Immune Response and Lipid Metabolism Gene Polymorphisms Are Associated with the Risk of Obesity in Middle-Aged and Elderly Patients

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Abstract: More than two billion people around the world are overweight or obese. Even in apparently healthy people, obesity has a potent effect on their quality of life. Experimental data indicate the role of infectious agents in systemic inflammation, revealing a correlation between the dietary habits of people with obesity and the level of systemic inflammation mediators, serum lipid concentration, and hormonal and immune status. This study aimed to determine the association of immune response and lipid metabolism gene polymorphisms with the risk of obesity. This study included 560 Caucasian patients living in Western Siberia (Russian Federation). A total of 52 polymorphic sites in 20 genes were analyzed using the 5′ TaqMan nuclease assay. Four risk-associated polymorphic variants were discovered—two variants in immune response genes (IL6R rs2229238, OR = 1.92, 95% CI = 1.36–2.7; p = 0.0002 in the dominant model; IL18 rs1946518, OR = 1.45, 95% CI = 1.03–2.04, p = 0.033 in the over-dominant model) and two variants in lipid metabolism genes (LP A rs10455872, OR = 1.86, 95% CI = 1.07–3.21, p = 0.026 in the log-additive model; LEPR rs1137100, OR = 2.88, 95% CI = 1.52–5.46, p = 0.001 in the recessive model). Thus, polymorphisms in immune response and lipid metabolism genes are potentially associated with the modification of obesity risk in the Caucasian population.

Keywords: genetic polymorphism; obesity; inflammation; immunity; lipid metabolism

1. Introduction

Despite specific public health policies targeting the obesity epidemic, more than two billion people around the world are overweight or obese [1]. It is predicted that one-fifth of the working-age population will be obese by 2025; the increase in the number of obese patients is accompanied by significant socio-economic losses [2], which determine the improvement of treatment and diagnosis of this pathology, as well as the assessment of individual susceptibility to its development.

Obesity is a multifactorial disease characterized by excessive accumulation of adipose tissue, accompanied by a low-grade chronic inflammation. The triggers of this inflammation are poorly studied, but it is known that the degree of inflammation correlates with the severity of obesity-associated pathologies, which suggests that understanding the inflammatory response may improve the treatment strategies of such diseases [3,4]. In addition to inflammation, impaired lipid metabolism can be a trigger of obesity [5,6].

It is known that the activity of molecules involved in inflammation and lipid metabolism is genetically determined. Genome-wide association studies (GWAS) allow identifying...
genetic variants associated with susceptibility to overweight and obesity [7]. In adults, the strongest associations with the risk of obesity were discovered for single-nucleotide polymorphisms (SNPs) in the FTO, MC4R, TMEM18, TNNI3K, SEC16B, GNPDA2, POMC, RPRGIP1L, IRX3, and IRX5 genes [8–11]. Moreover, it was shown that the genetic susceptibility to obesity is modified in a population-related manner [12–14]. Despite the previously obtained results, some issues related to the genetics of obesity, including the role of SNPs in genes involved in the inflammation and lipid metabolism pathways mediating obesity, are still poorly investigated, and the available results are contradictory. Therefore, understanding the role of genetic factors controlling the different pathways underlying the pathogenesis of obesity, particularly inflammation and lipid metabolism, plays a very important role in the development of personalized prevention strategies, especially in at-risk groups.

This study aimed to determine risk-associated polymorphic variants in immune response and lipid metabolism genes in obese middle-aged and elderly Caucasian patients.

2. Materials and Methods

2.1. Group Description

The present study included 560 Caucasian individuals aged 44 to 75 years (mean age of 59 years) that were long-term residents (at least three generations) in Western Siberia (Russian Federation) and undergoing a screening examination at the Research Institute for Complex Issues of Cardiovascular Diseases (Kemerovo, Russian Federation). Patients with cancer, autoimmune and mental diseases, and acute or exacerbated chronic infections associated with the inflammatory process were excluded from the study to avoid confounding effects. According to the World Health Organization age standards (2015), the patients included in this study were classified into two age groups: middle-aged (age ≤ 60 years) and elderly (age >60 years).

Obesity was defined as a body mass index (BMI) of 30 kg/m$^2$ or greater. In the studied group, the mean BMI was 28 kg/m$^2$, ranging from 17 kg/m$^2$ to 41 kg/m$^2$. According to this stratification, 220 individuals (39%) were obese, and 340 individuals (61%) had a normal BMI. The complete characteristics of patients included in this study are presented in Table 1.

| Index                                      | Number (%) |
|--------------------------------------------|------------|
| Male                                       | 319 (57)   |
| Female                                     | 241 (43)   |
| Age ≤60 years (middle-aged patients)       | 382 (68)   |
| Age >60 years (elderly patients)           | 178 (32)   |
| BMI ≥30 kg/m$^2$                           | 220 (39)   |
| BMI ≥30 kg/m$^2$ in middle-aged patients   | 156 (41)   |
| BMI ≥30 kg/m$^2$ in elderly patients       | 64 (36)    |

The design of this study was approved by the Local Ethical Committee of the Research Institute for Complex Issues of Cardiovascular Diseases (Kemerovo, Russian Federation). All individuals included in this study provided written informed consent to participate in the examination. This study was performed in accordance with the World Medical Association Declaration of Helsinki (ethical principles for medical research involving human participants with amendments in 2000) and Good Clinical Practice.
2.2. Molecular Genetic Testing

Genomic DNA was isolated using the routine phenol–chloroform extraction method from whole blood collected from the cubital vein in vacuum tubes with K3EDTA and stored at −80 °C until the next stage of the experiment.

