Research Paper

# *RAN/RANBP2* polymorphisms and neuroblastoma risk in Chinese children: a three-center case-control study

**Juxiang Wang[1,*], Zhenjian Zhuo[2,*], Min Chen[1], Jinhong Zhu[3], Jie Zhao[1], Jiao Zhang[4], Shanshan Chen[1], Jing He[1,5], Haixia Zhou[1]**

[1]Department of Hematology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325027, Zhejiang, China
[2]School of Chinese Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong 999077, China
[3]Department of Clinical Laboratory, Molecular Epidemiology Laboratory, Harbin Medical University Cancer Hospital, Harbin 150040, Heilongjiang, China
[4]Department of Pediatric Surgery, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China
[5]Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, Guangdong 510623, China
[*]Equal contribution

**Correspondence to:** Haixia Zhou, Jing He; **email:** zhxcrystal@126.com, hejing198374@gmail.com
**Keywords:** neuroblastoma, RAN, RANBP2, polymorphism, susceptibility
**Received:** March 27, 2018 **Accepted:** April 20, 2018 **Published:** April 28, 2018



## ABSTRACT

**The genetic etiology of sporadic neuroblastoma remains largely obscure. *RAN* and *RANBP2* genes encode Ras-related nuclear protein and Ran-binding protein 2, respectively. These two proteins form Ran-RanBP2 complex that regulate various cellular activities including nuclear transport. Aberrant functions of the two proteins are implicated in carcinogenesis. Given the unknown role of *RAN/RANBP2* single nucleotide polymorphisms (SNPs) in neuroblastoma risk, we performed a multi-center case-control study in Chinese children to assess the association of the *RAN/RANBP2* SNPs with neuroblastoma risk. We analyzed three potentially functional SNPs in *RAN* gene (rs56109543 C>T, rs7132224 A>G, rs14035 C>T) and one in *RANBP2* (rs2462788 C>T) in 429 cases and 884 controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to access the association between these four polymorphisms and neuroblastoma risk. No single variant was found to statistically significantly associate with neuroblastoma risk. However, individuals with 3 protective genotypes were less likely to develop neuroblastoma, in comparison to non-carriers (adjusted OR=0.33; 95% CI=0.12-0.96; *P*=0.042), as well as those with 0-2 protective genotypes (adjusted OR=0.33; 95% CI=0.11-0.94; *P*=0.038). Stratified analysis revealed no significant association for any of the four polymorphisms. Further studies are warranted to validate the weak impact of *RAN/RANBP2* SNPs on neuroblastoma risk.**

## INTRODUCTION

Neuroblastoma is a common extracranial solid tumor that derives from neural crest progenitor cells [1, 2]. Neuroblastoma mostly takes place in children younger than 1 year, and the average diagnosis time is about 17 months of age [3]. Neuroblastoma is characterized by a wide range of variable prognosis, spanning from spontaneous regression without chemotherapy to life-threatening tumor progression despite intensive treat-

ment [4-7]. Approximately 50% of neuroblastomas behave in highly malignant fashion, with distant metastasis at the time of diagnosis [8, 9]. Their 5-year survival rates remain less than 40% despite intensive, multi-modal therapy [10].

The affects of environmental factors on the risk of neuroblastoma have been investigated but remains undefined [11, 12]. Growing evidence has been directed to the genetic factors predisposing patients to neuroblastoma. Familial neuroblastoma is largely attributed to germline mutations in *PHOX2B* [13] or *ALK* [14, 15] gene. In contrast, the etiology of sporadic neuroblastoma, the most common type of neuroblastoma, remains partially unveiled. Several genome-wide association studies (GWASs) and the subsequent replication studies identified a number of neuroblastoma susceptibility alleles, including *BARD1*, *LIN28B*, *HACE1*, *LMO1*, *MMP20* and *CASC15* genes [16-23]. Moreover, candidate gene approaches also detected the genetic associations of *NEFL* [24] and *CDKN1B* [25] gene polymorphisms with neuroblastoma susceptibility.

Ran (Ras-related nuclear protein) is a small Ras-related GTP-binding protein. Ran mainly locates in the nucleus and cycles between the GDP-bound inactive and the GTP-bound active state [26]. It facilitates the movement of molecules in and out of the nuclear-pore complexes [27]. Dysregulated protein level of Ran could cause aberrant nuclear-cytoplasmic transport of tumor suppressors and oncogenes, which might lead to the initiation of cancer [28]. Moreover, Ran also mediates several crucial functions, such as promoting spindle assembly, regulating cell cycle, and facilitating pre-mRNA generation [29]. RanBP2 (Ran-binding protein 2) is the largest protein of the nuclear pore complex (350 kDa). It contains rich FG-repeats, four Ran-binding domains and binds to Ran GTP with high affinity [30]. RanBP2 was initially described to be implicated in regulating nuclear transport due to its linkage with Ran [31]. It was further identified to regulate numerous cellular activities [32-34]. *RAN/RANBP2* genes are reported to be associated with cancer development. However, the association of polymorphisms in the *RAN/RANBP2* genes and neuroblastoma risk has yet to be elucidated. To address this issue, we conducted a three-center case-control study in a Chinese population.

