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Research article

Estrogen Receptor 1 Gene Polymorphism and its Association with Idiopathic Short Stature in North Indian Population

Short title: ESR1 SNPs in ISS in North Indians

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Abstract
Background: In the hypothalamic-pituitary-gonadotrophin (HPG) axis, estrogen plays a key role in the bone maturation regulation and growth plates closure. This study was designed to explore the link between single nucleotide polymorphisms (SNPs) in estrogen receptor 1 (ESR1) gene with idiopathic short stature (ISS) susceptibility in the North Indian population.
Methods: Four SNPs of the ESR1 gene (rs543650, rs6557177, rs2234693 and rs9340799) were genotyped by Sanger sequencing in 52 ISS patients and 68 controls. Linkage disequilibrium (LD) and haplotyping were done by SNPstat and SHESISplus softwares. Extent of LD was determined by calculating D′ and r^2 values in SNPs paired combinations.
Results: A significant positive association was found between rs6557177 and rs543650 genotype and ISS susceptibility as compared to controls. The frequencies of the rs6557177 CC genotype (p=0.030; OR=0.13; 95% CI:0.01-1.10) and rs543650 genotype TT (p =0.043; OR=0.29; 95% CI: 0.09-0.92) were observed to be increased in ISS group as compared with the control group. However, no significant correlation was observed between clinical parameters of patients and these SNPs. rs543650 shown strong LD with rs2234693 and rs9340799, similarly rs2234693 and rs9340799.
Conclusion: Our study showed that CC genotype at rs6557177 and TT genotype of rs543650 of ESR1 constitutes risk factor for developing ISS in North Indian children. In the future, these findings may lead to a better understanding of the SNPs associated with ISS susceptibility.
Keywords: Genotype, Estrogen Receptor 1 gene, Haplotype, Idiopathic short stature, Linkage Disequilibrium, Single nucleotide polymorphism

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Background

Height less than 2 standard deviations from the corresponding mean for a given age, gender, and population is considered short stature.[1] The worldwide prevalence of short stature is approximately 3-5%.[2] Causes of short stature vary widely; physiological short stature can be familial or constitutional, while pathological causes can be systemic or secondary to environmental factors. Once these factors are ruled out or excluded, the condition is diagnosed as idiopathic short stature (ISS). Looking further into the genetic factors, numerous monogenic and syndromic causes for short stature have already been well established over the years, and genes concerned with growth regulators have been a major culprit. Among the growth regulators, the growth hormone (GH) performs a major and vital role and variation in its sequence may result in growth hormone deficiency (GHD) which subsequently leads to short stature.[3–6] There are many other genes (IGF1, SHOX, GHRHR and PROP1) sequence variations, chromosomal abnormalities, copy number changes and impaired genomic imprinting which also contribute to short stature.[5]
Two axes, namely the hypothalamic-pituitary-gonadal (HPG) axis and the growth hormone-insulin like growth factor (GH-IGF) axis were thought to play key roles in the regulation of growth, according to previously published data.[7] Any kind of abnormality in the latter GH-IGF axis is well known to result in short stature,[8] especially with mutations in genes like GHR,[9] IGFALS[10] and IGF-1R[11,12] but there is paucity of data on association of short stature risk with HPG axis. However, recent findings have revealed that the GH-IGF-I axis is just one of many regulatory systems that control chondrogenesis in the growth plate[13].
Estrogen is an important hormone in the HPG axis, that is associated with the regulation of bone maturation and growth. It is already known to be involved in male and female reproduction as well as in other systems including neuro endocrine, vascular, skeletal, and immune system. ESR1 gene codes for estrogen receptor and is located on chromosome 6 long arm. Size of ESR1 gene is 300Kb and it contains 8 exons. It has shown strong association with stature via Genome-wide linkage analysis. The role of estrogen is already well established in skeletal development and growth in females. Recently it has been recognised to affect body height in males as well.[14]
Single nucleotide polymorphisms (SNPs) in growth associated genes are considered an important cause of short stature. There is a possibility that other, more common variants of ESR1 with smaller effects could affect body height in the general population based on the powerful effects of rare sequence variations in the ESR1. Therefore, we hypothesized that ESR1 could be a factor that controls the tempo of growth and stature. Literature review was done for similar studies in the past which corroborated the hypothesis. El-Hefnawy et al[15] studied the rs827421 SNP in the ESR1 gene and found that the GG genotype and the G allele were significantly dominant among children with constitutional delay of growth and puberty (CDGP). Another study by Charmian et al[16] on the rs2234692 SNP in the same gene, showed similar results in children with ISS. Byung Ho Kang studied three SNPs in ESR1, namely rs3778609, rs12665044 and rs827421, and found positive results in the latest one.
We chose four rarely studied polymorphisms (rs543650, rs6557177, rs2234693 and rs9340799)[17] in the ESR1 gene for our study. We also assessed patterns of linkage disequilibrium (LD) of these selected SNPs.

