

Effect of the variant rs602662 of *FUT2* gene on anthropometric and metabolic parameters in a Caucasian obese population

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Abstract. – OBJECTIVE: Some obese subjects with genetic variants of *FUT2* gene could be implied in metabolic disorders. The aim of the present investigation was to evaluate the association between SNP rs602662 in *FUT2* gene with different obesity markers.

SUBJECTS AND METHODS: 166 Caucasian obese subjects were enrolled. Anthropometric parameters, blood pressure, fasting blood glucose, insulin concentration, insulin resistance (HOMA-IR), lipid profile, C reactive protein and prevalence of metabolic syndrome were recorded. The genotype of *FUT2* gene polymorphism (rs602662) was evaluated.

RESULTS: The genotype distribution of the rs602662 variant was the following: 29.5% (n=49) (GG), 47.6% (n=79) (GA) and 22.9% (n=38) (AA). We observed statistical differences between both genotypes (GG+GA vs. AA) in BMI (Delta: 0.4 ± 0.01 kg/m²; $p=0.04$), fat mass (Delta: 3.7 ± 0.3 kg; $p=0.02$), body weight (Delta: 5.9 ± 0.4 kg; $p=0.02$), waist circumference (Delta: 7.3 ± 0.9 cm; $p=0.03$), glucose (Delta: 5.5 ± 0.4 mg/dl; $p=0.04$), triglycerides (Delta: 25.9 ± 1.4 mg/dl; $p=0.01$), HDL-cholesterol (Delta: -5.7 ± 1.2 mg/dl; $p=0.02$), insulin (Delta: 5.0 ± 0.9 mU/L; $p=0.02$) and HOMA-IR (Delta: 1.4 ± 0.1 units; $p=0.03$) levels. Percentages of metabolic syndrome, central obesity, hypertriglyceridemia, low HDL cholesterol and hyperglycemia were lower in AA obese subjects than GG+GA. Logistic regression analysis showed a decreased risk of metabolic syndrome in AA subjects (OR=0.28, 95% CI=0.11-0.71, $p=0.01$).

CONCLUSIONS: AA genotype of *FUT2* rs602662 is associated with lower BMI and a better metabolic profile than subjects with GG+GA genotypes.

Key Words:

FUT2 gene, Obesity, Metabolic syndrome, Rs602662.

Introduction

Obesity is a heterogeneous disorder where multiple factors, especially related to exercise and diet

style, contribute to the pathogeny of this entity. Some genetic variants associated with obesity and related comorbidities have been identified¹⁻³. The physiological mechanisms underlying these genetic associations have not been fully elucidated. In these mechanisms, adipose tissue and central nervous system have been implied.

The *FUT2* gene, or *Se* gene, codes the enzyme alpha-1,2-L-fucosyltransferase2. This gene determines the formation of H antigens in the intestinal epithelial tissue⁴. Some individuals with genetic variants of *FUT2* gene are called non-secretors⁵⁻⁷. Non-secretors are unable to express them on mucosal surfaces or secrete histo-blood group antigens into fluids⁸. When *FUT2* gene is expressed in the intestines, the antigens produced by this gene participate in the regulation of saprophytic flora⁹. In addition, the type of the *FUT2* gene allele may be one of the main determinants of bacteria colonizing the intestine⁷.

Moreover, Metabolic Syndrome (MS) is a combination of risk factors, such as; glucose intolerance, abdominal obesity, dyslipemia (high triglyceride levels or low HDL-cholesterol levels), increased blood pressure and a pro-inflammatory status¹⁰. Environmental risk factors, genetic factors and microbiome are involved in the development of MS¹¹. In addition, MS is considered a polygenic and multifactorial disorder. This entity is secondary to the interaction of a number of different genes with environmental factors¹². In this context, adipose tissue is considered as an endocrine organ. This tissue plays a major role in the presence of MS¹³.

Finally, a single nucleotide variant (SNV) in *FUT2* gene (rs602662) has been found to be related with adiposity in a Danish population¹⁴. These results have not been replicated in the literature. Additionally, the effect of this SNV on the different components of metabolic syndrome has not been evaluated either.

