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Uncoupling the LRP6 V1062I Genetic Variant from Early-onset Insulin Resistance in a Healthy Pediatric Cohort

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Abstract

Childhood obesity has increased early-onset insulin resistance (IR), a key precursor to metabolic syndrome. The Wnt/β-catenin pathway regulates glucose homeostasis via the LRP6 co-receptor. While severe LRP6 mutations cause metabolic dysfunction, the common rs2302685 (V1062I) variant's role in pediatric IR is unknown. This cross-sectional study included 234 healthy children and adolescents (aged 9–18 years) from southern Iran. Genotyping of LRP6 rs2302685 was performed using PCR-RFLP. Seven surrogate indices of insulin sensitivity/resistance (HOMA-IR, QUICKI, insulin-to-glucose ratio, McAuley, revised McAuley, FIRI, Bennett's index) were assessed. Multivariate logistic regression adjusted for age, sex, BMI, and Tanner stage. Genotype frequencies were 73.1% (TT), 25.6% (CT), and 1.3% (CC), in Hardy-Weinberg equilibrium. Univariate analysis showed no significant differences in biochemical or insulin indices across genotypes. Multivariate regression under dominant and recessive models confirmed that rs2302685 was not independently associated with any of the seven indices (all $P > 0.05$), even after adjusting for adiposity and puberty. This comprehensive multi-index analysis demonstrates that LRP6 rs2302685 (V1062I) is not an independent genetic driver of early-onset insulin resistance in healthy Iranian children and adolescents. Despite prior links to dyslipidemia, its direct role in pediatric glucose homeostasis appears negligible. These findings help refocus future biomarker research on more relevant genetic targets for pediatric metabolic syndrome.

Keywords: Insulin Resistance; LRP6 gene; rs2302685 polymorphism; Pediatric Metabolic Syndrome; Wnt Signaling

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1. Introduction

The growing epidemic of childhood and adolescent obesity is one of the most important public health issues of the 21st century (1). It has been recently estimated by the International Diabetes Federation (IDF) and World Health Organization (WHO) that the prevalence of metabolic syndrome in children has increased and currently affects about 10% to 15% of all children worldwide (2, 3). In-

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sulin resistance (IR) is the pathophysiological core of this disease. IR is a systemic metabolic disorder characterized by a deficiency in the insulin-mediated glucose disposal in skeletal muscle and adipose tissue, with persistent hepatic gluconeogenesis (4, 5). Once regarded as a metabolic hallmark of aging and chronic visceral adiposity, the alarming downward shift in the age of IR onset has made it a crucial precursor to the premature development of type 2 diabetes (T2D) and early-onset atherosclerotic cardiovascular diseases (CVD) (1, 6-8).

While the obesogenic environment, characterized by a sedentary lifestyle and hypercaloric meals, is a major contributor, the progression of pediatric insulin resistance is becoming more dependent on the individual's underlying genetic architecture (9). The heritability estimates for insulin sensitivity and insulin secretion range from 25% to 50%, suggesting strong genetic involvement (10). Thus, identifying the exact genetic locus predisposing to early metabolic dysregulation is important not only for risk stratification but also for the development of innovative, personalized treatment targets before irreversible cardiometabolic disease occurs.

Metabolic research has undergone a paradigm shift in recent years, identifying the canonical Wnt/β-catenin signaling cascade as a key regulator of glucose and lipid homeostasis. In addition to its conventional roles in embryogenesis and oncogenesis, Wnt signaling also exhibits significant crosstalk with the insulin signaling pathway. In particular, activation of Wnt signaling has been shown to govern adipogenesis, modulate pancreatic β-cell proliferation, and directly influence the phosphorylation of key intracellular mediators, including glycogen synthase kinase-3β (GSK3β) and Akt (11, 12).

