3456–61. https://doi.org/10.1007/s12032-012-0255-3

28. Clark PE, Polosukhina D, Love H, Correa H, Coffin C, Perlman EJ, de Caestecker M, Moses HL, Zent R. β-Catenin and K-RAS synergize to form primitive renal epithelial tumors with features of epithelial Wilms’ tumors. Am J Pathol. 2011; 179:3045–55. https://doi.org/10.1016/j.ajpath.2011.08.006

29. Polosukhina D, Love HD, Correa H, Su Z, Dahlman KB, Pao W, Moses HL, Arteaga CL, Lovvorn HN 3rd, Zent R, Clark PE. Functional KRAS mutations and a potential role for PI3K/AKT activation in Wilms tumors. Mol Oncol. 2017; 11:405–21. https://doi.org/10.1002/1878-0261.12044

30. Dalpa E, Gourvas V, Soulitzis N, Spandidos DA. K-Ras, H-Ras, N-Ras and B-Raf mutation and expression analysis in Wilms tumors: association with tumor growth. Med Oncol. 2017; 34:6. https://doi.org/10.1007/s12032-016-0862-5

31. Chin LJ, Ratner E, Leng S, Zhai R, Nallur S, Babar I, Muller RJ, Straka E, Su L, Burki EA, Crowell RE, Patel R, Kulkarni T, et al. A SNP in a let-7 microRNA complementary site in the KRAS 3′ untranslated region increases non-small cell lung cancer risk. Cancer Res. 2008; 68:8535–40. https://doi.org/10.1158/0008-5472.CAN-08-2129

32. Ratner E, Lu L, Boeke M, Barnett R, Nallur S, Chin LJ, Pelletier C, Blitzblau R, Tassi R, Paranjape T, Hui P, Godwin AK, Yu H, et al. A KRAS-variant in ovarian...
cancer acts as a genetic marker of cancer risk. Cancer Res. 2010; 70:6509–15. https://doi.org/10.1158/0008-5472.CAN-10-0689

33. Paranjape T, Heneghan H, Lindner R, Keane FK, Hoffman A, Hollestelle A, Dorairaj J, Geyda K, Pelletier C, Nallur S, Martens JW, Hooning MJ, Kerin M, et al. A 3′-untranslated region KRAS variant and triple-negative breast cancer: a case-control and genetic analysis. Lancet Oncol. 2011; 12:377–86. https://doi.org/10.1016/S1470-2045(11)70044-4

34. Christensen BC, Moyer BJ, Avissar M, Ouellet LG, Plaza SL, McClean MD, Marsit CJ, Kelsey KT. A let-7 microRNA-binding site polymorphism in the KRAS 3′ UTR is associated with reduced survival in oral cancers. Carcinogenesis. 2009; 30:1003–07. https://doi.org/10.1093/carcin/bgp099

35. Wang WY, Chien YC, Wong YK, Lin YL, Lin JC. Effects of KRAS mutation and polymorphism on the risk and prognosis of oral squamous cell carcinoma. Head Neck. 2012; 34:663–66. https://doi.org/10.1002/hed.21792

36. Li ZH, Pan XM, Han BW, Guo XM, Zhang Z, Jia J, Gao LB. A let-7 binding site polymorphism rs712 in the KRAS 3′ UTR is associated with an increased risk of gastric cancer. Tumour Biol. 2013; 34:3159–63. https://doi.org/10.1007/s13277-013-0885-x

37. Fu W, Zhu J, Xiong SW, Jia W, Zhao Z, Zhu SB, Hu JH, Wang FH, Xia H, He J, Liu GC. BARD1 gene polymorphisms confer nephroblastoma susceptibility. EBioMedicine. 2017; 16:101–05. https://doi.org/10.1016/j.ebiom.2017.01.038

38. Fu W, Zhuo ZJ, Jia W, Zhu J, Zhu SB, Lin ZF, Wang FH, Xia H, He J, Liu GC. Association between TP53 gene Arg72Pro polymorphism and Wilms' tumor risk in a Chinese population. Onco Targets Ther. 2017; 10:1149–54. https://doi.org/10.2147/OTT.S131014

39. Jia W, Deng Z, Zhu J, Fu W, Zhu S, Zhang LY, Hu J, Wang F, Xia H, Liu GC, He J. Association between HACE1 gene polymorphisms and Wilms' Tumor risk in a Chinese population. Cancer Invest. 2017; 35:633–38. https://doi.org/10.1080/07357907.2017.1405016

40. Fu W, Li L, Xiong SW, Zhang T, Jia W, Zhu J, Zhao Z, Xia H, He J, Liu GC. miR-423 rs6505162 C>A polymorphism contributes to decreased Wilms tumor risk. J Cancer. 2018; 9:2460–65. https://doi.org/10.7150/jca.24916

