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ORIGINAL ARTICLE

Association of Growth Differentiation Factor-15 Polymorphisms and Growth Differentiation Factor-15 Serum Levels with Susceptibility to Multiple Myeloma in a Chinese Population

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SUMMARY

Background: **The aim of our study was to evaluate the relationship between growth differentiation factor-15 (GDF15) rs1058587, rs4808793, and rs1059369 polymorphisms, serum concentrations of GDF15, and International Staging System (ISS) staging or Durie-Salmon staging system (DS) staging in multiple myeloma patients and whether its polymorphism affects the expression of serum GDF15 in Chinese population.**
Methods: **A total of 120 patients with multiple myeloma and 119 healthy controls were included in the study. The SNaPshot technique was used to detect the GDF15 gene polymorphisms. Serum GDF15 levels were measured using an Enzyme-Linked ImmunoSorbent Assay (ELISA) kit.**
Results: **There was no significant difference in genotype distribution or allele frequency of three loci between multiple myeloma patients and healthy controls. However, the genotype distribution and allele frequencies of rs1059369 in ISS stage I were significantly different from those in ISS stage II ($p = 0.008$), and the distribution of rs1058587 genotype was different between ISS stage II and ISS stage III ($p = 0.014$). The overall serum concentration of GDF15 and the same genotype at the same locus (rs1058587: GC, GG; rs4808793: CC, GC; rs1059369: AA, AT, and TT) in patients with multiple myeloma was significantly higher than in the healthy control group (all $p < 0.05$).**
Conclusions: **Our results showed the genotype distribution and allele frequencies of rs1059369 and rs1058587 of GDF15 gene have some association with ISS and DS stage. But the polymorphism of GDF15 did not affect the expression of serum GDF15 in patients with multiple myeloma.**
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KEY WORDS

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INTRODUCTION

Multiple myeloma is a malignant blood disease characterized by abnormal proliferation of clonal plasma cells in the elderly [1]. Multiple myeloma is the second most common malignancy of the blood system, with an incidence of about 1/100,000 to 2/100,000 [2]. Due to a lack of understanding of multiple myeloma, most patients are seriously ill, with a poor therapeutic outcome by the time they are admitted to the hospital. Therefore, exploring the risk factors associated with multiple myeloma is essential for early prevention and early treatment. The etiology and pathogenesis of multiple myeloma are unknown. There are many causes of the disease, including ionizing radiation, environmental factors, and diet [3]. The results of Hosgood et al. [4] suggested that genetic variations played an important role in the risk of multiple myeloma.

Growth differentiation factor-15 (GDF15) which is derived from activated macrophages, is synthesized as pro-GDF15, and followed by protein cleavage and the release of N-terminal polypeptides, and finally secretion in the form of a 25 kD dimer [5]. The polymorphisms and expression of GDF15 have been associated with various cancers and blood system disease [6]. A previous study found no significant difference in GDF15 polymorphisms in patients with cardiovascular disease and controls [7]. However, a study on 1,383 prostate cancer patients and 780 controls showed that a genetic variation of GDF15 promoted prostate cancer susceptibility [8]. Katoh et al. demonstrated that a single nucleotide polymorphism of the GDF15 gene was associated with gastric cancer [9]. In a Chinese population, GDF15 gene mutations affected the development and progression of colorectal cancer patients [10]. Similarly, The GDF-15 gene rs1804826G/T polymorphism and GDF15 levels of serum in a Chinese population were associated with ischemic strokes and affected the development of ischemic strokes [11].

In 2007, Corre J et al. [12] first reported that GDF15 showed over-expression in bone marrow mesenchymal stem cells from patients with multiple myeloma, but it was not produced by the malignant cells themselves. Then, Corre J et al. [13,14], confirmed that the serum GDF15 level was about threefold higher in myeloma patients compared with healthy subjects. They further demonstrated that GDF15 was derived from the microenvironment, and its plasma concentration in patients with multiple myeloma is correlated to initial parameters of the disease and to patient survival. It is a key survival factor for multiple myeloma cells. Tarkun et al. [15] also reported that the serum level of GDF15 is a prognostic factor in multiple myeloma and may predict response to treatment. Indeed, GDF15 plays an important role in the progression of multiple myeloma, and it may become a major therapeutic target. However, its biological role in tumorigenesis, especially genetic function, is still not fully understood.