At the molecular crossroads lies the Low-Density Lipoprotein Receptor-Related Protein 6 (LRP6), a necessary transmembrane co-receptor for Wnt ligands (13). The structural and functional integrity of LRP6 is essential for metabolic homeostasis. LRP6 signaling has been shown in previous studies to promote glucose metabolism by inducing TCF7L2-dependent insulin receptor expression and stabilizing IGF receptors (14). Profound LRP6 mutations have been causally connected with profound metabolic abnormalities clinically. The groundbreaking discovery of the LRP6 R611C mutation highlighted its direct significance in familial early-onset coronary artery disease, severe hyperlipidemia, hypertension, and diabetes (15).

The metabolic effects of the most common single-nucleotide polymorphisms (SNPs) in the LRP6 gene, however, have not been studied well, especially in children. One such variant is the rs2302685 (V1062I) missense polymorphism in exon 14. The rs2302685 variant has been linked to alterations in body composition, increased LDL-C, and risk of myocardial infarction in recent adult and limited pediatric cohort studies (16, 17). Nevertheless, its exact functional role in impairing glucose homeostasis and promoting early-onset insulin resistance remains completely uncharacterized. The biological plausibility of the link between LRP6, insulin receptor expression, and GSK3β regulation is strong, offering a unique opportunity to examine the genetic basis of metabolic syndrome in youth through this specific polymorphism.

The present investigation was methodically designed to address this essential gap in the literature and to evaluate the association of the LRP6 rs2302685 (V1062I) polymorphism with various markers of insulin resistance and sensitivity. The study aims to test the V1062I substitution as an early genetic determinant of insulin resistance in a well-characterized cohort of healthy children and adolescents from Kawar, southern Iran, using a comprehensive set of 7 validated surrogate metabolic indices. Unraveling this relationship is a crucial step toward identifying vulnerable pediatric groups and applying timely genetics-based preventative treatments against the upcoming wave of adult cardiovascular and metabolic disorders.

2. Materials and Methods

2.1. Study design and participants

This cross-sectional study was nested within the Kawar cohort, which was conducted in an urban area 50 km east of Shiraz, Fars province, southern Iran. The first screening was performed on 478 healthy children and adolescents aged 9 to 18 years. Participants

were randomly chosen. Strict exclusion criteria were used; subjects with known underlying disease (e.g., thyroid disease, diabetes, renal failure, adrenal insufficiency), precocious or delayed puberty, and those on medications known to affect metabolic parameters (e.g., anticonvulsants or glucocorticoids) were excluded. Based on these criteria, 234 eligible participants (122 girls and 112 boys) were included in the final analysis. All participants and their grandparents were Iranian.

The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences (Ethics Code: IR.SUMS.REC.1400.019), and the research was conducted in compliance with the Declaration of Helsinki. Written informed consent was obtained from all participants and their parent/legal guardian before enrolment.

2.2. Anthropometric & Clinical Assessments

Anthropometric parameters were measured using standardized protocols. Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer, and body weight was measured to the nearest 0.1 kg using a calibrated scale. We calculated the Body Mass Index (BMI) as weight in kilograms divided by height in meters squared (kg/m²). Pubertal developmental stages were carefully examined by a pediatric endocrinologist using Tanner's classification (18). Participants were classified as prepubertal (Stage I), early pubertal (Stages II and III), and pubertal (Stages IV and V). Additional demographic and clinical data were obtained via standardized questionnaires.

2.3. Biochemical Studies

All subjects underwent an overnight fast of at least 12 hours, and venous blood samples (10 mL) were collected from them at the Endocrinology and Metabolism Research Center in Shiraz. Samples were split into EDTA tubes (5 mL for DNA extraction) and plain tubes (5 mL for serum separation) and stored at -80 °C until further analysis. Fasting blood glucose (FBS), total cholesterol, triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using standard enzymatic kits (BioSystems, Barcelona, Spain) in an A-25 automated clinical chemistry analyzer (BioSystems). Serum insulin concentrations were measured by radioimmunoassay (IZOTOP, Budapest, Hungary). Low-density lipoprotein cholesterol (LDL-C) was calculated (17).

