Associations of TP53 codon 72 polymorphism with complications and comorbidities in patients with type 1 diabetes

Bartosz Słomiński1 · Maria Skrzypkowska1 · Monika Ryba-Stanisławowska1 · Małgorzata Myśliwiec2 · Piotr Trzonkowski1

Received: 11 September 2020 / Revised: 22 December 2020 / Accepted: 29 December 2020 / Published online: 25 January 2021

© The Author(s) 2021

Abstract
Wild-type TP53 plays an important role in the regulation of immune response and systemic inflammation. In type 1 diabetes (T1D), TP53 pathways are upregulated and an increased susceptibility to apoptosis is observed. We hypothesize that TP53 codon 72 polymorphism could be associated with complications and comorbidities in patients with T1D. We have investigated the associations of the TP53 codon 72 polymorphism with the T1D complications and comorbidities (retinopathy, nephropathy, hypertension, dyslipidemia, autoimmune thyroiditis, and celiac disease) in 350 patients. The key results of our approach are as follows: (1) In diabetic subjects, the Pro/Pro genotype is associated with an increased risk of microvascular complications, dyslipidemia, and celiac disease; (2) the Arg/Arg variant is associated with a decreased risk of autoimmune thyroiditis and celiac disease; (3) the Pro allele is associated with an increased risk of dyslipidemia, autoimmune thyroiditis, and celiac disease. Although further studies are required, our results for the first time indicate that the TP53 codon 72 polymorphism could be considered a genetic marker to predict the increased susceptibility to some T1D complications and comorbidities.

Key messages
- We analyzed the TP53 codon 72 polymorphism in patients with T1D.
- Pro/Pro genotype is associated with an increased risk of microvascular complications, dyslipidemia, and celiac disease.
- The Arg/Arg variant is associated with a decreased risk of autoimmune thyroiditis and celiac disease.
- The Pro allele is associated with an increased risk of dyslipidemia, autoimmune thyroiditis, and celiac disease.

Keywords Type 1 diabetes · Diabetes complications · TP53 codon 72 polymorphism

Introduction
Wild-type TP53 (tumor protein p53, more commonly known as P53) has been established as a tumor suppressor in human cancer as it plays an important role in the control of cell proliferation and death. P53 is a transcription factor that protects the genome against a plethora of environmental and intracellular challenges [1]. The P53 protein is activated when DNA damage occurs by stress such as ultraviolet radiation, heat shock, growth factor withdrawal, hypoxia, and inflammation in various cells and tissues [2]. These stresses have an impact upon many tissue and organ functions and therefore can lead to many diverse disorders or even regulate normal organismic functions. The consequence of P53 positive regulation is the induction of pathways leading to cell cycle arrest, apoptosis, DNA repair, autophagy, and senescence [3]. Not only is P53 activated by stress signals, but it also seems to control energy metabolism under normal conditions [4]. P53 has been also found to be a critical factor governing immune responses and inflammation, aging, reproduction, development, and neurodegeneration [5]. A number of studies suggest that P53 plays a protective role against various autoimmune conditions by suppressing cytokine production as well as reducing the number of pathogenic cells [6].
**Materials and methods**

**Subjects**

This study was conducted with 350 Caucasian adolescents, including 171 boys and 179 girls (mean age 15.5 ± 3.5 years) with clinical and laboratory diagnosis of T1D, recruited from the Chair and Clinics of Pediatrics, Diabetology and Endocrinology, Medical University of Gdańsk. T1D diagnosis was based on the American Diabetes Association criteria [10]. All patients were treated with humanized insulin at doses of 0.87 ± 0.2 U/kg. At the time of sampling, lipid levels (total cholesterol – TC, triglycerides – TG, high-density lipoprotein cholesterol – HDL, low-density lipoprotein cholesterol – LDL) along with biochemical measurement of renal function, C-reactive protein (CRP), and glycated hemoglobin (HbA1c) were monitored. All of the subjects with diabetes-related complications and comorbidities were newly diagnosed and previously untreated.

Population control subjects consisted of 200 healthy volunteers from the same population. Neither signs of autoimmune and/or inflammatory disease at the time of sampling nor evidence of T1D in families were disclosed as confirmed by medical records and laboratory tests.

Written informed consent to participate in the study was obtained from all subjects or from their parents. This study was approved by the Ethics Committee of the Medical University of Gdańsk (NKEBN/2014/2009; 2009) and the investigation was carried out in accordance with the principles of the Declaration of Helsinki.

---

**Medical examinations**

Systolic and diastolic blood pressures (SBP and DBP, resp.) were measured using automatic 24-h ambulatory blood pressure monitoring (ABPM) by the Holter method. All the average values of the blood pressure were expressed in the centile charts. Arterial hypertension was diagnosed when the blood pressure value reached at least the 95th percentile for the corresponding age, gender, and height on at least three separate occasions [11].

Ophthalmologic investigation was performed in all T1D patients. Diabetic retinopathy was determined by visual acuity, intraocular pressure measurement, anterior segment examination by slit lamp (TOPCON SL-82, Japan), and fluorescein angiography (digital camera-Topcon IMAGEnet2000, Japan). The eye fundus examination was performed with the +90D lens (Ocular Instruments Inc., Bellevue, WA, USA). Each image was graded for retinopathy according to the Early Treatment for Diabetic Retinopathy Study (ETDRS) severity level and was dichotomized as having retinopathy (level 15 and above) or not having retinopathy (≤ 14) [12].

Renal function was determined by estimated glomerular filtration rate (eGFR), which was evaluated by using the Zappitelli equation: eGFR (ml/min/1.73 m²) = (507.76 * e(0.3 * height (cm))/serum cystatin C (mg/l) 0.635 * serum creatinine (μmol/l) 0.547) [13].

