Genetic and Biochemical Parameters

Table 5.1: Table summarizes the demographic Information of Case and Control.

Parameters	Case (n=36)	Control (n=50)	p-value
Gender (Male)	27	22	0.000034
Gender (Female)	9(25%)	28(56%)	0.000167
Age	56.80±11.19	39.38±12.54	<0.00001
RLS severity (mean)	14.44±6.46	---	--

p-value <0.05 is statistically significant

Table 5.1 shows the demographic information of case and control studied. For this study we included 36 cases and 50 controls. There is a statistically significant difference between age and gender for case and control. Mean IRLSSG rating was 14.44±6.46.

Table 5.2: Table summarizes the Clinical Parameters and their association with case and control.

Parameter	Case (n=36)	Control (n=50)	p-value	reference range
Iron(mcg/dl)	58.23±18.235	111.99±31.262	< 0.00001	60 - 180
TIBC (mcg/dL)	363.21±60.147	325.61±58.047	0.004563	250 - 400
Iron Saturation (%)	15.23±5.29	33.89±11.61	<0 .00001	15 - 50
Ferritin(ng/mL)	67.91±42.01	199.23±85.73	< 0.00001	10 - 291
ALT(U/L)	26.48±14.15	26.33±7.94	0.948521	< 35
AST(U/L)	26.58±9.39	19.58±5.32	0.000034	< 35
ALP(U/L)	89.59±48.68	82.50±20.02	0.355851	30 - 120
GGT(U/L)	24.93±9.1	22.11±9.26	0.164516	< 38
In-direct bilirubin (mg/dL)	0.60±0.31	0.62±0.14	0.689789	0.20 - 1.0
Direct Bilirubin(mg/dL)	0.17±0.10	0.19±0.05	0.187581	< 0.2
Total Protein(g/dL)	7.14±0.56	7.15±0.55	0.914356	6.6 - 8.3
Albumin(g/dL)	4.26±0.28	4.4±0.22	0.019478	3.5 - 5.2

Globulin(g/dL)	2.87±0.42	2.75±0.55	0.288785	1.9 - 3.7
A:G ratio	1.34±0.35	1.66±0.40	0.000234	1 - 1.7
Urea(mg/dL)	21.19±6.36	24.32±8.06	0.056575	20.0 - 40.0
Creatinine(mg/dL)	0.94±0.15	1.05±0.09	0.000029	0.5 - 0.9
Sodium(mmol/L)	135.94±4.03	133.78±3.54	0.010041	136 - 145
Chloride(mmol/L)	101.36±4.85	101.74±3.05	0.658636	98 - 107
Potassium(mmol/L)	4.56±0.50	4.42±0.47	0.176666	3.5 - 5.1

p-value <0.05 is statistically significant

Table 5.2 shows the biochemical parameters of case and controls. In this we find that differences between serum Iron profile for case and control which includes serum iron (p-value <0.05), TIBC (p-value <0.05), Iron Saturation (p-value <0.05) and Ferritin (p-value <0.05) were all statistically significant.

a. MEIS1				
Parameter	**TT**	**TG**	**GG**	**p-value**
Iron	56.25±16.90	59.1±19.05	-	0.672738
TIBC	343.74±48.77	371.77±63.51	-	0.202219
Iron Saturation	16.43±5.03	14.70±5.42	-	0.374118
Ferritin	54.54±19.51	73.79±47.93	-	0.209894
RLS Rating	14.45±5.95	14.44±6.80	-	0.995149

Table 5.3 summarizes the RLS SNP and their correlation with clinical parameters. Where,

p-value <0.05 is statistically significant

b. TOX3				
Parameter	**TT**	**TG**	**GG**	**p-value**
Iron	57.11±19.04	58.83±11.55	57.94±26.78	0.977203
TIBC	352.62±62.42	366.35±60.90	364.80±62.59	0.878567
Iron Saturation	15.34±6.19	14.72±3.11	15.99±7.58	0.826636
Ferritin	64.85±35.75	74.39±53.10	59.25±21.30	0.640598
RLS Rating	14±7.50	13.88±5.64	15.63±7.50	0.774026

p-value <0.05 is statistically significant

c. PTPRD			
Parameter	**TT+TC**	**CC**	**p-value**

Iron	58.58±19.65	56.46±9.07	0.799409
TIBC	358.15±60.12	388.49±58.64	0.265423
Iron Saturation	15.22±5.75	15.27±2.00	0.982538
Ferritin	66.54±41.08	74.76±50.02	0.668117
RLS Rating	14.43±6.36	14.5±7.63	0.982014

