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Genetic Variants in the MicroRNA Machinery Gene *GEMIN4* Are Associated with Risk of Prostate Cancer: A Case-control Study of the Chinese Han Population

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Single-nucleotide polymorphisms located in the microRNA biogenesis pathway could alter the risk for developing prostate cancer. The present study was intended to identify common genetic variants responsible for prostate cancer susceptibility in the *GEMIN4* gene. The high-resolution melting method was used to genotype seven polymorphisms (rs7813, rs4968104, rs3744741, rs2740348, rs1062923, rs910925, and rs910924) in the *GEMIN4* gene in 300 prostate cancer patients and 244 matched controls. The encouraging discovery in this study was in the rs2740348. Patients carrying the variant heterozygote GC genotype in the rs2740348 were at a 36% decreased risk of prostate cancer (odds ratio [OR] = 0.64; 95% confidence interval [CI] = 0.42, 0.99). Similarly, this variant allele carrier showed significant risk for prostate cancer (OR = 0.64). In addition, subjects carrying the homozygote TT genotype in the rs7813 had a significantly increased risk of prostate cancer (OR = 2.53, 95% CI = 1.07, 6.28). Two common haplotypes were found to be associated with decreased risk of prostate cancer. In the subgroup analysis, higher risk of more severity of prostate cancer (clinical stage III and IV) was observed in individuals with the rs7813 TT genotype (OR = 2.64, 95% CI = 1.02, 7.64), while lower risk of more severity of prostate cancer was observed in individuals with the rs3744741 T allele (OR = 0.69, 95% CI = 0.50, 0.96). Overall, our study provides substantial support for the association between the *GEMIN4* gene and the risk of prostate cancer.

Introduction

Prostate cancer accounts for ~14% of the total new cancer cases and ranks as the 6th cancer death in men (Jemal *et al.*, 2011). Prostate cancer is recognized as a complex and heterogeneous disease. Evidence obtained from a family-based linkage analysis and a pathway-based association study has identified a large scale of genetic variants contributing to the risk of prostate cancer. However, it will still take time to elucidate the highly specific mechanisms of the gene interaction and network in the development and progression of prostate cancer.

MicroRNAs (miRNAs) are a kind of noncoding RNA molecules about 20 nucleotides in length. It is known that miRNAs induce gene silence in the posttranscriptional regulation through Watson–Crick-based bonding to the 3′ untranslated regions (3′ UTR) of target messenger RNAs (Esquela-Kerscher and Slack, 2006; Wu *et al.*, 2006). MiRNAs regulate about 30% of human genes, and alternations in their processing might lead to cancer occurrence and progress (Chiosea *et al.*, 2006; Thomson *et al.*, 2006; Kumar *et al.*, 2007). It has been proved that pre-miRNA gene polymorphisms might predict the risk of prostate cancer, indicating the potential association between variations in the miRNA biogenesis pathway and the development of prostate cancer (George *et al.*, 2011).

GEMIN4 protein is accepted as a key member of the GEMIN protein family that is involved in multiple pathological processes. It was reported that this protein was a shared part of the survival of motor neuron complex and a 15S ribonucleoprotein complex (miRNPs). MiRNPs, containing a protein in the AGO protein, Eif2C, are essential in the miRNA splicing and mature (Charroux *et al.*, 1999, 2000; Hutvagner and Zamore, 2002). The GEMIN4 protein was also referred to as an important molecule in the RNA-induced silencing complex (RISC) that participated in the mature process of miRNAs, the target RNA recognition and repression (Hannon, 2002; Nelson *et al.*, 2004). Other independent studies provided similar evidence that the involvement of the GEMIN4 protein in the RISC played a

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critical role in the processing of miRNAs (Dostie *et al.*, 2003; Nelson, *et al.*, 2004). Thus, abnormity in the GEMIN4 protein might result in the differential expression of some specific miRNAs that are related to the malignant tumors.