2.4. Evaluation of insulin resistance and sensitivity indices

To offer an extensive assessment of glucose homeostasis, seven validated surrogate indices for insulin resistance and sensitivity were computed together with the fasting biochemical parameters for each subject:

HOMA-IR (Homeostatic Model Assessment of Insulin Resistance): (Fasting Insulin [μU/mL] × Fasting Glucose [mmol/L]) / 22.5

QUICKI (Quantitative insulin sensitivity check index): 1 / (log Fasting Insulin [μU/mL] + log Fasting Glucose [mg/dL])

Insulin to glucose ratio: Fasting insulin (μU/mL) / Fasting glucose (mmol/L)

McAuley Index: exp [2.63 - 0.28 × ln (Insulin [μU/mL]) - 0.31 × ln (Triglycerides [mmol/L])]

Modified McAuley Index: exp [3.29 − (0.25 × ln Insulin [μU/mL]) − (0.22 × ln BMI [kg/m²]) − (0.28 × ln Triglycerides [mmol/L])]

FIRI (Fasting Insulin Resistance Index): (Fasting Insulin [μU/mL] × Fasting Glucose [mmol/L]) / 25

Bennett's Index: 1/(log(Fasting Glucose [mmol/L]) × log(Fasting Insulin [uU/mL])) (19)

2.5. DNA Extraction and Genotyping (PCR-RFLP)

Genomic DNA was isolated from peripheral blood cells using a conventional salting-out procedure. The DNA concentration and purity were determined spectrophotometrically (absorbance at 260 and 280 nm). Samples with an OD260/OD280 ratio of 1.5-2.0 were considered suitable for further investigation.

The LRP6 rs2302685 (V1062I) polymorphism was genotyped by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) approach (17). The surrounding genomic

area of the polymorphism was amplified using the following primer pair: Forward 5′-AGTCTGGGCAGTGAAATCAG-3′, Reverse 5′-TCCAGTTTAGAGAAGATAGTTGCC-3′. The PCR reaction mixture (total volume 20 µL) contained 14.9 µL of distilled water, 2.0 µL of PCR buffer, 0.75 µL of MgCl2, 0.5 µL of dNTPs, 0.3 µL of primer, 0.25 µL of Taq DNA polymerase, and 1.0 µL of template DNA.

The PCR protocol consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94 °C for 35 s, annealing at 53 °C for 30 s, and extension at 72°C for 30 s. The reaction was terminated with a final extension at 72 °C for 5 min. The PCR products were digested with the BceAI rapid-digest restriction enzyme (specific to the target sequence) at 37 °C for 24h. The digested fragments were separated by electrophoresis on a 2% agarose gel, stained with Green Viewer fluorescent dye, and visualized under UV light using a gel documentation system.

2.6. Statistical Analysis

Statistical analyses were performed using SPSS (version 22.0; SPSS Inc., Chicago, IL, USA). Continuous variables were assessed for normality using the Kolmogorov-Smirnov test. Data with normal distribution were reported as mean ± standard deviation (SD), and data with non-normal distribution were presented as median and interquartile range (IQR). The differences between genotype groups were tested with a One-Way ANOVA or a Student's t-test for parametric variables, and with a Kruskal-Wallis test or a Mann-Whitney U test for nonparametric variables.

Genotype and allele frequencies were calculated, and departures from Hardy-Weinberg equilibrium were tested with the Arlequin program (v. 3.5.2.2). The allele and genotype frequencies between the genders were compared by Chi-square ($\chi 2$) test or Fisher's exact test. Multivariate logistic regression analyses were conducted to assess the independent association of the LRP6 rs2302685 polymorphism with measures of insulin resistance. These analyses were stratified into four models, with potential confounders incrementally adjusted for. Model 1 was adjusted for age; Model 2 for age and sex; Model 3 for age, sex, and BMI; and Model 4 for age, sex, BMI, and Tanner pubertal stage. Both dominant and recessive genetic models were tested. For all analyses, a P-value of <0.05 was considered statistically significant.

