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Polymorphisms of insulin receptor substrate 2 are putative biomarkers for pediatric medulloblastoma: considering the genetic susceptibility and pathological diagnoses

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ABSTRACT

Molecular profiling subgrouped medulloblastoma (MB) into four subtypes featured by distinct footprints. However, germline studies on genetic susceptibility in Chinese population have not been reported. To investigate the correlation of polymorphisms involved in the AKT signaling pathway with clinicopathological parameters in pediatric MB, and their contribution to the clinical outcome, we performed a case-controlled cohort consisting of 48 patients with pediatric MB and 190 healthy controls from Han population. Significant association in rs7987237 of insulin receptor substrate 2 (IRS2) was identified as risk allele/genotype between MB patients and control group (P<0.05). The allele "C" of rs7987237 in IRS2 gene was associated with an increased risk of MB (P=0.025; OR=2.95, 95%CI 1.43–6.11) after Bonferroni correction. Among 48 patients, various genotypes of rs7987237 show significant association with pathological diagnosis and metastases risk (P<0.05). Furthermore, the survival curve of patients with genotype "CC" of rs7987237 was confirmed with better outcome (P<0.001). Combined with previous results, our study suggests that polymorphisms of IRS2 putatively participated in the development of pediatric MB development. Therefore, it may benefit the early diagnosis and indicate the prognosis of patients with MB in Han population.

Key Words: pediatric, medulloblastoma, pathology, susceptibility, prognosis

INTRODUCTION

Medulloblastoma is the most occurred pediatric malignant brain tumor. Recent studies have shown significant correlations between specific polymorphisms with malignant brain tumors.[1)] Miscellaneous studies integrating mRNA expression profile and genetic variants in medulloblastoma showed series of invaluable signature genes.[2)] Previous studies on these signature genes such as *PTCH1*, *SUFU*, *APC*, and *TP53* indicated a significant correlation between germline gene variants as well as familiar cancer syndrome with occurrence of medulloblastoma.[3-6)] Northcott[7)]

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reported, from a screening of the most common genes in the top list of somatic copy number aberrations (SCNAs) in 1087 unique medulloblastoma, high frequencies of SCNAs were identified in genes like *IRS2*, *PTEN*, *AKT3*, *PIK3C2B* that were enriched in the AKT signaling pathway. Further, as described by the study conducted by Wu *et al.*[8] on medulloblastoma mouse model, the frequency of insertions in the region of such key genes like *PTEN* and *AKT* was much higher than other background genes. Thus, genetic aberration affecting AKT signaling pathway may serve an important role in the pathogenesis of medulloblastoma.[9,10] A series of SNPs located in the region of *IRS2*, *PTEN* and *AKT3* was reported to be associated with tumor risk. Previous reports indicated a significant association between these genetic variation and the risk of breast cancer[11] and colorectal cancer.[12] The significant association of genetic polymorphism of *PTEN* with risk of esophageal squamous cell carcinoma was noted in Chinese population.[13] However, the association between certain SNPs in the AKT pathway with the risk, the clinical parameters and the prognosis of medulloblastoma is yet to be determined in China.

In the present case-control study, we recruited samples from 48 pediatric patients with medulloblastoma and 190 healthy adults as control in the Han population. 11 tag-SNPs in the region of *IRS2*, *PTEN*, and *AKT3* were screened through the high-throughout Sequenom MassArray genotypic approach to decipher the contribution of these SNPs in predicting the risk of susceptibility and the prognosis of medulloblastoma.

SUBJECTS AND METHODS

Patients and Controls

The study was approved by the Ethics Review Board of Shanghai Xinhua Hospital according to the Helsinki Declaration with written permission from the patients and control group. A total of 48 patients with histopathologically confirmed pediatric medulloblastoma between 2008 and 2013 were recruited from the Department of Pediatric Neurosurgery in Xinhua Hospital. Peripheral blood samples in ethylenediamine tetra acetic acid (EDTA) tubes from 190 healthy individuals with no family history of cancer in first degree relatives were recruited as controls. The control samples were appropriate for gender with the patient samples. All tumor samples were independently reviewed by two experienced pathologists and the samples were graded histologically using published criteria.1[4]

SNP Selection and Genotyping

The Tag-SNPs were selected based on the following criteria: firstly based on the genes in AKT pathway with the most common somatic copy number aberrations reported previously.[7] Based on that, SNPs of five genes (*IRS2*, *PTEN AKT3*, *PIK3C2B* and *PIK3C2G*) were selected and the pathogenic polymorphisms being associated with cancer or other diseases were screened out according to studies reported previously (http://www.ncbi.nlm.nih.gov/SNP).[15-19] We finally pooled 11 tag-SNP derived from the International HapMap Project Database (http://www.hapmap.org). Minor allele frequency of each polymorphism was at least 5% in a Chinese population.