41. Fu W, Liu GC, Zhao Z, Zhu J, Jia W, Zhu SB, Hu JH, Wang FH, He J, Xia H. The correlation between LIN28B gene potentially functional variants and Wilms tumor susceptibility in Chinese children. J Clin Lab Anal. 2018; 32:e22200. https://doi.org/10.1002/jcla.22200

42. Zhu J, Fu W, Jia W, Xia H, Liu GC, He J. Association between NER pathway gene polymorphisms and Wilms Tumor risk. Mol Ther Nucleic Acids. 2018; 12:854–60. https://doi.org/10.1016/j.omtn.2018.08.002

43. Zhu J, Jia W, Wu C, Fu W, Xia H, Liu G, He J. Base excision repair gene polymorphisms and Wilms Tumor susceptibility. EBioMedicine. 2018; 33:88–93. https://doi.org/10.1016/j.ebiom.2018.06.018

44. Zhuo ZJ, Liu W, Zhang J, Zhu J, Zhang R, Tang J, Yang T, Zou Y, He J, Xia H. Functional polymorphisms at ERCC1/XPF genes confer neuroblastoma risk in Chinese children. EBioMedicine. 2018; 30:113–19. https://doi.org/10.1016/j.ebiom.2018.03.003

45. He J, Wang MY, Qiu LX, Zhu ML, Shi TY, Zhou XY, Sun MH, Yang YJ, Wang JC, Jin L, Yang YN, Li J, Yu HP, Wei QY. Genetic variations of mTORC1 genes and risk of gastric cancer in an Eastern Chinese population. Mol Carcinog. 2013 (Suppl 1); 52:E70–79. https://doi.org/10.1002/mc.22013
Supplemental Table 1. Frequency distribution of selected variables in Wilms tumor patients and controls.

| Variables          | Cases (n=355) | Controls (n=1070) | \(P^a\) |
|--------------------|---------------|-------------------|---------|
| Age                |               |                   |         |
| Range, months      | 1-148.63      | 0.03-156          | 0.131   |
| Mean ± SD, months  | 30.67 ± 23.96 | 32.27 ± 26.89     |         |
| ≤18 months         | 125           | 425               |         |
| >18 months         | 230           | 645               |         |
| Gender             |               |                   | 0.182   |
| Female             | 163           | 448               |         |
| Male               | 192           | 622               |         |
| Clinical stages    |               |                   |         |
| I                  | 119           |                   |         |
| II                 | 92            |                   |         |
| III                | 79            |                   |         |
| IV                 | 47            |                   |         |
| NA                 | 18            |                   |         |

SD, standard deviation; NA, not available.

\(^a\) Two-sided \(\chi^2\) test for distributions between Wilms tumor patients and cancer-free controls.
Supplemental Table 2. SNPs captured by the four selected potentially functional SNPs as predicted by SNPInfo software.