Materials and Methods

Subjects: This was a prospective study done in a tertiary care hospital from July 2021 to June 2023. This study was approved by Institutional Ethics Committee (Ref: NK/7784/MD/527). Power of the study was calculated using Quanto (http://biostats.usc.edu/Quanto.html). Children (n=52) with idiopathic short stature (ISS) i.e., they have heights that are less than two standard deviations (SD) below the mean height for their age, gender, and population, with physiological, environmental, systemic and genetic causes ruled out, were enrolled. This included normal growth hormone (GH) levels.

Control group:

Control group included 68 children who were having height within +/- 2 SD of the mean height of normal. The control group was comprised of children who came to our hospital in the same time frame for routine immunization or for OPD management of transient viral illnesses and siblings of admitted children.

Inclusion criteria

1. A height that is less than 2 SDs above or below the mean height of kids of the same age and gender
2. Normal routine investigation of blood, thyroid function, liver, and kidney function
3. The weight and length at birth should fall within the normal range
4. There shouldn't be any additional inherited metabolic diseases, congenital skeletal anomalies, chromosomal abnormalities, SHOX mutation, or chronic illnesses

Exclusion criteria:

Short stature with an identified etiology

Informed consent: For children fulfilling inclusion and exclusion criteria, parents / guardian was approached for enrolment in the study. Prior to enrolment in the study, written informed consent was obtained after providing a thorough information sheet. Assent was sought from children above 8 years of age.

Evaluation: After taking fully informed consent, all individuals diagnosed with ISS were included in the study.

1. A detailed case review was performed including review of family history, previous medical records and investigation reports.
2. A thorough anthropometric evaluation was performed, including measurements of the body mass index, height, arm span, upper-to-lower segment ratio, arm span to height, and sitting height to height.
3. Detailed physical examination was done to look for any deformity or dysmorphism, specifically for skeletal abnormalities and facial features.

Sample collection: After written consent, 2-4 ml EDTA blood was collected for DNA extraction, from the patients and controls.

Molecular analysis: Genomic DNA was extracted from peripheral blood by QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany).

Genotyping:

Sanger sequencing was done for genotyping and the primers used can be shared on request. Applied Biosystems' BigDye® Terminator Cycle Sequencing kit v3.1 was used to perform direct DNA sequencing on purified amplicons using forward and/or reverse primers in a PCR reaction. The results were analysed using ABI-3500xl Genetic Analyzer.

Statistical analysis

The Statistical Package for Social Sciences for Windows (SPSS version 14.0, Chicago, Illinois, USA) was used to conduct the statistical analysis. The frequencies of alleles and genotypes were calculated. For each polymorphism under consideration, the Hardy Weinberg equilibrium (HWE) was calculated. Fisher's exact test and ANOVA were used to compare gene polymorphisms within groups and between subgroups, respectively. Subgroups were further examined using the t test and Wilcoxon-Mann-Whitney analysis, depending on whether the data is parametric or not. Calculating odds ratios and 95% confidence intervals, a p-value of 0.05 was set as cut-off for statistical significance. For genotype frequencies, logistic regression was used and for allele frequency, and cross-tabular calculation of odds ratio and chi-square values were calculated, using SPSS, version 25. To rule out the effect of confounding factors on analysis, all the results were adjusted by the confounding factors (age, gender, height, BMI). Haplotype analysis was done using SNPstats online software[18] for all four SNPs. The extent of LD was determined in SNPs combinations by D′ and r2 values. The SHESIS plus (http://shesisplus.bio-x.cn/SHEsis.html) and SNPstat (https://www.snpstats.net/start.htm) online tools were used to assess the LD between ESR1 haplotypes (rs543650, rs6557177, rs2234693 and rs9340799).

Results:

Among cases, 53.8% of the participants in the group were male and 46.2% of the participants in the group were female. Among controls, 64.7% of the participants were male. 35.3% of the participants were female. The age of cases with ISS is 11 (8-13) years and controls are 8 (5.75-11) years. However, there were no significant differences in BMI in the control and patient group. Mean height standard deviation score (SDS) in ISS group was significantly less than that of the control group ($P < 0.001$), as expected (Table 1).