The urinary albumin excretion (UAE) was expressed as the average of three 24-h collections. Cases were classified as microalbuminuria when in at least two out of three urine samples, UAE ratio was > 30–300 mg/24 h. Diabetic nephropathy was defined as persistent microalbuminuria in two out of three consecutive urine samples without clinical or laboratory evidence of other kidney or urinary tract disease.

Dyslipidemia was defined by the presence of one or more abnormal serum lipid concentrations: TC ≥ 5.17 mmol/l (200 mg/dl); HDL < 1.03 mmol/l (40 mg/dl); LDL ≥ 2.6 mmol/l (100 mg/dl); TG ≥ 1.69 mmol/l (150 mg/dl) [14]. Further analyses were performed after controlling for age and pubertal stage to avoid differences in lipid values [15].

In all of the patients, the diagnosis of celiac disease (CD) was made in accordance with the revised criteria of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition [16]. To be defined as celiac sufferer, each subject was required to have (1) positive celiac-specific antibodies (IgA-AGA/IgG-AGA; IgA-EmA/IgG-EmA and IgA-anti-tTG); (2) or a proximal small intestinal biopsy compatible with celiac disease; and (3) either clinical and/or histological improvement with a gluten-free diet. Celiac patients fulfilled all three criteria.

Screening for autoimmune thyroiditis (TA) was performed using measurements of thyroid antiperoxidase antibody (TPOAb), antithyroglobulin antibody (TGAAb), and thyroid-stimulating hormone (TSH) receptor antibodies (TSHRAb).
and sonographic signs of the disease [17]. Free thyroxine and TSH were also measured. TA was defined as the presence of at least one thyroid autoantibody.

**Methods**

Venous blood samples were withdrawn after 12–14 h overnight fasting. Serum and plasma samples were collected from T1D patients by centrifugation at 500g for 15 min and stored at −70 °C until analysis.

Concentrations of TNF-α, ICAM-1, VCAM-1, IL-6, and IL-10 were determined using commercial enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s protocol.

Plasma TC, TG, and HDL-C concentrations were measured in an independent, ISO-certified laboratory. LDL-C was estimated by the Friedewald equation [18].

**Genotyping protocol**

Genomic DNA from all subjects was isolated from EDTA-stabilized blood using the EXTRACTME DNA BLOOD kit (Blirt, Poland). DNA was stored at −20 °C until the time of use.

The genotyping of TP53 codon 72 polymorphism (rs1042522) was carried out using tetra-primer amplification refractory mutation system–polymerase chain reaction (ARMS–PCR). In this assay, confronting pairs of primers (outers and inners) were used as shown below:

- **forward outer**: 5′ − ACAAGGTTGGGCTGGGACCTGGAGGG − 3′.
- **reverse outer**: 5′ − CAGCCCCCTCAGGGCAACTGACCGTGCCA − 3′.
- **forward inner**: 5′ − CTCCCAAGATGCCAGGCTGCTCCCAGAATGCCAGAGGCT − 3′.
- **reverse inner**: 5′ − GTAGGAGCTGCTGGTGTCAGGGCCAGG − 3′.

The region containing TP53 codon 72 polymorphism was amplified in a total volume of 15 μl, containing 20 ng of DNA template, 1.65 mM MgCl₂ (Thermo Fisher Scientific, MA, USA), 200 μM dNTP (Thermo Fisher Scientific, MA, USA), 250 nM of each primer (Sigma-Aldrich, MO, USA), and 0.75 U FIREPol DNA polymerase with 1x buffer (Solis BioDyne, Tartu, Estonia). The procedure consisted of denaturation at 96 °C for 15 min, followed by 35 cycles of 96 °C for 30 s, 68 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 1 min. PCR products were visualized on a 2% agarose gel with ethidium bromide staining. Genotyping was performed as follows: 403, 249 bp for Pro/Pro (GG) genotype; 403, 249, 210 bp for Pro/Arg (GC) genotype; and 403, 210 bp for Pro/Pro (CC) genotype.

DNA samples were first sequenced to establish three TP53 gene polymorphic variants as a quality control. Afterwards, DNA samples of the Arg/Arg, Arg/Pro, and Pro/Pro individuals were routinely added to the examined ones to ensure genotype accuracy.

**Statistical analysis**

The results were analyzed using Statistica, ver. 12 (StatSoft, Inc., USA). Conformation of the allele frequencies to the Hardy-Weinberg equilibrium (HWE) proportions was tested by the χ² test. The genotypes and allele frequencies of the TP53 codon 72 polymorphism were compared using Pearson’s χ² test. Differences between groups were analyzed by ANOVA for normally distributed values or the Kruskal–Wallis test for nonparametric values (the post hoc NIR test was applied to assess statistical significance) and by the χ² Pearson test for dichotomous variables. Correlation between variables was evaluated using Spearman’s correlation coefficient. To deal with multiple testing, Benjamini-Hochberg’s correction was used for statistical significance. The level of significance was set at p ≤ 0.05. Logistic regression model was used to examine the association between TP53 codon 72 polymorphism and diabetes-related complications and comorbidities.

**Results**

**TP53 codon 72 genotype distribution**

TP53 codon 72 genotypes were analyzed in T1D patients and healthy controls. The occurrence of each genotype and allele frequencies is shown in Table 1. The genotype distributions for both, healthy group and T1D patients, were in Hardy-Weinberg equilibrium (p = 0.73 and 0.69, resp.). Comparison of the frequencies of TP53 codon 72 genotypes between healthy group and the T1D patients revealed lack of significant differences (p = 0.11) but the presence of Arg/Pro variant was connected with a some increased risk of T1D (OR = 1.457, p = 0.04). In case of the allele frequencies among both groups, no differences were found (p = 0.15).

**TP53 codon 72 polymorphism and clinical characteristics of patients**

Characteristics of T1D patients included in this study differing in the TP53 codon 72 polymorphism are shown in Table 2. There were no statistically significant differences in sex, age, age of T1D onset, duration of T1D, BMI, HbA1c, and values of blood pressure between subjects with different TP53 genotypes. However, individuals with Pro/Pro variant had lowest
values of eGFR \((p = 0.02)\). There were no statistically significant differences in all clinical parameters between Arg and Pro alleles.