SNPs were selected according to the following criteria: (i) location within immune response and lipid metabolism genes; (ii) minor allele frequency >5% in Caucasian populations; (iii) functional consequences and related studies on their role in obesity pathogenesis. Accordingly, we selected 52 SNPs in 20 genes. The complete characteristics of the selected SNPs are presented in Table 2.

Table 2. Characteristics of the studied polymorphic variants.

| Gene     | Reference SNP Number | Chromosomal Position | Nucleotide Change | Variant Type         |
|----------|----------------------|----------------------|-------------------|----------------------|
| TLR1     | rs5743611            | chr4:38798593        | C > G             | Missense variant     |
|          | rs5743551            | chr4:38806033        | T > A, C, G       | 5’ UTR variant       |
| TLR2     | rs5743708            | chr4:153705165       | G > A             | Missense variant     |
| TLR4     | rs4986791            | chr9:117713324       | C > T             | Missense variant     |
|          | rs4986790            | chr9:117713024       | A > G, T          | Missense variant     |
| TLR6     | rs5743810            | chr4:38828729        | A > C, G, T       | Missense variant     |
|          | rs3775073            | chr4:38828211        | T > C, G          | Missense variant     |
| IL1RL1   | rs4988956            | chr2:102351547       | G > A             | Missense variant     |
|          | rs11685424           | chr2:102310521       | G > A             | Upstream transcript variant |
| IL1B     | rs1143634            | chr2:112832813       | G > A             | Synonymous variant   |
|          | rs16944              | 2:112837290          | A > G             | Upstream transcript variant |
| IL6R     | rs2228145            | chr1:154454494       | A > C, T          | Missense variant     |
|          | rs2229238            | chr1:154465420       | T > A, C          | 3’ UTR variant       |
| IL6      | rs1800796            | chr7:22726627        | G > A, C          | Intron variant       |
|          | rs1554606            | chr7:22729088        | T > A, G          | Intron variant       |
|          | rs2069827            | chr7:22725837        | G > C, T          | Upstream transcript variant |
| CXCL8    | rs2227306            | chr4:73741338        | C > T             | Intron variant       |
|          | rs4073               | chr4:73740307        | A > C, G, T       | Upstream transcript variant |
| IL10     | rs1800871            | chr1:206773289       | A > G             | Upstream transcript variant |
|          | rs1800872            | chr1:206773062       | T > G             | Upstream transcript variant |
|          | rs1800896            | chr1:206773552       | T > C             | Upstream transcript variant |
| IL12RB1  | rs375947             | chr19:18069641       | A > G             | Missense variant     |
| IL12B    | rs3212227            | chr5:159315942       | T > G             | 3’ UTR variant       |
| IL18RAP  | rs917997             | chr2:102454108       | T > A, C, G       | Not announced        |
|          | rs2058659            | chr2:102430896       | G > A             | Intron variant       |
| IL18R1   | rs13015714           | chr2:102355405       | G > A, T          | Upstream transcript variant |
|          | rs1974675            | chr2:102369915       | G > A             | Intron variant       |
|          | rs6758936            | chr2:102374909       | G > A             | Intron variant       |
|          | rs3755276            | chr2:102361999       | C > T             | Intron variant       |
| Gene | Reference SNP Number | Chromosomal Position | Nucleotide Change | Variant Type |
|------|----------------------|----------------------|------------------|--------------|
| IL18 | rs187238 | chr11:112164265 | C > A, G | Upstream transcript variant |
|      | rs360719 | chr11:112165426 | A > G | Upstream transcript variant |
|      | rs1946518 | chr11:112164735 | T > G | Upstream transcript variant |
| IL33 | rs7025417 | chr9:6240084 | T > C, G | Intron variant |
| TNF  | rs1799964 | chr6:31574531 | T > C | Upstream transcript variant |
|      | rs361525 | chr6:31575324 | G > A | Upstream transcript variant |
|      | rs1800629 | chr6:31575254 | G > A | Upstream transcript variant |
| CRP  | rs3093077 | chr1:159709846 | A > C, G, T | Not announced |
|      | rs1800947 | chr1:159713648 | C > A, G, T | Synonymous variant |
|      | rs1130864 | chr1:159713301 | G > A | Intron variant |
|      | rs1205 | chr1:159712443 | C > T | 3’ UTR variant |
|      | rs429358 | chr19:44908684 | T > C | Missense variant |
|      | rs769452 | chr19:44907853 | T > A, C | Missense variant |
|      | rs7412 | chr19:44908822 | C > T | Missense variant |
| APOE | rs1042031 | chr2:21002881 | C > A, T | Missense variant/Stop gained |
|      | rs6752189 | chr2:20996129 | G > T | Not announced |
|      | rs10455872 | chr6:160589086 | A > G | Intron variant |
| LIPC | rs1800588 | chr15:58431476 | C > G, T | Intron variant |
| CXCR1| rs16858811 | chr2:218165120 | A > C | Missense variant |
|      | rs1126579 | chr2:218136011 | T > C | 3’ UTR variant |
| INS  | rs689 | chr11:2160994 | A > G, T | Intron variant |
| IGF1R| rs2229765 | chr15:98934996 | G > A, T | Missense variant |
| LEP  | rs7799039 | chr7:128238730 | G > A, C | Not announced |
| LEPR | rs1137101 | chr1:65592830 | A > G, T | Missense variant |
|      | rs1137100 | chr1:65570758 | A > G, T | Missense variant |
| IL1F9| rs17659543 | chr2:112958729 | C > T | Not announced |