## RESULTS

### Characteristics of study population

The detailed characteristics of subjects from Guangzhou and Zhengzhou were provided in the previous publications [35-37]. The detailed demographic characteristics in neuroblastoma patients and controls for Wenzhou, Guangdong and Henan subjects were presented in Supplementary Table 1. There were no significant differences between cases and controls from Wenzhou regarding age (20.25 ± 20.73 vs. 23.58 ± 15.36 months old, $P$=0.496) and gender ($P$=1.000).

**Table 1. Association of *RAN* and *RANBP2* polymorphisms with neuroblastoma risk.**

| Genotype | Cases (N=429) | Controls (N=884) | $P$ [a] | Crude OR (95% CI) | $P$ | Adjusted OR (95% CI) [b] | $P$ [b] |
|---|---|---|---|---|---|---|---|
| *RAN* rs56109543 (HWE=0.587) | | | | | | | |
| CC | 304 (70.86) | 620 (70.14) | | 1.00 | | 1.00 | |
| CT | 118 (27.51) | 238 (26.92) | | 1.01 (0.78-1.31) | 0.933 | 1.01 (0.78-1.31) | 0.942 |
| TT | 7 (1.63) | 26 (2.94) | | 0.55 (0.24-1.28) | 0.165 | 0.55 (0.24-1.29) | 0.168 |
| Additive | | | 0.363 | 0.93 (0.74-1.16) | 0.504 | 0.93 (0.74-1.16) | 0.502 |
| Dominant | 125 (29.14) | 264 (29.86) | 0.787 | 0.97 (0.75-1.24) | 0.787 | 0.97 (0.75-1.24) | 0.781 |
| Recessive | 422 (98.37) | 858 (97.06) | 0.155 | 0.55 (0.24-1.27) | 0.161 | 0.55 (0.24-1.28) | 0.164 |
| *RAN* rs7132224 (HWE=0.289) | | | | | | | |
| AA | 227 (52.91) | 479 (54.19) | | 1.00 | | 1.00 | |
| AG | 170 (39.63) | 335 (37.90) | | 1.07 (0.84-1.37) | 0.581 | 1.07 (0.84-1.36) | 0.596 |
| GG | 32 (7.46) | 70 (7.92) | | 0.97 (0.62-1.51) | 0.875 | 0.96 (0.62-1.51) | 0.870 |
| Additive | | | 0.823 | 1.02 (0.85-1.22) | 0.828 | 1.02 (0.85-1.22) | 0.842 |
| Dominant | 202 (47.09) | 405 (45.81) | 0.665 | 1.05 (0.84-1.33) | 0.665 | 1.05 (0.83-1.32) | 0.680 |
| Recessive | 397 (92.54) | 814 (92.08) | 0.771 | 0.94 (0.61-1.45) | 0.771 | 0.94 (0.61-1.45) | 0.770 |
| *RAN* rs14035 (HWE=0.800) | | | | | | | |
| CC | 285 (66.43) | 590 (66.74) | | 1.00 | | 1.00 | |
| CT | 135 (31.47) | 263 (29.75) | | 1.06 (0.83-1.37) | 0.635 | 1.06 (0.83-1.37) | 0.641 |
| TT | 9 (2.10) | 31 (3.51) | | 0.60 (0.28-1.28) | 0.187 | 0.60 (0.28-1.29) | 0.191 |
| Additive | | | 0.338 | 0.96 (0.78-1.19) | 0.731 | 0.96 (0.78-1.19) | 0.727 |
| Dominant | 144 (33.57) | 294 (33.26) | 0.912 | 1.01 (0.79-1.30) | 0.911 | 1.01 (0.79-1.29) | 0.918 |
| Recessive | 420 (97.90) | 853 (96.49) | 0.164 | 0.59 (0.28-1.25) | 0.169 | 0.59 (0.28-1.26) | 0.173 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *RANBP2* rs2462788 (HWE=0.194) | | | | | | | |
| CC | 402 (93.71) | 810 (91.63) | | 1.00 | | 1.00 | |
| CT | 27 (6.29) | 74 (8.37) | | 0.74 (0.47-1.16) | 0.187 | 0.74 (0.47-1.16) | 0.188 |
| TT | 0 (0.00) | 0 (0.00) | | / | / | / | / |
| Additive | | | 0.185 | 0.74 (0.47-1.16) | 0.187 | 0.74 (0.47-1.16) | 0.188 |
| Dominant | 27 (6.29) | 74 (8.37) | 0.185 | 0.74 (0.47-1.16) | 0.187 | 0.74 (0.47-1.16) | 0.188 |
| Combined effect of protective genotypes for *RAN* [c] | | | | | | | |
| 0 | 394 (91.84) | 814 (92.08) | 0.073 [d] | 1.00 | | 1.00 | |
| 1 | 26 (6.06) | 38 (4.30) | | 1.41 (0.85-2.36) | 0.186 | 1.41 (0.84-2.36) | 0.189 |
| 2 | 5 (1.17) | 7 (0.79) | | 1.48 (0.47-4.68) | 0.509 | 1.48 (0.47-4.70) | 0.507 |
| 3 | 4 (0.93) | 25 (2.83) | | **0.33 (0.11-0.96)** | **0.041** | **0.33 (0.12-0.96)** | **0.042** |
| 0-2 | 425 (99.07) | 859 (97.17) | | 1.00 | | 1.00 | |
| 3 | 4 (0.93) | 25 (2.83) | 0.028 | **0.32 (0.11-0.94)** | **0.037** | **0.33 (0.11-0.94)** | **0.038** |

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

[a] $\chi^2$ test for genotype distributions between neuroblastoma patients and controls.