As far as we know, there are no studies analyzing the role of GDF15 polymorphisms in multiple myeloma and no studies have explored the relationship between GDF15 gene polymorphisms and ISS/DS stage and GDF15 levels of serum in patients with multiple myeloma. In this study, for the first time, we evaluated the relationship between three GDF-15 gene loci (rs1058587, rs4808793 and rs1059369) and susceptibility with multiple myeloma in the Chinese population. We also analyzed the relationship between GDF-15 gene polymorphisms and ISS/DS stage and the GDF15 levels of serum.

MATERIALS AND METHODS

Study population

This was a case-control study of 239 subjects, including 120 patients with multiple myeloma and 119 healthy controls. Blood samples were collected from multiple myeloma patients who attended the Hematology Department of the First Affiliated Hospital of Guangxi Medical University from January 2018 to January 2019. The diagnosis of multiple myeloma was determined according to the guidelines of the National Comprehensive Cancer Network. Patients with multiple myeloma who met any of the following criteria were excluded: (i) Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), or other viral infections; (ii) neoplastic disease; and (iii) other hematological diseases. Multiple myeloma staging was based on the International Staging System (ISS) and Durie-Salmon staging system (DS). Based on a physical examination conducted at our hospital, 119 matched healthy controls were identified.

This study was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University. All the participants have signed informed consent.

DNA extraction and genotyping

Peripheral blood samples for genomic DNA extraction were collected using an AxyPrep Blood Genomic DNA Kit (AXYGEN Biosciences, Hangzhou, China) according to the manufacturer's instructions. The primer sequences for the three gene loci (rs1058587, rs4808793, and rs1059369) of GDF15 used in this study are summarized in Table 2. A 10 μL PCR reaction system was used containing 10 x buffer, 10 mmol primer, 10 mmol dNTP, 0.5U HotStart Taq polymerase, 1 μL of DNA sample, and 25 mmol Mg^{2+}. The steps of the multiplex PCR reaction were as follows: 95°C for 15 minutes; 15 cycles of 94°C for 40 seconds, 63°C for 60 seconds, and 72°C for 90 seconds; 25 cycles of 94°C for 40 seconds, 56°C for 40 seconds, and 72°C for 90 seconds; followed by 72°C for 8 minutes, and finally stayed at 4°C. The

purified PCR product was sequenced using an ABI 3730XL genetic analyzer (ABI Prism SNaPShot multiplex kit; Applied Biosystems Inc., USA) in accordance with the manufacturer's instructions. ABI GeneMapper 4.0 software was used to analyze the data collected by the sequencing analyzer. Figure 1 describes the sequencing map for alleles of rs1058587, rs4808793 and rs1059369 polymorphisms in multiple myeloma patients and healthy controls.

Measurement of serum GDF15 concentrations

Due to insufficient peripheral blood, serum samples were only collected from 71 multiple myeloma patients before treatment and 104 healthy controls. They were allowed to clot for 30 minutes. The samples were then centrifuged at approximately 1,000 x g for 15 minutes. Subsequently, the serum was separated and stored at -80°C until further use. The serum GDF15 concentration was detected using a sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA) kit (Human GDF-15 ELISA Kit; SAB Biotech Company, Shanghai, China) in accordance with the manufacturer's instructions. The concentration of GDF15 was obtained using a standard curve established by the kit standard in the range of 15.6 - 1,000 pg/mL.