The human *GEMIN4* gene is located at 17q13 with a large number of the identified polymorphisms. Wan *et al.* found that single-nucleotide polymorphisms (SNPs) in the *GEMIN 4* gene had a potential effect on the DNA repair in the heptacellular carcinoma cells and contributed to the development of heptacellular cancer (Wan *et al.*, 2004). Recent studies indicated that polymorphisms in the *GEMIN4* gene were associated with the etiology and clinical outcome of cancers, such as bladder cancer (Yang *et al.*, 2008), renal cancer (Horikawa *et al.*, 2008; Lin *et al.*, 2010), and ovarian cancer (Liang *et al.*, 2010). Although it is obvious that variations in the *GEMIN 4* gene may take part in the carcinogenesis, the concrete mechanism of their effects on prostate cancer remains largely unknown. Therefore, we conducted a case-control study that evaluates the association between common polymorphisms in the *GEMIN 4* gene and the risk of prostate cancer to provide genetic evidence of miRNA machinery genes for prostate cancer. This is, to our knowledge, the first genetic association study for miRNA machinery genes for prostate cancer.

Materials and Methods

Study subjects

Three hundred prostate cancer patients (mean age: 71.43 ± 9.38) were recruited from the inpatient unit of the West China Hospital and Sichuan Provincial People's Hospital in Chengdu, China. There were no age or cancer stage strictures on recruitment. All participants were individuals in Chinese Han ethnicity with pathologically confirmed diagnosis of prostate cancer from Jan 2003 to May 2010. Consensus diagnosis of each patient and evaluation of the clinical stage were made by two independent urologists. Evaluation of the clinical stage was based on the combination of Gleason scores and tumor-node-metastasis (TNM) stage (drafted by AJCC and UICC in 1997). All the patients were followed till November 2011. Two hundred and forty-four age-matched healthy male subjects (mean age:71.15 ± 9.64) in the Chinese Han population who had received a health examination in the same period were chosen as the control group. They were interviewed in an attempt to exclude cancer history. Their total PSA level was lower than 2 ng/mL. Informed consent was obtained from all the participants.

SNP selection

Seven functional SNP loci in the *GEMIN4* gene, consisting of one in the 3′ UTR region and six in the exons, were identified in the International HapMap Project (www.hapmap .org) and dbSNP (www.ncbi nlm nih gov/projects/SNP) databases (Table 1).

Genomic DNA extracting and genotyping

DNA was extracted from peripheral blood samples (EDTA anticoagulant) by using the QIAamp® DNA Blood mini kit (Qiagen) according to the manufacturer's instructions. Genotyping of the samples was performed in the LightCycler® 480 Real-Time polymerase chain reaction (PCR) System (Roche Diagnostics), based on the high-resolution melting (HRM) method. The PCR primer design was done through Primer Priemer 5.0, Oligo 11.0, and Blast in the (pubmed) Pubmed database (Table 1). The PCR mixture for the seven polymorphisms contained the ingredient, namely 2.0 μL purified genomic DNA (10 ng/μL), 0.4 μL forward primer (10 pmol/μL), 0.4 μL reverse primer (10 pmol/μL), 1.0 μL 20× EVA-GREEN, 1.5 μL dNTP (2.5 mM), 0.1 μL Hot Star Taq® Plus DNA Polymerase, 2.0 μL 10× buffer (with Mg2+), and 12.6 μL water. The amplification condition of 50 cycles after denaturation was set as follows: 95°C for 10 s, 60°Cfor 15 s, and 72°C for 25 s. Then, the products underwent a process of denaturation for 1 min at 95°C and cooling for 1 min at 40°C. HRM analysis was performed by slowly heating from 65°C to 95°C at a rate of 0.01°C /s, followed by cooling down to 40°C. Standard DNA samples representing different genotypes of each SNP were identified by DNA sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit and ABI 3130 genetic analyzer (Applied Biosystems) with the same primers used in the previous PCR system.

