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

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### What is already known on this topic?

In the hypothalamic-pituitary-gonadotrophin (HPG) axis, estrogen plays a key role in bone maturation regulation and growth plate closure. Estrogen is an important hormone in the HPG axis, and because of its association with bone maturation, modulates growth. Estrogen is involved in male and female reproduction but also affects other systems, including the neuroendocrine, vascular, skeletal, and immune systems. Single nucleotide polymorphisms in estrogen receptor 1 *(ESR1)* gene are known to be involved in growth and development.

### What this study adds?

We investigated correlations between growth/height-related genetic polymorphisms in the *ESR1* gene and the phenotype of idiopathic short stature (ISS) in order to better understand the etiology of this enigmatic growth disorder that accounts for a significant portion of pediatric endocrine practice. Our study showed that the CC genotype at rs6557177 and also TT genotype of rs543650 of *ESR1* individually constituted risk factor for developing ISS in North Indian children. We have shown that rs543650 is in linkage disequilibrium (LD) with rs2234693 and rs9340799, rs2234693 and rs9340799 also showed strong LD (D': 0.89).

## Abstract

**Objective:** In the hypothalamic-pituitary-gonadotrophin axis, estrogen plays a key role in the regulation of bone maturation and growth plate closure. This study was designed to explore the link between single nucleotide polymorphisms (SNPs) in the estrogen receptor 1 *(ESR1)* gene with idiopathic short stature (ISS) susceptibility in a North Indian population.

**Methods:** Four SNPs of *ESR1* (rs543650, rs6557177, rs2234693 and rs9340799) were genotyped by Sanger sequencing in ISS patients and controls. Linkage disequilibrium (LD) and haplotyping were done by SNPStat and SHEsisPlus software. The extent of LD was determined by calculating D' and $R^2$ values in SNP paired combinations.

**Results:** Fifty-two ISS patients were compared with 68 controls. A significant positive association was found between rs6557177 and rs543650 genotype and ISS susceptibility. The frequencies of the rs6557177 CC genotype [p = 0.030; odds ratio (OR) = 0.13; 95% confidence interval (CI): 0.01-1.10] and rs543650 genotype TT (p = 0.043; OR = 0.29; 95% CI: 0.09-0.92) were increased in the ISS

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group compared with controls. However, no significant correlation was observed between clinical parameters of patients and these SNPs. rs543650 showed strong LD with rs2234693 and rs9340799, similarly rs2234693 and rs9340799.
**Conclusion:** Our study showed that the CC genotype at rs6557177 and TT genotype at rs543650 of *ESR1* constituted a risk factor for developing ISS in North Indian children. 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

## Introduction

Height of less than two standard deviations (SD) 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 been well established and genes concerned with growth regulators have emerged as major culprits. Among the growth regulators, growth hormone (GH) performs a major and vital role and variation in its sequence may result in GH deficiency which subsequently leads to short stature (3,4,5,6). There are many other genes, such as *IGF1*, *SHOX*, *GHRHR* and *PROP1*, in which sequence variations, chromosomal abnormalities, copy number changes and impaired genomic imprinting are known to contribute to short stature (5).

Two axes, namely the hypothalamic-pituitary-gonadal (HPG) axis and the GH-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 the genes *GHR* (9), *IGFALS* (10) and *IGF-1R* (11,12) but there is paucity of data on association of short stature risk with the HPG axis. However, recent findings have revealed that the GH-IGF-1 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. Estrogen is involved in male and female reproduction but also affects other systems, including the neuroendocrine, vascular, skeletal, and immune systems. The *ESR1* gene codes for the estrogen receptor (ER) and is located on the long arm of chromosome. The size of *ESR1* is 300Kb and it contains eight 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 *ESR1*. Therefore, we hypothesized that *ESR1* could be a factor that controls the tempo of growth and stature. A literature review was performed for similar earlier studies, which corroborated the hypothesis. El-Hefnawy et al. (15) studied the rs827421 SNP in *ESR1* 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 Quigley et al. (16) on the rs2234692 SNP in the same gene, showed similar results in children with ISS. Kang et al. (17) studied three SNPs in *ESR1*, namely rs3778609, rs12665044 and rs827421, and found positive results in SNP rs827421 only. 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.

## Methods

### Subjects

This was a prospective study done in a tertiary care hospital from July 2021 to June 2023. This study was approved by Postgraduate Institute of Medical Education and Research Institutional Ethics Committee (ref: NK/7784/MD/527, date: 28.09.2021). The power of the study was calculated using Quanto (http://biostats.usc.edu/Quanto.html). Children with ISS i.e., with heights that are less than two SDs below the mean height for their age, gender, and population, with physiological, environmental, systemic and genetic causes ruled out, were enrolled. This included normal GH levels.