Statistical analysis

Statistical analysis of all data was performed using SPSS software, version 17.0 (SPSS Inc., Chicago, IL, USA). The distribution type of the data was determined by the Kolmogorov-Smirnov test. The mean and standard deviation were used to describe data with a normal distribution, and the median and interquartile range were used to describe data with a skewed distribution. The Mann-Whitney U test was applied for comparisons of serum GDF15 levels in the multiple myeloma group and control group according to the different genotypes. The Kruskal-Wallis test was used to compare the serum GDF15 levels of those with different genotypes at the same locus. The clinical features of the multiple myeloma patients are represented by numbers and proportions. The genotype and allele frequencies of rs1058587, rs4808793, and rs1059369 were calculated by direct counting. The Hardy-Weinberg equilibrium was tested by the goodness of fit χ^2 test in the control group. The allele or genotype frequencies of the three sites in the multiple myeloma group and the control group were compared using a chi-square test or Fisher's exact probability method, as appropriate. Similarly, chi-square test or Fisher's exact probability method is also used to compare genotypes or gene frequencies of different ISS or Durie-Salmon classifications. To evaluate the relative risks of specific alleles and genotypes, binary logistic regression was used to calculate the odds ratios, with their 95% confidence intervals when controlling for gender and age as covariates. A p-value < 0.05 (two-tailed) was considered statistically significant.

RESULTS

Basic characteristics of the multiple myeloma patients

The clinical manifestations of the included multiple myeloma patients were summarized in Table 1. The average age of multiple myeloma patients was 57.68 years old. The study included 62 male patients and 58 female patients, accounting for 51.67% and 48.33%, respectively. Among the patients we included, IgG immunoglobulin subtypes were the most common, accounting for 46.67%, followed by IgA subtypes, accounting for 22.50%. Regardless of the ISS staging or the DS staging, patients with multiple myeloma in the stage III accounted for the largest proportion.

Correlation between the rs1058587, rs4808793, and rs1059369 polymorphisms and the risk of multiple myeloma

Table 3 describes the genotype and allele frequencies of rs1058587, rs4808793 and rs1059369 of the GDF15 gene in the multiple myeloma patients and healthy controls. The genotype distribution of rs1058587, rs4808793, and rs1059369 in the control group were all consistent with the Hardy-Weinberg equilibrium hypothesis (all $p > 0.05$). However, the results showed no significant difference in the genotype distribution or allele frequency of rs1058587 in the multiple myeloma patients versus the healthy controls. GDF15 gene rs4808793 and rs1059369 have the same discovery. By adjusting for age and gender, a binary logistic regression analysis result showed that the GDF15 rs1058587 polymorphism was not associated with the risk of multiple myeloma (all $p > 0.05$). The same finding was observed when the correlation between the rs4808793, rs1059369 polymorphism, and multiple myeloma was evaluated (Table 3).

Comparison of the rs1058587, rs4808793, and rs1059369 polymorphism between different ISS staging or Durie-Salmon staging

As shown in Table 4, the results showed that the genotype distribution and allele frequencies of rs1059369 in ISS stage I were significantly different from those in ISS stage II ($p = 0.008$). The distribution of rs1058587 genotype was different between ISS stage II and ISS stage III ($p = 0.014$). However, there were no differences in the genotype distribution or allele frequency of the three loci (rs1058587, rs4808793, and rs1059369) between ISS stage I and ISS stage III.

Similarly, as shown in Table 5, there was no difference in genotype distribution and gene frequency distribution between the three loci (rs1058587, rs4808793, and rs1059369), whether in the comparative Durie-Salmon Stage I and Durie-Salmon Stage II or in the comparative Durie-Salmon Stage I and Durie-Salmon Stage III. But the genotype distribution and allele frequencies of rs1058587 in Durie-Salmon stage II were significantly different from those in Durie-Salmon stage III ($p =$

Table 1. Clinical characteristics of patients with multiple myeloma.

Characteristics		Mean ± SD or number (%) (n:120)
Age (years)		57.68 ± 10.46
Gender	male	62 (51.67)
	female	58 (48.33)
Immunoglobulin subtype	IgG	56 (46.67)
	IgA	27 (22.50)
	IgM	5 (4.17)
	IgD	7 (5.83)
	non-secretory	3 (2.50)
	unknown	22 (18.33)
ISS staging	I	20 (16.67)
	II	34 (28.33)
	III	63 (52.50)
	unknown	3 (2.50)
Durie-Salmon staging	I	5 (4.17)
	II	20 (16.67)
	III	92 (76.67)
	unknown	3 (2.50)

Table 2. Primer sequences used for detecting the different GDF15 SNPs.

