Impact of the myosin heavy chain 9 gene single nucleotide polymorphism on inflammatory bowel disease

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Research Article

Keywords: IBD, MYH9, SNP

Posted Date: May 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-248913/v1

Abstract

Background

To date, the cause of inflammatory bowel disease (IBD) remains a mystery. A balance between cell proliferation and apoptosis maintains intestinal tissue homeostasis. Dissociation-induced myosin-actin contraction results in stem cell apoptosis. This study aiming to evaluate the influence of the myosin heavy chain 9 (MYH9) gene single nucleotide polymorphism (SNP) on inflammatory bowel disease.

Subjects and methods:

The study carried on eighty patients with IBD and seventy controls. All participants subjected to history taking, thorough physical examination examination, colonoscopy and laboratory investigations. Genotyping performed for rs3752462 and rs4821480 by SNP assay real-time PCR methods.

Results

On analyzing rs4821480, The TG and GG genotypes have significant increased distribution among the IBD patients as compared to the controls with 5.3 fold increase in the risk of IBD and higher prevalence of GG genotype in patients with low hemoglobin level and higher BMI. While on analyzing rs3753462 CT and TT genotypes were significantly more frequent in the in the IBD patients as compared to the controls with 4.6 fold increase in the risk of IBD.

Conclusion

The allele G of rs4821480 and T of rs3753462 of MYH9 gene associated with more susceptibility to IBD.

Summary

IBD is a multifactorial disease, affected by a combination of environmental and genetic factors. The genetic etiology of IBD is still unclear. This is the first study carried out to reveal if there is any association between two SNPs of MYH9 gene and IBD. This study concluded that MYH9 gene polymorphism might be associated with the pathogenesis of IBD.

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disease affecting the intestine. It affecting many patients in Egypt and the Middle East. However, starting a disease registry is highly crucial for IBD, and establishing a specific unit for IBD is extremely important for better diagnosis, treatment, and patients to care (1, 2).

The intestinal mucosa acts as a barrier to protect the host from dangerous pathological organisms and it is the site at which the interactions with commensal organisms take place. These interactions modify by the immune system in the intestine and play a part in immune homeostasis. IBD can take place in the condition of disruption of this homeostasis (3).

There are 2 types of stem cells of the GIT mucosa: the rapid proliferating leucine-rich repeat having G protein-coupled receptor 5 + ve (Lgr5 þ) cells which preserve the intestinal homeostasis, and the Bmi1 þ (þ 4) cells which play a part in the intestinal regeneration after injury. These 2 stem cell types can interconvert in the mucosa lining the intestine (4).

The MYH9 gene encodes the heavy chain of the non-muscle myosin IIA (NMM-IIA) protein (5). This protein is included in many significant functions, involving the motility of the cell, cytokinesis, cell shape maintenance, and specific functions like secretion. The MYH9 mutations are also, correlated with the occurrence of other disorders like the giant platelet syndrome (6).

The non-muscle myosin protein is found in large quantities in the liver, platelets, and kidney and smaller quantities in the intestine, cochlea, spleen, and thymus (7, 8)

The NMM-IIA produced force for cell motility through catalyzing ATP hydrolysis and plays a role in a broad range of cellular functions in several cells, like mitosis, cell migration, and cell adhesion (9). Many studies documented that the dissociation-induced myosin-actin contraction resulted in induced and embryonic stem cell apoptosis (10).

This study carried out to reveal if there is an association between the SNP of the MYH9 gene and IBD.

Subjects And Methods

This prospective case-control study conducted at Menoufia University Hospitals. The subjects included in the study were divided into two groups; group I included 80 cases diagnosed with inflammatory bowel disease and group II which included age and sex-matched 70 healthy subjects as a control group. Informed consent obtained from all the participants before starting the study. Besides, the local ethical committee of Menoufia University approved the study. All patients subjected to complete history taking, thorough physical examination, fecal occult colonoscopy and routine laboratory investigation include occult blood tests. Whole blood samples were taken from patients and controls to detect the SNP of the MYH9 gene.

This done in two main steps, first, whole blood DNA extraction by Quick-genomic DNA™ MiniPrep kit, Zymo Research. Second, the MYH9 SNPs (rs 3753462& rs4820480) were genotyped using Real-Time PCR Instrument, Applied Biosystems®7500.