[b] Adjusted for age and gender.

[c] Protective genotypes were rs56109543 TT, rs7132224 GG and rs14035 TT.

[d] For additive model.

### *RAN/RANBP2* polymorphisms and neuroblastoma risk

The genotype frequencies of *RAN/RANBP2* genes polymorphisms (Supplementary Table 2) and neuroblastoma susceptibility between all cases and controls were presented in Table 1 and Supplementary Table 3. All genotype frequencies in controls were in Hardy-Weinberg equilibrium (HWE) (rs56109543, *P*=0.587; rs7132224, *P*=0.289; rs14035, *P*=0.800; rs2462788, *P*=0.194). In single locus analysis, no statistically significant association were found regarding all the four SNPs and neuroblastoma risk. We further investigated the combined effect of protective genotypes of *RAN* in neuroblastoma risk. We observed that individuals with 3 protective genotypes were at significantly lower risk of developing neuroblastoma than those without protective genotypes [adjusted odds ratio (OR)=0.33; 95% confidence interval (CI)=0.12-0.96; *P*=0.042]. Moreover, subjects with 3 combined risk genotypes of *RAN* have a significant decreased risk of neuroblastoma (adjusted OR=0.33; 95% CI=0.11-0.94; *P*=0.038), compared with those with 0-2 protective genotypes.

### Stratification analysis

Stratification analysis was further adopted to assess the effects of the *RAN* polymorphisms on neuroblastoma risk among different strata (Table 2). However, we failed to detect significant association for any of the four polymorphisms in single locus analysis. Moreover, the cumulative effects of protective genotypes were also insignificant.

## DISCUSSION

In the current study, we performed the first investigation into the impact of SNPs in *RAN/RANBP2* genes on the risk of neuroblastoma in Chinese Han children. Our data revealed that the single *RAN* or *RANBP2* gene polymorphism might not be strong enough to confer the neuroblastoma susceptibility in Chinese children. However, three protective *RAN* genotypes were observed to cumulatively reduce the risk of neuroblastoma.

Overexpression of Ran has been observed in several human malignancies, including lung, prostate, breast, colon cancer, and neuroblastoma [38, 39]. Conditional knockdown of *RAN* gene reduced the viability of activated K-Ras-transformed cells, through inducing S-phase arrest [40]. Barrès et al. found that Ran protein is highly expressed in invasive serous epithelial ovarian cancers and overexpression of Ran is associated with poor patient outcome [41]. They also detected that silencing Ran could impair tumor growth *in vitro* and *in vivo* [42]. Xia et al. showed that RNA interference-mediated knockdown of *RAN* induces aberrant mitotic formation and apoptosis in cancer cells [38]. Silencing *RAN* causes abnormal nucleocytoplasmic transportation of transcription factors in tumor cells [43]. RanBP2 protein also plays critical roles in cellular processes. Knockdown of *RANBP2* results in an aberrant metaphase, mitotic arrest in $G_2$/M phase and mitotic cell death [44]. A study by Dawlaty *et al*. demonstrated that RanBP2 acts as a novel tumor suppressor in lung cancer through regulating TopoII by sumoylation [45]. In addition, RanBP2 hypomorphic mice are more suscep-