Genotypic and genetic data analyses were performed at Bio-X Institutes of Shanghai Jiao Tong University in China. Genomic DNA was supplied from peripheral blood lymphocytes of participants using the Qiagen Blood Kit (Qiagen, Basel, Switzerland). Genotypic was performed using the Sequenom MassARRAY iPLEXTM Platform (http://www.sequenom.com/seq.genotyping.html). The PCRs were carried out on the Gene Amp® PCR System 9700 (Applied Biosystems, CA, USA). The iPLEX Gold assay was detected using 10–20 ng of genomic DNA in 384-well plate format for the MassARRAY System. The quality-control analyses included the genotypic

of internal positive-control samples, the use of no template controls, and replicates for 10% of the samples.

Statistical Methods

For each polymorphism, deviation of the observed genotype frequencies from those expected under Hardy-Weinberg equilibrium (HWE) was evaluated in the Chi-square test in the SHEsis analysis platform (http://analysis.bio-x.cn/myAnalysis.php). We compared allele and genotype frequencies, Odds Ratios (ORs) and 95% confidence intervals (95% CIs) were calculated by SHEsis analyses between cases and healthy controls. The association between genotypes and the risk of medulloblastoma was analyzed by Chi-square test after stratifying the subjects according to the clinicopathological parameters. Statistical significance was set at $P<0.05$. Survival analyses and plots were performed using PASW Statistics version 18 (SPSS Inc, Chicago, IL, USA).

RESULTS

Sample Characteristic

A group of 48 medulloblastoma patients and 190 control subjects were recruited for the present study. The mean age of the patient and control group was 4.8 years and 42.4 years (patients ranged from 0.4 to 9 years and controls varied from 22 to 58 years), respectively. The patient and control samples comprised 66.7% males and 55.8% males, respectively ($P>0.05$). 30 (62.5%) patients had classic medulloblastoma, 5 (10.5%) cases had larger cell/anaplastic medulloblastoma, 13 (27.1%) kids were with desmoplastic medulloblastoma. In the present cohort of medulloblastoma, 19 (39.6%) were clinically listed as metastatic status, and 25 (52.1%) patients were younger than 3 years old.

Individual SNP Association Analysis

After statistical case-control analysis, carriers of four variant SNPs in *AKT3* and *IRS2* genes were linked to the risk of pediatric medulloblastoma. Allele frequency and genotype distribution of these remarkable SNPs were presented in Table 1. IRS2 rs7987237, rs913949 and AKT3rs897959, rs4590656 demonstrated a significant association with the risk of pediatric medulloblastoma before Boferroni corretion. Our results showed that both the allele “C ”and the genotype “CC” of rs7987237 in IRS2 gene were associated with an increased risk of medulloblastoma ($P=0.0023$ and $P=0.009$, respectively). Bonferroni correction effaced most of the statistically significant association with pediatric medulloblastoma. The allele “C” of IRS2 rs7987237 still shows an increased risk ($P=0.025$; OR=2.95, 95%CI 1.43–6.11).

Genotype SNP and Clinicopathological Parameters of Medulloblastoma

In these children with medulloblastoma, genotype of significant rs7987237 might affect clinical parameter of medulloblastoma such as pathological diagnosis, metastatic status, age of onset. As showed in Table 2, the clinicopathological parameters of patients displayed significant differences between various genotype groups of rs7987237 ($P<0.05$). Furthermore, we described different survival curve for various genotype of rs7987237 in Kaplan-Meier analysis. The significant difference was found in Figure 1 ($P<0.001$).

Table 1 Genotype and Allele Frequencies of Significant SNPs in the patient-control Analysis.