Molecular genetic testing was performed by allele-specific real-time polymerase chain reaction (real-time PCR) with fluorescently labeled TaqMan probes (Applied Biosystems, Waltham, MA, USA). Per each analyzed sample, 10 μL of reaction mixture containing 1.25 μL of appropriate TaqMan probe (Applied Biosystems, Waltham, MA, USA), 5 μL of TaqMan™ Universal PCR Master Mix (Applied Biosystems, Waltham, MA, USA), 1.75 μL of DNase-free water, and 2 μL of 100 ng genome DNA template was prepared. The amplification was performed using the Viia 7 Real-Time PCR System (Applied Biosystems, Waltham, MA, USA) in 96-well PCR plates as follows: 10 min at 95 °C (one cycle), 15 s at 95 °C (one cycle), and 60 s at 60 °C (40 cycles). As a negative control, a reaction mixture without the genomic DNA template was used. Results of genotyping were analyzed using the QuantStudio™ Real-Time PCR Software v.1.3 (Applied Biosystems, Waltham, MA, USA). The quality of the PCR was evaluated by repeated genotyping of 10% of the samples.

2.3. Statistical Analysis

Statistical analysis was performed using STATISTICA 10.0 Software (StatSoft, Tulsa, OK, USA). Quantitative data were tested using the Yates’ chi-square test or the Fisher exact test. The genotyping results were analyzed using the SNPStats web tool. The most likely inheritance model for each specific gene polymorphism was determined using Akaike’s
information criterion (AIC). The results are presented as the odds ratio (OR) and the 95% confidence interval (CI) calculated using five inheritance models (codominant, dominant, recessive, over-dominant, and log-additive). The differences were considered statistically significant at $p < 0.05$.

3. Results

Four SNPs associated with an increased risk of obesity were discovered—two variants in immune response genes ($IL6R$ rs2229238, OR = 1.92, 95% CI = 1.36–2.71, $p = 0.0002$ in the dominant model; $IL18$ rs1946518, OR = 1.45, 95% CI = 1.03–2.04, $p = 0.033$ in the over-dominant model) and two variants in lipid metabolism genes ($LPA$ rs10455872, OR = 1.86, 95% CI = 1.07–3.21, $p = 0.026$ in the log-additive model; $LEPR$ rs1137100, OR = 2.88, 95% CI = 1.52–5.46, $p = 0.001$ in the recessive model). It was inferred that the A/A genotype (recessive model) of the $TNF$ gene (rs1800629) was associated with a high risk of presenting an obesity phenotype (OR = 10.29, 95% CI 1.22–86.59, $p = 0.0081$). However, reliable conclusions concerning the pathogenetic effect of this SNP could not be drawn, since this genotype was discovered in only 0.3% of nonobese patients. Moreover, the recessive models of the $CXCL8$ gene (rs4073 and rs2227306) were characterized by a protective effect (OR = 0.56, 95% CI = 0.37–0.86, $p = 0.0065$ and OR = 0.49, 95% CI = 0.31–0.79, $p = 0.0025$, respectively (Table 3).