p-value <0.05 is statistically significant

Table 5.4: SNP (wild vs. mutant) and their p-value and OR

Gene	**SNP**	**ALLELE**	**p-value**	**OR**	**95% CI**
MEIS1	**rs2300478**	GG* vs TG	0.9702	1.0784	0.0206 to 56.3943
		GG vs TT	0.7251	2.0435	0.0381 to 109.6845
		GG vs TG+TT	0.8718	1.3836	0.0268 to 71.3588
		TG vs TT	0.1508	1.9360	0.7862 to 4.7677
BTBD9	**rs9296249**	CC* vs TC	0.8796	1.3562	0.0263 to 69.9594
		CC* vs TT	0.6705	3.0000	0.0190 to 473.1018
		CC vs TC+TT	0.8718	1.3836	0.0268 to 71.3588
		TC vs TT	0.6299	2.2121	0.0876 to 55.8717
TOX3	**rs3104767**	TT* vs TG	0.9121	0.9398	0.3122 to 2.8296
		TT vs GG	0.2583	0.4773	0.1324 to 1.7210
		TT vs TG+GG	0.6160	0.7644	0.2675 to 2.1843
		TG vs GG	0.2099	0.5078	0.1761 to 1.4647
PTPRD	**rs1975197**	AA* vs GA	0.3318	3.3571	0.2910 to 38.7359
		AA vs GG	0.783	0.6667	0.0372 to 11.9362
		AA vs GA+GG	0.3951	2.884	0.2512 to 33.0706
		GA vs GG	0.0574	0.1986	0.0375 to 1.0521
MAP2K5	**rs1026732**	AA* vs GA	1.0000	1	0.0136 to 73.2695
		AA vs GG	0.8656	1.4058	0.0272 to 72.5906
		AA vs GA+GG	0.8718	1.3836	0.0268 to 71.3588
		GA vs GG	0.7365	1.4118	0.1894 to 10.5221

***Indicate wild type allele of SNP; p-value <0.05 is statistically significant**

Table 5.5: The table summarizes the allele frequency found in polymorphs studied. It shows predominant presence of T allele.

Gene	Allele Frequency	Global Allele frequency *
MEIS1 (rs2300478)	T: 70%, G:30%	T:75.8%, G:24.2% [a]
BTBD9 (rs9296249)	T: 50%, C:50%	T:75%, G:25% [b]
TOX3 (rs3104767)	T:49%, G:51%	T:45%, G:55% [c]
PTPRD (rs1975197)	T: 47%, C:53%	G:83%, A:17% [d]
MAP2K5 (rs1026732)	G: 98%, A:2%	G:64%, A:36% [e]

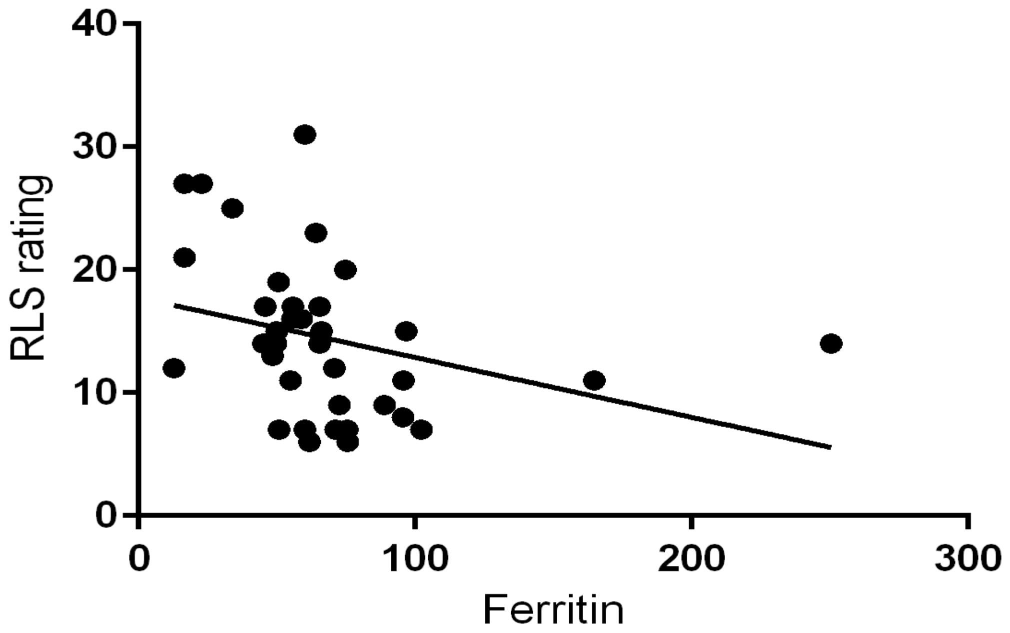

Figure 5.1: The figure demonstrates an interrelationship between RLS rating and Ferritin.