3. Results

3.1. Clinical, anthropometric, and demographic characteristics

An initial screening was conducted among 478 children and adolescents in the Kavar cohort. After careful application of exclusion criteria (i.e., exclusion of persons with known endocrine problems, delayed or precocious puberty, and use of interfering medications), a total of 234 healthy participants (122 girls and 112 boys) were included in the final analysis. The baseline clinical, anthropometric, and biochemical characteristics of the study population was shown in table 1. The mean age of the study population was 13.78±2.69 years, with a mean Body Mass Index (BMI) of 17.72±3.18 kg/m2. Baseline biochemical profiling revealed that the group was within the normal physiological ranges. The mean FBS was 92.76±11.93 mg/dL, and the median fasting serum insulin was 8.8 (IQR: 3.7 - 13.5) µU/mL. Seven indices were derived for each participant in an attempt to represent a holistic view of glucose homeostasis: 1) HOMA-IR median: 1.70, IQR: 1.5-1.83; 2) QUICKI mean: 0.37±0.04. Table 1 shows the anthropometric and biochemical characteristics. Finally, the crucial influence of hormonal changes on insulin sensitivity prompted careful staging of pubertal development using the Tanner staging system, with the distribution across stages I-V shown in Table 2.

3.2. LRP6 rs2302685 (V1062I) Genotype and Allele Distribution

Genotype and allele frequencies of lrp6 rs2302685 (V1062I) stratified by gender was shown in table 3. Genotype distribution of LRP6 rs2302685 polymorphism by PCR-RFLP showed a dominant wild-type allele. The most common genotype was TT, which was observed in 171 (73.1%) of 234 subjects. The heterozygous CT genotype was present in 60 participants (25.6%), and only 3 individu-

Table 1. Baseline Clinical, Anthropometric, and Biochemical Characteristics of the Study Population

Variable	Mean±SD	Median (IQR)
Age (years)	13.78±2.69	14.0 (25.4)*
Height (cm)	154.1±34.14	154.0 (20)
Weight (kg)	41.13±17.43	42.0 (20)
BMI (kg/m²)	17.72±3.18	17.2 (3.4)
Total Cholesterol (mg/dL)	156.6±92.30	154.0 (39)
HDL-C (mg/dL)	47.24±68.15	45.0 (8.15)
LDL-C (mg/dL)	94.35±26.8	—
Triglycerides (mg/dL)	99.74±92.51	62.0 (57)
Fasting Blood Glucose (mg/dL)	92.76±11.92	76.0 (17)
Fasting Insulin (µU/mL)	8.8±13.5	7.7 (5.3)
QUICKI index	0.37±0.04	0.4 (0.03)
HOMA-IR	1.70±1.18	1.5 (0.83)
Insulin-to-Glucose ratio	2.01±1.13	1.8 (0.97)
McAuley index	2.34±0.79	2.2 (0.88)
Revised McAuley index	2.88±0.90	2.7 (1.05)
FIRI	1.54±1.05	1.3 (0.75)
Bennett's index	1.89±2.37	1.8 (0.55)

Notes: BMI: Body Mass Index; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; FIRI: Fasting Insulin Resistance Index; IQR: Interquartile Range.

als (1.3%) had the homozygous mutant CC genotype.

The T allele frequency was 85.9%, and the C allele was 14.1% at the allelic level. Analyses of gender stratification showed no statistically significant variations in the genotype distribution (P=0.878) or allele frequencies (P=0.875) between boys and girls. Significantly, the genotype frequencies in our study population were in strict compliance with the Hardy-Weinberg Equilibrium (P=0.37), supporting the genetic stability and representativeness of the cohort.

3.3. Effect of LRP6 rs2302685 on Biochemical Parameters and Insulin Indices

Biochemical parameters and insulin resistance/sensitivity indices were compared among the three genotype groups to assess the functional effect of the LRP6 rs2302685 variant (Table 4). Variables with normal distribution (FBS, QUICKI, McAuley, revised McAuley and Bennett's index) were analyzed by One-way ANOVA and independent T-tests, and variables with non-normal distribution (serum insulin, HOMA-IR, FIRI, and insulin to glucose ratio) were evaluated using Kruskal-Wallis and Mann-Whitney U tests.