### Control Group

The control group included children who had a height within +/- 2 SD of the mean height for normal. The control group was comprised of children who came to our hospital

in the same time frame for routine immunization or for outpatient management of transient viral illnesses and siblings of admitted children.

### Inclusion Criteria for ISS

1. A height less than 2 SDs below the mean height of children of the same age and gender;

2. Normal routine investigation of blood (complete blood count), 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 detailed information sheet. Assent was sought from children above eight 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 (BMI), 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 of 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 are available 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 polymerase chain reaction. The results were analysed using ABI-3500xL Genetic Analyzer (Applied Biosystems, California).

### Statistical Analysis

The Statistical Package for Social Sciences (SPSS) for Windows version 14.0, was used for statistical analysis (Chicago, IL, USA). 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 was parametric or not. Calculating odds ratios (ORs) and 95% confidence intervals (CI), 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 OR 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, including age, gender, height, and BMI. Haplotype analysis was done using SNPStats online software (18) for all four SNPs. The extent of LD was determined in SNP combinations by calculating D' and $R^2$ values. The SHEsisPlus (http://shesisplus.bio-x.cn/SHEsis.html) and SNPStats (https://www.snpstats.net/start.htm) online tools were used to assess the LD between *ESR1* haplotypes (rs543650, rs6557177, rs2234693 and rs9340799).

## Results

There were 52 patients with ISS and the control group included 68 children (Table 1). Among the ISS cases, 53.8% were male, while in the control group 64.7% of the participants were male. The median age of cases with ISS was 11 (8-13) years and controls was 8 (5.75-11) years. However, there were no significant differences in BMI in the control and patient group. Mean height SD score (SDS) in the 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 HWE, in cases and controls, for all four SNPs investigated.

**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 |

AS: arm span, SD: standard deviation, US to LS: upper segment to lower segment, U/L: upper limb, L/L: lower limb, WNL: within normal limits, bold values are representing significant p values, MPH: mid-parental height, NS: not significant, BMI: body mass index

### 1. Genotypic and Allelic Frequencies of *ESR1* SNPs Among Cases and Controls

#### rs543650

TT, GT and GG genotypes of rs543650 were found in 12 (23.1%), 15 (28.8%) and 25 (48.1%) ISS cases and in 5 (7.4%), 27 (39.7%) and 36 (52.9%) 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 in 6 (11.5%), 7 (13.5%) and 39 (75%) cases and 1 (1.5%), 16 (23.5%) and 51 (75%) 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 in 21 (40.4%), 24 (46.2%) and 7 (13.5%) cases and 27 (39.7%), 23 (33.8%) and 18 (26.5%) controls, respectively. There was no significant association between the various cases and controls in terms of genotypic or allelic frequency of rs2234693 (Table 2).

#### rs9340799

The AA, AG and GG genotypes of rs9340799 were found in 24 (46.2%), 23 (44.2%) and 5 (9.6%) cases and 30 (44.1%), 28 (41.2%) and 10 (14.7%) 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 mid-parental height (MPH) were summarized (Table 1). Further significant SNPs (rs6557177 and rs543650) were compared for baseline

**Table 2. Comparison of genotype and allele frequencies of the four SNPs investigated between ISS 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) | $\chi^2 = 2.886$ 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) | $\chi^2 = 1.145$ 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) | $\chi^2 = 1.146$ 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) | $\chi^2 = 0.335$ p value-0.563 |
| **A** | 33 (31.7%) | 48 (35.3%) | | |

SNPs: single nucleotide polymorphisms, ISS: idiopathic short stature, CI: confidence interval, OR: odds ratio

variables. The differences in age, height, body weight, BMI, and MPH of the wild type genotype compared to heterozygous and mutant genotypes at loci rs6557177 and rs543650 of ISS were not significant ($p > 0.05$) (Table 3).

**Association between response to "GH therapy" and ESR1 SNPs (rs6557177 and rs543650) in patients:** The association between the response to GH therapy and *ESR1* SNPs (rs6557177 and rs543650) was done using Fisher's exact test. However, no significant association was found (Table 4).

## Linkage Disequilibrium

Measures of 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 $R^2$ 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 $R^2$ 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 was evident that the rs543650 was in LD with rs2234693 and rs9340799 (D' value 0.73 and 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 $R^2$ values (0.01, 0.0 and 0.03

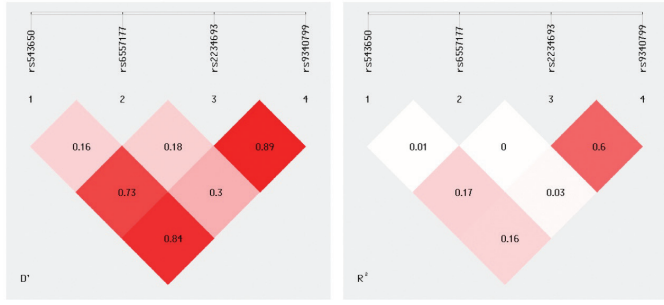


**Figure 1.** Plot showing linkage disequilibrium (D' and $R^2$ values) between the four SNPs. Increasing intensity of the red color indicates a stronger linkage disequilibrium