**Genotype and allele distribution of TP53 codon 72 in T1D patients considering complications and comorbidities**

We have compared the distribution of genotypes and alleles between individuals with and without T1D complications and comorbidities (Table 3). None deviated significantly from HWE in all studies. There were no differences in the genotypic and allelic distributions with respect to retinopathy \((p = 0.57 \text{ and } 0.60, \text{ resp.})\), nephropathy \((p = 0.26 \text{ and } 0.37, \text{ resp.})\), and hypertension \((p = 0.25 \text{ and } 0.72, \text{ resp.})\). However, we have observed alterations in the frequencies of TP53 codon 72 genotypes, but not alleles, due to diabetic microvascular complications (both retinopathy and nephropathy). Genotype distributions in patients with nephropathy and retinopathy were different in comparison to complication-free group \((p = 0.02)\). We have also found differences in the genotypic and allelic distributions with respect to dyslipidemia \((p = 0.002 \text{ and } 0.03, \text{ resp.})\), autoimmune thyroiditis \((p = 0.03 \text{ and } 0.01, \text{ resp.})\), and celiac disease \((p < 0.000 \text{ and } 0.000, \text{ resp.})\).

**Associations of TP53 codon 72 polymorphism with complications and comorbidities in patients with type 1 diabetes**

Among the variables reported in Table 3 that were found to be significantly different between individuals with and without T1D complications and comorbidities, the logistic regression

| Clinical parameter | TP53 genotypes |  \(p^1\) |  \(p^2\) |  \(p^3\) |  \(p^4\) | TP53 alleles |
|-------------------|----------------|--------|--------|--------|--------|---------------|
| N (%)             | Arg/Arg        | 166    | 152    | 32     | -      | 484           |
| Sex (male/female) | Arg/Arg        | 81/85  | 79/73  | 11/21  | 0.19   | 241/243       |
| Age (years)       | Arg/Arg        | 15.6±3.4 | 15.3±3.1 | 16.5±3.2 | 0.14   | 15.6±3.2      |
| Age of onset of diabetes (years) | Arg/Arg | 8.9±3.1 | 8.3±3.1 | 9.1±2.8 | 0.20   | 8.6±3.1       |
| Duration of diabetes (years) | Arg/Arg | 6.8±2.8 | 6.9±2.8 | 7.6±3.5 | 0.35   | 7.1±3.0       |
| BMI (kg/m²)       | Arg/Arg        | 20±2   | 20±2   | 21±3   | 0.16   | 20±3          |
| HbA1c (%) (mmol/mol) | Arg/Arg | 8.5±1.7 | 8.7±1.6 | 8.6±1.4 | 0.60   | 8.7±1.5       |
| eGFR (ml/min/1.73 m²) | Arg/Arg | 121±26 | 128±26 | 116±27 | 0.02   | 124±27        |

**Table 1** Distribution of genotype and allele frequencies of TP53 codon 72 polymorphism in healthy group and patients with T1D

| TP53 genotypes | Healthy (N = 200) | T1D (N = 350) | \(\chi^2\)     | Odds ratio analysis |
|----------------|-------------------|---------------|----------------|---------------------|
| \(N\)           | \%                | \(N\)         | \%             |         | P         |
| Arg/Arg         | 112               | 166           | 56.0           | 4.39    | 0.098    |
| Arg/Pro         | 69                | 152           | 34.5           | 0.11    | 1.457    |
| Pro/Pro         | 19                | 32            | 9.5            | 0.89    | 0.958    |

| Allele frequency | \(\%\) | \(\%\) | \(\chi^2\) | \(OR\) | 95% CI |
|------------------|-------|-------|------------|-------|-------|
| Arg              | 293   | 484   | 2.07       | 0.818 | 0.622–1.076 |
| Pro              | 107   | 216   | 30.8       | 1.222 | 0.929–1.607 |

Bold \(p\) values indicate that the differences are statistically significant

\(N\) number of patients, \(OR\) odds ratio, 95% CI 95% confidence interval

**Table 2** Selected clinical characteristics of T1D patients stratified according to TP53 codon 72 genotypes and alleles

| Clinical parameter | Arg/Arg | Arg/Pro | Pro/Pro | \(p^1\) | \(p^2\) | \(p^3\) | \(p^4\) | TP53 alleles |
|--------------------|---------|---------|---------|--------|--------|--------|--------|---------------|
| N (%)              | 166     | 152     | 32      | -      | -      | -      | -      | 484           |
| Sex (male/female)  | 81/85   | 79/73   | 11/21   | 0.19   | -      | -      | -      | 241/243       |
| Age (years)        | 15.6±3.4 | 15.3±3.1 | 16.5±3.2 | 0.14 | 0.31   | 0.17   | 0.05 | 15.6±3.2      |
| Age of onset of diabetes (years) | 8.9±3.1 | 8.3±3.1 | 9.1±2.8 | 0.20 | 0.12   | 0.65   | 0.18 | 8.6±3.1       |
| Duration of diabetes (years) | 6.8±2.8 | 6.9±2.8 | 7.6±3.5 | 0.35 | 0.69   | 0.15   | 0.23 | 7.1±3.0       |
| BMI (kg/m²)        | 20±2    | 20±2    | 21±3    | 0.16   | 0.25   | 0.24   | 0.07 | 20±3          |
| HbA1c (%) (mmol/mol) | 8.5±1.7 | 8.7±1.6 | 8.6±1.4 | 0.60   | 0.32   | 0.76   | 0.78 | 8.7±1.5       |
| eGFR (ml/min/1.73 m²) | 121±26 | 128±26 | 116±27 | 0.02 | 0.02   | 0.36   | 0.02 | 124±27        |
| Systolic blood pressure (mmHg) | 116±8  | 115±8  | 113±8  | 0.28 | 0.61   | 0.11   | 0.19 | 115±7         |
| Diastolic blood pressure (mmHg) | 72±6   | 73±6   | 72±5   | 0.76   | 0.55   | 0.79   | 0.55 | 72±6          |