SNP ID	Primer sequence
rs1058587	F: 5'-CGTGTGCAGACGGCAGCAA-3'
	R: 5'-TGCTGGCAGAATCTTCGTCC-3'
	EF: 5'-gactgactgaAGAGCGCGTGCGCGCAACGGGGAC-3'
rs4808793	F: 5'-CAGGGATGAATCTGCTCTTGTCT-3'
	R: 5'-CCTTGTGTCTCTTACCCACGCA-3'
	EF: 5'-GTCTCTTACCCACGCATGCCTGTCACATGCAGACACCCACACACACCCA-3'
rs1059369	F: 5'-AAGATTTCACTTACCTTCTGGCGT-3'
	R: 5'-AACTCAGGACGGTGAATGGCTC-3'
	EF: 5'-gacTTAGCAGGTCCTCGTAGCGTTTCCGCAACTCTCGGAATCTGGAGTCTTCGG-3'

F - forward, R - reverse, E - extension.

0.000).

Serum GDF15 levels

As shown in Table 6, 104 healthy controls and 71 multiple myeloma patients were included in the analysis of serum GDF15 levels. The results revealed that the overall serum GDF15 concentrations of the multiple myeloma patients were significantly higher than those in the healthy control group (527.84 ± 524.19 vs. 255.66 ± 134.84, $p < 0.001$). The levels of GDF15 of the same genotype at the same locus in the multiple myeloma group were significantly higher than those in the healthy control group, regardless of GC and GG genotypes of rs1058587, CC and GC genotypes of rs4808793, or AA, AT, and TT genotypes of rs1059369. However, there was no significant difference in the GDF15 levels of the different genotypes at the same locus in the multiple myeloma group (rs1058587, $p = 0.908$; rs4808793, $p = 0.120$; rs1059369, $p = 0.733$). Moreover, there was no difference in serum GDF15 levels between different

Table 3. Genotype and allele frequencies of GDF15 rs1058587, rs4808793, and rs1059369 polymorphisms between MM and healthy controls.

Polymorphisms	Healthy controls (n = 119)	MM (n = 120)	OR (95% CI)	p-value
rs1058587				
CC	10	15	1.0^{Ref}	
CG	63	42	1.448 (0.580 - 3.614)	0.427
GG	46	63	1.434 (0.592 - 3.472)	0.424
C allele	83	72	1.0^{Ref}	
G allele	155	168	1.859 (0.758 - 1.698)	0.294
P^{HWE}	0.071			
rs4808793				
CC	52	42	1.0^{Ref}	
CG	55	60	0.671 (0.378 - 1.189)	0.171
GG	12	18	0.524 (0.226 - 1.216)	0.132
C allele	159	144	1.0^{Ref}	
G allele	79	96	2.332 (0.495 - 1.053)	0.135
P^{HWE}	0.646			
rs1059369				
AA	39	54	1.0^{Ref}	
AT	61	49	0.698 (0.308 - 1.583)	0.389
TT	19	17	1.010 (0.457 - 2.232)	0.981
A allele	139	157	1.0^{Ref}	
T allele	99	83	0.856 (0.549 - 1.333)	0.491
P^{HWE}	0.548			

Table 4. Genotype and allele frequencies of GDF15 rs1058587, rs4808793, and rs1059369 polymorphisms between different ISS staging.

SNP	ISS Stage I	ISS Stage II	ISS Stage III	p-value (I II)	p-value (I III)	p-value (II III)
rs1058587						
CC	4	8	3	0.442	0.129	0.014
CG	8	8	26			
GG	8	18	34			
C allele	16	24	32	0.625	0.076	0.147
G allele	24	44	94			
rs4808793						
CC	5	11	26	0.840	0.386	0.668
CG	12	18	28			
GG	3	5	9			
C allele	22	40	80	0.698	0.336	0.523
G allele	18	28	46			
rs1059369						
AA	12	12	28	0.025	0.076	0.228
AT	8	13	27			
TT	0	9	8			
A allele	32	37	83	0.008	0.092	0.117
T allele	8	31	43			

Table 5. Genotype and allele frequencies of GDF15 rs1058587, rs4808793, and rs1059369 polymorphisms between different Durie-Salmon staging.