| rs    | Chr. | Allele | LDsnp   | Pop/LD | TFBS   | Splicing (ESE or ESS) | miRNA (miRanda) | nsSNP | Nearby Gene | Distance (bp) | Allele | Asian CHB |
|-------|------|--------|---------|--------|--------|------------------------|-----------------|-------|-------------|--------------|--------|-----------|
| rs10842466 | 12 | A/G | rs12587 | CHB/0.856 | -- | -- | -- | -- | LRMP | 46140|9888 | G | 0.217 | 0.274 |
| rs10842492 | 12 | G/T | rs12587 | CHB/0.818 | -- | -- | -- | -- | CASC1 | 45785|41086 | T | 0.237 | 0.287 |
| rs10842494 | 12 | C/T | rs12587 | CHB/0.842 | -- | -- | -- | -- | CASC1 | 48236|38635 | T | 0.767 | 0.700 |
| rs10842496 | 12 | G/T | rs12587 | CHB/0.831 | -- | Y | -- | Y | CASC1 | 50266|36605 | G | 0.758 | 0.720 |
| rs10842498 | 12 | C/T | rs12587 | CHB/1 | -- | -- | -- | -- | CASC1 | 76131|10740 | C | 0.225 | 0.267 |
| rs10842501 | 12 | C/T | rs12587 | CHB/1 | Y | -- | -- | -- | CASC1 | 82293|4578 | T | 0.781 | 0.756 |
| rs10842502 | 12 | C/T | rs12587 | CHB/0.941 | Y | -- | -- | -- | CASC1 | 82484|4387 | T | 0.762 | 0.716 |
| rs10842505 | 12 | A/G | rs12587 | CHB/1 | -- | -- | -- | -- | LYRM5 | 5442|4357 | A | 0.791 | 0.731 |
| rs11047824 | 12 | A/G | rs12587 | CHB/0.887 | -- | -- | -- | -- | LRMP | 40512|15516 | G | 0.204 | 0.273 |
| rs11047865 | 12 | C/G | rs12587 | CHB/0.833 | -- | -- | -- | -- | CASC1 | 45284|41587 | C | 0.236 | 0.284 |
| rs11047887 | 12 | A/C | rs12587 | CHB/1 | Y | -- | -- | -- | LYRM5 | 522|9277 | A | 0.222 | 0.244 |
| rs11047888 | 12 | C/T | rs12587 | CHB/1 | Y | -- | -- | -- | LYRM5 | 666|9133 | T | 0.778 | 0.756 |
| rs11047894 | 12 | C/G | rs12587 | CHB/1 | -- | -- | -- | -- | Kras | 7490|38179 | C | 0.778 | 0.733 |
| rs11047901 | 12 | A/G | rs12587 | CHB/1 | -- | -- | -- | -- | KRAS | 18149|27525 | A | 0.219 | 0.267 |
| rs11047902 | 12 | C/T | rs12587 | CHB/1 | -- | -- | -- | -- | KRAS | 21613|24061 | C | 0.193 | 0.267 |
| rs1137188 | 12 | G/A | rs12587 | CHB/1 | -- | -- | Y | -- | Kras | 1172|44502 | A | 0.778 | 0.727 |
| rs11611468 | 12 | A/C | rs12587 | CHB/1 | -- | -- | -- | -- | CASC1 | 79900|6971 | C | 0.785 | 0.757 |
| rs11832421 | 12 | C/T | rs12587 | CHB/0.831 | -- | -- | -- | -- | LRMP | 42465|13563 | T | 0.787 | 0.720 |
| rs12368504 | 12 | C/T | rs12587 | CHB/1 | -- | -- | -- | -- | KRAS | 19512|26162 | T | 0.772 | 0.756 |
| rs12423443 | 12 | C/T | rs12587 | CHB/0.807 | -- | -- | -- | -- | CASC1 | 69228|17643 | T | -- | 0.714 |
| rs12579073 | 12 | A/C | rs12587 | CHB/1 | -- | -- | -- | -- | KRAS | 17619|28055 | C | 0.116 | 0.244 |
| rs12579942 | 12 | C/T | rs12587 | CHB/1 | -- | -- | -- | -- | KRAS | 25014|20660 | T | 0.810 | 0.756 |
| **rs12587** | **12** | **T/G** | **rs12587** | **1** | -- | -- | Y | -- | KRAS | 648|45026 | G | **0.807** | **0.756** |
| rs12810577 | 12 | A/G | rs12587 | CHB/0.91 | Y | -- | -- | -- | CASC1 | 84940|1931 | G | 0.222 | 0.262 |
| rs12815546 | 12 | C/T | rs12587 | CHB/1 | -- | -- | -- | -- | KRAS | 24362|21312 | T | 0.778 | 0.756 |
| rs12822857 | 12 | A/G | rs12587 | CHB/1 | -- | -- | -- | -- | KRAS | 11437|34237 | G | 0.775 | 0.727 |
| rs13096 | 12 | T/C | rs12587 | CHB/1 | -- | Y | -- | -- | KRAS | 1661|44013 | T | 0.190 | 0.244 |
| rs17329025 | 12 | A/G | rs12587 | CHB/0.91 | -- | -- | -- | -- | KRAS | 25633|20041 | A | 0.193 | 0.262 |
| rs1908946 | 12 | G/C | rs12587 | CHB/0.891 | -- | -- | Y | LRMP | 37874|18154 | G | 0.214 | 0.278 |
| rs2352782 | 12 | G/A | rs12587 | CHB/0.806 | -- | -- | -- | -- | CASC1 | 24735|62136 | G | 0.219 | 0.289 |
| rs4246229 | 12 | A/G | rs12587 | CHB/1 | -- | -- | -- | -- | KRAS | 9489|36185 | A | 0.807 | 0.759 |
| SNP   | Genotype | rsID   | Chrm | Position | MAF  | Populations | Method | p-value | MAF  | Populations | Method | p-value |
|-------|----------|--------|------|----------|------|-------------|--------|---------|------|-------------|--------|---------|
| rs4963859 | A/C      | rs12587 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs4963860 | C/T      | rs12587 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs712    | C/A      | rs12587 | CHB/1 | Y        | --   | --          | --     | --      | --   | --          | --     | --      |
| rs7299998 | C/T      | rs12587 | CHB/0.882 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs7302922 | C/T      | rs12587 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs7306769 | A/G      | rs12587 | CHB/0.856 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs7308865 | A/C      | rs12587 | CHB/0.841 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs9266   | G/A      | rs12587 | CHB/1 | Y        | --   | --          | --     | --      | --   | --          | --     | --      |
| rs9634100 | C/T      | rs12587 | CHB/0.837 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs2273267 | T/A      | rs2273267 | Y    | Y        | --   | --          | --     | --      | --   | --          | --     | --      |