Genotyping:

The Genotypes were found to be in Hardy-Weinberg equilibrium, in cases and controls, for all four SNPs.

1. Genotypic and Allelic frequencies of ESR1 SNPs among cases and controls

rs543650

TT, GT and GG genotypes of rs543650 were found to be 12 (23.1%), 15 (28.8%) and 25(48.1%) among cases and 5 (7.4%), 27 (39.7%) and 36 (52.9%) among controls respectively. There was a significant association between the various cases and controls in terms of genotypic frequency of rs543650 (OR=3.46; 95%CI: 1.08-11.04; **p = 0.036**) (Table 2).

rs6557177

The CC, CT and TT genotypes of SNP rs6557177 were found to be 6 (11.5%), 7 (13.5%) and 39 (75%) among cases and 1 (1.5%), 16 (23.5%) and 51 (75%) among controls respectively. There was a significant association between the various cases and controls in terms of genotypic frequency of rs6557177, when compared between the wild and homozygous mutant genotypes (OR=0.073; 95%CI: 0.01-0.072; **p = 0.025**) (Table 2). The allelic frequencies were however similar in both groups.

rs2234693

The TT, CT and CC genotypes of rs2234693 were found to be 21 (40.4%), 24 (46.2%) and 7 (13.5%) among cases and 27 (39.7%), 23 (33.8%) and 18 (26.5%) among controls respectively. There was no significant association between the various cases and controls in terms of genotypic nor allelic frequency of rs2234693 (Table 2).

rs9340799

The AA, AG and GG genotypes of rs9340799 were found to be 24 (46.2%), 23 (44.2%) and 5 (9.6%) among cases and 30 (44.1%), 28 (41.2%) and 10 (14.7%) among controls respectively. There was no significant association between the various cases and controls in terms of genotypic frequency of rs9340799 (Table 2).

Analyses of baseline variables by genotype:

The clinical characteristics of the participants, including age, height, body weight, BMI, and MPH were summarized (Table 1). Further significant SNPs (rs6557177 and rs543650) were compared for baseline variables. The differences in age, height, body weight, BMI, and MPH of the wild type genotype compared to hetero and mutant genotypes at loci rs6557177 and rs543650 of ISS were not statistically significant (P>0.05) (Table 3).

Association Between 'Growth Hormone Therapy' and ESR1 SNPs (rs6557177 and rs543650) in patients:

The association between Growth Hormone Therapy and ESR1 SNPs (rs6557177 and rs543650) was done using Fisher's exact test, however, could not get significant association (Table 4)

Linkage disequilibrium:

Measures of linkage disequilibrium (LD) play a key role in a wide range of applications from disease association to demographic history estimation. To search for LD between pairs of SNPs, pooled genotyping data for patients and controls were analyzed. Both the global statistic R2 and the statistic D′, which takes into consideration the limitations on R imposed by the different allele frequencies of the marker pair, were developed. A stronger disequilibrium between the alleles is suggested when the D' and R2 values are closer to 1, indicating a high likelihood of the alleles co-inheriting. The values are suggestive of allele independence if they are nearer or equal to zero. It is evident that the rs543650 is in linkage disequilibrium with rs2234693 and rs9340799 (D’ value: 0.73, 0.84 respectively). rs2234693 and rs9340799 also showed strong LD (D’: 0.89). A matrix with each LD statistic selected is shown in figure 1. Weak LD between rs543650-rs6557177; rs6557177-rs2234693 and rs6557177-rs9340799 of ESR1 were observed, as suggested by low D’ values (0.16, 0.18 and 0.3 respectively). Low R2 values (0.01,0.0 and 0.03 respectively) contradict the above alleles' coinheritance. The low allele frequencies may be the cause of the observed low R2 values.

Haplotype:

A haplotype analysis of all the SNPs was performed using haplotype frequencies predicted by the Shesisplus. However, there were no significant differences between cases and controls (Table 5).

Discussion:

We investigated correlations between growth/height-related genetic polymorphisms in the ESR1 gene and the phenotype of ISS in order to better understand the etiology of the enigmatic growth disorder that accounts for a significant portion of pediatric endocrine practice. A study with animal model mice with knocked out ESR1 gene have shown that ESR1 gene plays a role in early growth plate fusion.[19] Emons et al have shown that estradiol level stimulates the local VEGF at growth plate during puberty,[20] although actual mechanism is not known but it is a matter of research.