**Table 2. Stratification analysis for the association between *RAN* gene genotypes and neuroblastoma susceptibility.**

| Variables | rs56109543 (case/control) | | AOR (95% CI) [a] | *P* [a] | rs14035 (case/control) | | AOR (95% CI) [a] | *P* [a] | Protective genotypes (case/control) | | AOR (95% CI) [a] | *P* [a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CC/CT | TT | | | CC/CT | TT | | | 0-2 | 3 | | |
| Age, month | | | | | | | | | | | | |
| ≤18 | 145/327 | 1/13 | 0.17 (0.02-1.32) | 0.091 | 144/326 | 2/14 | 0.33 (0.07-1.45) | 0.140 | 145/328 | 1/12 | 0.19 (0.02-1.45) | 0.109 |
| >18 | 277/531 | 6/13 | 0.89 (0.33-2.35) | 0.806 | 276/527 | 7/17 | 0.79 (0.32-1.92) | 0.597 | 280/531 | 3/13 | 0.44 (0.12-1.55) | 0.200 |
| Gender | | | | | | | | | | | | |
| Female | 181/365 | 4/11 | 0.70 (0.22-2.24) | 0.550 | 183/366 | 2/10 | 0.38 (0.08-1.77) | 0.219 | 183/366 | 2/10 | 0.38 (0.08-1.77) | 0.219 |
| Male | 241/493 | 3/15 | 0.42 (0.12-1.46) | 0.170 | 237/487 | 7/21 | 0.68 (0.29-1.63) | 0.392 | 242/493 | 2/15 | 0.28 (0.06-1.22) | 0.090 |
| Sites of origin | | | | | | | | | | | | |
| Adrenal gland | 163/858 | 1/26 | 0.22 (0.03-1.61) | 0.134 | 160/853 | 4/31 | 0.71 (0.25-2.03) | 0.518 | 164/859 | 0/25 | / | / |
| Retroperitoneal | 94/858 | 2/26 | 0.68 (0.16-2.92) | 0.604 | 93/853 | 3/31 | 0.85 (0.25-2.83) | 0.785 | 94/859 | 2/25 | 0.71 (0.16-3.03) | 0.638 |
| Mediastinum | 119/858 | 4/26 | 1.08 (0.37-3.16) | 0.887 | 121/853 | 2/31 | 0.46 (0.11-1.96) | 0.295 | 121/859 | 2/25 | 0.56 (0.13-2.39) | 0.432 |
| Others | 38/858 | 0/26 | / | / | 38/853 | 0/31 | / | / | 38/859 | 0/25 | / | / |
| Clinical stage | | | | | | | | | | | | |
| I+II+4s | 175/858 | 4/26 | 0.73 (0.25-2.13) | 0.567 | 176/853 | 3/31 | 0.47 (0.14-1.56) | 0.217 | 177/859 | 2/25 | 0.38 (0.09-1.62) | 0.190 |
| III+IV | 224/858 | 3/26 | 0.47 (0.14-1.59) | 0.226 | 221/853 | 6/31 | 0.76 (0.31-1.86) | 0.550 | 225/859 | 2/25 | 0.33 (0.08-1.39) | 0.129 |

AOR, adjusted odds ratio; CI, confidence interval.

[a] Adjusted for age and gender, omitting the corresponding stratification factor.

tible to spontaneous and carcinogen-induced lung tumors. Consistently, two independent studies also demonstrated that RanBP2 level was downregulated in human lung cancers [46, 47].

Herein, for the first time we investigated whether *RAN/RANBP2* SNPs could contribute to the risk of neuroblastoma in Chinese children. However, our findings found no significant relationship between all the analyzed *RAN/RANBP2* polymorphisms and neuroblastoma risk. Such null relationship might be attributed to the relatively small sample size, although we tried to expand the sample by recruiting subjects from three centers. To be highlighted, a study conducted by Luo et al. explored the association between sumoylation-related genes polymorphisms and risk of gastric cancer [48]. They are the first group investigating the role of *RANBP2* gene polymorphism in cancer risk. Their study included 1021 gastric cancer cases and 1304 controls from Chinese population. However, they failed to obtain a significant association between *RANBP2* gene intron variant rs12614691 and gastric cancer risk. In the combined analysis of our study, subjects carrying 3 protective genotypes tend to have decreased neuroblastoma risk in comparison to those without risk genotypes or those with 0-2 protective genotypes. This phenomenon was quite biologically plausible as each single variant in each gene might not be strong enough to influence the risk of cancer.

The current study was the first investigation on the association of *RAN/RANBP2* genes SNPs with neuroblastoma risk. Another merit of this study was that this is a three-center case-control study. Several limitations exist in the current study. First, because of the low incidence rate of neuroblastoma, the recruitment of eligible patients was a great challenge for us. Even though we enrolled participants from three hospitals, the sample size is still moderate. This limited sample size inevitably impaired the strength of the statistical power. Second, this study only incorporated four SNPs in the *RAN/RANBP2* genes. Future studies should investigate

more potentially functional polymorphisms in *RAN/RANBP2* genes. Third, as all the participants included were of Chinese origin, conclusions should be taken with caution when extrapolated to other populations. Fourth, functional analysis is warranted to justify the described associations, which would illustrate the underlying mechanisms of how theses SNPs modify neuroblastoma susceptibility. Additionally, we only assessed the possible association of the SNPs with neuroblastoma risk. Other environmental factors, such as dietary habit, childhood exposure, and health situation, would help to provide further insight into the influence of *RAN/RANBP2* polymorphisms on neuroblastoma risk.

In all, here we demonstrate that common variants at the *RAN/RANBP2* genes are associated with the risk of neuroblastoma in the Chinese children in a low-impact manner. Future larger-sample, functional studies are warranted to address the mechanism by which *RAN/RANBP2* SNPs impacts tumorigenesis of neuroblastoma.