Giving its potential role in adiposity markers and lack of data in the literature, the current study was

designed to evaluate SNP rs602662 in *FUT2* gene and its association with obesity, MS and metabolic parameters.

Subjects and Methods

Subjects and Clinical Investigation

The study was realized with patients who were referred from Primary Care Physicians to our Outpatient Clinic to evaluate their obesity (BMI: body mass index ≥ 30 kg/m²) with an age ≥ 18 years. A total of 166 subjects agreed to participate in the study. All subjects gave informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of HCUVA (No. 06/2016). The inclusion criteria for the study group included the following: body mass index ≥ 30 kg/m² and age ≥ 18 years. The exclusion criteria were: a history of cardiovascular events, history of alcoholism, malignant tumor and drugs known to influence lipid or glucose levels.

During the first visit, anthropometric data (weight, height, BMI, fat mass by impedance, waist circumference) and blood pressure were measured.

For biochemical assays, 5 ml of venous blood were aliquoted in ethylenediaminetetraacetic acid EDTA-coated tubes. The following parameters were measured: insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and C-reactive protein. Dietary intake was also recorded.

Metabolic syndrome (MS) was defined according to the criteria of Adult Treatment Panel III (ATPIII)¹⁰. The patients needed to meet at least 3 of the following criteria to be diagnosed with MS: elevated fasting glucose or treatment for diabetes, elevated triglycerides (>150 mg/dl) or treatment for dyslipidemia, low HDL cholesterol < 40 mg/dl (males) or < 50 mg/dl (females), elevated systolic or diastolic blood pressure ($>130/85$ mmHg or antihypertensive treatment) and increased waist circumference (> 94 cm for males or > 80 cm for females).

Anthropometric Parameters and Blood Pressure

Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences were measured using a non-elastic measuring tape (Omrom, California, CA, USA). Both parameters were used to derive waist-to-hip ratio (WHR). Body weight was determined while the subjects were minimally unclothed and not wearing shoes, using digital scales (Omrom, California,

CA, USA). Based on these parameters, body mass index was calculated with the following formula: [body weight (kg) divided by square of height (m)]. Fat mass was determined by impedance with an accuracy of 5 g¹⁵ (EFG BIA 101 Anniversary, Akern, Italy). The following equation was used: $(0.756 \text{ Height}^2/\text{Resistance}) + (0.110 \text{ Body mass}) + (0.107 \text{ Reactance}) - 5.463$. Mean systolic and diastolic blood pressures were calculated by averaging three measurements (Omrom, California, CA, USA), after the subjects sat for 10 minutes.

Biochemical Procedures

In order to assess lipid profile, we measured total cholesterol, HDL-cholesterol and triglyceride levels using the COBAS INTEGRA 400 analyzer (Roche Diagnostic, Montreal, Canada). LDL cholesterol was calculated using Friedewald's formula (LDL cholesterol = total cholesterol - HDL cholesterol - triglycerides/5)¹⁶. Glucose levels and insulin were measured by electrochemiluminescence assay (COBAS INTEGRA 400 analyzer, Roche Diagnostic, Montreal, Canada). Based on these parameters, homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using these values: $\text{glucose} \times \text{insulin}/22.5$ ¹⁷. C-reactive protein (CRP) was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany).

Dietary Intakes and Physical Activity

Records of daily dietary intake for 3 previous days (2 days during the week and one during the weekend) were recorded with a computer-based data evaluation system (Dietosource[®], Geneva, Switzerland). In order to calculate these parameters, national composition food tables were used as reference¹⁸. All subjects with a self-reported questionnaire recorded daily physical activity. They recorded the daily activity in minutes per day.

Genotyping FUT2 Gene

A method based on quantitative DNA polymerase chain reaction (qPCR) was used to evaluate the polymorphism rs602662 of the *FUT2* gene. The genetic material was amplified in the real time polymerase chain reaction with the QuantStudio 12K Flex Real-Time qPCR instrument (Thermo Fisher, Pittsburgh, PA, USA).