Univariate analysis showed that the LRP6 rs2302685 polymorphism did not significantly affect any of the metabolic parameters analyzed. The rare CC genotype was asso-

Table 2. Distribution of Pubertal Development According to Tanner Staging.

Pubertal Stage	Girls (n=122)	Boys (n=112)	Total (n=234)
Stage 1 (Pre-pubertal)	24	29	53
Stage 2 (Early pubertal)	19	17	36
Stage 3 (Early pubertal)	23	13	36
Stage 4 (Pubertal)	9	33	42
Stage 5 (Pubertal)	47	20	67
Total	122	112	234

Table 3. Genotype and Allele Frequencies of LRP6 rs2302685 (V1062I) Stratified by Gender.

Parameter	Total (n=234)	Boys (n=112)	Girls (n=122)	P-value
Genotype, n (%)				0.878
TT (Wild-type)	171 (73.1%)	82 (73.2%)	89 (73.0%)	
CT (Heterozygous)	60 (25.6%)	29 (25.9%)	31 (25.4%)	
CC (Homozygous mutant)	3 (1.3%)	1 (0.9%)	2 (1.6%)	
Allele, n (%)				0.875
T allele	402 (85.9%)	193 (86.16%)	209 (85.66%)	
C allele	66 (14.1%)	31 (13.84%)	35 (14.34%)	

Notes: P-values derived from Chi-square or Fisher's exact test. Hardy-Weinberg Equilibrium: P = 0.37.

ciated with somewhat lower mean and median FBS values and particular sensitivity indices (McAuley and Bennett's indices), but these differences were minor and did not reach statistical significance (all P>0.05). The detailed comparison of these parameters by genotype is shown in Tables 4.

3.4. Multivariate Logistic Regression analysis to robustly analyze the independent relationship between the LRP6 polymorphism and insulin resistance

We performed a rigorous multivariate logistic regression analysis (Table 5). This analysis was intended to address the complicated multifactorial nature of metabolic syndrome by correcting for key physiological variables in four separate models in a sequential manner:
Model 1: Adjusted for age
Model 2: Age and sex adjusted.
Model 3: Age-, sex- and BMI-adjusted.
Model 4: Adjusted for age, sex, BMI, and Tanner pubertal stage.

The connections were evaluated under two genetic models: The dominant model (CC+CT vs. TT) and the Recessive model (CT vs. CC+TT).

As shown in Tables 5, our logistic regression analysis indicated clearly that the LRP6 rs2302685 polymorphism is not an independent predictor of insulin resistance in this cohort. In the fully adjusted Model 4, which robustly controls for the profound effects of the adiposity (BMI) and pubertal hormonal shifts (Tanner stage), the Odds Ratios (ORs) of all 7 examined indices (HOMA-IR, QUICKI, Insulin-to-glucose ratio, McAuley, revised McAuley, FIRI and Bennett's index) did not reach statistical significance (all P > 0.05) in both dominant and recessive inheritance models.

Table 4. Comparison of Metabolic Parameters Across LRP6 rs2302685 Genotypes.

Parameter	TT Genotype (n=171)	CT Genotype (n=60)	CC Genotype (n=3)	P-value
FBS (mg/dL) *	78.32 ± 11.48	76.3 ± 13.41	70.33 ± 8.62	0.32
QUICKI index *	0.36 ± 0.05	0.36 ± 0.03	0.35 ± 0.01	0.94
McAuley index *	2.39 ± 0.86	2.32 ± 0.64	2.04 ± 0.16	0.69
Revised McAuley index *	2.94 ± 0.98	2.87 ± 0.73	2.52 ± 0.21	0.67
Bennett's index *	1.97 ± 2.14	1.93 ± 0.61	1.75 ± 0.16	0.98
Insulin (µU/mL) #	8.3 (4.65)	8.1 (3.45)	9.5	0.55
HOMA-IR #	1.54 (1.05)	1.52 (0.87)	1.69	0.77
Insulin-to-Glucose ratio #	1.76 (1.21)	1.98 (1.00)	2.38	0.40
FIRI #	1.39 (0.93)	1.34 (0.75)	1.52	0.60

Notes: * Data are expressed as Mean ± SD. P-values derived from One-way ANOVA.
Data are expressed as Median (IQR). P-values derived from Kruskal-Wallis test.
Abbreviations: FBS, Fasting Blood Glucose; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; FIRI, Fasting Insulin Resistance Index.