Bold \(p\) values indicate that the differences are statistically significant

\(N\) number of patients, \(p^1\) the comparison between all genotypes, \(p^2\) the post hoc comparison Arg/Arg vs. Arg/Pro, \(p^3\) the post hoc comparison Arg/Arg vs. Pro/Pro, \(p^4\) the post hoc comparison Arg/Pro vs. Pro/Pro, \(p^5\) the comparison Arg vs. Pro
model was performed. Table 4 shows the results of TP53 genotype and allele interaction effects for microvascular complications, dyslipidemia, autoimmune thyroiditis, and celiac disease. No significant interaction effect of genotype was observed in the other studied variables (data not shown).

Logistic regression analysis revealed a tendency of the Pro/Pro variant to associate with microvascular complications (OR = 2.535, \( p = 0.01 \)), dyslipidemia (OR = 4.015, \( p = 0.001 \)), and celiac disease (OR = 4.714, \( p < 0.000 \)) where the minor genotype increases the risk of these conditions. Simultaneously, there were characteristic features linking Arg/Arg carriers and autoimmune thyroiditis (OR = 0.553, \( p = 0.02 \)) and celiac disease (OR = 0.262, \( p = 0.004 \)) where the major genotype decreases the risk of these conditions.

Logistic regression analysis also revealed a significant associations between Pro allele and dyslipidemia (OR = 1.429, \( p = 0.03 \)), autoimmune thyroiditis (OR = 1.608, \( p = 0.01 \)), and celiac disease (OR = 2.836, \( p < 0.000 \)) with this variant increasing the risk of these conditions.

### Table 3  Genotype and allele distribution of TP53 codon 72 polymorphism in T1D patients considering complications and comorbidities

| T1D complications and comorbidities | TP53 genotypes | \( p^1 \) | HWE | TP53 alleles | \( p^2 \) |
|-------------------------------------|----------------|----------|------|--------------|----------|
|                                     | Arg/Arg | Arg/Pro | Pro/Pro |              | Arg | Pro |
| N | % | N | % | N | % | N | % | N | % |
|----------------------|---------|---------|---------|------------------|------|------|
| Microvascular complications | No (\( N =225 \)) | 106 | 47.1 | 105 | 46.7 | 14 | 6.2 | 0.02 | 0.70 | 317 | 70.4 | 133 | 29.6 | 0.32 |
| Yes (\( N =125 \)) | 60 | 48.0 | 47 | 37.6 | 18 | 14.4 | 0.67 | 167 | 66.8 | 83 | 33.2 |
| Retinopathy | No (\( N =284 \)) | 136 | 47.6 | 126 | 44.0 | 24 | 8.4 | 0.57 | 0.69 | 398 | 69.6 | 174 | 30.4 | 0.60 |
| Yes (\( N =64 \)) | 30 | 46.9 | 26 | 40.6 | 8 | 12.5 | 0.67 | 86 | 67.2 | 42 | 32.8 |
| Nephropathy | No (\( N =270 \)) | 129 | 47.8 | 120 | 44.4 | 11 | 13.8 | 0.26 | 0.70 | 378 | 70.0 | 162 | 30.0 | 0.37 |
| Yes (\( N =80 \)) | 37 | 46.2 | 32 | 40.0 | 11 | 13.8 | 0.66 | 106 | 66.3 | 54 | 33.7 |
| Hypertension | No (\( N =284 \)) | 136 | 47.8 | 119 | 42.0 | 29 | 10.2 | 0.25 | 0.69 | 398 | 69.6 | 174 | 30.4 | 0.72 |
| Yes (\( N =66 \)) | 30 | 45.5 | 33 | 50.0 | 3 | 4.5 | 0.70 | 93 | 70.4 | 39 | 29.6 |
| Dyslipidemia | No (\( N =160 \)) | 94 | 49.5 | 88 | 46.3 | 8 | 4.2 | 0.002 | 0.73 | 276 | 72.6 | 104 | 27.4 | 0.03 |
| Yes (\( N =190 \)) | 72 | 45.0 | 64 | 40.0 | 24 | 15.0 | 0.65 | 208 | 65.0 | 112 | 35.0 |
| Autoimmune thyroiditis | No (\( N =265 \)) | 135 | 51.0 | 110 | 41.5 | 20 | 7.5 | 0.03 | 0.72 | 380 | 71.7 | 150 | 28.3 | 0.01 |
| Yes (\( N =85 \)) | 31 | 36.5 | 42 | 49.4 | 12 | 14.1 | 0.61 | 104 | 61.2 | 66 | 38.8 |
| Celiac disease | No (\( N =321 \)) | 160 | 49.8 | 137 | 42.7 | 24 | 7.5 | <0.000 | 0.71 | 457 | 71.2 | 185 | 28.8 | <0.000 |
| Yes (\( N =29 \)) | 6 | 20.7 | 15 | 51.7 | 8 | 27.6 | 0.47 | 27 | 46.5 | 31 | 53.5 |

Bold \( p \) values indicate that the differences are statistically significant. Microvascular complications = retinopathy and nephropathy. \( N \) number of patients, \( p^1 \) the comparison between all genotypes, \( p^2 \) the comparison Arg vs. Pro, HWE Hardy-Weinberg equilibrium.