| Gene | Model | Genotype | No Obesity, $N$ (%) | Obesity, $N$ (%) | OR (95% CI) | $p$ | AIC |
|------|-------|----------|---------------------|------------------|-------------|-----|-----|
| $IL6R$ rs2229238 | Codominant | C/C | 189 (55.9) | 88 (40) | 1.00 | 0.0009 | 741.5 |
| | | T/C | 123 (36.4) | 112 (50.9) | 1.97 (1.37–2.83) | 0.0006 | 739.8 |
| | | T/T | 26 (7.7) | 20 (9.1) | 1.66 (0.88–3.14) | 0.56 | 753.3 |
| | Dominant | C/C | 189 (55.9) | 88 (40) | 1.00 | 0.0065 | 747.3 |
| | | T/C-T/T | 149 (44.1) | 132 (60) | 1.92 (1.36–2.71) | 1.53 (1.17–2.01) | 0.0016 | 743.7 |
| | Recessive | C/C-T/C | 312 (92.3) | 200 (90.9) | 1.00 | 0.0005 | 741.9 |
| | | T/T | 26 (7.7) | 20 (9.1) | 1.20 (0.65–2.21) | 0.74 (0.58–0.94) | 0.013 | 748.3 |
| | Over-dominant | C/C-T/T | 215 (63.6) | 108 (49.1) | 1.00 | 0.32 | 753.5 |
| | | T/C | 123 (36.4) | 112 (50.9) | 1.83 (1.29–2.58) | 0.56 (0.37–0.86) | 0.0065 | 747.4 |
| $CXCL8$ rs4073 | Codominant | A/T | 154 (45.4) | 109 (49.5) | 0.90 (0.61–1.34) | 0.022 | 748.8 |
| | | A/A | 94 (27.7) | 40 (18.2) | 0.53 (0.33–0.86) | 0.015 | 752.4 |
| | Dominant | T/T | 91 (26.8) | 71 (32.3) | 1.00 | 0.0065 | 747.2 |
| | | A/T-A/A | 248 (73.2) | 149 (67.7) | 0.76 (0.53–1.11) | 0.56 (0.37–0.86) | 0.32 | 753.5 |
| | Recessive | T/T-A/T | 245 (72.3) | 180 (81.8) | 1.00 | 0.74 (0.58–0.94) | 0.013 | 748.3 |
| Gene          | Model        | Genotype | No Obesity, N (%) | Obesity, N (%) | OR (95% CI)       | p   | AIC  |
|--------------|--------------|----------|-------------------|---------------|------------------|-----|------|
| CXCL8        | Codominant   | C/C      | 103 (30.3)        | 77 (35)       | 1.00             |     |      |
|              |              | C/T      | 160 (47.1)        | 115 (52.3)    | 0.94 (0.64–1.38) | 0.0098 | 748  |
|              |              | T/T      | 77 (22.6)         | 28 (12.7)     | 0.48 (0.28–0.81) |     |      |
|              | Dominant     | C/C      | 103 (30.3)        | 77 (35)       | 1.00             |     | 753.7|
|              |              | C/T     | 237 (69.7)        | 143 (65)      | 0.79 (0.55–1.14) | 0.21 |      |
|              | Recessive    | C/C-C/T  | 263 (77.3)        | 192 (87.3)    | 1.00             |     | 746.1|
|              |              | C/T     | 77 (22.6)         | 28 (12.7)     | 0.49 (0.31–0.79) | 0.0025 |      |
|              | Over-dominant| C/C-T/T  | 180 (52.9)        | 105 (47.7)    | 1.00             |     | 754  |
|              |              | C/T     | 160 (47.1)        | 115 (52.3)    | 1.22 (0.87–1.71) | 0.25 |      |
|              | Log-additive | -        | -                 | -             | 0.73 (0.57–0.94) | 0.012 | 749  |
| TNF          | Codominant   | G/G      | 261 (77)          | 167 (75.9)    | 1.00             |     | 749.2|
|              |              | G/A      | 77 (22.7)         | 47 (21.4)     | 0.93 (0.62–1.41) | 0.028 |      |
|              |              | A/A      | 1 (0.3)           | 6 (2.7)       | 10.14 (1.20–85.48) |     |      |
|              | Dominant     | G/G      | 261 (77)          | 167 (75.9)    | 1.00             |     | 754.3|
|              |              | G/A-A/A  | 78 (23)           | 53 (24.1)     | 1.05 (0.70–1.56) | 0.83 |      |
|              | Recessive    | G/G-G/A  | 338 (99.7)        | 214 (97.3)    | 1.00             |     | 747.3|
|              |              | A/A      | 1 (0.3)           | 6 (2.7)       | 10.29 (1.22–86.59) | 0.0081 |      |
|              | Over-dominant| G/G-A/A  | 262 (77.3)        | 173 (78.6)    | 1.00             |     | 754.1|
|              |              | G/A      | 77 (22.7)         | 47 (21.4)     | 0.90 (0.60–1.37) | 0.63 |      |
|              | Log-additive | -        | -                 | -             | 1.17 (0.81–1.69) | 0.41 | 753.6|
| IL18         | Codominant   | G/G      | 111 (32.6)        | 63 (28.9)     | 1.00             |     | 748.3|
|              |              | T/G      | 158 (46.5)        | 122 (56)      | 1.32 (0.89–1.95) | 0.066 |      |
|              |              | T/T      | 71 (20.9)         | 33 (15.1)     | 0.78 (0.46–1.31) |     |      |
|              | Dominant     | G/G      | 111 (32.6)        | 63 (28.9)     | 1.00             |     | 751.2|
|              |              | T/G-T/T  | 229 (67.3)        | 155 (71.1)    | 1.15 (0.79–1.68) | 0.45 |      |
|              | Recessive    | G/G-T/G  | 269 (79.1)        | 185 (84.9)    | 1.00             |     | 748.3|
|              |              | T/T      | 71 (20.9)         | 33 (15.1)     | 0.65 (0.41–1.03) | 0.062 |      |
|              | Over-dominant| G/G-T/G  | 182 (53.5)        | 96 (44)       | 1.00             |     | 747.2|
|              |              | T/G      | 158 (46.5)        | 122 (56)      | 1.45 (1.03–2.04) | 0.033 |      |
|              | Log-additive | -        | -                 | -             | 0.93 (0.73–1.20) | 0.59 | 751.5|
| LPA          | Codominant   | A/A      | 315 (92.7)        | 191 (87.6)    | 1.00             |     | 746.6|
|              |              | A/G      | 25 (7.3)          | 25 (11.5)     | 1.64 (0.92–2.95) | 0.032 |      |
|              |              | G/G      | 0 (0)             | 2 (0.9)       | NA (0.00-NA)     |     |      |
|              | Dominant     | A/A      | 315 (92.7)        | 191 (87.6)    | 1.00             |     | 747.6|
|              |              | A/G-G/G  | 25 (7.3)          | 27 (12.4)     | 1.79 (1.00–3.17) | 0.049 |      |
|              | Recessive    | A/A-A/G  | 340 (100)         | 216 (99.1)    | 1.00             |     | 747.3|
|              |              | G/G      | 0 (0)             | 2 (0.9)       | NA (0.00-N/A)    | 0.042 |      |
Table 3. Cont.