Table 5. Multivariate Logistic Regression Analysis for the Association Between LRP6 rs2302685 and All Seven Metabolic Indices.

Metabolic Index	Genetic Model	Model 1 (P / OR [95% CI])	Model 2 (P / OR [95% CI])	Model 3 (P / OR [95% CI])	Model 4 (P / OR [95% CI])
QUICKI	Dominant	0.77 / -0.02	0.80 / -0.02	0.83 / -0.02	0.79 / -0.02
	(CC+CT vs. TT)	(-0.01, -0.01)	(-0.01, -0.01)	(-0.01, -0.01)	(-0.01, -0.01)
	Recessive	0.81 / -0.02	0.86 / -0.01	0.88 / -0.01	0.82 / -0.02
	(CT vs. CC+TT)	(-0.02, -0.01)	(-0.02, -0.01)	(-0.02, -0.01)	(-0.02, -0.01)
HOMA-IR	Dominant	0.19 / -0.10	0.18 / -0.09	0.18 / -0.09	0.28 / -0.08
	(CC+CT vs. TT)	(-0.65, 0.13)	(-0.63, 0.12)	(-0.64, 0.12)	(-0.60, 0.18)
	Recessive	0.18 / -0.10	0.17 / -0.10	0.17 / -0.10	0.28 / -0.08
	(CT vs. CC+TT)	(-0.71, 0.14)	(-0.70, 0.12)	(-0.71, 0.12)	(-0.66, 0.19)
Insulin-to-Glucose Ratio	Dominant	0.85 / -0.01	0.83 / -0.02	0.68 / -0.03	0.62 / -0.03
	(CC+CT vs. TT)	(-0.34, 0.28)	(-0.35, 0.28)	(-0.37, 0.24)	(-0.39, 0.23)
	Recessive	0.77 / -0.02	0.73 / -0.02	0.59 / -0.04	0.54 / -0.04
	(CT vs. CC+TT)	(-0.39, 0.29)	(-0.40, 0.28)	(-0.43, 0.24)	(-0.45, 0.24)
McAuley Index	Dominant	0.40 / -0.06	0.41 / -0.06	0.54 / -0.04	0.51 / -0.05
	(CC+CT vs. TT)	(-0.32, 0.13)	(-0.32, 0.13)	(-0.29, 0.15)	(-0.31, 0.15)
	Recessive	0.45 / -0.05	0.46 / -0.05	0.61 / -0.04	0.56 / -0.04
	(CT vs. CC+TT)	(-0.33, 0.15)	(-0.33, 0.15)	(-0.30, 0.18)	(-0.33, 0.18)
FIRI	Dominant	0.18 / -0.09	0.15 / -0.10	0.14 / -0.10	0.20 / -0.09
	(CC+CT vs. TT)	(-0.55, 0.11)	(-0.55, 0.08)	(-0.56, 0.08)	(-0.54, 0.12)
	Recessive	0.18 / -0.09	0.14 / -0.10	0.13 / -0.10	0.20 / -0.09
	(CT vs. CC+TT)	(-0.59, 0.11)	(-0.60, 0.08)	(-0.61, 0.08)	(-0.59, 0.12)
Bennett's Index	Dominant	0.75 / -0.02	0.73 / -0.02	0.77 / -0.02	0.68 / -0.03
	(CC+CT vs. TT)	(-0.59, 0.42)	(-0.59, 0.42)	(-0.59, 0.43)	(-0.64, 0.42)
	Recessive	0.74 / -0.02	0.71 / -0.03	0.75 / -0.02	0.64 / -0.03
	(CT vs. CC+TT)	(-0.64, 0.45)	(-0.65, 0.44)	(-0.64, 0.46)	(-0.72, 0.44)
Revised McAuley Index	Dominant	0.38 / -0.06	0.38 / -0.06(-	0.53 / -0.04	0.51 / -0.05
	(CC+CT vs. TT)	(-0.36, 0.14)	0.36, 0.14)	(-0.32, 0.17)	(-0.34, 0.17)
	Recessive	0.43 / -0.05	0.44 / -0.05(-	0.59 / -0.04	0.56 / -0.04
	(CT vs. CC+TT)	(-0.38, 0.16)	0.38, 0.16)	(-0.34, 0.19)	(-0.36, 0.20)