### Table 4  Odds ratio analysis for complications and comorbidities in T1D patients

| T1D complications and comorbidities | TP53 genotypes | \( p^1 \) | TP53 alleles | \( p^2 \) |
|-------------------------------------|----------------|----------|--------------|----------|
|                                     | Arg/Arg | Arg/Pro | Pro/Pro | Arg | Pro |
| OR | 95% CI | \( p \) | OR | 95% CI | \( p \) | OR | 95% CI | \( p \) |
|----------------------|---------|---------|---------|------|------|
| Microvascular complications | 1.036 | 0.666–1.612 | 0.87 | 0.689 | 0.440–1.078 | 0.10 | 2.535 | 1.211–5.307 | 0.01 | 1.185 | 0.849–1.652 | 0.32 |
| Dyslipidemia | 0.835 | 0.547–1.276 | 0.40 | 0.773 | 0.504–1.185 | 0.23 | 4.015 | 1.745–9.238 | 0.0001 | 1.429 | 1.035–1.973 | 0.03 |
| Autoimmune thyroiditis | 0.553 | 0.333–0.916 | 0.02 | 1.376 | 0.841–2.251 | 0.20 | 2.014 | 0.937–4.325 | 0.07 | 1.608 | 1.119–2.310 | 0.01 |
| Celiac disease | 0.262 | 0.104–0.664 | 0.004 | 1.439 | 0.670–3.089 | 0.35 | 4.714 | 1.883–11.801 | <0.000 | 2.836 | 1.645–4.888 | <0.000 |

Bold \( p \) values indicate that the differences are statistically significant. Microvascular complications = retinopathy and nephropathy. Arg\(^1\)–Arg = reference allele. OR odds ratio, 95% CI 95% confidence interval.
Serum concentrations of different variables in patients with T1D differing in the TP53 codon 72 polymorphism

Table 5 describes the association between TP53 genotypes and serum concentrations of different variables in T1D patients. There was no statistically significant difference in serum concentrations of TNF-α, IL-6, IL-10, and triglycerides between subjects with different TP53 genotypes. However, Pro/Pro carriers had the lowest concentrations of ICAM-1 ($p<0.000$), VCAM-1 ($p<0.000$), and HDL-C ($p=0.04$) and the highest levels of total cholesterol ($p<0.000$) and LDL-C ($p<0.000$). Simultaneously, the Arg/Arg carriers had increased serum concentrations of CRP ($p=0.03$) and decreased IL-6/IL-10 ratio ($p=0.001$) when compared to holders bearing other genotypes.

There were also considerable differences between the alleles. Pro carriers had the lowest concentrations of CRP ($p=0.03$) and total cholesterol ($p=0.006$) and the highest of IL-6/ IL-10 ratio ($p=0.002$) and LDL-C ($p=0.001$).

Discussion

Linkage between TP53 codon 72 polymorphism and type 2 diabetes (T2D) has been described in various studies. Results from the latest meta-analysis revealed that the Arg variant is one of the strongest genetic risk factors for T2D [19]. To the best of our knowledge, there were only three genetic investigations to establish the role of TP53 codon 72 polymorphism in T1D. Spitsina et al. [20] have not found any associations, and Bitti et al. [21] have suggested that the Arg/Arg genotype predispose to T1D in a sex-specific and age-specific manner, whereas Gloria-Bottini et al. [22] have found increase in the Arg/Arg genotype in T1D vs. control subjects. In our study, T1D seemed to have little association ($p=0.04$) with Arg/Pro carriers but not with the other genotypes nor alleles. This controversy might be due to the fact that the genotype distribution of the TP53 codon 72 polymorphism varies among racial and ethnic groups. Interestingly, the frequency of this TP53 variant is also associated with UV exposure, increasing latitude or colder winter temperatures [23].

In the currently available literature, there are no studies investigating the associations of TP53 codon 72 polymorphism with T1D complications and comorbidities such as retinopathy, nephropathy, hypertension, dyslipidemia, autoimmune thyroiditis, and celiac disease. Our results indicate for the first time that this polymorphism may affect the abovementioned conditions. The key results of our approach are as follows:

1) In diabetic subjects, the Pro/Pro genotype is associated with an increased risk of microvascular complications (OR = 2.535), dyslipidemia (OR = 4.015), and celiac disease (OR = 4.714);
2) The Arg/Arg variant is associated with a decreased risk of autoimmune thyroiditis (OR = 0.553) and celiac disease (OR = 0.262);
3) The Pro allele is associated with an increased risk of dyslipidemia (OR = 1.429), autoimmune thyroiditis (OR = 1.608), and celiac disease (OR = 2.836).

Table 5  Serum concentrations of different variables in patients with T1D differing in the TP53 codon 72 polymorphism