In this study, we have taken 4 SNPs of ESR1 gene rs2234693, rs9340799, rs543650 and rs6557177 and analyzed in ISS population and control population presenting in north Indian tertiary care center. For the SNP rs543650, the wild allele (T) is found to be in lower frequency (0.3568) than the alternate allele (0.6432) in South Asian population, as per dbSNP.[21]

Out of these 4 SNPs, we have found significant association of rs6557177 and rs543650 with ISS in our cohort. ESR1 SNP rs6557177 CC genotype (OR=0.073, p-value=0.025) and rs543650 genotype TG (OR=3.46, p- value=0.036) was found to be significantly associated with ISS, the former being a protective factor while the latter is a risk factor. This means that people with TG genotype of rs543650 were more likely to have ISS than people with other genotypes and the people with CC genotype of rs6557177 are less likely to be affected. SNP rs6557177 T>C was found to be increased in ISS group in a study from Chinese population.[22] SNP rs543650T>G was also found to be associated with decreased bone marrow density by Scalco et al.[23] .

Our findings that the "tall" (T) and (C) alleles at rs543650 and rs6557177, respectively, are significantly more common in our ISS cohort compared to controls provides independent evidence for an association between ER and stature, indicating that variations in estrogen sensitivity may play a role in the impaired growth that characterizes ISS. Additionally, we looked for associations with response to GH treatment with selected ESR1 SNPs. However, in our ISS cohort, we did not find a relationship between ESR1 alleles and GH treatment response. Sowińska-Przepiera et al. have shown an association of ESR1 rs2234693 and rs9340799 with bone mass gain in lumbar spine after the onset of estrogen replacement in Turner syndrome patient in adult cohort.[24] A study by Harlid et al have shown that women with SNP rs851987 in ESR1 tend to have taller stature.[25] Dahlgren et al have found that ESR1 SNP rs2179922 is associated with height in cohort of 2 Swedish population.[26] The ESR1 rs2234693 polymorphism (PVUII intron 1) has been linked to height during puberty[10,27] and adulthood[28,29]. However, we could not find any association of rs2234693, rs9340799 SNPs with ISS in our cohort. Additionally, two unrelated patients have been reported to have recessive germline ESR1 mutations that result in estrogen resistance and significant pubertal growth delays.[26,30]According to this study's findings, ESR1 gene polymorphisms may contribute to the emergence of ISS. Further studies are needed to confirm these findings and to investigate the underlying mechanisms by which ER1 gene polymorphisms contribute to ISS. However, the findings regarding the association between ESR1 gene polymorphisms and idiopathic short stature are still inconclusive and inconsistent. The genetic basis of idiopathic short stature is likely to be multifactorial, involving interactions between multiple genes and environmental factors. Therefore, it is important to consider that ESR1 gene polymorphisms alone may not fully explain the development of idiopathic short stature.

Further research on larger sample size is needed to better understand the role of ESR1 gene polymorphisms in idiopathic short stature. Larger and more comprehensive studies, including diverse populations, are necessary to provide more definitive conclusions regarding the genetic factors contributing to this condition. It's also important to note that genetic factors are just one aspect of a complex interplay of factors involved in growth and development.

Limitations:

Our study has several limitations, including a small sample size that limits our ability to detect associations, especially when genetic effects are small, and an even smaller sample size when we divide our sample by sex, which prevents us from conducting sex-specific analyses. A smaller sample size can make it more difficult to detect statistically significant results, and can also make it more difficult to generalize the results of the study to the wider population.

Strengths:

Being a prospective study, children were subjected to assessment of phenotype and then enrolled for Sanger sequencing for ESR1 gene SNPs. Hence, odds of picking the wrong population for sample analysis was very less. Also, SNPs of ESR1 gene are not widely studied as a cause of idiopathic short stature in our part of the world and the study would be a stepping stone in this direction. This study has provided valuable data that can be used to further investigate the role of SNPs in ESR1 gene in idiopathic short stature. The phenotypic genotypic correlation would also be an area of interest in the upcoming years. It will help to find alternate etiology in cases of ISS and will be helpful to give direction for new research in field of treatment of idiopathic short stature. This study has provided valuable data that can be used to design new studies to investigate the role of SNPs in ESR1 gene in ISS.