		Allele		Odds Ratio OR						HWB-*P*	
SNP_ID	Gene	(Frenquency n)		(95%CI)	*P*-alle	*P**	Genotype			*P*-geno	case/control
rs7987237	*IRS2*	C	T				C/C	C/T	T/T		
	case/control	87/291	9/89	2.95 (1.43–6.11)	0.002	0.025	39/111	9/69	0/10	0.009	0.47/0.86
rs913949	*IRS2*	A	G				A/A	A/G	G/G		
	case/control	81/279	13/101	2.25 (1.20–4.22)	0.009	>0.05	34/90	13/99	0/1	0.008	0.27/0.07
rs4590656	*AKT3*	C	T				C/C	C/T	T/T		
	case/control	69/215	25/153	1.96 (1.18–3.24)	0.007	>0.05	26/67	17/81	4/36	0.038	0.61/0.20
rs897959	*AKT3*	C	T				C/C	C/T	T/T		
	case/control	68/225	24/147	1.85 (1.11–3.08)	0.016	>0.05	28/74	12/77	6/35	0.035	0.02/0.06
rs2735343	*PTEN*	C	G				C/C	C/G	G/G		
	case/control	44/183	50/197	–	0.8	–	10/40	24/103	13/47	0.9	–
rs2248293	*PTEN*	C	T				C/C	C/T	T/T		
	case/control	40/174	42/184	–	0.9	–	12/43	16/88	13/48	0.5	–
rs12357281	*PTEN*	G	–				G/G				
	case/control	94/380	–	–	1	–	47/190			1	–
rs4773092	*IRS2*	A	C				A/A	A/G	G/G		
	case/control	51/235	43/145	–	0.17	–	14/68	23/99	10/23	0.25	–
rs2289046	*IRS2*	A	G				A/A	A/G	G/G		
	case/control	46/218	48/156	–	0.10	–	11/65	24/88	12/34	0.26	–
rs754204	*IRS2*	C	T				C/C	C/T	T/T		
	case/control	59/246	37/130	–	0.46	–	17/81	25/84	6/23	0.6	–
rs4132509	*AKT3*	A	C				A/A	A/C	A/A	C/C	
	case/control	39/124	55/256	–	0.10	–	10/22	19/80	18/88	0.2	–

Global chi2 is 94.65 while df=15 (frequency<0.03 in both control & case has been dropped.) Global Fisher's *p* value is 1.90e–013; Global Pearson's *p* value is 1.33e–013. *P**: Boferroni correction *P* value; HWB-*P*: *P* value of Hardy-Weinberg equilibrium for the patients and control population.

Table 2 Correlation between SNP genotypes of IRS2 and clinicopathological parameters of medulloblastoma patients

Variable		rs7987237			rs913949		
		C/C	C/T	*P*	A/A	A/G	*P*
Age group	≤3years	25	5	0.63	19	10	0.37
	>3years	14	4		14	4	
Gender	Female	15	4	0.74	12	6	0.67
	Male	24	5		21	8	
Histological type	Classic	25	5	**0.02**	19	10	0.06
	Desmoplastic	12	1		12	1	
	Large cell/Anaplastic	2	3		2	3	
Metastatic risk group	M0	27	2	**0.006**	22	7	0.28
	M1-3	12	7		11	7	

P values obtain using $\chi 2$ or Fisher's exact tests.

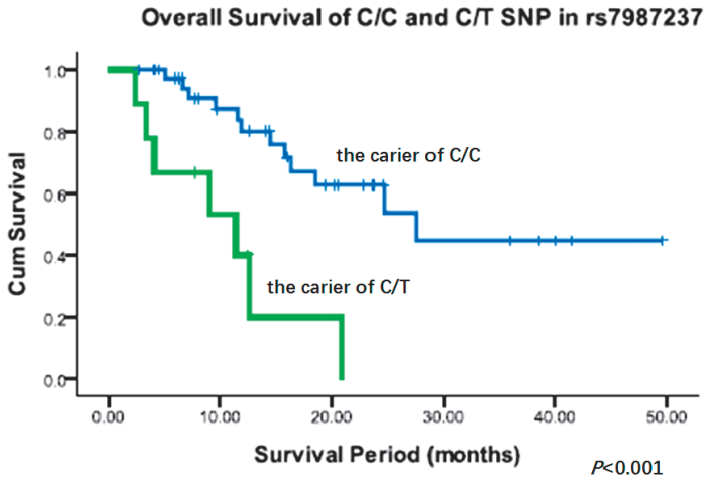

Fig. 1 Different survival curve for various genotype of rs7987237 in Kaplan-Meier analysis

DISCUSSION

In the current study, we identified novel SNPs of susceptibility genes like *AKT*, *IRS2* and their correlation with clinic-pathological parameters from the case-control study consisting of 48 medulloblastoma patients matched with 190 controls. Our findings suggest that rs7987237 may harbor association with clinical parameters of pediatric medulloblastoma, such as tendency to metastasis, clinical prognosis. This is the first study showing this association with pediatric medulloblastoma in the Han Chinese population.