Table 1. The Characteristics of the Seven Polymorphisms

SNP ID	*Position*	*Major/minor allele*	*MAF (%)*	*PCR primers*
rs7813	*Arg1033Cys*	C>T	30.0	5′GACTTTGAGCAAGACCAACCCTTCTGTCA3′(forward) 5′CCGATGCCCTCAGCAATGGACTTTA3′(reverse)
rs4968104	*Glu593Val*	T>A	16.2	5′TGAAAGTGGCAGATGAATTGGGACC3′(forward) 5′AAATGTGCAGCCTGGCTGTGGT 3′(reverse)
rs3744741	*Arg684Gln*	C>T	23.4	5′ACGGGGAGCAGGTCTGGAGC3′(forward) 5′CCTGCCTTTCTTGAGGTTAGATGTTG3′(reverse)
rs2740348	Gln450Glu	G>C	11.0	5′GAAGAAGTGGGCCTTCTCGGACG3′(forward) 5′TCTATCACTGTTTCCAGCAGCCTCAA3′(reverse)
rs1062923	*Ile739Thr*	T>C	8.8	5′TCCCAAGGAGAAGCGGTGCC3′(forward) 5′CCGGGGAGAAGGTCTCAGCATT3′(reverse)
rs910925	*Ala579Gly*	C>G	30.2	5′TGGTCAATCTCGGCACCCACAAG3′(forward) 5′ATGACACCATGAAAGTGGCAGATGAATT3′(reverse)
rs910924	-	C>T	17.1	5′GCAAGCTCGGGGTCCAGCGTAAA3′(forward) 5′CCAGACAGCAGCGTTCCGGATCCTAG3′(reverse)

SNP, single-nucleotide polymorphism; MAF, minor allele frequency; PCR, polymerase chain reaction.

Statistical analysis

Statistical analysis was performed by SPSS 13.0. The exact probability method was used to test the Hardy–Weinberg equilibrium in controls. p-value ≤ 0.01 was considered the statistically significant disequilibrium. The statistical significance for the differences in genotypes was determined by Pearson χ^2 analysis. Haplotype analysis was done by the SHEsis software (Shi and He, 2005; Li *et al.*, 2009). The calculation of odds ratio (OR) and 95% confidence interval (95% CI) was conducted with the risk option of crosstabs to estimate the prostate cancer risk. All p-values were two sided. The threshold of statistical significance for the prostate cancer risk tests was $p \leq 0.05$.

Result

Demographic characteristics

The characteristics of 300 prostate cancer patients and 244 healthy controls recruited in our study are presented in Table 2. No significant difference was detected with regard to age distribution between cases and controls ($t = 0.35$, $p = 0.73$). The adenocarcinoma was the pathological character for all the patients. Overall, 48.33% of the patients were in moderate differentiation degree, the rest of whom were in high (35.00%) and low differentiation degree (16.67%), respectively. There were 81 (27.00%) patients in clinical stages I and II, whereas 219 (73.00%) patients were in stages III and IV. Genotype distributions for all the polymorphisms in the control group were in Hardy–Weinberg equilibrium.

The main effect of individual SNP on the risk of prostate cancer

Table 3 presents the data obtained from the genotype of individual polymorphisms in all subjects. Considerable differences between cases and controls were observed in two SNPs (rs2740348 and rs7813). The rs2740348 polymorphism was found to be associated with a decreased risk of prostate cancer, in which the subjects carrying the variant heterozygote GC genotype and C allele were at a 36% lower risk for prostate cancer than the wild genotype GG and G allele (OR = 0.64; 95% CI = 0.42, 0.99). In addition, the rs7813 polymorphism was associated with an increased risk of prostate cancer, among which the subjects carrying the TT genotype were at a 2.53-fold elevated risk for prostate cancer when compared with the CC genotype (OR = 2.53; 95% CI = 1.07, 6.28). The power calculation values were 0.65 and 0.61 for rs7813 and rs2740348, respectively. However, no significant associations with the prostate cancer risk were found among the remaining five polymorphisms.

Table 2. Demographic Characteristics in Cases and Controls

Variables	*Subgroups*	*Cases (N = 300) (%)*	*Controls (N = 244)*	p
Age (years)		71.43 ± 9.38	71.15 ± 9.64	0.73
Clinical stage	I	34 (11.33)		
	II	47 (15.67)		
	III	75 (25.00)		
	IV	144 (48.00)		
Differentiation degree	Low	50 (16.67)		
	Moderate	145 (48.33)		
	High	105 (35.00)		

Haplotypes analysis

Frequencies of the seven common haplotypes are listed in Table 4. Taking the most common type in the study population, MWWWWMW (in the order of rs7813-rs4968104-rs3744741-rs2740348-rs1062923-rs910925-rs910924) as the reference, subjects carrying the MWWMWMW haplotype had a 50% lower risk for prostate cancer (OR = 0.50, 95% CI = 0.25, 0.99). The other haplotype (MWMMWMW) was also associated with the development of prostate cancer, conferring a much lower risk (OR = 0.44, 95% CI = 0.19, 1.00).