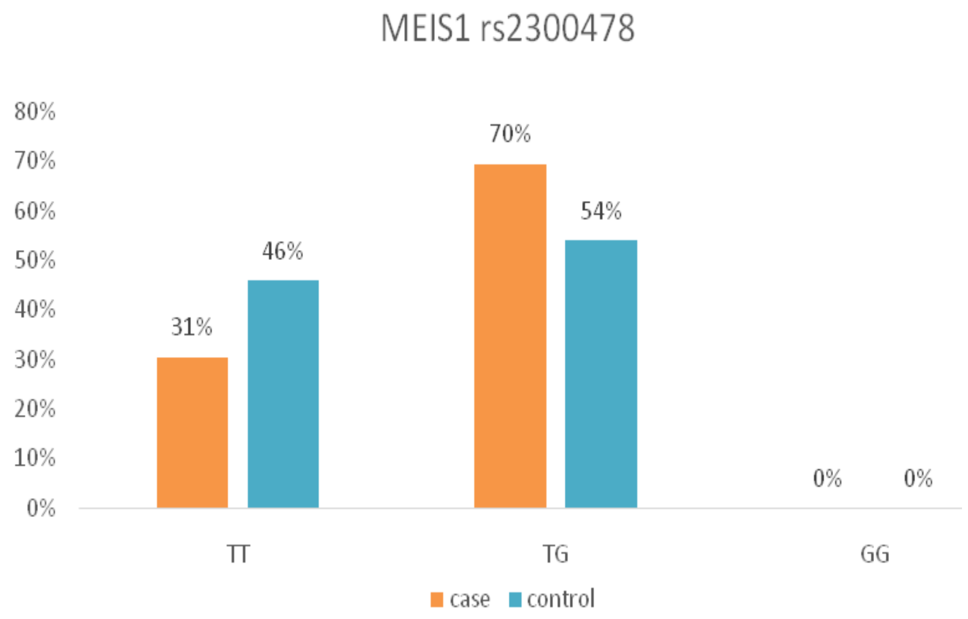

Figure 5.2 A: Genotype distribution of MEIS 1 SNP among the healthy and case subjects' analysis. MEIS1: the analysis indicates that the T/G heterozygous mutation was mostly present in cases (70% vs. 54%) while T/T homozygous mutation was mostly distributed among controls (30% vs. 46%), whereas no case or control showed any wild type G/G allele.

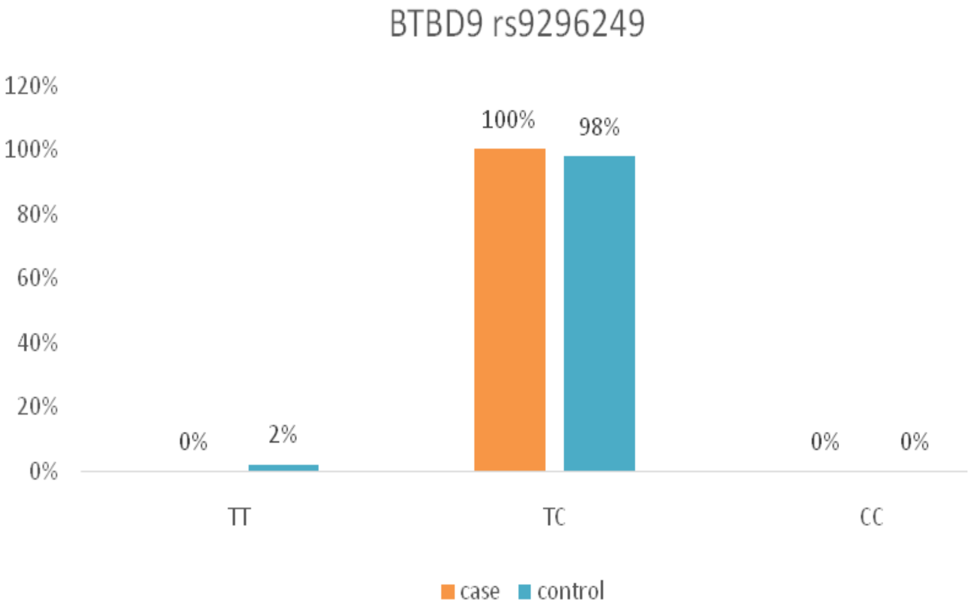

Figure 5.2 B: Genotype distribution of BTBD9 SNP among the healthy and case subjects' analysis.BTBD9: the analysis indicates that wild and mutant type alleles were mostly distributed equally among case and control T/C heterozygous allele (100% vs 98%).