The IRS-AKT pathway has turned out to be aberrantly activated in malignant brain tumors.[20] Elizabeth identifies *IRS2* as a driver oncogene and *IRS2* activated by PI3K-AKT pathway may represent an optional mechanism in the process of carcinogenesis.[21] It is equally a vital mediator triggering tumor metastasis[22] and affected clinical prognosis.[23] The association between polymorphic changes of *IRS2* with pediatric medulloblastoma has yet to be unclear. Diverse teams provided evidences that the insulin-like growth factor may contribute to medulloblastoma transformation.[24,25] In our present results, the difference of genotype and allele frequencies of rs7987237 in *IRS2* between the case and control groups revealed a significant association with medulloblastoma susceptibility. Polymorphisms of *IRS2* correlated with the severity of breast cancer[26] and the clinical outcome in pancreatic cancer.[27] Feigelson HS genotyped 648 cases of cancers and 659 controls finding that some SNPs in *IRS2* regions may harbor risk alleles for breast cancer (Table 3).[11] Combining with those previous investigations, our study suggests a potential role of polymorphisms of *IRS2* in the development of pediatric medulloblastoma. Although, the functional aspect of polymorphisms of *IRS2* still requires further investigation.

On the other hand, the polymorphism impact of *IRS2* may be covered beneath the clinical manifestations. Present study further explored possible clue between genotype of rs2987237 and patients' clinical phenotype the pathological characteristics of pediatric medulloblastoma. In the present cohort, the incidence of medulloblastoma metastasis including M1-M3 was 39.58% (19/48), which is the same as 40% as reported by previous reports.[8] Clinicopathological variables may portend high risk of poor prognosis in addition to larger cell/anaplastic histology. Half of patients are under the age of 3 years old (24/48), which is slightly higher than previously

Table 3 Summary of the significant SNPs in the present study and in previous literature.

		in the present study		in previous study		
Gene	SNPs	Susceptibility of Medulloblastoma	*P value* (allele/genotype)	Various of Diseases	Risk of development	Reference
IRS2	rs7987237	+	0.002/0.009	Breast cancer	+	Feigelson HS (2008)
AKT3	rs4590656	+	0.007/0.035	High altitude sickness	+	Buroker NE (2012)
AKT3	rs897959	+	0.016/0.038	Diabete Mellitus type2	+	Sookoian S (2009)

reported studies.[28] 60% of patients in our study were confirmed to be classic histology (29/48). In the present study, the genotype C/T of rs7987237 in patients shown closer association with larger cell/ anaplastic medulloblastoma and the risk of metastatic, both of which increased the likelihood of a poor prognosis. Furthermore, Kaplan-Meier survival curve of these patients with the genotype C/T of rs7987237 showed significantly poorer overall survival than genotype C/C. These results suggest polymorphism of rs7987237 could serve as a useful bio-marker predicting the risk of metastasis and clinical outcome.

Although this is the first study focusing on SNP of *IRS2* involved in the pediatric medulloblastoma susceptibility and clinical parameters in the Han Chinese population, the number of patients was limited. Future study would definitely incorporate larger population which is essential for addressing a definitive conclusion of certain germline polymorphisms. The exact function of the polymorphisms and regulatory mechanism for gene expression has not meant to research clearly. The molecular mechanisms still have yet to be investigated in detail.

CONCLUSIONS

Our finding suggests that the polymorphisms of *IRS2* has a potential role in the development of pediatric medulloblastoma. Furthermore, they may also correlate with the clinicopathologic feature of medulloblastoma which may suggest a putatively functional involvement of *IRS2* polymorphisms in the pathogenesis of medulloblastma. As a novel liquid biopsy approach, the germ line variants of *IRS2* status may be helpful in aiding the diagnosis and predicting the prognosis of medulloblastoma in a minimal invasive way. It would also shed light in the forthcoming investigation in deciphering the contribution of germ line variants to the etiology of medulloblastoma.

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