| Clinical parameter | TP53 genotypes | $p^1$ Arg/Arg | $p^2$ Arg/Pro | $p^3$ Pro/Pro | $p^4$ | TP53 alleles | $p^5$ Arg | $p^5$ Pro |
|--------------------|----------------|---------------|---------------|---------------|-------|----------------|-------|---------|
| TNF-α (pg/ml)      | 1.02 ± 0.90    | 1.06 ± 0.96   | 1.25 ± 0.85   | 0.44          | 0.68  | 0.20           | 0.30  | 1.03 ± 0.91| 1.12 ± 0.93| 0.26 |
| CRP (mg/l)         | 2.17 ± 1.58    | 1.78 ± 1.18   | 1.84 ± 1.08   | 0.03          | 0.01  | 0.21           | 0.82  | 2.05 ± 1.48| 1.80 ± 1.15| 0.03 |
| ICAM-1 (ng/ml)     | 514 ± 100      | 532 ± 134     | 439 ± 49      | <0.00         | 0.15  | <0.00          | <0.00 | 520 ± 112 | 505 ± 123 | 0.11 |
| VCAM-1 (ng/ml)     | 812 ± 169      | 903 ± 196     | 745 ± 91      | <0.00         | <0.00 | <0.00          | <0.00 | 841 ± 183 | 856 ± 186 | 0.30 |
| IL-6 (pg/ml)       | 1.45 ± 0.98    | 1.59 ± 1.12   | 1.25 ± 1.10   | 0.21          | 0.26  | 0.32           | 0.10  | 1.49 ± 1.03| 1.49 ± 1.12| 0.92 |
| IL-10 (pg/ml)      | 2.09 ± 1.93    | 2.45 ± 2.28   | 2.40 ± 2.37   | 0.31          | 0.14  | 0.45           | 0.90  | 2.20 ± 2.05| 2.43 ± 2.29| 0.19 |
| IL-6/IL-10         | 1.58 ± 1.04    | 2.30 ± 2.33   | 2.20 ± 1.79   | 0.001         | <0.00 | 0.08           | 0.78  | 1.81 ± 1.59| 2.27 ± 2.18| 0.002 |
| Total cholesterol  | 4.53 ± 0.63    | 4.36 ± 0.58   | 5.25 ± 0.69   | <0.00         | 0.02  | <0.00          | <0.00 | 4.48 ± 0.62| 4.63 ± 0.73| 0.006 |
| HDL-C (mmol/l)     | 1.54 ± 0.29    | 1.62 ± 0.29   | 1.54 ± 0.15   | 0.04          | 0.02  | 0.92           | 0.14  | 1.57 ± 0.29| 1.59 ± 0.25| 0.23 |
| LDL-C (mg/l)       | 2.46 ± 0.49    | 2.40 ± 0.57   | 3.05 ± 0.75   | <0.00         | 0.39  | <0.00          | <0.00 | 2.44 ± 0.52| 2.60 ± 0.69| 0.001 |
| Triglycerides (mmol/l) | 1.05 ± 0.51 | 1.02 ± 0.39   | 1.13 ± 0.34   | 0.46          | 0.59  | 0.36           | 0.22  | 1.04 ± 0.47| 1.05 ± 0.38| 0.72 |

Bold $p$ values indicate that the differences are statistically significant

$N$ number of patients, $p^1$ the comparison between all genotypes, $p^2$ the post hoc comparison Arg/Arg vs. Arg/Pro, $p^3$ the post hoc comparison Arg/Arg vs. Pro/Pro, $p^4$ the post hoc comparison Arg/Pro vs. Pro/Pro, $p^5$ the comparison Arg vs. Pro
There is much evidence that inflammation is an important player in the T1D complications and comorbidities. Our data imply various effects of TP53 codon 72 polymorphism on the inflammatory status in patients. The Arg/Arg homozygotes and Arg carriers had the highest concentrations of CRP and Pro/Pro carriers had the lowest levels of proinflammatory ICAM-1 and VCAM-1. Therefore, Pro/Pro carriers seem to express weakened inflammatory response. On the other hand, individuals bearing Arg/Arg variant exhibited the lowest values of IL-6/IL-10 ratio as opposed to Pro carriers. In the light of the foregoing, patients carrying the Pro allele are more privileged.

Leaving aside our differences, accumulating evidence strongly indicates that P53 plays a significant role in the inflammation and autoimmunity [24]. The mechanisms underlying P53-mediated inflammation have been extensively investigated in animal models. Murine p53 can directly repress the activation of IL-6 promoter [25]. In addition, p53 inhibits the transcription of TNF-inducible genes and NF-κB-dependent promoters and, consistently, p53 deficiency in macrophages or mast cells enhances the production of proinflammatory cytokines such as IL-1, IL-6, IL-12, and TNF-α [26]. Furthermore, Kawashima et al. [27] have found that p53 enhances the transcription of Foxp3 gene and induces the differentiation of T regulatory cells, deficiency of which causes systemic autoimmune diseases. Moreover, p53−/− mice treated with low-dose streptozotocin showed a higher rate of T1D incidence and higher levels of proinflammatory cytokines [28]. Consistent with P53 putative regulatory effect in autoimmunity, the presence of anti-P53 antibodies has been described in patients with some autoimmune disorders [29] including T1D [30]. Furthermore, upregulated TP53 pathways and increased susceptibility to apoptosis of CD4+CD25high T regulatory cells have been observed in T1D [31].

Some studies have investigated the associations between TP53 codon 72 polymorphism and susceptibility to inflammatory and autoimmune diseases. The TP53 codon 72 Arg/Arg polymorphism has been associated with higher risk of inflammatory bowel disease [32]. On the other hand, Pro/Pro homozygotes demonstrate increased risk of endometriosis [33] and the Pro allele may be involved in the development of coronary artery disease [34]. Ruggeri et al. [35] and Chen at al. [36] showed an increased prevalence of the homozygous genotype Arg/Arg in Hashimoto’s thyroiditis patients. We obtained some conflicting results. In our study, we observed that the Arg/Arg variant is associated with decreased risk of autoimmune thyroiditis. The main reason for this discrepancy may be the fact that our patients had T1D and TA, not only Hashimoto’s thyroiditis. Moreover, population age distribution was different in our cohort and it is known that age of Hashimoto’s thyroiditis onset may influence other autoimmune disease clustering [37].

Diabetes is often associated with dysregulation of lipid metabolism and subsequently dyslipidemia. In the present study, we have observed that the Pro/Pro genotype and Pro allele are associated with an increased risk of dyslipidemia. Moreover, we have found that Pro/Pro homozygotes and Pro carriers had the highest concentrations of total and LDL cholesterol. Mounting evidence suggests that P53 plays a crucial role in normal as well as disturbed lipid metabolism [38]. Therefore, one may speculate that TP53 codon 72 polymorphism constitutes an additional modulator of this process.

Some previous findings suggested that Pro/Pro carriers are more privileged during events contributing to inflammation and metabolic homeostasis, probably due to better retention of correct setting of inflammatory and metabolic parameters even in the presence of severe disturbance [39]. As the Arg variant is a stronger apoptosis inducer while the Pro variant is a stronger transcriptional activator, tissue-specific differences between these phenotypes have been observed [40]. While unraveling this issue, we have to bear in mind that, as for many transcription factors, the overall effect of the protein comes from its expression in leukocytes as well as non-immune cells such as the pancreatic islets. Moreover, effector T cell dysregulation is observed upon p53 activation in T1D patients [41]. Intriguingly, multiple studies have shown that the TP53 codon 72 polymorphism can affect apoptosis in the context of not only the wild-type TP53 sequence but also the TP53 with sustained somatic mutations. Interestingly, some reports suggest the association of TP53 codon72 Pro isoform with higher levels of apoptosis [42]. Furthermore, there are still many unmeasured genetic and environmental factors that may modulate the effect of P53 on cell fates and as such, can contribute to the T1D complication and comorbidity development.