Association with the risk of prostate cancer severity

Table 5 lists the genetic frequencies of individual SNP in the subgroups according to different clinical stages. Patients were divided into two subgroups according to the clinical stages: group1 (patients in clinical stages I and II) and group2 (patients in clinical stages III and IV). A higher risk for prostate cancer progression (clinical stages III and IV) was observed in subjects with the rs7813 TT genotype when compared with those with the CC genotype (OR = 2.64, 95% CI = 1.02, 7.64, Power = 0.59). Meanwhile, a lower risk for severity was found in the rs3744741 T-allele carriers (OR = 0.69, 95% CI = 0.50, 0.96, Power = 0.64). Moreover, in the case of the rs2740348 polymorphism, the lower risk was also found for the genotype GC (OR = 0.46, 95% CI = 0.21, 0.95, Power = 0.61).

Discussion

The link between the miRNAs and prostate cancer has been highlighted by many previous studies (Linsley *et al.*, 2007; Raveche *et al.*, 2007; Sooryanarayana *et al.*, 2008; Ruping *et al.*, 2009). A number of polymorphisms including two SNPs (hsa-mir196a2 and hsa-mir499) in pre-miRNA genes have been identified as potential risk factors for prostate cancer in different races (Amundadottir *et al.*, 2006; Eeles *et al.*, 2008; Thomas *et al.*, 2008; George *et al.*, 2011). However, until recently, there are gaps in our knowledge of the genetic resources in the differential expression of the miRNAs, resulting in the development of prostate cancer. In this study, significant associations were found between polymorphisms in the *GEMIN4* gene and the risk of prostate cancer. This result would lead to the further elucidation of the precise mechanisms in the distinguished expression of miRNAs and the relationship between the genetic variants in the miRNA biogenesis pathway genes and the susceptibility of prostate cancer.

This study investigated seven polymorphisms in an important miRNA machinery gene *GEMIN4* gene in a total of 544 subjects. As main observations of our study, the rs2740348 and rs7813 could cause changes in the prostate cancer risk. The possible explanation is that the two SNP loci are located in the exons, the functional region of the *GEMIN4* gene, which may play a potential role in the regulation of protein expression. The expression of the GEMIN4 protein is tightly associated with the biogenesis of related miRNAs, which may alter the risk of prostate cancer. Further

Table 3. The Polymorphisms of the *GEMIN4* Gene and the Risk of Prostate Cancer

		Patients (N = 300)		*Controls (N = 244)*		
Polymorphism		N	%	n	%	*OR (95% CI)*
rs7813	CC	10	3.33	19	7.79	1
	CT	98	32.67	81	33.20	2.29 (0.95, 5.84)
	TT	192	64.00	144	59.02	**2.53 (1.07, 6.28)**
	C	118	19.67	119	24.39	1
	T	482	80.33	369	75.61	1.32 (0.98, 1.78)
rs4968104	TT	259	86.33	201	82.37	1
	TA/AA	41	13.67	43	17.62	0.74 (0.45, 1.21)
	T	559	93.17	445	91.19	1
	A	41	6.83	43	8.81	0.76 (0.47, 1.22)
rs3744741	CC	188	62.67	142	58.20	1
	CT	92	30.67	82	33.61	0.85 (0.58, 1.25)
	TT	20	6.67	20	8.20	0.76 (0.37, 1.54)
	C	468	78.00	366	75.00	1
	T	132	22.00	122	25.00	0.85 (0.63, 1.13)
rs2740348	GG	246	82.00	182	74.60	1
	GC/CC	54	18.00	62	25.40	**0.64 (0.42, 0.99)**
	G	546	91.00	426	87.30	1
	C	54	9.00	62	12.70	**0.64 (0.42, 0.99)**
rs1062923	TT	298	99.33	240	98.36	1
	TC/CC	2	0.67	4	1.64	0.40 (0.04, 2.84)
	T	598	99.67	484	99.18	1
	C	2	0.33	4	0.82	0.40 (0.04, 2.84)
rs910925	CC	23	7.67	18	7.37	1
	CG	135	45.00	103	42.21	1.03 (0.49, 2.10)
	GG	142	47.33	123	50.40	0.90 (0.44, 1.84)
	C	181	30.17	139	28.48	1
	G	419	69.83	349	71.52	0.92 (0.70, 1.21)
rs910924	CC	240	80.00	190	77.87	1
	CT/TT	60	20.00	54	22.13	0.88 (0.57, 1.36)
	C	540	90.00	434	88.93	1
	T	60	10.00	54	11.07	0.89 (0.59, 1.34)