The genotype reaction mix was prepared using TaqMan universal master mix II (2x), supplied by Applied Biosystems, Foster City, USA, 2010. The manufacturer described probes: [VIC/FAM] for **SNP1** (rs3752462)

was: AGGTGTGAGGTCAAAGCAAGCCTGG[C/T]ACTCACTGGCTTCTCAATGAGGTCG. For **SNP2**(rs4821480) was:

TTTTCCTAGATCAAAGGATAATTTT[G/T]AAAGGTCACGAGCTCCCCTGAAACA. Both primers and probes purchased from an Applied Biosystem, Foster City, USA, 2010. The fluorescence generated by PCR amplification indicates which alleles are present in the sample. Figures (1) shows the allelic discrimination plot of rs3752462 SNP of the MYH 9 gene and figure (2) shows the allelic discrimination plot of rs4820480 SNP of MYH 9 gene.

Statistical analysis

Data entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS 21.0, IBM/SPSS Inc., Chicago, IL) software for analysis. Baseline characteristics of the study population were presented as frequencies and percentages (%) or mean values and standard deviations (SD) or median and range (IQR) (after testing of normality by Kolmogorov-Smirnov and Shapiro-Wilk's tests).

For comparison of data, the Chi-Square test (or Fisher's exact test) was used to compare two independent groups of qualitative data. For quantitative data, one-way analysis of the variance (ANOVA) and Kruskal Wallis test were used to compare more than two groups of parametric and non-parametric quantitative data respectively.

Results

Starting with demographics, age and gender were not significantly different between cases and controls ($p > 0.05$). However, there was a statistically significant difference between cases and controls regarding BMI ($p < 0.001$). Table (1) illustrates these data.

Regarding the presentations reported, a fecal occult blood test was positive in all cases while it was negative in all controls included in the current study ($p < 0.001$). As regards CBC parameters analyzed in the included subjects, it was evident that hemoglobin, leucocytes count, and platelets were significantly higher in controls compared to IBD cases ($p < 0.05$). These data are not shown.

With a comparison of the different genotype distribution of rs4821480 in the

IBD cases and control group, The TG, GG genotypes, and the combination of TG + GG genotypes were significantly more prevalent in the IBD group as compared to the control group. The odds ratio of the G allele in the IBD group was 5.93 fold Table (2) illustrates these results.

With a comparison of the different genotype distribution of rs3753462 in the IBD and control group, The CT, TT genotype, and the combination of CT + TT genotypes were significantly more frequent in the IBD group as compared to the control group. The odds ratio of the T allele in the IBD group was 3.792 fold. Table (3) illustrates these results.

On analyzing rs4821480 in IBD cases, although the GG genotype had significantly lower hemoglobin levels compared to the other two genotypes, genotype, it was significantly associated with higher BMI,)data not shown(.

Discussion

IBD is a multifactorial disease that affected by a combination of environmental and genetic factors; the definite IBD etiology is still unrecognized (1).

This study carried out to reveal if there is an association between the SNP of the MYH9 gene and IBD. The study conducted on 150 subjects including 70 healthy controls, and 80 cases with IBD.

In this study, age and gender were not significantly different between cases and controls ($p > 0.05$). However, there was a statistically significant difference between cases and controls regarding BMI ($p < 0.001$). BMI was significantly higher in the IBD group compared to controls.

The continuous blood loss in addition to tenesmus, diarrhea, and other associated symptoms of IBD are responsible for decrease body weight as compared to the controls.

However, based upon the disease severity this change in the BMI could be insignificant. As reported by Hanafy et al. (2018) who reported that the mean values of the body mass index for the IBD and controls (11).

These current results were comparable to El-Hodhod et al. (2013) who reported that hemoglobin concentration was significantly lower in IBD patients during flare compared to controls (12)

In this study, regarding the distribution of rs3753462 of MYH9 in the IBD and control group, The CT and TT genotypes were significantly higher prevalence in the IBD group; T allele has 3.79-fold increase risk of IBD. In addition, on analyzing rs4821480 of MYH9, It was evident that the GG genotype had a significantly higher prevalence in IBD cases; G allele has 5.93-fold increase risk of IBD. Moreover, the GG genotype had significantly lower hemoglobin levels and higher BMI compared to the other two types. To the best of our knowledge, the polymorphisms in the MYH9 gene concerning IBD not tested before.