DNA was isolated from buccal swabs using QIAamp[®]. A total volume of 10 μ l with 2.5 μ l TaqMan OpenArray Master Mix (Applied Biosystems, Foster City, California, CA, USA) and 2.5 μ l human DNA sample were loaded and amplified on arrays following the manufacturer's instructions (Termocicler Life Technologies, Carlsbad, CA,

Table I. Anthropometric variables and blood pressure.

Parameters	GG n=49	GA n=79	AA n=38	p-value	GG+GA n=128	AA n=38	p-value
Age (years)	45.1±3.2	44.9±1.9	45.5±2.1	0.23	45.0±4.1	45.5±2.1	0.43
Range	27-54	26-51	28-52		26-54	28-52	
BMI	39.5±1.2	39.2±1.3	39.0±1.1	0.07	39.4±1.1	39.0±1.1*	0.03
Weight (kg)	104.8±3.1	102.4±1.1	97.9±2.8 ^{#,§}	0.03	103.8±2.1	97.9±2.8*	0.02
Fat mass (kg)	50.1±2.0	49.7±1.1	46.1±2.0 ^{#,§}	0.03	49.8±2.1	46.1±2.0*	0.02
WC (cm)	120.7±3.1	118.2±2.0	111.8±6.1 ^{#,§}	0.01	119.1±4.0	111.8±3.1*	0.03
WHR	1.1±0.1	1.0±0.02	0.8±0.1 ^{#,§}	0.01	1.1±0.04	0.8±0.1*	0.03
SBP (mmHg)	126.8±2.8	126.5±2.1	126.9±4.1	0.43	126.7±2.1	126.9±4.1	0.34
DBP (mmHg)	80.4±5.0	80.2±3.1	80.2±4.2	0.39	80.3±4.1	80.2±4.2	0.36

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference; WHR: waist to hip ratio. ^{#,§}*p*<0.05 among three genotypes with ANOVA test and Bonferroni post-hoc test. [#](AA vs. GA), [§](AA vs. GG). **p*<0.05 between genotypes in a recessive model (GG+GA vs. AA).

USA). Genotype calling and sample clustering for Open Array assays were performed in TaqMan Genotyper (Life Technologies, Carlsbad, CA, USA). Hardy Weinberg equilibrium was calculated with a statistical test (Chi-square). The variant of *FUT2* gene was in Hardy Weinberg equilibrium (*p*=0.34).

Statistical Analysis

Statistical analyses were performed with the statistical software SPSS for Windows, version 23.0 software package (IBM Corp., Armonk, NY, USA). *p*-values below 0.05 were considered statistically significant. Sample size was determined to detect differences over 3 kg of body weight with 90% power and 5% significance. Bonferroni test was applied for multiple testing to reduce Type I error in association analysis.

The *FUT2* rs602662 genotype was analyzed using a recessive model (GG+GA vs. AA). Descriptive statistics of all variable values were presented as mean±standard deviation for continuous variables. Descriptive statistics of categorical variables were presented as percentages. Variables were analyzed with ANOVA test with Bonferroni post-hoc test and Student's *t*-test (for normally distributed variables) or Kruskal-Wallis' test (for non-normally-distributed variables). Logistic regression analyses adjusted by age, gender and BMI were used to calculate odds ratio (OR) and 95% confidence interval (CI) to estimate the association between the rs602662 SNV and the components of MS.

Results

A total of 166 Caucasian obese subjects were enrolled. Individuals were middle-aged with an

average age of 45.3±5.1 years (range: 26-54). The mean (BMI) was 39.2±3.1 kg/m² (range: 36.4-41.4). Gender distribution was 119 women (71.7%) and 47 men (28.3%). The distribution of the rs602662 polymorphism in this Caucasian obese population was 29.5% (n=49) (GG), 47.6% (n=79) (GA) and 22.9% (n=38) (AA). The allele frequency was G (0.60) and A (0.40).