SNP	Durie-Salmon Stage I	Durie-Salmon Stage II	Durie-Salmon Stage III	p-value (I II)	p-value (I III)	p-value (II III)
rs1058587						
CC	0	8	7	0.106	0.565	0.000
CG	3	8	31			
GG	2	4	54			
C allele	3	24	45	0.178	0.985	0.000
G allele	7	16	139			
rs4808793						
CC	2	3	37	0.481	1.000	0.078
CG	3	13	42			
GG	0	4	13			
C allele	7	19	116	0.358	0.914	0.069
G allele	3	21	68			
rs1059369						
AA	3	12	37	1.000	0.466	0.273
AT	1	6	41			
TT	1	2	14			
A allele	7	30	115	1.000	0.887	0.134
T allele	3	10	69			

Table 6. The association of GDF15 polymorphisms with serum GDF15 levels (median ± IQR, pg/mL) in MM and healthy controls.

Groups	Overall	rs1058587				rs4808793				rs1059369			
		CC	GC	GG	p-values	CC	GC	GG	p-values	AA	AT	TT	p-values
Controls (n = 104)	255.66 ± 134.84	188.47 ± 381.14	186.22 ± 103.67	254.03 ± 88.60	0.025	221.18 ± 131.25	236.31 ± 123.06	280.76 ± 153.98	0.221	238.86 ± 204.08	244.45 ± 106.03	214.03 ± 55.45	0.955
MM (n = 71)	527.84 ± 524.19	600 ± 250.73	571.98 ± 618.39	511.61 ± 442.72	0.908	441.27 ± 322.58	676.49 ± 733.79	351.38 ± 370.12	0.120	585.17 ± 576.04	519.72 ± 641.18	393.17 ± 317.61	0.733
p-values	< 0.001	0.077	< 0.001	< 0.001		< 0.001	< 0.001	0.271		< 0.001	< 0.001	0.038	

ISS stage (Figure 2) or Durie-Salmon stage (Figure 3).

DISCUSSION

GDF15, as a member of transforming growth factor (TGF) beta superfamily proteins, has been shown to be involved in regulating the development or progression of various neoplastic diseases [16]. A genome-wide association study demonstrated that the GDF15 gene was associated with the serum GDF15 concentration and that genetic factors affected the expression level of serum GDF15 [17]. Multiple myeloma is a malignant disease with abnormal proliferation of bone marrow plasma cells, accompanied by overproduction of monoclonal immunoglobulin or light-chain [18]. Athiyarath et al. [19] studied the role of GDF15 gene mutations in regulating iron overload in β-thalassemia and found that genetic polymorphisms of eight GDF15 loci were significantly associated with the blood disorder. Ärlestig et