Many studies utilized genetic and pharmacologic inhibition of the heavy chains NM II to evaluate their importance in the monolayers model of the intestinal mucosa. These studies concluded that first, the motor activity of NM II is critical for the standard barrier properties preservation of these layers. Second, NM II has a significant responsibility in regulating the junctional remodeling by motivating 2 phases against each other: junctional assembly and reassembly. Third, the heavy chain of NM IIA works as a vital AJ/TJ functions regulator (13–15).

In several previous studies, the expression of MYH9 proved to be as an indicator to observe the progression and prognosis of gastrointestinal tract (16–21).

Another study found that MYH9 down-regulated many small interfering RNAs (siRNAs) in pancreatic cancer patients, and the down-regulation of tumor suppressor genes led to the occurrence of tumors (22).

A modern study on mice evaluating the specific NM IIA knockout in the epithelium of the intestine documented that the NM IIA loss was reasonable to increase the GIT barrier permeability and to stimulate low-grade inflammation in the intestine and elevates the sensitivity of the animal to colitis, which lead to severe erosion of the epithelium and more barrier breakdown exacerbation. (23).

The interactions of heavy chain with proteins, which bind to myosin, as well as specific heavy chain of NM IIA phosphorylation may have a relation with abnormal cytoskeleton organization in the intestinal epithelium lead to inflammation development in the intestine (24).

Outside the GIT, the polymorphisms of the (*MYH9*) gene have been claimed in dissimilar kidney diseases, as well as in diabetic nephropathy (25).

Several studies imply that MYH9/NMHC-IIA plays an important role in the invasive behavior of cancer cells. For instance, the EGF-dependent heavy chain phosphorylation of the myosin-IIA has a clear responsibility in mediating chemotaxis and motility (26). Myosin IIA seems to be the main mts1target (27), the metastasis- 1, and co-localizes with mts1 to the migrating tumor cells leading edge (28); mts1 affects both the myosin IIA phosphorylation and its assembly behavior (29, 30). A modern study associates MYH9 as an SRF target, that plays a role in cancer cell invasion and metastasis (31).

Further studies are required to explain the mechanism regulate functions of NM IIA in the epithelium of the intestine in the state of health and inflammation of GIT.

Conclusion

The MYH9 gene polymorphism may be associated with the pathogenesis of IBD. The allele G of rs4821480 and T of rs3753462 of MYH9 gene associated with more susceptibility to IBD and GG genotypes of rs4821480 associated with anemia and overweight.

Declarations

Acknowledgement

We acknowledge the central laboratory unit, faculty of Medicine, Menoufia University for providing us with the necessary instruments for completion of the study.

Author's Contribution

Elsayed El-Shayeb, Abd El-naser Gadallah and Ahmed Ezz El-Arab design study, Eman badr do the lab investigation, Elsayed El-Shayeb analyzes the results, Ahmed Megahed Taman collect the sample and

follow the patients. All authors write and revise of the manuscript and approve the final manuscript for submission.

Conflict of Interest:

The authors declare that they have no conflict of interest.

Funding sources: No sources of funding

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Tables

Table (1): Comparison between the three studied groups according to demographic data.

	IBD (n= 80)		Control (n = 70)		Test of Sig.	p
	No.	%	No.	%		
Sex						
Male	40	50.0	41	58.5	c^2= 0.453	0.798
Female	40	50.0	29	41.5		
Age (years)					H=3.126	0.210
Mean ± SD.	31.77 ± 10.05		29.10 ± 9.07			
Median (IQR)	30.0(27.0 – 35.0)		29.0(21.0 – 34.0)			
BMI (Kg/m^2)					H=16.033*	<0.001*
Mean ± SD.	23.98 ± 2.43		27.95 ± 4.39			
Median (IQR)	24.0(22.0 – 26.0)		27.34(24.79 – 29.76)			

Table (2): Comparison between IBD and Control according to SNP (rs4821480)

rs4821480	IBD (n= 80)		Control (n = 70)		p	OR (95%CI)
	No.	%	No.	%		
SNP						
TT®	44	55	60	85.7	1.000	–
TG	24	30	10	14.3	0.038*	OR 3.706(1.075 – 12.772)
GG	12	15	0	0.0	–	–
TG + GG	36	45	10	14.3	0.005*	OR 5.353(1.640 – 17.473)
Allele						
T®	112	70	130	92.8	1.000	–
G	48	30	10	7.2	0.001*	OR 5.930(2.044 – 17.208)