The averages of age and BMI were similar in both above-mentioned genotype groups (Table I). Mean values of age (GG+GA; 45.0±4.1 years vs. AA; 45.5±2.1 years: ns) were similar between both genotype groups. The ratio females/males (GG+GA 26.1% males vs. 73.9% females vs. AA; 19.2% males vs. 80.8% females) was also similar.

Table I reports adiposity parameters and blood pressure (GG+GA vs. AA). Analyzing the three genotypes separately, weight, fat mass and waist circumference resulted to be lower in AA subjects than in GA or GG patients. Applying the recessive genetic model (GG+GA vs. AA), we observed statistical differences between both genotype groups in BMI (Delta: 0.4±0.01 kg/m²: *p*=0.04), fat mass (Delta: 3.7±0.3 kg: *p*=0.02), weight (Delta: 5.9±0.4 kg: *p*=0.02) and waist circumference (Delta: 7.3±0.9 cm: *p*=0.03).

Biochemical characteristics are shown in Table II. Analyzing the three genotypes separately, triglycerides, insulin and HOMA-IR were lower in AA subjects than in GA or GG patients. Glucose levels were lower in AA than in GG. Finally, HDL-cholesterol levels were higher in AA patients than in GG or GA patients (Table II). Applying the recessive genetic model (GG+GA vs. AA), we observed statistical differences between both genotype groups in glucose (Delta: 5.5±0.4 mg/dl: *p*=0.04), triglycerides (Delta: 25.9±1.4 mg/dl: *p*=0.01), HDL-cholester-

Table II. Biochemical parameters (mean±SD).

Parameters	GG n=49	GA n=79	AA n=38	p-value	GG+GA n=128	AA n=38	p-value
Fasting Glucose (mg/dl)	111.7±3.1	105.4±4.1	102.9±3.1 ^{\$}	0.04	108.4±2.1	102.9±3.1*	0.04
Total cholesterol (mg/dl)	203.7±9.8	201.6±10.1	199.4±9.1	0.43	202.3±11.8	199.4±9.1	0.21
LDL-cholesterol (mg/dl)	115.5±4.9	112.3±4.1	110.4±4.1	0.24	113.6±5.1	110.4±4.1	0.39
HDL-cholesterol (mg/dl)	51.3±3.0	52.7±2.1	57.9±3.0 ^{#\$}	0.02	52.2±2.1	57.9±3.0*	0.02
Triglycerides (mg/dl)	156.1±9.1	138.8±8.1	115.7±8.1 ^{#\$}	0.01	140.1±11.0	115.7±8.1*	0.01
Insulin (mUI/l)	23.7±2.9	20.5±1.3	16.6±2.1 ^{#\$}	0.02	21.6±3.0	16.6±2.1*	0.02
HOMA-IR	6.4±0.9	5.4±0.2	4.3±0.2 ^{#\$}	0.03	5.7±0.4	4.3±0.2*	0.03
CRP	4.8±0.9	4.6±0.2	4.3±0.9	0.49	4.7±0.4	4.3±0.9	0.43

HOMA-IR (homeostasis model assessment of insulin resistance). CRP (C reactive protein). ^{#\$} $p < 0.05$ among three genotypes with ANOVA test and Bonferroni post-hoc test. * (AA vs. GA), \$ (AA vs. GG). ^{*} $p < 0.05$ between genotypes in a recessive model (GG+GA vs. AA).

ol (Delta: -5.7 ± 1.2 mg/dl; $p = 0.02$), insulin (Delta: 5.0 ± 0.9 mUI/L; $p = 0.02$) and HOMA-IR (Delta: 1.4 ± 0.1 units; $p = 0.03$).

Table III shows dietary intakes and self-reported physical activity. Caloric intake, macronutrient intake, type of dietary fat and vitamin B12 intakes were similar in both genotypes. Physical activity was also similar in both groups.