4. Discussion

The worldwide epidemic of childhood obesity has drastically accelerated the onset of insulin resistance (IR) and metabolic syndrome, which were formerly limited to adults. As insulin resistance in children tracks strongly into adulthood, the ability to detect and prevent its development during the pediatric window is of utmost clinical significance to lessen the future burden of type 2 diabetes (T2D) and cardiovascular disease Saklayen (2, 6, 10). Environmental variables are important, but genetic susceptibility to early-onset IR is a key area of research (19).

Our hypothesis of a relationship between the LRP6 gene and pediatric insulin resistance was based on the complex biochemical crosstalk between the Wnt/β-catenin and insulin signaling pathways. LRP6, a co-receptor required for Wnt signaling, is particularly abundant in metabolic organs. The consequence of Wnt signaling dysregulation is altered phosphorylation of downstream targets (e.g., GSK3β, Akt, mTOR), which are common nodes in the insulin signaling cascade (11, 12). First investigations have shown that LRP6 mutations (R611C) strongly affect

glucose tolerance by down-regulating insulin receptor expression via TCF7L2-dependent mechanisms (14, 15). Given the strong biological rationale, we hypothesized that the common missense variant LRP6 rs2302685 (V1062I) may serve as an early genetic biomarker of impaired glucose homeostasis and insulin resistance in a vulnerable pediatric population.

Quantifying insulin resistance in youth is difficult (20). The gold standard is the hyperinsulinemic-euglycemic clamp, which is highly invasive and not practicable for large pediatric groups. To resolve this issue, our study employed a rigorous, multidimensional analytical approach, using seven distinct validated surrogate measures.

We analyzed HOMA-IR (a measure of hepatic insulin resistance) and QUICKI (a logarithmic transformation with high sensitivity) rather than a single basal measure. Furthermore, given the lipotoxic roots of metabolic syndrome, we also integrated McAuley and revised McAuley indices, which uniquely combine serum triglycerides and BMI to predict lipid-driven insulin resistance (21, 22). We also considered FIRI, the Bennett index, and the insulin-to-glucose ratio to capture other physiological aspects of the glucose-insulin feedback loops.

This phenotypic framework was robust, as it was derived by dissecting the data using these different metabolic views across 4 stringent regression models, adjusted for age, sex, obesity, and pubertal hormonal changes. The robustness of our conclusion lies in the consistent absence of statistical significance across all seven indices, rather than relying on a single, potentially biased marker. It unequivocally shows that the V1062I variant does not affect the insulin signaling pathways assessed by these global markers, either subtly or overtly.

We found no association between the LRP6 rs2302685 polymorphism and insulin resistance as an independent risk factor in healthy Iranian children and adolescents. Indeed, our original idea was supported by robust biochemical arguments. The diverse functional outcome of the several LRP6 mutations gives a very good explanation for this result.