## MATERIALS AND METHODS

### Study populations

This case-control study was conducted in three centers: Guangzhou Women and Children's Medical Center, The First Affiliated Hospital of Zhengzhou University and The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University. The study was approved by the Institutional Review Board of the above three hospitals. In total, 429 neuroblastoma cases and 884 controls from three centers were included in this study. To be specific, 275 cases and 531 controls were enrolled from Guangzhou [35-37], 118 cases and 281 controls were recruited from Zhengzhou [49, 50], and 36 cases and 72 controls were enrolled from Wenzhou (Supplementary Table 1). The recruitment period lasts from December 2007 to June 2017. All the participants' parents provided signed informed consent before the study. Selection criteria of the included participants were accessible in our previous publication [51].

### SNP selection and genotyping

We chose potentially functional polymorphisms in the *RAN/RANBP2* genes from dbSNP database (http://www.ncbi.nlm.nih.gov/). An online tool, SNP info (http:// snpinfo.niehs.nih.gov/) was used to predict the functions of SNPs. In brief, we searched the potentially functional candidate SNPs located in the 5'-flanking region, 5' untranslated region, 3' untranslated region, and exon of *RAN/RANBP2* genes. Three potentially functional SNPs in *RAN* gene (rs56109543 C>T, rs7132224 A>G, rs14035 C>T) and one SNP in *RANBP2* (rs2462788 C>T) were chosen for analysis that captured nine additional SNPs with LD>0.8 (Supplementary Table 2). Three SNPs (rs56109543, rs7132224, rs2462788) are located in transcription factor binding sites (TFBS) and one SNP rs14035 might affect the microRNA binding site activity. As shown in Supplementary Figure 1, there was no significant LD ($R^2$<0.8) between each *RAN* SNP pair ($R^2$=0.488 between rs56109543 and rs7132224, $R^2$=0.582 between rs14035 and rs7132224), except for the rs56109543 and rs14035 ($R^2$=0.838).

The peripheral blood was used to extract genomic DNA. We genotyped the gene polymorphisms using Taqman real-time PCR [52-54]. On each 384-well plate, eight negative controls with water were used as quality control samples. The randomized and blinded process method was adopted to genotype all case and control samples. 10% random selection samples were re-genotyped and the genotype concordance rate was 100%.

### Statistical analysis

Departures from HWE for the selected SNPs in controls were evaluated using goodness-of-fit $\chi 2$ test. Allele frequencies and demographic variables between the two groups were assessed by chi-square test. The ORs, 95% CIs, and the corresponding *P* value for each SNP were calculated with adjustment for age and gender. Risk associations between genotypes and neuroblastoma were determined from logistic regression analysis. All calculations were performed using SAS software version 9.4 (SAS Institute, Cary, NC). All statistical tests were two-sided, and significant threshold was set using $P< 0.05$.

## CONFLICTS OF INTEREST

The authors have no competing interests to declare.

## FUNDING

This work was supported by grants from the Pearl River S&T Nova Program of Guangzhou (No: 201710010086), Scientific Research Foundation of Wenzhou (No: 2015Y0492), Zhejiang Provincial Medical and Health Science and Technology plan (No: 2009A148), and Zhejiang Provincial Science and Technology Animal Experimental Platform Project (No: 016C37113).

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## SUPPLEMENTARY MATERIAL

**Supplementary Table 1. Frequency distribution of selected characteristics in neuroblastoma patients and controls.**

| Variables | Wenzhou | | | | | Guangdong province | | | | | Henan province | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases (N=36) | | Controls (N=72) | | $P$ [a] | Cases (N=275) | | Controls (N=531) | | $P$ [a] | Cases (N=118) | | Controls (N=281) | | $P$ [a] |
| | No. | % | No. | No. | | No. | % | No. | % | | No. | % | No. | % | |
| Age range, month | 0.05-72 | | 8-72 | | 0.496 | 0-132 | | 0.07-156 | | 0.079 | 0-131.1 | | 0.1-144.0 | | 0.189 |
| Mean ± SD | 20.25±20.73 | | 23.58±15.36 | | | 31.50±25.43 | | 29.73±24.86 | | | 46.24±29.98 | | 44.97±33.23 | | |
| ≤18 | 20 | 55.56 | 35 | 48.61 | | 103 | 37.45 | 233 | 43.88 | | 23 | 19.49 | 72 | 25.62 | |
| >18 | 16 | 44.44 | 37 | 51.39 | | 172 | 62.55 | 298 | 56.12 | | 95 | 80.51 | 209 | 74.38 | |
| Gender | | | | | 1.000 | | | | | 0.510 | | | | | 0.196 |
| Female | 17 | 47.22 | 34 | 47.22 | | 114 | 41.45 | 233 | 43.88 | | 54 | 45.76 | 109 | 38.79 | |
| Male | 19 | 52.78 | 38 | 52.78 | | 161 | 58.55 | 298 | 56.12 | | 64 | 54.24 | 172 | 61.21 | |
| Clinical stages | | | | | | | | | | | | | | | |
| I | 15 | 41.67 | / | / | | 54 | 19.64 | | | | 15 | 12.71 | | | |
| II | 2 | 5.56 | / | / | | 62 | 22.55 | | | | 31 | 26.27 | | | |
| III | 9 | 25.00 | / | / | | 49 | 17.82 | | | | 19 | 16.10 | | | |
| IV | 7 | 19.44 | / | / | | 94 | 34.18 | | | | 49 | 41.53 | | | |
| 4s | 3 | 8.33 | / | / | | 8 | 2.91 | | | | 3 | 2.54 | | | |
| NA | | | | | | 8 | 2.91 | | | | 1 | 0.85 | | | |
| Sites of origin | | | | | | | | | | | | | | | |
| Adrenal gland | 11 | 30.56 | / | / | | 64 | 23.27 | | | | 89 | 75.42 | | | |
| Retroperitoneal region | 9 | 25.00 | / | / | | 87 | 31.64 | | | | / | / | | | |
| Mediastinum | 14 | 38.89 | / | / | | 90 | 32.73 | | | | 19 | 16.10 | | | |
| Other region | 2 | 5.56 | / | / | | 26 | 9.45 | | | | 10 | 8.47 | | | |
| NA | | | | | | 8 | 2.91 | | | | / | / | | | |