Declarations

- **Availability of data and materials:** All data generated or analyzed during this study are included in this article

- **Ethics approval and consent to participate:** The study protocol was approved by Institutional Thesis Committee and Institutional Ethics Committee (Approval no.: NK/6656/MS/315).
- **Consent for publication:** Written informed consent was obtained from the participants.
- **Conflict of interest:** The authors declare that they have no competing interests.
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Table 1. Descriptive clinical and laboratory data of studied cases and controls

Parameters	Group		p value
	Cases (n = 52)	Controls (n = 68)	
Gender			0.229
Male	28 (53.8%)	44 (64.7%)	
Female	24 (46.2%)	24 (35.3%)	
Median Age (Years)	11 (8-13)	8 (5.75-11)	NS
Weight (Kg)	24.88 ± 10.58	28.41 ± 13.41	0.312
Weight for Age (SDs)	-1.91 ± 1.24	-0.40 ± 1.16	**<0.001**
Height (cm)	120.40 ± 18.71	127.35 ± 21.94	0.064
Height for Age (SDs)	-2.78 ± 1.15	-0.42 ± 0.97	**<0.001**
BMI (Kg/m²)	16.36 ± 3.20	16.66 ± 3.34	0.721
Arm Span (cm)	118.14 ± 19.15	127.93 ± 21.84	**0.010**
AS to Height Difference (cm)			**<0.001**
Significant	12 (23.1%)	0 (0.0%)	
Not Significant	40 (76.9%)	68 (100.0%)	
AS-to-Height Ratio	0.98 ± 0.03	1.00 ± 0.01	**<0.001**
US-to-LS Ratio	1.00 ± 0.08	1.09 ± 0.11	**<0.001**
US-to-LS Ratio for Age			**<0.001**
WNL	24 (46.2%)	68 (100.0%)	
Abnormal	28 (53.8%)	0 (0.0%)	
MPH (cm)	156.77 ± 6.69	167.18 ± 7.88	**<0.001**
Region: U/L			1.000
Normal	52 (100.0%)	68 (100.0%)	
Abnormal	0 (0.0%)	0 (0.0%)	
Region: Wrist and Hands			0.433
Normal	51 (98.1%)	68 (100.0%)	
Abnormal	1 (1.9%)	0 (0.0%)	
Region: L/L and feet			1.000
Normal	52 (100.0%)	68 (100.0%)	
Abnormal	0 (0.0%)	0 (0.0%)	
Region: Thoracolumbar spine			1.000
Normal	52 (100.0%)	68 (100.0%)	
Abnormal	0 (0.0%)	0 (0.0%)	
Growth Hormone Therapy (Yes)	11 (21.2%)	0 (NaN%)	1.000
Parents Affected (Yes)	16 (30.8%)	0 (0.0%)	**<0.001**
Sibling/ Relative Affected (Yes)	10 (19.2%)	0 (0.0%)	**<0.001**
Micrognathia	2 (3.8%)	0 (0.0%)	0.186
High- Arched Palate	6 (11.5%)	0 (0.0%)	**0.006**
Short Arms and Forearms	24 (46.2%)	0 (0.0%)	**<0.001**
Cubitus Valgus	2 (3.8%)	0 (0.0%)	0.186
Madlung Deformity	2 (3.8%)	0 (0.0%)	0.186
Short Leg and Feet	20 (38.5%)	0 (0.0%)	**<0.001**
Genu Valgum/ Bowing of Tibia	0 (0.0%)	0 (0.0%)	1.000
Muscular Hypertrophy	0 (0.0%)	0 (0.0%)	1.000

Table 2. Comparison of Genotype and Allele frequencies of 4 SNPs between cases and controls