In contrast to the R611C mutation that directly affects intracellular glucose metabolism (15), the metabolic consequences of the rs2302685 (V1062I) variant remain incompletely understood. Previous studies have reported associations with lipid-related traits, including hyperlipidemia and elevated LDL-C levels; however, the present study was not designed to evaluate lipid metabolism and therefore cannot draw conclusions regarding such associations. For example, Xu et al. reported that rs2302685 was associated with a higher risk of myocardial infarction in a Chinese population via raised LDL-C (16). In a similar Iranian pediatric population, Montazeri-Najafabady et al. reported that the CC genotype of rs2302685 was a significant predictor of hyperlipidemia (17).

Biochemically, we hypothesize that the V1062I amino acid substitution might affect the structural affinity of the LRP6 extracellular domain for certain Wnt ligands or lipid complexes (e.g., in LDL clearance mechanisms) but not the ability of the intracellular domain to regulate GSK3β or to interact with insulin receptor substrates. Moreover, the strong compensatory mechanisms of the young (e.g., increased β-cell hypersecretion) may effectively offset minor genetic abnormalities in glucose sensing, thereby maintaining euglycemia and appropriate insulin sensitivity during childhood.

We also discuss these findings in the context of our cohort's genetic architecture. The MAF of the C allele was 14.1%, which is very close to the 13.8% found in the Chinese HapMap population (17) and ~18.5% in European cohorts (23). This is in sharp contrast to Japanese populations, where the MAF is dramatically lower (5-6%) (24). We observed no correlation with insulin parameters in the Iranian cohort, consistent with the findings of Zenibayashi et al., who found no association between other LRP6 variations and T2D in Japan (24). This suggests that LRP6 polymorphisms may have population-specific metabolic effects.

The present study benefits from a carefully screened homogenous group and careful correction for physiological variables, most

notably Tanner staging of puberty and BMI. Surrogate measures of insulin activity have been used to assess overall insulin activity. There are, however, limitations. A cross-sectional design is not conducive to causal inferences. The sample size was statistically powered for the larger cohort, but resulted in a low proportion of homozygous mutant (CC) individuals (1.3%), which would limit the ability to detect recessive genetic effects. Finally, PCR-RFLP is a common genotyping method, but future studies should also include direct DNA sequencing and genome-wide transcriptomic analysis to further elucidate the underlying mechanisms. Finally, although PCR-RFLP is a widely used and cost-effective genotyping method, it may be susceptible to technical errors such as incomplete restriction enzyme digestion. Confirmatory genotyping by direct DNA sequencing was not performed in the present study. Therefore, future investigations should validate these findings using sequencing-based approaches and larger cohorts to further strengthen the reliability of genotype assignment and elucidate the underlying biological mechanisms. Another limitation of this study is the lack of detailed assessment of lifestyle-related factors, including dietary intake and physical activity. Because validated questionnaires evaluating these variables were not administered, residual confounding by environmental influences on insulin sensitivity cannot be excluded. Future studies should incorporate comprehensive lifestyle assessments to better characterize gene–environment interactions affecting pediatric insulin resistance.

5. Conclusion

In conclusion, whereas the Wnt/LRP6 pathway is known to be involved in metabolic control, the LRP6 rs2302685 (V1062I) polymorphism does not independently contribute to insulin resistance in Iranian children. Our extensive multi-index study results are consistent with findings that this variation, a biomarker of dyslipidemia and cardiovascular risk, has a modest direct effect on early-onset insulin resistance. Publishing this well-powered uncoupling result is crucial focusing future genetic screening efforts on more relevant diagnostic targets. Ultimately, knowledge of the exact genetic architecture of pediatric metabolic syndrome will provide clinicians with methods to screen patients and enable them to intervene in a personalized, preventive manner to protect the metabolic health and lifespan of future generations.

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AUTHOR CONTRIBUTIONS

AG involved in study design, data collection, data analysis, data interpretation, literature search, generation of figures, and writing of the manuscript. NMN involved in study design, data collection, data analysis, data interpretation, literature search, generation of figures, and writing of the manuscript. MHD involved in study design, data collection, data analysis, data interpretation, and literature search. ZD involved in study design, data collection, data analysis, data interpretation.

Conflict of Interest

The authors declare that they have no conflict of interest.

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