SD, standard deviation; NA, not available.

[a] Two-sided $\chi^2$ test for distributions between neuroblastoma cases and cancer-free controls.

**Supplementary Table 2. Polymorphisms captured by the four selected functional polymorphisms in *RAN/RANBP2* genes as predicted by SNPinfo (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html).**

| rs | Chr. | Allele | LDsnp | Pop/LD | TFBS | miRNA(miRanda) | Nearby Gene | Allele | Asian | CHB |
|---|---|---|---|---|---|---|---|---|---|---|
| rs10773832 | 12 | C/T | rs14035 | CHB/0.957 | -- | -- | *RAN\|\|GPR133* | T | 0.747 | 0.833 |
| rs10773833 | 12 | C/G | rs14035 | CHB/0.957 | -- | -- | *RAN\|\|GPR133* | C | 0.772 | 0.833 |
| rs10848236 | 12 | A/G | rs14035 | CHB/0.87 | Y | -- | *STX2\|\|RAN* | G | 0.788 | 0.857 |
| rs11061209 | 12 | A/G | rs14035 | CHB/0.957 | -- | -- | *RAN\|\|GPR133* | G | 0.772 | 0.833 |
| rs11061222 | 12 | C/T | rs14035 | CHB/0.957 | -- | -- | *RAN\|\|GPR133* | T | 0.783 | 0.833 |
| **rs14035** | **12** | **C/T** | **rs14035** | **1** | **--** | **Y** | ***RAN*** | **C** | **0.779** | **0.839** |
| rs3809142 | 12 | C/T | rs14035 | CHB/0.828 | Y | -- | *STX2\|\|RAN* | C | -- | 0.863 |
| rs7958223 | 12 | A/C | rs14035 | CHB/1 | -- | -- | *RAN* | C | 0.781 | 0.843 |
| **rs2462788** | **2** | **T/C** | **rs2462788** | **1** | **Y** | **--** | ***LOC644911\|\|RANBP2*** | **T** | **--** | **0.081** [a] |
| **rs56109543** | **12** | **C/T** | **rs56109543** | **1** | **Y** | **--** | ***STX2\|\|RAN*** | **T** | **--** | **0.129** [a] |
| rs10848218 | 12 | C/T | rs7132224 | CHB/0.819 | -- | -- | *STX2\|\|RAN* | C | 0.344 | 0.250 |
| **rs7132224** | **12** | **A/G** | **rs7132224** | **1** | **Y** | **--** | ***STX2\|\|RAN*** | **A** | **0.663** | **0.738** |
| rs7307055 | 12 | G/T | rs7132224 | CHB/0.936 | -- | -- | *STX2\|\|RAN* | G | 0.683 | 0.759 |

SNP, single nucleotide polymorphism; LD, linkage disequilibrium; TFBS, transcription factor binding sites; CHB, Han Chinese in Beijing, China.

[a] Southern Han Chinese, using data from 1000 Genomes (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/).