| Gene    | Model       | Genotype  | No Obesity, N (%) | Obesity, N (%) | OR (95% CI) | p     | AIC   |
|---------|-------------|-----------|-------------------|----------------|-------------|-------|-------|
|         | Over-dominant | A/A-G/G   | 315 (92.7)        | 193 (88.5)     | 1.00        | 0.1   | 748.8 |
|         |             | A/G       | 25 (7.3)          | 25 (11.5)      | 1.62 (0.91–2.92) |       |       |
|         | Log-additive |           |                    |                | 1.86 (1.07–3.21) | 0.026 | 746.5 |
| LEPR    | Codominant  | A/A       | 181 (53.4)        | 97 (44.5)      | 1.00        | 0.0021| 740.4 |
|         |             | A/G       | 141 (41.6)        | 94 (43.1)      | 1.24 (0.87–1.78) |       |       |
|         |             | G/G       | 17 (5)            | 27 (12.4)      | 3.19 (1.64–6.18) |       |       |
|         | Dominant    | A/A       | 181 (53.4)        | 97 (44.5)      | 1.00        | 0.036 | 746.2 |
|         |             | A/G-G/G   | 158 (46.6)        | 121 (55.5)     | 1.44 (1.02–2.03) |       |       |
|         | Recessive   | A/A-A/G   | 322 (95)          | 191 (87.6)     | 1.00        | 0.001 | 739.8 |
|         |             | G/G       | 17 (5)            | 27 (12.4)      | 2.88 (1.52–5.46) |       |       |
|         | Over-dominant | A/A-G/G   | 198 (58.4)        | 124 (56.9)     | 1.00        | 0.76  | 750.6 |
|         |             | A/G       | 141 (41.6)        | 94 (43.1)      | 1.06 (0.75–1.49) |       |       |
|         | Log-additive |           |                    |                | 1.53 (1.16–2.00) | 0.0021| 741.2 |

Note: Statistically significant results after applying Akaike’s information criterion (AIC) are highlighted in bold.

After stratification by gender, we found that the T/C genotype in the IL6R gene (rs2229238) and the G/G genotype in the LEPR gene (rs1137100) were associated with an increased risk of obesity only in males (OR = 2.27, 95% CI = 1.40–3.70, \( p = 0.0003 \) and OR = 2.80, 95% CI = 1.27–6.17, \( p = 0.028 \), respectively), while the T/G genotype in the IL18 gene (rs1946518) was associated with an increased risk of obesity only in females (OR = 2.02, 95% CI = 1.07–3.83, \( p = 0.03 \)). A protective effect was shown for the T/T genotype in the CXCL8 gene (rs2227306) in females (OR = 0.44, 95% CI = 0.20–0.95, \( p = 0.04 \)) and the G/G genotype in the IL1RL1 gene (rs11685424) in males (OR = 0.46, 95% CI = 0.23–0.94, \( p = 0.023 \)) (Table 4).

Table 4. Association of SNPs with risk of obesity in groups stratified by gender.

| Gene     | Gender | Genotype | No Obesity, N | Obesity, N | OR (95% CI) | p     |
|----------|--------|----------|---------------|------------|-------------|-------|
| IL6R     | Male   | C/C      | 112           | 43         | 1.00        |       |
|          |        | T/C      | 73            | 64         | 2.27 (1.40–3.70) | 0.002 |
|          |        | T/T      | 15            | 10         | 1.74 (0.73–4.17) |       |
|          | Female | C/C      | 77            | 48         | 1.65 (0.96–2.83) | 0.08  |
|          |        | T/T      | 11            | 10         | 1.57 (0.62–4.00) |       |
| CXCL8    | Male   | C/C      | 66            | 42         | 1.00        |       |
|          |        | C/T      | 92            | 61         | 1.04 (0.63–1.72) | 0.5   |
|          |        | T/T      | 44            | 14         | 0.51 (0.25–1.04) |       |
|          | Female | C/C      | 37            | 35         | 1.00        |       |
|          |        | C/T      | 68            | 54         | 0.83 (0.46–1.49) | 0.04  |
|          |        | T/T      | 33            | 14         | 0.44 (0.20–0.95) |       |
### Table 4. Cont.