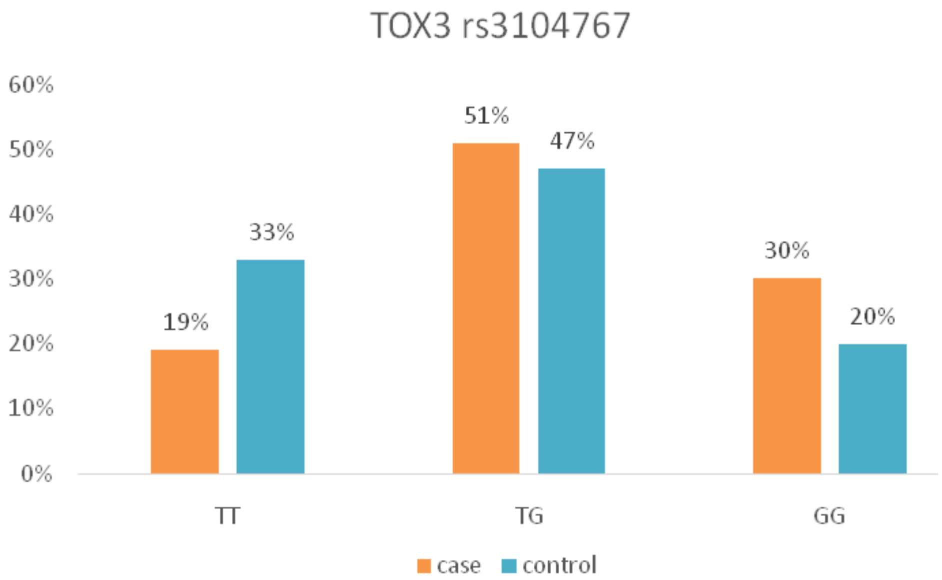

Figure 5.2 C: Genotype distribution of TOX3 SNP among the healthy and case subjects' analysis.TOX3: the analysis shows of (19% vs 33%) distribution of case and control in T/T homozygous allele, (51% vs 47%) distribution of case and control in T/G heterozygous allele and (30% vs 20%) distribution in wild G/G allele.

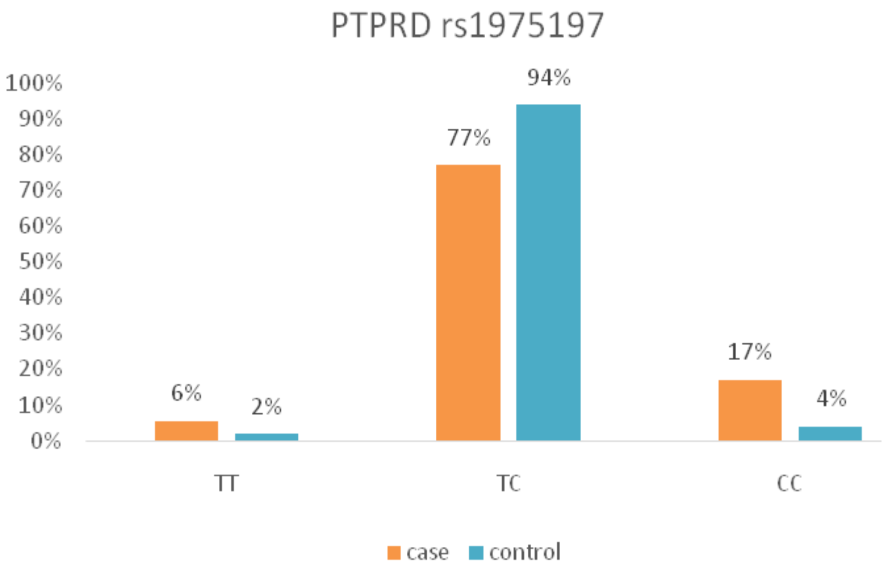

Figure 5.2 D: Genotype distribution of PTPRD SNP among the healthy and case subjects' analysis PTPRD: here the majority distribution of case and control is in T/C heterozygous allele (77% vs 94%) while T/T homozygous allele has (6% vs 2%) distribution for case and control and (17% vs 4%) distribution in C/C wild type allele.