SNP	Cases n (%)	Controls n (%)	OR (95% CI)	p-value
rs543650				
TT	12 (23.1%)	5 (7.4%)		
TG	15 (28.8%)	27 (39.7%)	**3.46 (1.08-11.04)**	**0.036**
GG	25 (48.1%)	36 (52.9%)	0.80 (0.36-1.80)	0.590
T	39 (37.5%)	37 (27.2%)	1.61(0.93-2.78)	$\chi2 = 2.886$ p-value – 0.089
G	65 (62.5%)	99 (72.8%)		
rs6557177				
TT	39 (75%)	51 (75%)		
TC	7 (13.5%)	16 (23.5%)	0.13 (0.02-1.10)	0.127
CC	6 (11.5%)	1 (1.5%)	**0.073 (0.01-0.72)**	**0.025**
T	85 (81.7%)	118 (86.8%)	0.68 (0.34-1.38)	$\chi2 = 1.145$ p-value – 0.285
C	19 (18.3%)	18 (13.2%)		
rs2234693				
TT	21 (40.4%)	27 (39.7%)		
TC	24 (46.2%)	23 (33.8%)	2.00(0.71-5.67)	0.193
CC	7 (13.5%)	18 (26.5%)	2.68 (0.95-7.62)	0.064
T	66 (63.5%)	77 (56.6%)	1.33 (0.79-2.25)	$\chi2 = 1.146$ p-value – 0.284
C	38 (36.5%)	59 (43.4%)		
rs9340799				
AA	24 (46.2%)	30 (44.1%)		
AG	23 (44.2%)	28 (41.2%)	1.60(0.48-5.31)	0.443
GG	5 (9.6%)	10 (14.7%)	1.64(0.49-5.49)	0.420
A	71 (68.3%)	88 (64.7%)	1.17 (0.68-2.02)	$\chi2 = 0.335$ p-value – 0.563
A	33 (31.7%)	48 (35.3%)		

Table 3: Baseline data by genotypes of rs6557177 and rs543650

Parameters	rs6557177				rs543650			
	CC (n = 6)	TC (n = 7)	TT (n = 39)	p value	GG (n=25)	GT (n=15)	TT (n=12)	p value
Age (Years)	13.17 ± 2.14	11.00 ± 3.27	9.90 ± 3.72	0.097	10.68 ± 3.56	10.20 ± 3.69	10.17 ± 3.97	0.881
Gender				0.176				0.113
Male	5 (83.3%)	2 (28.6%)	21 (53.8%)		13 (52.0%)	11 (73.3%)	4 (33.3%)	
Female	1 (16.7%)	5 (71.4%)	18 (46.2%)		12 (48.0%)	4 (26.7%)	8 (66.7%)	
Weight for Age (SDs)	-1.65 ± 0.61	-2.03 ± 1.66	-1.93 ± 1.25	0.660	-1.58 ± 1.15	-2.31 ± 1.15	-2.11 ± 1.43	0.129
Height for Age (SDs)	-2.84 ± 0.50	-2.56 ± 1.11	-2.81 ± 1.24	0.660	-2.59 ± 0.80	-3.15 ± 1.19	-2.70 ± 1.64	0.199
BMI (Kg/m²)	17.60 ± 2.32	15.89 ± 4.25	16.26 ± 3.14	0.239	17.08 ± 3.61	15.65 ± 2.35	15.77 ± 3.09	0.487
MPH (cm)	162.98 ± 7.75	154.27 ± 4.59	156.26 ± 6.43	0.071	156.03 ± 6.94	158.65 ± 5.98	155.96 ± 7.09	0.355

Table 4: ESR1 SNPs (rs543650 and rs6557177) and growth hormone therapy in cases

ESR1 SNPs	Growth Hormone Therapy		Fisher's Exact Test
	Yes	No	p-value
rs543650			
GG	4 (36.4%)	21 (51.2%)	0.511
GT	3 (27.3%)	12 (29.3%)	
TT	4 (36.4%)	8 (19.5%)	
rs6557177			
CC	1 (9.1%)	5 (12.2%)	0.833
CT	2 (18.2%)	5 (12.2%)	
TT	8 (72.7%)	31 (75.6%)	

Table 5. Haplotype association of 4 selected SNPs (adjusted by age and sex)

	rs543650	rs6557177	rs2234693	rs9340799	Freq	OR (95% CI)	P-value
1	G	T	T	A	0.2925	1.00	---
2	T	T	T	A	0.2546	0.53 (0.25 - 1.13)	0.1
3	G	T	C	G	0.2239	0.90 (0.40 - 2.04)	0.81
4	G	T	C	A	0.0647	0.62 (0.19 - 2.06)	0.43
5	T	T	C	G	0.0399	0.73 (0.18 - 2.89)	0.65
6	G	C	C	G	0.0394	0.54 (0.13 - 2.32)	0.41
7	T	C	T	A	0.034	0.66 (0.12 - 3.60)	0.63
8	T	T	T	G	0.0232	0.65 (0.09 - 4.95)	0.68
9	G	C	T	A	0.016	0.44 (0.03 - 7.67)	0.57
10	T	C	C	G	0.006	0.00 (-Inf - Inf)	1

Figure 1. The plot showing linkage disequilibrium (D' and R^2 values) between the four SNPs. Red colour shows more strong LD