OR: Odds ratio CI: Confidence interval LL: Lower limit UL: Upper Limit

Table (3): Comparison between IBD and control according to SNP (rs3753462)

rs3753462	IBD (n= 80)		Control (n = 70)		p	OR (95%CI)
	No.	%	No.	%		
SNP						
CC®	26	32.5	49	70.0	1.000	–
CT	32	40.0	15	21.4	0.022*	OR 3.733(1.211 – 11.513)
TT	22	27.5	6	8.6	0.009*	OR 7.467(1.648 – 33.821)
CT + TT	54	67.5	21	30.0	0.003*	OR 4.667(1.689 – 12.898)
Allele						
C®	84	52.5	113	80.7	1.000	–
T	76	47.5	27	19.3	0.001*	OR 3.792(1.779 – 8.080)

OR: Odds ratio CI: Confidence interval LL: Lower limit UL: Upper Limit

Figures

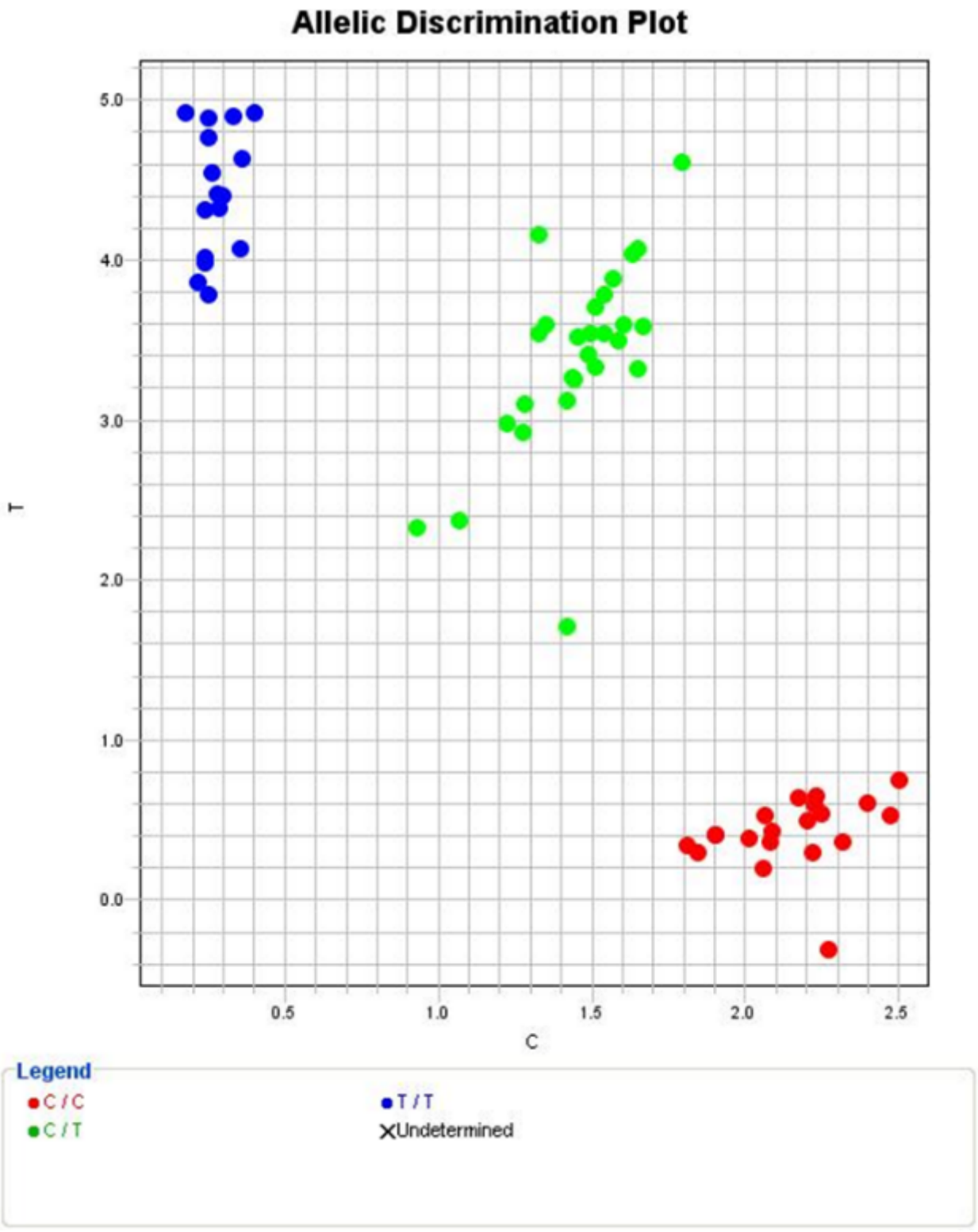

Figure 1

Allelic discrimination plot of rs3752462 SNP of MYH 9 gene.