The bold numbers mean the *p*-value is <0.05.
OR, odds ratio; CI, confidence interval.

functional studies are required to investigate the underlying mechanisms of these two polymorphisms in the synthesis of the GEMIN4 protein in the Chinese Han population. In addition, our findings are consistent with the results that these two polymorphisms, rs2740348 and rs7813, have potential roles in carcinogenesis, such as renal cell carcinoma (Horikawa *et al.*, 2008), heptacellular carcinoma (Wan *et al.*, 2004), bladder cancer (Yang *et al.*, 2008), and ovarian cancer (Liang *et al.*, 2010) in other studies. No significant associations between the other five polymorphisms and prostate cancer risk were observed in our study. Thus, they may play less important roles in the pathogenesis of prostate cancer.

Table 4. Haplotypes and the Risk of Prostate Cancer

	Cases (N = 300)		*Controls (N = 244)*			
Haplotype	n	%	n	%	*OR (95% CI)*	P
H1 (MWWWWMW)	107	35.67	60	24.59		1
H2 (MWMWWMW)	65	21.67	50	20.49	0.73(0.44, 1.22)	0.20
H3 (MWWMWMW)	23	7.67	26	10.66	**0.50(0.25, 0.99)**	**0.03**
H4 (MMWWWMM)	19	6.33	19	7.79	0.56(0.26, 1.22)	0.11
H5 (MWMMWMW)	14	4.67	18	7.38	**0.44(0.19, 1.00)**	**0.03**
H6(MMMWWMM)	13	4.33	12	4.92	0.61(0.24, 1.56)	0.25
H7 (MWWWWMM)	8	2.67	4	1.64	1.12(0.29, 5.30)	0.86
Others	51	17.00	55	22.54	-	-

The haplotype analysis was in the order of rs7813, rs4968104, rs3744741, rs2740348, rs1062923, rs910925, and rs910924.
The bold numbers mean the *p*-value is <0.05.
W, wild genotypes; M, heterozygous and mutant homozygous genotypes.

Table 5. The Polymorphisms of the *GEMIN4* Gene and the Risk of Prostate Cancer in Different clinical Stages

Polymorphism	*Genotype and allele*	*Controls (N = 244)* n	*Clinical stage I and II (N = 81)* n	*OR*	*Clinical stage III and IV (N = 219)* n	*OR*	*Clinical stage III and IV vs. Clinical stage I and II* *OR*
rs7813	CC	19	3	1	7	1	1
	CT	81	26	2.03 (0.53, 11.52)	72	2.41 (0.90, 7.17)	1.19 (0.18, 5.68)
	TT	144	52	2.29 (0.63, 12.52)	140	**2.64 (1.02, 7.64)**	1.15 (0.19, 5.28)
	C	119	32	1	86	1	1
	T	369	130	1.31 (0.83, 2.10)	352	1.32 (0.95, 1.83)	1.01 (0.62, 1.61)
	TT	201	73	1	186	1	1
	TA/AA	43	8	0.51 (0.20, 1.17)	33	0.83 (0.49, 1.40)	1.62 (0.69, 4.25)
rs4968104	T	445	154	1	405	1	1
	A	43	8	0.54 (0.21, 1.19)	33	0.84 (0.51, 1.39)	1.57 (0.69, 4.02)
	CC	142	41	1	147	1	1
	CT	82	30	1.27 (0.71, 2.26)	62	0.73 (0.48, 1.11)	0.58 (0.32, 1.05)
	TT	20	10	1.73 (0.67, 4.24)	10	0.48 (0.20, 1.13)	0.28 (0.10, 0.81)
rs3744741	C	366	112	1	356	1	1
	T	122	50	1.34 (0.88, 2.01)	82	**0.69 (0.50, 0.96)**	**0.52 (0.34, 0.80)**
	GG	182	70	1	176	1	1
	GC/CC	62	11	**0.46 (0.21, 0.95)**	43	0.72 (0.50, 1.14)	1.55 (0.73, 3.54)
rs2740348	G	426	151	1	395	1	1
	C	62	11	**0.50 (0.23, 0.99)**	43	0.75 (0.48, 1.15)	1.55 (0.73, 3.54)
	TT	240	81	1	217	1	1
	TC/CC	4	0	-	2	0.55 (0.05, 3.91)	-
rs1062923	T	484	162	-	436	1	1
	C	4	0	-	2	0.56 (0.05, 3.90)	-
	CC	18	9	1	14	1	1
	CG	103	32	0.62 (0.24, 1.74)	103	1.29 (0.57, 2.95)	2.07 (0.72, 5.69)
	GG	123	40	0.65 (0.25, 1.78)	102	1.07 (0.47, 2.44)	1.64 (0.58, 4.44)
rs910925	C	139	50	1	131	1	1
	G	349	112	0.89 (0.60, 1.34)	307	0.93 (0.70, 1.25)	1.05 (0.69, 1.57)
	CC	190	65	1	175	1	1
	CT/TT	54	16	0.87 (0.43, 1.67)	44	0.88 (0.55, 1.42)	1.02 (0.52, 2.08)
rs910924	C	434	146	1	394	1	1
	T	54	16	0.88 (0.46, 1.62)	44	0.90 (0.57, 1.40)	1.02 (0.54, 2.00)