Thus, although further studies are required, the TP53 codon 72 polymorphism could be considered a genetic marker to predict increased susceptibility to some T1D complications and comorbidities.

The present study has strengths and limitations that need to be briefly addressed. The advantages include the use of a pure Caucasian population from the north region of Poland to eliminate false positive results due to population stratification. Regarding limitations, although the study cohort is homogeneous and well-characterized, it may be considered relatively small. Therefore, conducting further studies on a larger group, especially with the consideration of its genetic substructure, is needed to confirm our results. In spite of these limitations, our findings emphasize the role of the TP53 codon 72 polymorphism in T1D complications and comorbidities.

Authors’ contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by B. Słomiński, M. Skrzypkowska, and M. Ryba-Stanislawowska. P. Trzonkowski and M. Myśliwiec were involved in the acquisition of patients and supervised the study. The first draft of the manuscript was written by B. Słomiński. All authors read and approved the final manuscript.
References

1. Ashley AK, Kemp CJ (2018) DNA-PK, ATM, and ATR: PIKKing on p53. Cell Cycle 17:275–276
2. Green DR, Kroemer G (2009) Cytoplasmic functions of the tumour suppressor p53. Nature 458:1127–1130
3. Sionov RV, Hauy P (1998) Apoptosis by p53: mechanisms, regulation, and clinical implication. Springer Semin Immunopathol 19:345–362
4. Vousden KH, Ryan KM (2009) p53 and metabolism. Nat Rev Cancer 9:691–700
5. Kung CP, Leu JJ, Basu S, Khaku S, Anokye-Danso F, Liu Q, George DL, Ahima RS, Murphy ME (2016) The P2R polymorphism of p53 predisposes to obesity and metabolic dysfunction. Cell Rep 14:2413–2425
6. Takatori H, Kawashima H, Suzuki K, Nakajima H (2014) Role of p53 in systemic autoimmune diseases. Crit Rev Immunol 34:509–516
7. Bojesen SE, Nordestgaard BG (2008) The common germline Arg72Pro polymorphism of p53 and increased longevity in humans. Cell Cycle 7:158–163
8. Thomas M, Kalita A, Labrecque S, Pati D, Ban L (1999) Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol 19:1092–1100
9. Jeong BS, Hu W, Belyi V, Rabada R, Levine AJ (2010) Differential levels of transcription of p53-regulated genes by the arginine/proline polymorphism: p53 with arginine at codon 72 favors apoptosis. FASEB J 24:1347–1355
10. American Diabetes Association (2010) Diagnosis and classification of diabetes mellitus. Diabetes Care 33:S62–S69
11. National High Blood Pressure Program Working Group on High Blood Pressure in Children and Adolescents (2004) The fourth report on the diagnosis, evaluation and treatment of high blood pressure in children and adolescent. Pediatrics 114:555–576
12. Early Treatment Diabetic Retinopathy Study Research Group (1991) Classification of diabetic retinopathy from fluorescein angiograms. Ophthalmology 98:807–822
13. Fadrowski JJ, Neu AM, Schwartz GJ, Furh SL (2011) Pediatric GFR estimating equations applied to adolescents in the general population. Clin J Am Soc Nephrol 6:1427–1435
14. American Diabetes Association (2003) Management of dyslipidemia in children and adolescents with diabetes. Diabetes Care 26:2194–2197
15. Cook S, Auinger P, Huang TT (2009) Growth curves for cardiometabolic risk factors in children and adolescents. J Pediatr 155:S6–S26
16. Walker-Smith JA, Guandalini S, Schmitz J, Shmerling DH, Visakorpi JK (1990) Revised criteria for diagnosis of coeliac disease. Arch Dis Child 65:909–911 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1792502/?page=1
17. Slatsky J, Shipton B, Wahaba H (2000) Thyroiditis: differential diagnosis and management. Am Fam Physician 61:1047–1052 http://clinchem.aaccjnls.org/content/18/6/499.long
18. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma, without the use of preparative ultra. Clin Chem 18:499–502 http://clinchem.aaccjnls.org/content/18/6/499.long
19. Burgdorf KS, Grarup N, Justesen JM, Harder MN, Witte DR, Jorgensen T, Sandberg A, Lauritzen T, Madsbad S, Hansen T, DIAGRAM Consortium, Pedersen O (2011) Studies of the association of Arg72Pro of tumor suppressor protein p53 with type 2 diabetes in a combined analysis of 55,521 Europeans. PLoS One 6:e15813
20. Spitsina EV, Yakunina NY, Chudakova DA, Nikitin AG, Svetlova GN, Soluyanova TN, Stirokov IA, Nosikov VV (2007) The association of the TP53 polymorphisms Pro72Arg and C(−)594CC with diabetic polyneuropathy in Russian Muscovites with type 1 diabetes mellitus. Mol Biol 41:901–905
21. Bitti ML, Saccucci P, Capasso F, Piccinini S, Angelini F, Raponi N, Porcari M, Arcano S, Petrelli A, Del Duca E, Bottini E, Gloria-Bottini F (2011) Genotypes of p53 codon 72 correlate with age at onset of type 1 diabetes in a sex-specific manner. J Pediatr Endocrinol Metab 24:437–439
22. Gloria-Bottini F, Bottini E (2015) Genetic factors adaptive in a malarial environment may increase the risk of type 1 diabetes. J Diabetes 7:430–432
23. Shi H, Tan S-J, Zhong H, Hu W, Levine A, Xiao C-J, Peng Y, Qi X-B, Shou W-H, Ma R-LZ, Li Y, Su B, Lu X (2009) Winter temperature and UV are tightly linked to genetic changes in the p53 tumor suppressor pathway in Eastern Asia. Am J Hum Genet 84:1130–1135
24. Muñoz-Fontela C, Mandinova A, Aaronson SA, Lee SW (2016) The P72R polymorphism of p53 predisposes to obesity and metabolic dysfunction. Cell Cycle 7:158–163
25. Santhanam U, Ray A, Sehgal PB (1991) Repression of the interleukin-6 gene promoter by p53 and the retinoblastoma susceptibility gene product. Proc Natl Acad Sci U S A 88:7605–7609
26. Komarova EA, Krivokrysenko V, Wang K, Neznanov N, Chernov AV, Komarov PG, Brennan ML, Golovkina TV, Rokhlin OW, Kundrash DV, Nedosposav SA, Hazen SL, Feinstein E, Gudkov AV (2005) p53 is a suppressor of inflammatory response in mice. FASEB J 19:1030–1032
27. Kawashima H, Takatori H, Suzuki K, Iwata A, Yokota M, Suto A, Minamino T, Hirose K, Nakajima H (2013) Tumor suppressor p53 inhibits systemic autoimmune diseases by inducing regulatory T cells. J Immunol 191:3614–3623
28. Zheng S-J, Lamhamedi-Cherradi S-E, Wang P, Xu L, Chen YH (2005) Tumor suppressor p53 inhibits autoimmune inflammation and macrophage function. Diabetes 54:1423–1428