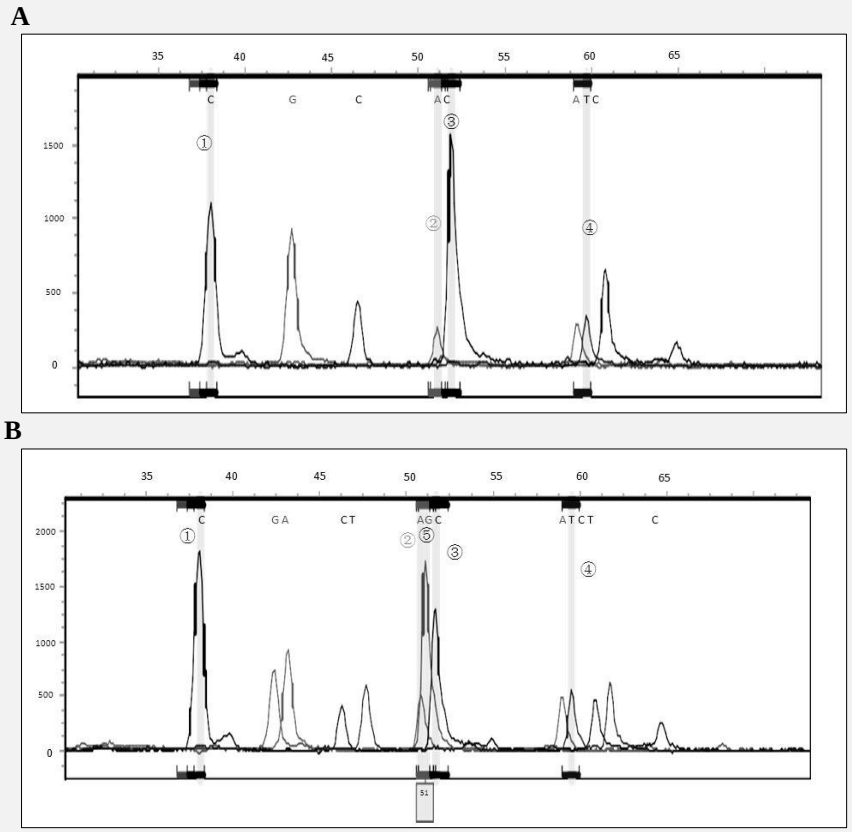

Figure 1. Sequencing map for genotypes of rs1058587, rs4808793, and rs1059369 polymorphisms.

(A) The peaks ① - ④ show patients with multiple myeloma rs1058587-C, rs1059369-A, rs4808793-C, and rs1059369-T allele, respectively; (B) The peaks ① - ⑤ show healthy controls rs1058587-C, rs1059369-A, rs4808793-C , rs1059369-T, and rs4808793-G allele, respectively.

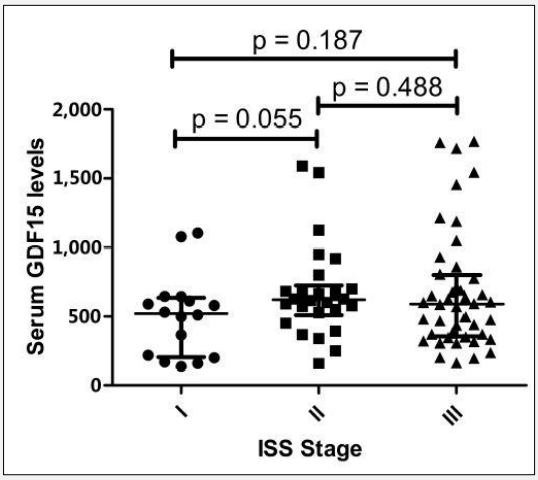

Figure 2. The association of serum GDF15 levels with different ISS stage in MM.

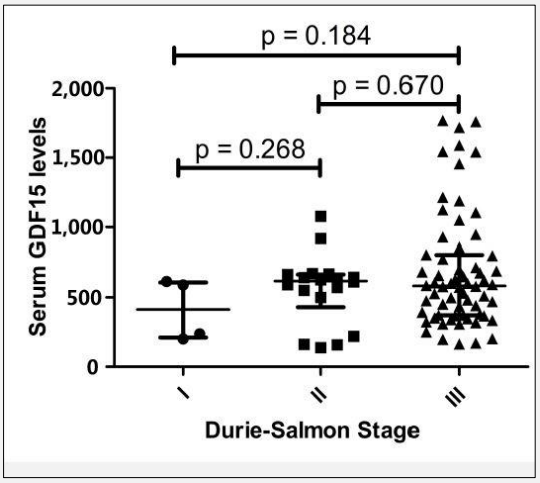

Figure 3. The association of serum GDF15 levels with different Durie-Salmon Stage in MM.

al. [20] found that GDF15 gene polymorphisms were associated with rheumatoid arthritis in a cross-sectional study involving 681 patients with rheumatoid arthritis. However, there were only a few studies on serum levels of GDF15 in multiple myeloma. For example, Westhrin et al. reported that serum GDF15 levels in multiple myeloma patients were also significantly higher than those in controls [21]. A case-control study in China also showed that serum GDF15 levels were significantly higher in 24 patients of multiple myeloma as compared with 20 healthy controls. Our findings are consistent with those of these previous studies [21].

Many causes, such as iron content, oxygen content, and the environment, as well as genetic variations, influence the expression level of serum GDF15 [22,23]. Our study just explored the relationship between GDF15 gene polymorphisms and serum GDF15 concentrations. However, the results revealed no significant difference between the GDF15 levels of the different genotypes (rs1058587, rs4808793, and rs1059369) of the same locus in the multiple myeloma group. As noted earlier, GDF15 is derived from macrophages, and activated macrophages affect the expression of GDF15 [20]. Therefore, causes other than genetic variations affect the expression of GDF15, which may have a greater impact on GDF15 expression.