| Gene     | Gender | Genotype | No Obesity, N | Obesity, N | OR (95%CI) | p   |
|----------|--------|----------|---------------|------------|------------|-----|
| IL1RL1   | Male   | A/A      | 58            | 37         | 1.00       |     |
|          |        | G/A      | 91            | 65         | 1.12 (0.67–1.89) | 0.023 |
|          |        | G/G      | 51            | 15         | **0.46 (0.23–0.94)** |     |
|          | Female | A/A      | 45            | 30         | 1.00       |     |
|          |        | G/A      | 67            | 49         | 1.11 (0.61–2.01) | 0.43  |
|          |        | G/G      | 26            | 24         | 1.39 (0.68–2.87) |     |
| IL18     | Male   | G/G      | 70            | 43         | 1.00       |     |
|          |        | T/G      | 94            | 59         | 1.01 (0.61–1.66) | 0.48  |
|          |        | T/T      | 38            | 15         | 0.63 (0.31–1.28) |     |
|          | Female | G/G      | 41            | 20         | 1.00       |     |
|          |        | T/G      | 64            | 63         | **2.02 (1.07–3.83)** | 0.03  |
|          |        | T/T      | 33            | 18         | 1.10 (0.50–2.41) |     |
| LEPR     | Male   | A/A      | 105           | 53         | 1.00       |     |
|          |        | A/G      | 83            | 45         | **1.07 (0.66–1.75)** | 0.028 |
|          |        | G/G      | 13            | 18         | **2.80 (1.27–6.17)** |     |
|          | Female | A/A      | 76            | 44         | 1.00       |     |
|          |        | A/G      | 58            | 49         | **1.48 (0.87–2.53)** | 0.004 |
|          |        | G/G      | 4             | 9          | **4.04 (1.17–13.94)** |     |

Note: Statistically significant results are highlighted in bold.

In the group of middle-aged patients (age ≤60 years), the G/G genotype in the LEPR gene (rs1137100) was associated with a fourfold increased risk of obesity (OR = 4.23, 95% CI = 1.74–10.28, \( p = 0.03 \)). The same tendency was shown for the T allele in the IL6R gene (rs2229238). Among elderly patients (age >60 years), a risk association was shown for the G/A genotype in the CRP gene (rs1130864) (OR = 1.98, 95% CI = 1.03–3.83, \( p = 0.01 \)) and for the C allele in the TLR2 gene (rs3804099). Furthermore, the middle-aged patients with the A/A and T/T genotypes in the CXCL8 gene (rs4073 and rs2227306, respectively) had a twofold decreased risk of obesity (Table 5). In contrast, the C allele in the IL6R gene (rs2228145) was associated with a decreased risk of obesity in middle-aged patients, whereas this allele acquired was associated with an increased risk of obesity development in elderly patients (Table 5).

### Table 5. Association of SNPs with obesity risk in groups stratified by age.

| Gene     | Age     | Genotype | No Obesity, N | Obesity, N | OR (95%CI) | p   |
|----------|---------|----------|---------------|------------|------------|-----|
| IL6R     | ≤60 years| A/A      | 94            | 88         | 1.00       |     |
|          |         | A/C      | 107           | 59         | **0.58 (0.38–0.90)** | 0.004 |
|          |         | C/C      | 24            | 9          | **0.40 (0.18–0.90)** |     |
|          | >60 years| A/A      | 63            | 25         | 1.00       |     |
|          |         | A/C      | 40            | 32         | **2.03 (1.05–3.91)** | 0.027 |
|          |         | C/C      | 10            | 7          | 1.70 (0.58–4.98) |     |
Table 5. Cont.