Funding This work was supported by The State Committee for Scientific Research ST49 (Medical University of Gdańsk).

Data availability All data generated or analyzed during this study are included in this published article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This study was approved by the Ethics Committee of the Medical University of Gdańsk (NKEBN/2014/2009; 2009).

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© Springer
29. Kuhn HM, Kromminga A, Flammann HT, Frey M, Layer P, Arndt R (1999) p53 autoantibodies in patients with autoimmune diseases: a quantitative approach. Autoimmunity 31:229–235

30. Di Cesare E, Previti M, Lombardo F, Di Benedetto A, Mazzù N, Romano G, De Luca F, Lasco A, Cucinotta D (2001) Serum anti-p53 autoantibodies in patients with type 1 diabetes. Ann Clin Lab Sci 31:253–258 https://www.ncbi.nlm.nih.gov/pubmed/11508828

31. Jailwala P, Waukau J, Gisic S, Jana S, Ehlenbach S, Hessner M, Alemzadeh R, Matsuysama S, Laud P, Wang X, Ghosh S (2009) Apoptosis of CD4+ CD25(high) T cells in type 1 diabetes may be partially mediated by IL-2 deprivation. PLoS One 4:e6527

32. Volodko N, Salla M, Eksteen B, Fedorak RN, Huynh HQ, Baksh S (2015) TP53 codon 72 Arg/Arg polymorphism is associated with a higher risk for inflammatory bowel disease development. World J Gastroenterol 21:10358

33. Safan MA, Ghanem AA (2018) Association between polymorphisms of XRCC1 and TP53 genes and endometriosis. JAMMR 6:999–1007

34. Omrani-Nava V, Hedayatizadeh-Omran A, Alizadeh-Navaei R, Mokhberi V, Jalalian R, Janbabaei G, Amjadi O, Rahmatpour G, Mozaffari A (2018) TP53 single nucleotide polymorphism (rs1042522) in Iranian patients with coronary artery disease. Biomed Rep 9:259–265

35. Ruggeri RM, Vicchio TM, Giovinazzo S, Certa R, Alibrandi A, Trimarchi F, Benvena S, Trovato M (2015) TP53 polymorphism may contribute to genetic susceptibility to develop Hashimoto’s thyroiditis. J Endocrinol Investig 38:1175–1182

36. Chen RH, Chang CT, Wang TY, Huang WL, Tsai CH, Tsai FJ (2008) p53 codon 72 proline/arginine polymorphism and autoimmune thyroid diseases. J Clin Lab Anal 22:321–326

37. Ruggeri RM, Trimarchi F, Giuffrida G, Certa R, Cama E, Campenni A, Alibrandi A, De Luca F, Wsniewska M (2017) Autoimmune comorbidities in Hashimoto’s thyroiditis: different patterns of association in adulthood and childhood/adolescence. Eur J Endocrinol 176:133–141

38. Berkers CR, Maddocks OD, Cheung EC, Mor I, Vousden KH (2013) Metabolic regulation by p53 family members. Cell Metab 18:617–633

39. Bonfigli AR, Sirolla C, Testa R, Cucchi M, Spazzafumo L, Salvioli S, Cerillo A, Olivieri F, Festa R, Procopio AD, Brandoni G, Boemi M, Marra M, Franceschi C (2013) The p53 codon 72 (Arg72Pro) polymorphism is associated with the degree of insulin resistance in type 2 diabetic subjects: a cross-sectional study. Acta Diabetol 50:429–436

40. Azzam GA, Frank AK, Hollstein M, Murphy ME (2011) Tissue-specific apoptotic effects of the p53 codon 72 polymorphism in a mouse model. Cell Cycle 10:1352–1355

41. Pellegrino M, Traversi G, Arena A, Cappa M, Rosado MM, Andreoni M, Delfino DV, Moretti F, Fierabracci A (2020) Effect of p53 activation through targeting MDM2/MDM4 heterodimer on T regulatory and effector cells in the peripheral blood of Type 1 diabetes patients. PLoS One 15:e0228296

42. Grochola LF, Zeron-Medina J, Mériaux S, Bond GL (2020) Single-nucleotide Polymorphisms in the p53 Signaling Pathway. Cold Spring Harb Perspect Biol 2:a001032

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.