| rs10842508 | C/T     | rs7312175 | CHB/0.956 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs10842509 | C/G     | rs7312175 | CHB/0.92 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs11047826 | C/T     | rs7312175 | CHB/0.848 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs11047880 | A/G     | rs7312175 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs11047918 | A/G     | rs7312175 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs12228277 | A/T     | rs7312175 | CHB/0.919 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs12229161 | C/T     | rs7312175 | CHB/0.809 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs12230737 | A/G     | rs7312175 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs12423489 | C/T     | rs7312175 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs12424283 | A/G     | rs7312175 | CHB/0.92 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs12427141 | A/G     | rs7312175 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs2970532  | C/T     | rs7312175 | CHB/0.956 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs3782188  | A/G     | rs7312175 | CHB/0.85 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs3924649  | G/A     | rs7312175 | CHB/0.842 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs4623993  | C/T     | rs7312175 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs7312175  | A/G     | rs7312175 | Y    | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs7973746  | C/G     | rs7312175 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs7979296  | G/T     | rs7312175 | CHB/0.956 | Y   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs10505959 | C/T     | rs7973450 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs10771166 | C/T     | rs7973450 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs10771174 | C/T     | rs7973450 | CHB/0.887 | --   | --   | --          | --     | --      | --   | --          | --     | --      |
| rs10771175 | C/G     | rs7973450 | CHB/1 | --       | --   | --          | --     | --      | --   | --          | --     | --      |
| rs    | 12   | SNP   | Genotype | CHB/1 | \(1\) | -- |  -- | LRMP | CASC1 | 51497|35374 | T | 0.876 | 0.917 |
|-------|------|-------|----------|-------|-------|----|-----|------|-------|-------|-------|------|----------|
| rs7973450 | 12 | C/T   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 44502|11526 | T | 0.101 | 0.091 |
| rs7960092 | 12 | A/C   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 4980|81891 | T | 0.888 | 0.917 |
| rs7960428 | 12 | C/G   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 45380|14191 | C | 0.872 | 0.868 |
| rs7973450 | 12 | C/T   | rs7973450 | CHB/0.877 | -- | -- | -- | LRMP | CASC1 | 41847|14181 | T | 0.895 | 0.907 |
| rs12220196 | 12 | T/G   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 29567|57304 | T | 0.867 | 0.878 |
| rs1497253 | 12 | G/A   | rs7973450 | CHB/0.887 | -- | -- | -- | LRMP | CASC1 | 81407|5464 | A | 0.891 | 0.917 |
| rs11047858 | 12 | A/C   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 40930|15098 | G | 0.893 | 0.909 |
| rs11047825 | 12 | C/T   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 44450|42421 | G | 0.895 | 0.893 |
| rs12220196 | 12 | A/G   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 21281|65590 | G | 0.101 | 0.101 |
| rs12220196 | 12 | A/G   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 50110|36761 | G | 0.886 | 0.916 |
| rs12220196 | 12 | T/C   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 35383|33288 | A | 0.878 | 0.889 |
| rs12220196 | 12 | T/C   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 43484|43387 | A | 0.878 | 0.889 |
| rs12220196 | 12 | A/G   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 23249|63622 | G | 0.903 | 0.900 |
| rs12220196 | 12 | T/C   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 77006|9865  | T | 0.907 | 0.895 |
| rs12220196 | 12 | A/G   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 40363|15665 | T | 0.894 | 0.910 |
| rs12220196 | 12 | T/C   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 53583|33288 | A | 0.878 | 0.889 |
| rs12220196 | 12 | C/T   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 46937|9091  | C | 0.889 | 0.899 |
| rs12220196 | 12 | C/T   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 47047|8981  | G | 0.889 | 0.893 |
| rs12220196 | 12 | C/T   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 3466|42208 | T | 0.882 | 0.917 |
| rs12220196 | 12 | C/T   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 38938|17090 | T | 0.893 | 0.909 |
| rs12220196 | 12 | C/T   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 36490|50381 | T | 0.888 | 0.898 |
| rs12220196 | 12 | A/G   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 81407|94271 | A | 0.890 | 0.917 |
| rs12220196 | 12 | A/G   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 51113|4915  | C | 0.878 | 0.916 |
| rs12220196 | 12 | A/G   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 39142|16886 | A | 0.889 | 0.911 |
| rs12220196 | 12 | C/T   | rs7973450 | CHB/1 | -- | -- | -- | LRMP | CASC1 | 77720|9151  | T | 0.878 | 0.917 |

SNP, single nucleotide polymorphism; LD, linkage disequilibrium; TFBS, transcription factor binding sites; ESE, exonic splicing enhancer; ESS, exonic splicing silencer; rsSNP, nonsynonymous single nucleotide polymorphism; CHB, Han Chinese in Beijing, China.