| Gene     | Age       | Genotype | No Obesity, N | Obesity, N | OR (95%CI)    | p    |
|----------|-----------|----------|---------------|------------|---------------|------|
|          | ≤60 years |          |               |            |               |      |
|          |           | C/C      | 130           | 59         | 1.00          |      |
|          |           | T/C      | 82            | 81         | **2.21 (1.43–3.41)** | 0.015|
|          |           | T/T      | 14            | 16         | **2.53 (1.16–5.53)** |      |
| IL6R     | >60 years | C/C      | 59            | 29         | 1.00          |      |
| rs2229238|           | T/C      | 41            | 31         | 1.54 (0.80–2.93) | 0.49 |
|          |           | T/T      | 12            | 4          | 0.67 (0.20–2.25) |      |
|          | ≥60 years |          |               |            |               |      |
|          |           | T/T      | 59            | 50         | 1.00          |      |
|          |           | A/T      | 100           | 79         | 0.93 (0.58–1.51) | 0.027|
| CXCL8    | rs4073    | A/A      | 67            | 27         | **0.46 (0.26–0.83)** |      |
|          | >60 years | T/T      | 32            | 21         | 1.00          |      |
|          |           | A/T      | 54            | 30         | 0.84 (0.41–1.71) | 0.07 |
|          |           | A/A      | 27            | 13         | 0.74 (0.31–1.75) |      |
|          | ≤60 years | C/C      | 65            | 54         | 1.00          |      |
|          |           | C/T      | 110           | 82         | 0.89 (0.56–1.40) | 0.04 |
|          |           | T/T      | 51            | 20         | **0.45 (0.24–0.86)** |      |
|          | >60 years | C/C      | 38            | 23         | 1.00          |      |
|          |           | C/T      | 50            | 33         | 1.08 (0.55–2.14) | 0.05 |
|          |           | T/T      | 26            | 8          | 0.52 (0.20–1.36) |      |
| CRP      | rs1130864 | G/G      | 115           | 79         | 1.00          |      |
|          | ≤60 years | G/A      | 98            | 62         | 0.91 (0.59–1.39) | 0.19 |
|          |           | A/A      | 13            | 15         | 1.70 (0.77–3.78) |      |
|          | >60 years | G/G      | 55            | 22         | 1.00          |      |
|          |           | G/A      | 47            | 37         | **1.98 (1.03–3.83)** | 0.01 |
|          |           | A/A      | 12            | 5          | 1.04 (0.33–3.29) |      |
| LEPR     | rs1137100 | A/A      | 125           | 70         | 1.00          |      |
|          | ≤60 years | A/G      | 93            | 67         | 1.29 (0.84–1.98) | 0.03 |
|          |           | G/G      | 8             | 18         | **4.23 (1.74–10.28)** |      |
|          | >60 years | A/A      | 56            | 27         | 1.00          |      |
|          |           | A/G      | 48            | 27         | 1.14 (0.59–2.20) | 0.15 |
|          |           | G/G      | 9             | 9          | 2.12 (0.75–5.98) |      |
| TLR2     | rs3804099 | T/T      | 86            | 64         | 1.00          |      |
|          | ≤60 years | T/C      | 106           | 68         | 0.86 (0.55–1.34) | 0.36 |
|          |           | C/C      | 34            | 24         | 0.95 (0.52–1.77) |      |
|          | >60 years | T/T      | 56            | 16         | 1.00          |      |
|          |           | T/C      | 41            | 30         | **2.55 (1.23–5.28)** | 0.01 |
|          |           | C/C      | 17            | 17         | **3.35 (1.39–8.04)** |      |

Note: Statistically significant results are highlighted in bold.
4. Discussion

Chronic inflammation is involved in the pathogenesis of many diseases including obesity, type 2 diabetes mellitus, and atherosclerosis [15]. Danger signals caused by molecular patterns of microbial agents and endogenous damage factors (PAMPs and DAMPs) trigger the assembly of innate immunity intracellular sensors, which leads to the activation of caspase-1 and the production of proinflammatory cytokines IL1β and IL18 [16]. Interleukin-18 (IL18) is an important proinflammatory cytokine involved in the pathogenesis of acute coronary events and type 2 diabetes mellitus [17], and it is associated with the modification of obesity and metabolic syndrome risk, although the underlying mechanisms remain unclear [18]. It is known that IL18R and IL18 expression in adipose tissue is enhanced in nondiabetic obesity, and it is associated with a proinflammatory gene signature and insulin resistance in such patients [19]. Polymorphic variant rs1946518 in the IL18 gene is located in the promoter region and is associated with type 1 and 2 diabetes mellitus [20,21]. In was shown that the NLRP3 inflammasomes regulate adipose tissue metabolism via promoting IL18 secretion [22]. Despite the fact that this SNP was not associated with a metabolic syndrome in a northern Iranian population [23], we found an association of this polymorphic variant with a risk of obesity in females. We suppose that the T/G genotype of the IL18 gene (rs1946518) is associated with increased activity of proinflammatory IL18, which interacts with the IL18Ra/β heterodimer receptor complex expressed mainly by immune cells (e.g., macrophages, dendritic cells, T and B lymphocytes), in addition to endothelial and smooth muscle cells, thus stimulating these cells in an autocrine/paracrine manner [19,24]. An increased number of these cells, especially macrophages, can be found in the expanding adipose tissue in obese patients [25].

Interleukin-6 (IL6) is a pleiotropic cytokine involved in both immune and nonimmune events in numerous cells and tissues outside of the immune system [26]. IL6 activates an intracellular signaling cascade leading to inflammation via binding to its receptor IL6R [27]. It was reported that IL6R gene polymorphism is associated with BMI and obesity [28–30]. The results herein describing an increased risk of obesity in middle-aged males carrying the C/T genotype of the IL6R gene (rs2229238) are consistent with the findings of an association between the C/T genotype of this gene and an increased risk of obesity in schoolboys from Taiwan [31]. We suppose that the C/T genotype is associated with an elevated serum IL6R concentration and an increased level of the IL6/IL6R complex, resulting in greater IL6 signal transduction and IL6 production, with an effect on adipocytes and immune cells in adipose tissue, as well as on insulin-targeting cells in peripheral tissues [7,31].