Ärlestig et al. [20] showed that the GDF15 rs1058587 polymorphism was associated with deep vein thrombosis/pulmonary embolism. Freedman et al. [24] explored the relationship between genetic variations and the overall survival rate of prostate cancer patients and found that the rs1058587 polymorphism of the GDF15 gene was associated with survival rates. A study involving a Chinese population demonstrated that the GDF15 gene rs4808793 polymorphism was not associated with coronary heart disease or disease severity [25]. However, a study reported a relationship between GDF15 gene variants and left ventricular hypertrophy in patients with essential hypertension and showed that the rs4808793G allele of GDF15 was associated with a significant increase in left ventricular hypertrophy, whereas the rs1059369 of GDF15 polymorphism was not associated with left ventricular hypertrophy [26]. No previous studies examined the relationship between GDF15 rs1058587, rs4808793, and rs1059369 polymorphisms and multiple myeloma. In our study, there was no significant difference in the genotype distribution or allele frequency of rs1058587, rs4808793, and rs1059369 of the multiple myeloma patients and healthy controls. Similarly, by adjusting for age and gender, the results of the binary logistic regression statistical analysis showed that the GDF15 rs1058587, rs4808793, and rs1059369 polymorphisms were not associated with the risk of multiple myeloma (all $p > 0.05$). However, the genotype distribution and allele frequencies of rs1059369 in ISS stage I were significantly different from those in ISS stage II ($p = 0.008$), and the distribution of rs1058587 genotype was different between ISS stage II and ISS stage III ($p = 0.014$). Moreover, the serum levels of GDF15 of the same genotype at the same locus (rs1058587: GC, GG; rs4808793: CC, GC; rs1059369: AA, AT, and TT) in the multiple myeloma group were significantly higher than those in the healthy control group. However, there was no significant difference in the GDF15 levels of the different genotypes at the same locus in the multiple myeloma group (rs1058587, $p = 0.908$; rs4808793, $p = 0.120$; rs1059369, $p = 0.733$), also with different ISS stage (Figure 2) or Durie-Salm-

on stage (Figure 3).

These findings are negative in the sense that, apart from the sub-analysis of the disease stage, GDF15 gene polymorphism has no correlation with multiple myeloma. This study has a number of limitations. First, the sample size was small, and a larger sample size is required to verify the results. Second, blood samples were collected only from patients in the Guangxi region, which does not represent the whole Chinese population. Thus, a multicenter study is required. Finally, we studied only three gene loci polymorphisms. Therefore, it is also possible that the results do not accurately reflect the relationship between GDF15 polymorphisms and serum GDF15 concentrations because they are based on only a small number of samples and only three gene loci. Then, the relationship between other loci of GDF15 and multiple myeloma needs to be explored. In summary, this study is the first to study the relationship between GDF15 rs1058587, rs4808793, and rs1059369 polymorphisms, serum concentrations of GDF15 and ISS staging or Durie-Salmon staging in multiple myeloma patients. The results suggested that polymorphisms of three GDF15 loci (rs1058587, rs4808793, and rs1059369) were not associated with multiple myeloma. However, the genotype distribution and allele frequencies of rs1059369 and rs1058587 have some association with ISS stage and Durie-Salmon stage. Furthermore, in patients with multiple myeloma, serum GDF15 levels were not associated with these GDF15 polymorphisms, ISS stage, and Durie-Salmon stage. Nevertheless, the levels of GDF15 of the same genotype at the same locus in the multiple myeloma group were significantly higher than healthy control patients, regardless of GC and GG genotypes of rs1058587, CC and GC genotypes of rs4808793, or AA, AT, and TT genotypes of rs10593 69.

Author Contributions:

Ruolin Li and Chengbin Wang drafted the overall design of this paper. Zuojian Hu, Shanzi Qin, Chunni Huang and Yibin Yao collected resources of blood sample. Ruolin Li, Zuojian Hu, Yu Lu conducted experiments. Ruolin Li, Zuojian Hu, Yu Lu and Xue Qin conducted data curation and analyzed the data by using Software. Ruolin Li wrote the original draft article. Xue Qin and Chengbin Wang reviewed and edited the original draft.

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Declaration of Interest:

None.

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