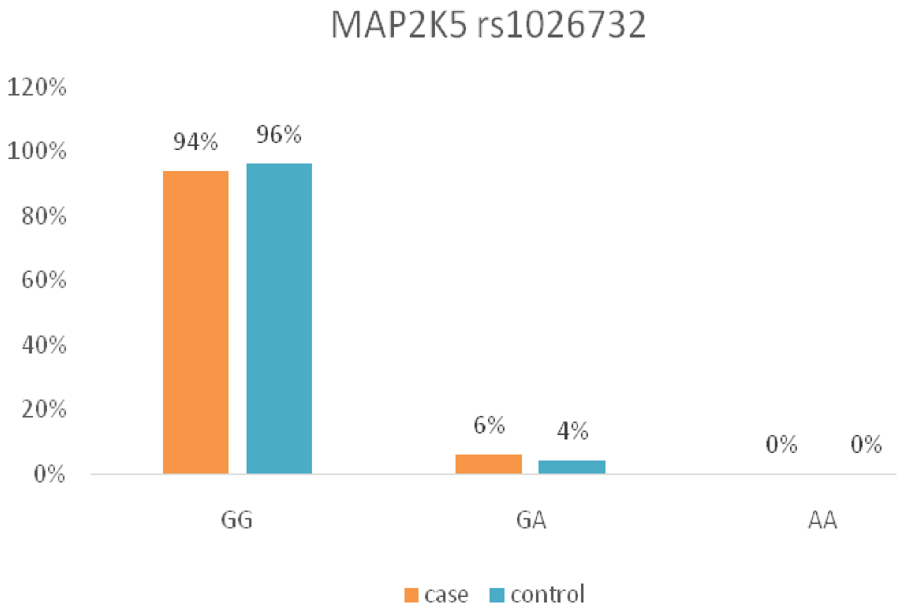

Figure 5.2 E: Genotype distribution of MAP2K5 SNP among the healthy and case subjects' analysis.MAP2K5: here the distribution is predominantly in G/G homozygous allele (94% vs 96%) with some distribution in G/A heterozygous allele (6% vs 4%). Table summarizes the RLS SNP and their correlation with clinical parameters (Table 5.3 a,b,c) .

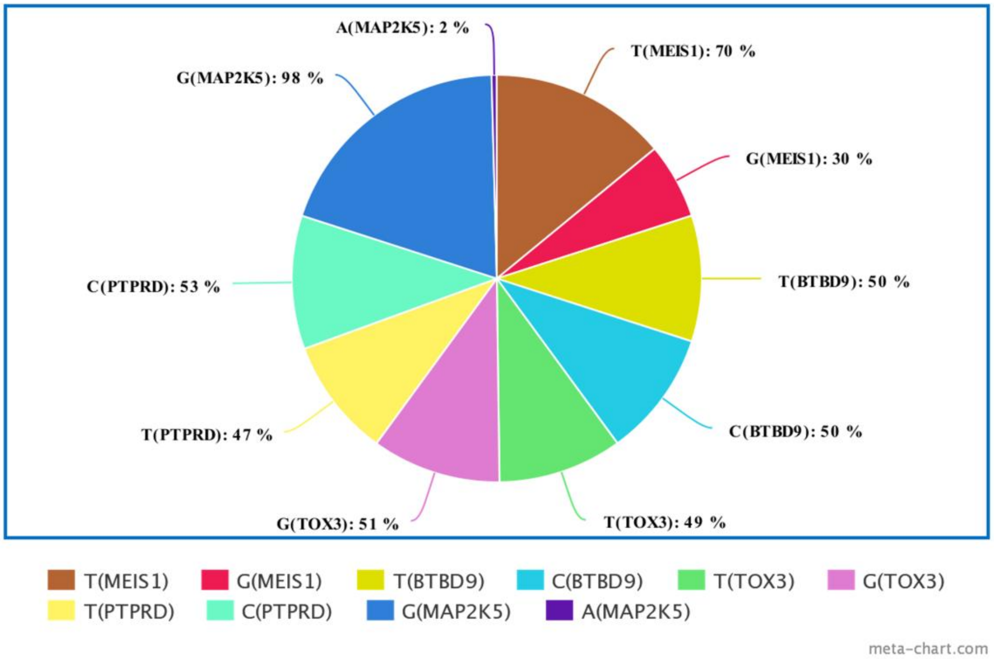

Figure 5.3: Pie chart showing allele frequency of SNP under study,