Lipoprotein(a), encoded by the LPA gene, is a serine protease with inhibition activity toward tissue plasminogen activator I. The encoded protein is proteolytically cleaved, resulting in fragments that can attach to atherosclerotic lesions and promote thrombogenesis. Elevated plasma levels of this protein are linked to atherosclerosis [32]. LPA genetic polymorphism is associated with different cardiovascular pathologies, e.g., coronary artery disease, aortic valve stenosis, and valvular calcification [33–36]. In the presented research, we determined for the first time an association between LPA gene polymorphism (rs10455872) and obesity risk according to the log-additive inheritance model, regardless of gender and age. We hypothesize that the log-additive inheritance model was characterized by some defects in the expression of lipoprotein(a) linked to apoprotein B-100, thus leading to an increase in its synthetic rate and, consequently, an elevated obesity risk.

The protein encoded by the LEPR gene is a receptor for leptin (an adipocyte-specific hormone regulating lipid metabolism). Mutations in this gene are associated with obesity and pituitary dysfunction. LEPR gene polymorphism is associated with early onset of severe obesity and hyperphagic eating behavior [37]. The G/G genotype of the LEPR gene (rs1137100) is potentially associated with increased expression of the leptin receptor located in hypothalamic tissue, which has a significant role in controlling energy homeostasis and lipid metabolism. The increased expression of the leptin receptor results in more active binding to leptin, whose elevated secretion by adipocytes is associated with the increased obesity risk. Our results are consistent with the literature data showing that the
A minor allele of the \textit{LEPR} gene (rs1137100) is more frequent in obese patients from different populations \cite{38,39}.

An increased serum level of inflammatory markers and acute phase proteins, including C-reactive protein (CRP), is observed in obese patients \cite{40}. It has been suggested that CRP has a direct role in the regulation of adiposity via affecting the action of adipokines \cite{41}. Human CRP can dissociate into a physiologically active and proinflammatory monomeric form, which can bind to cell surface receptors \cite{42} and is potentially involved in the pathogenesis of inflammatory diseases \cite{43}. An association was revealed between CRP and leptin level \cite{44}, and a direct effect of leptin on CRP production by hepatocytes was discovered. Therefore, CRP is potentially involved in lipid metabolism via an adipohepato axis (leptin produced by adipocytes enhances CRP expression, which in turn may antagonize leptin action by limiting its tissue availability) \cite{45}. Our results demonstrate that genetically determined changes in CRP production can affect the adipo-hepato axis, leading to the defects in lipid metabolism and an increased risk of obesity, but only in elderly individuals.

Defects in the relationship between adipocytes and macrophages play an important role in the initiation of adipose tissue inflammation, thereby triggering obesity \cite{46–48}. Metabolic disorders lead to disbalance between pro- and anti-inflammatory regulators of macrophages toward the formation of proinflammatory M1-macrophages, which is linked to adipocyte dysfunction and the development of chronic inflammation in adipose tissue \cite{48}. Toll-like receptors (TLRs) represent a possible pathophysiological link between obesity and inflammation. TLRs are widely represented on the surface of immune cells (macrophages, dendritic cells, neutrophils, basophils, B and T lymphocytes, natural killer cells) and nonimmune cells (fibroblasts, epithelial cells, keratinocytes) \cite{49}. Moreover, adipocytes also express TLRs that actively participate not only in antibacterial defense, but also in the initiation of chronic inflammation of adipose tissue \cite{50}. Enhanced lipolysis in adipocytes leads to an increase in the level of unsaturated fatty acids, which, through TLRs, promote the differentiation of macrophages into an M1 phenotype \cite{51}. We found that the C allele of the \textit{TLR2} gene (rs3804099) was associated with a threefold increase in risk of obesity in elderly patients due to more active TLR-promoted inflammation.

Our most interesting results were the sex- and age-specific associations of immune response and lipid metabolism gene polymorphisms with the risk of obesity in the studied cohort. The gender dimorphism of biological and physiological functions is caused by gender-based chromosomal differences in gonadal hormone secretion. In humans, the level of gonadal hormones not only varies between males and females, but also changes depending on age, and this physiological alteration can influence the function of gonadal hormone-sensitive genes \cite{52}. Gonadal hormones can significantly modulate cell signaling pathways and control gene regulation and expression. It was shown that gonadal hormones may modify the immune response via regulating the production of pro- and anti-inflammatory cytokines and TLR expression \cite{53–56}. Moreover, lipid metabolism also varies according to gender and age. Recently, a gender-specific association of \textit{FTO} gene polymorphism with risk of obesity was revealed \cite{57}. Therefore, the gender- and age-modulated associations of SNPs in the inflammatory response and lipid metabolism genes with obesity risk identified in the present study suggest the role of gene–gender interactions in the development of this pathology. It should be noted that our results need to be replicated in different populations with a larger sample size.

5. Conclusions

Genetic polymorphisms in the immune response and lipid metabolism genes are associated with increased obesity risk in middle-aged and elderly Caucasian patients in a gender- and age-depended manner. The obtained results can be used to assess the personalized risk of obesity in healthy donors during medical examination or screening, as well as to develop appropriate early prevention strategies targeting obesity in at-risk groups.
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