*SNPs: single nucleotide polymorphisms*

**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 |

MPH: mid-parental height, SDs: standard deviations, BMI: body mass index

**Table 4. ESR1 SNPs (rs543650 and rs6557177) and response to growth hormone therapy in cases**

| *ESR1* SNPs | Response to 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%) | 0.853 |
| CT | 2 (18.2%) | 5 (12.2%) | |
| TT | 8 (72.7%) | 31 (75.6%) | |

SNPs: single nucleotide polymorphisms

**Table 5. Haplotype association of the four 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 | 0.2249 | 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 |

SNPs: single nucleotide polymorphisms, CI: confidence interval, OR: odds ratio

respectively) contradict the above alleles' coinheritance. The low allele frequencies may be the cause of the observed low $R^2$ values.

### Haplotype

A haplotype analysis of all the SNPs was performed using haplotype frequencies predicted by 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 this 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* plays a role in early growth plate fusion (19). Emons et al. (20) have shown that estradiol level stimulates the local vascular endothelial growth factor level at the growth plate during puberty, although the actual mechanism is not known and is under investigation.

In the present study, we investigated four SNPs of *ESR1* (rs2234693, rs9340799, rs543650 and rs6557177) and analyzed frequencies in an ISS population and a control population presenting in a North Indian tertiary care center. For the SNP rs543650, the wild allele (T) was found to be in lower frequency (0.3568) than the alternate allele (0.6432) in a South Asian population, as per dbSNP (21).

Of these four SNPs, two (rs6557177 and rs543650) had significant association with ISS in our cohort. *ESR1* SNP rs6557177 CC genotype and rs543650 genotype TG were found to be significantly associated with ISS, the former being a protective factor while the latter was 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 were less likely to be affected. The rs6557177 T > C SNP was increased in an ISS group in a study from a Chinese population (22). Moreover, rs543650T > G was reported 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 the ER and stature, indicating that variations in estrogen sensitivity may play a role in the impaired growth that characterizes ISS.

In addition, 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. (24) have shown an association between *ESR1* rs2234693 and rs9340799 with bone mass gain in lumbar spine after the onset of estrogen replacement in Turner syndrome patient in an adult cohort. A study by Harlid et al. (25) showed that women with SNP rs851987 in *ESR1* tend to have taller stature. Dahlgren et al. (26) reported that *ESR1* SNP rs2179922 was associated with height in a cohort of two Swedish populations. 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 between rs2234693 or rs9340799 SNPs with ISS in our cohort. Furthermore, 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 *ESR1* gene polymorphisms contribute to ISS. However, the findings regarding the association between

*ESR1* gene polymorphisms and ISS are still inconclusive and inconsistent. The genetic basis of ISS 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 ISS.

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

### Study 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 will make it more difficult to detect statistically significant results, and will also limit the generalizability of the results to the wider population.

## Conclusion

Being a prospective study, children were subjected to assessment of phenotype and then enrolled for Sanger sequencing for *ESR1* gene SNPs. Hence, the risk of selecting the wrong population for sample analysis was very low. In addition, SNPs of *ESR1* gene are not widely studied as a cause of ISS 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 *ESR1* SNPs in ISS. The phenotypic/genotypic correlation would also be an area of interest in the upcoming years. It will help to identify alternate etiologies in cases of ISS and will be helpful to give direction for new research in the field of treatment for ISS. We believe that this study has provided valuable initial data that can be used to design new studies to investigate the role of *ESR1* SNPs in ISS.

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### Ethics

**Ethics Committee Approval:** This study was approved by Postgraduate Institute of Medical Education and Research Institutional Ethics Committee (ref: NK/7784/MD/527, date: 28.09.2021).

**Informed Consent:** Written informed consent was obtained from the families of the participants.

### Authorship Contributions

Concept: Ravi Shankar Patel, Devi Dayal, Anupriya Kaur, Inusha Panigrahi, Harvinder Kaur, Priyanka Srivastava, Design: Ravi Shankar Patel, Priyanka Srivastava, Data Collection or Processing: Ravi Shankar Patel, Roshan Daniel, Chitra Bhardwaj, Anu Kumari, Pratibha Bawa, Ankita Tyagi, Devi Dayal, Anupriya Kaur, Inusha Panigrahi, Harvinder Kaur, Analysis or Interpretation: Ravi Shankar Patel, Roshan Daniel, Chitra Bhardwaj, Anu Kumari, Pratibha Bawa, Ankita Tyagi, Literature Search: Ravi Shankar Patel, Roshan Daniel, Chitra Bhardwaj, Anu Kumari, Pratibha Bawa, Writing: Roshan Daniel, Priyanka Srivastava.

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