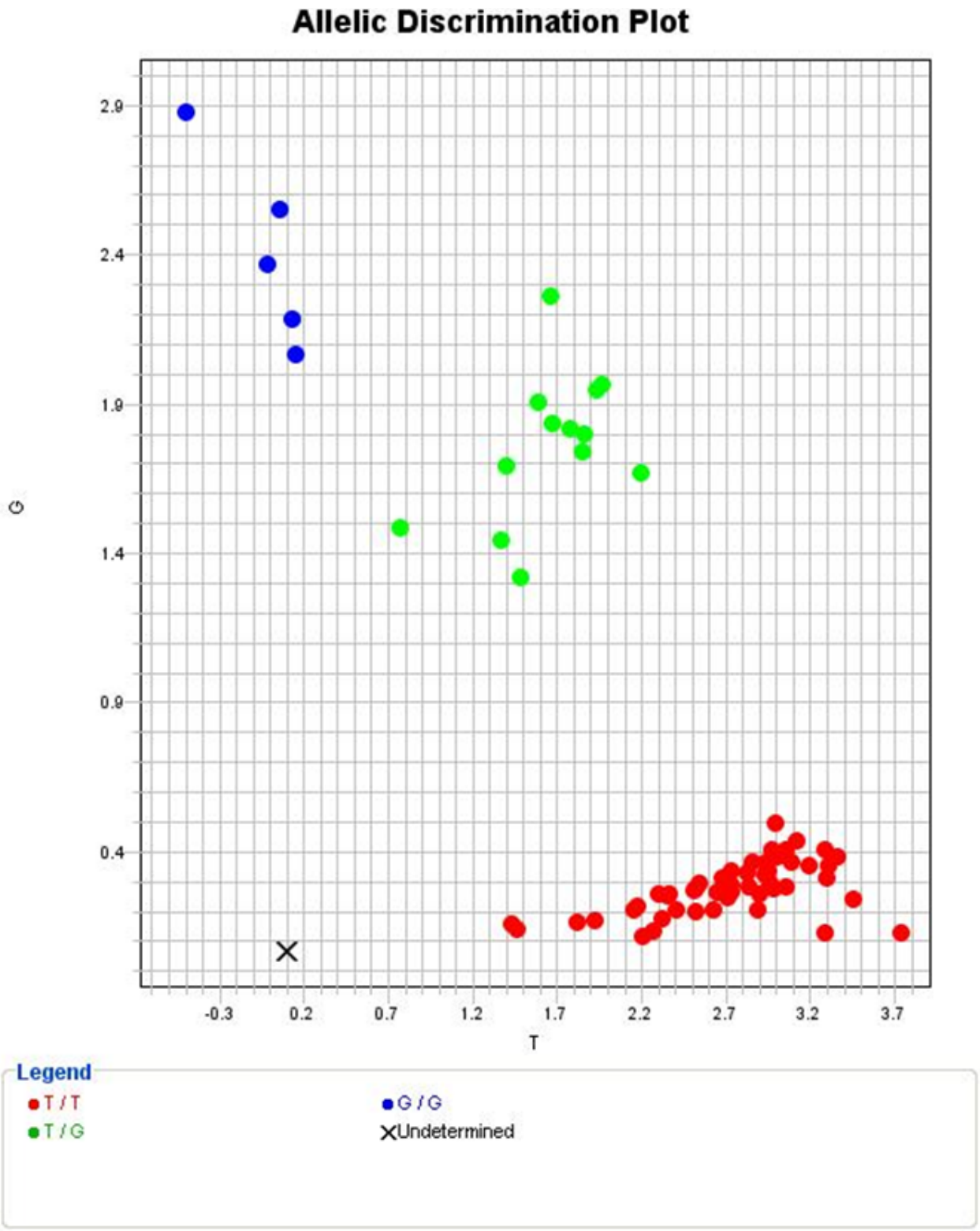

Figure 2

Allelic discrimination plot of rs4820480 SNP of MYH 9 gene.