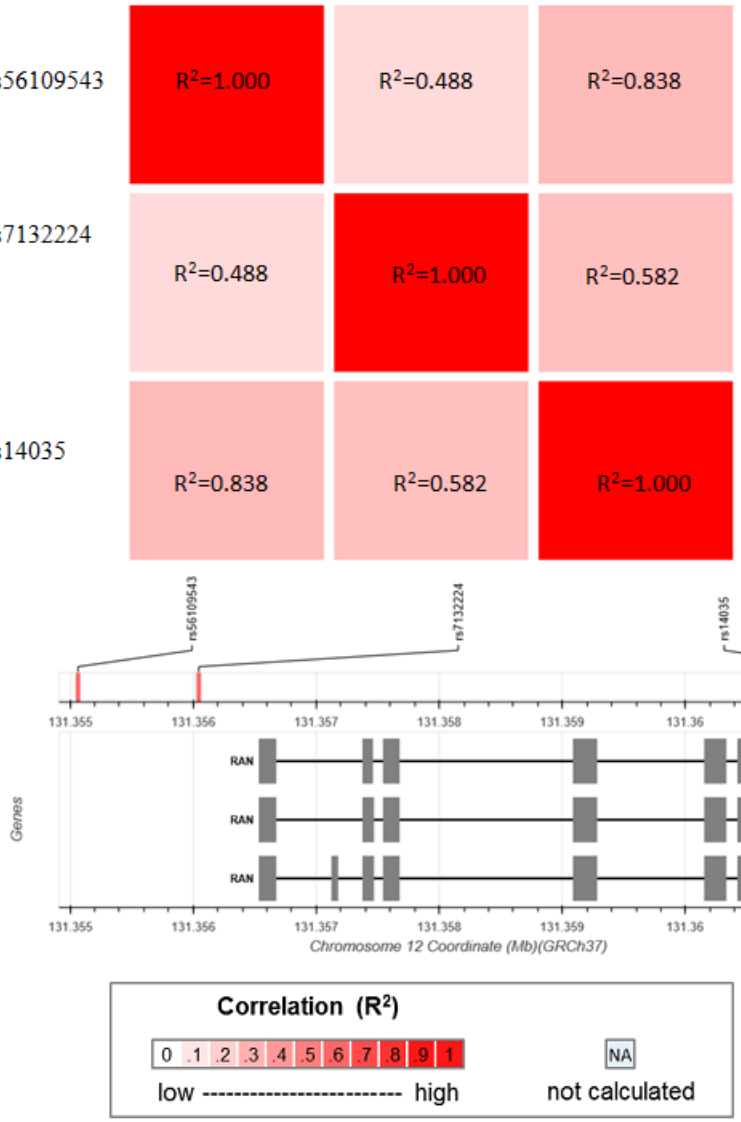
**Supplementary Table 3. Association of *RAN* and *RANBP2* polymorphisms with neuroblastoma risk (Divided subjects)**