The bold numbers mean the *p*-value is < 0.05.

Nevertheless, the lack of association may be due to the limited sample size. Two polymorphisms exhibited such a low minor allele frequency that the testing efficiency could not be high enough to avoid type II error completely. So, case-control studies with large samples should be performed to testify the associations with these five SNPs of negative results in our studies, and meta-analysis may also be necessary in further studies.

In the haplotype analysis, we found that H3 (MWWM WMW) and H5 (MWMMWMW) might contribute to a decreased risk of prostate cancer in the Chinese Han population (rs7813, rs4968104, rs3744741, rs2740348, rs1062923, rs910925, and rs910924). Previous studies evaluated the seven SNPs (rs7813, rs4968104, rs3744741, rs2740348, rs1062923, rs910925, and rs910924) and found that the haplotype MWWMWMW was associated with a lower risk of bladder cancer in the Caucasian population (Yang *et al.*, 2008). In addition, the MWWMWW haplotype consisting of six SNPs in *GEMIN4* (rs7813, rs4968104, rs3744741, rs2740348, rs1062923, and rs910924) conferred the decreased risk of renal cell carcinoma in Caucasians, African Americans, and Mexican Americans (Horikawa *et al.*, 2008).

MiRNA profiling has identified some miRNAs with distinguished expression in the invasiveness and metastasis of prostate cancer, such as miR-224, miR-21, miR-let7, miR-100, and miR-218 (Zhang *et al.*, 2007; Prueitt *et al.*, 2008; Leite *et al.*, 2011). The distinguished expression may be caused by variations in the miRNA biogenesis. Alterations of the miRNA processing pathways could potentially impose on the miRNA transcription, splicing, and transcriptional regulation of genes that affect cell proliferation and apoptosis. Thus, polymorphisms in the miRNA biogenesis pathways may also contribute to disease progresses. A study has pointed out that the rs3744741 polymorphism might be an independent factor for the decreased risk of death in renal cell carcinoma in Caucasians (hazard ratio = 0.39; 95% CI = 0.19, 0.77) (Lin *et al.*, 2010). In consistence with the previous study, significant associations with prostate cancer progression were also identified for the rs3744741 in our study. Besides, the rs7813 and rs2740348 might also potentially affect the severity of prostate cancer.

In summary, this study demonstrates that the *GEMIN4* gene, one of the miRNA machinery genes, contributes to the alternative risk of prostate cancer. Additional studies with a larger sample size should be performed to validate our results, especially in the Caucasian population with a much higher prevalence of prostate cancer.

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Authors' Contributions

Liu JM and Liu JN finished genomic DNA extraction, HRM analysis, statistical analysis, and prepared the article. Wei MT, He YZ, Liao BH, and Liao G participated in collecting blood samples. Li H and Huang J conceived of the study, participated in its design and coordination, and helped prepare the article. All authors read and approved the final manuscript.

Disclosure Statement

The authors declare that they have no competing interests.

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