| Genotype | Guangdong province | | | | Henan province | | | | Wenzhou | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cases (N=275) | Controls (N=531) | AOR (95% CI) [a] | *P* [a] | Cases (N=118) | Controls (N=281) | AOR (95% CI) [a] | *P* [a] | Cases (N=36) | Controls (N=72) | AOR (95% CI) [a] | *P* [a] |
| *RAN* rs56109543 C>T | | | | | | | | | | | | |
| CC | 198 (72.00) | 372 (70.06) | 1.00 | | 81 (68.64) | 190 (67.62) | 1.00 | | 25 (69.44) | 58 (80.56) | 1.00 | |
| CT | 71 (25.82) | 141 (26.55) | 0.95 (0.68-1.32) | 0.745 | 36 (30.51) | 84 (29.89) | 0.99 (0.62-1.58) | 0.955 | 11 (30.56) | 13 (18.06) | 2.32 (0.87-6.3118) | 0.094 |
| TT | 6 (2.18) | 18 (3.39) | 0.64 (0.25-1.64) | 0.353 | 1 (0.85) | 7 (2.49) | 0.34 (0.04-2.85) | 0.322 | 0 (0.00) | 1 (1.39) | / | / |
| Additive | | | 0.90 (0.68-1.19) | 0.438 | | | 0.89 (0.58-1.37) | 0.602 | | | 1.96 (0.77-4.97) | 0.156 |
| Dominant | 77 (28.00) | 159 (29.94) | 0.91 (0.66-1.26) | 0.576 | 37 (31.36) | 91 (32.38) | 0.94 (0.59-1.50) | 0.790 | 11 (30.56) | 14 (19.44) | 2.23 (0.84-5.95) | 0.108 |
| Recessive | 269 (97.82) | 513 (96.61) | 0.65 (0.26-1.66) | 0.367 | 117 (99.15) | 274 (97.51) | 0.35 (0.04-2.85) | 0.323 | 36 (100.00) | 71 (98.61) | / | / |
| *RAN* rs7132224 A>G | | | | | | | | | | | | |
| AA | 148 (53.82) | 291 (54.80) | 1.00 | | 59 (50.00) | 142 (50.53) | 1.00 | | 20 (55.56) | 46 (63.89) | 1.00 | |
| AG | 109 (39.64) | 199 (37.48) | 1.08 (0.79-1.47) | 0.627 | 48 (40.68) | 115 (40.93) | 0.99 (0.63-1.56) | 0.975 | 13 (36.11) | 21 (29.17) | 1.58 (0.65-3.85) | 0.316 |
| GG | 18 (6.55) | 41 (7.72) | 0.87 (0.48-1.57) | 0.649 | 11 (9.32) | 24 (8.54) | 1.10 (0.50-2.39) | 0.814 | 3 (8.33) | 5 (6.94) | 1.37 (0.29-6.50) | 0.691 |
| Additive | | | 1.00 (0.79-1.26) | 0.991 | | | 1.03 (0.74-1.43) | 0.882 | | | 1.32 (0.70-2.50) | 0.389 |
| Dominant | 127 (46.18) | 240 (45.20) | 1.04 (0.78-1.40) | 0.775 | 59 (50.00) | 139 (49.47) | 1.01 (0.66-1.56) | 0.961 | 16 (44.44) | 26 (36.11) | 1.54 (0.67-3.53) | 0.314 |
| Recessive | 258 (93.45) | 490 (92.28) | 0.85 (0.48-1.50) | 0.566 | 107 (90.68) | 257 (91.46) | 1.10 (0.52-2.33) | 0.801 | 33 (91.67) | 67 (93.06) | 1.18 (0.26-5.38) | 0.834 |
| *RAN* rs14035 C>T | | | | | | | | | | | | |
| CC | 188 (68.36) | 349 (65.73) | 1.00 | | 74 (62.71) | 187 (66.55) | 1.00 | | 23 (63.89) | 54 (75.00) | 1.00 | |
| CT | 81 (29.45) | 159 (29.94) | 0.95 (0.69-1.31) | 0.755 | 41 (34.75) | 87 (30.96) | 1.17 (0.74-1.86) | 0.507 | 13 (36.11) | 17 (23.61) | 1.97 (0.80-4.87) | 0.142 |
| TT | 6 (2.18) | 23 (4.33) | 0.48 (0.19-1.20) | 0.117 | 3 (2.54) | 7 (2.49) | 1.17 (0.29-4.66) | 0.828 | 0 (0.00) | 1 (1.39) | / | / |
| Additive | | | 0.85 (0.65-1.12) | 0.248 | | | 1.14 (0.76-1.71) | 0.516 | | | 1.74 (0.73-4.13) | 0.213 |
| Dominant | 87 (31.64) | 182 (34.27) | 0.89 (0.65-1.22) | 0.463 | 44 (37.29) | 94 (33.45) | 1.17 (0.75-1.84) | 0.497 | 13 (36.11) | 18 (25.00) | 1.92 (0.78-4.73) | 0.159 |
| Recessive | 269 (97.82) | 508 (95.67) | 0.49 (0.20-1.21) | 0.123 | 115 (97.46) | 274 (97.51) | 1.11 (0.28-4.39) | 0.884 | 36 (100.00) | 71 (98.61) | / | / |
| *RANBP2* rs2462788 C>T | | | | | | | | | | | | |
| CC | 258 (93.83) | 489 (92.09) | 1.00 | | 112 (94.92) | 256 (91.10) | 1.00 | | 32 (88.89) | 65 (90.28) | 1.00 | |
| CT | 17 (6.18) | 42 (7.91) | 0.76 (0.43-1.37) | 0.364 | 6 (5.08) | 25 (8.90) | 0.56 (0.22-1.40) | 0.214 | 4 (11.11) | 7 (9.72) | 1.22 (0.33-4.52) | 0.770 |
| TT | 0 (0.00) | 0 (0.00) | / | / | 0 (0.00) | 0 (0.00) | / | / | 0 (0.00) | 0 (0.00) | / | / |
| Additive | | | 0.76 (0.43-1.37) | 0.364 | | | 0.56 (0.22-1.40) | 0.214 | | | 1.22 (0.33-4.52) | 0.770 |
| Dominant | 17 (6.18) | 42 (7.91) | 0.76 (0.43-1.37) | 0.364 | 6 (5.08) | 25 (8.90) | 0.56 (0.22-1.40) | 0.214 | 4 (11.11) | 7 (9.72) | 1.22 (0.33-4.52) | 0.770 |
| Combined effect of protective genotypes for *RAN* [b] | | | | | | | | | | | | |
| 0 | 256 (93.09) | 490 (92.28) | 1.00 | | 105 (88.98) | 257 (91.46) | 1.00 | | 33 (91.67) | 67 (93.06) | 1.00 | |
| 1 | 12 (4.36) | 17 (3.20) | 1.38 (0.65-2.93) | 0.406 | 11 (9.32) | 17 (6.05) | 1.56 (0.70-3.45) | 0.275 | 3 (8.33) | 4 (5.56) | 1.40 (0.29-6.84) | 0.676 |
| 2 | 3 (1.09) | 7 (1.32) | 0.79 (0.20-3.08) | 0.729 | 2 (1.69) | 0 (0.00) | / | / | 0 (0.00) | 0 (0.00) | / | / |
| 3 | 4 (1.45) | 17 (3.20) | 0.46 (0.15-1.38) | 0.165 | 0 (0.00) | 7 (2.49) | / | / | 0 (0.00) | 1 (1.39) | / | / |
| 0-2 | 271 (98.55) | 514 (96.80) | 1.00 | | 118 (100.00) | 274 (97.51) | 1.00 | | 36 (100.00) | 71 (98.61) | 1.00 | |
| 3 | 4 (1.45) | 17 (3.20) | 0.45 (0.15-1.34) | 0.150 | 0 (0.00) | 7 (2.49) | / | / | 0 (0.00) | 1 (1.39) | / | / |

AOR, adjusted odds ratio; CI, confidence interval.

[a] Adjusted for age and gender.

[b] Protective genotypes were rs56109543 TT, rs7132224 GG and rs14035 TT from combined subjects.




**Supplementary Figure 1.** Linkage disequilibrium analysis for the three selected polymorphisms in the RAN gene in Han Chinese population consisting of CHB (Han Chinese in Beijing, China) and CHS (Southern Han Chinese) subjects.