The percentage of individuals who had metabolic syndrome (MS) was 37.8% (n=63) and 62.2% patients without MS (n=103). The frequency of subjects with metabolic syndrome and different components of MS (central obesity, hypertriglyceridemia, hypertension or hyperglycemia) have been reported in Table IV. According to the results of metabolic characteristics, the percentages of patients who had Metabolic syndrome (OR=0.31, 95% CI=0.13-0.75; $p = 0.01$), central obesity (OR=0.37, 95% CI=0.03-0.97; $p = 0.03$), hypertriglyceridemia (OR=0.34,

95% CI=0.12-0.88; $p = 0.02$), low HDL cholesterol (OR=0.39, 95% CI=0.09-0.94; $p = 0.03$) and hyperglycemia (OR=0.36, 95% CI=0.10-0.94; $p = 0.02$) were lower in AA obese subjects than in GG+GA subjects.

Logistic regression analysis showed a low risk of metabolic syndrome cholesterol in AA subjects (OR=0.28, 95% CI=0.11-0.71, $p = 0.01$) after adjusting by dietary fatty acid intakes, gender, BMI and age (Table V).

Discussion

Our study identifies a relationship between *FUT2* SNV (rs602662) and adiposity markers, lipid profile, insulin resistance and metabolic syndrome. We report that the AA genotype is associated with lower BMI and a better metabolic profile than G allele carriers.

Table III. Dietary Intakes and physical activity (mean±SD).

Parameters	GG n=49	GA n=79	AA n=38	p-value	GG+GA n=128	AA n=38	p-value
Calories (cal/day)	1,687.3±204.2	1,631.5±200.5	1,648.5±133.5	0.29	1,651.5±203.5	1,648.5±133.5	0.28
Carbohydrates (g/day)	195.3±32.1	200.2±25.1	201.2±29.1	0.35	199.2±19.1	201.2±29.1	0.34
Proteins (g/day)	77.9±9.2	79.6±8.4	80.6±7.4	0.41	79.6±5.4	80.6±7.4	0.49
Lipids (g/day)	64.6±8.1	66.7±7.1	66.7±7.1	0.51	65.9±7.8	66.7±7.1	0.53
Fiber (g/day)	14.4±3.1	14.9±3.1	14.8±5.1	0.49	14.5±5.9	14.8±5.1	0.45
Cholesterol (mg/day)	307.6±120.1	308.7±192.8	318.1±197.8	0.29	308.1±181.8	318.1±197.8	0.25
Saturated fatty acids (g/day)	17.5±3.1	16.8±4.1	15.9±2.1	0.49	17.2±2.9	15.9±2.1	0.39
Monounsaturated fatty acids (g/day)	28.1±4.1	27.2±3.1	28.2±4.8	0.41	27.9±4.0	28.2±4.8	0.45
Polyunsaturated fatty acids (g/day)	6.1±4.1	7.0±3.9	7.1±3.2	0.28	6.8±3.9	7.1±3.2	0.23
B12 vitamin (ug/day)	2.4±0.3	2.3±0.9	2.6±0.8	0.28	2.3±0.5	2.6±0.8	0.23
Physical activity (minutes/week)	99.9±11.7	101.3±9.1	101.4±10.1	0.23	100.4±6.1	101.4±10.1	0.33

No statistical differences were found.

Table IV. Metabolic syndrome and components of MS.

Parameters	GG n=49	GA n=79	AA n=38	p-value	GG+GA n=128	AA n=38	p-value
Percentage of MetS	43.8%	43.0%	18.9%	0.02	43.3%	18.9%*	0.01
Percentage of central obesity	54.2%	51.9%	31.6% ^{#,§}	0.04	52.8%	31.6%*	0.04
Percentage of hypertriglyceridemia	35.3%	36.7%	16.2% ^{#,§}	0.03	36.2%	16.2%*	0.02
Low HDL cholesterol	31.3%	34.1%	13.5% ^{#,§}	0.03	33.1%	13.5%*	0.03
Percentage of hypertension	71.3%	67.1%	73.0%	0.49	72.4%	73.0%*	0.65
Percentage of hyperglycemia	34.2%	27.1%	13.5% [§]	0.09	31.5%	13.5%*	0.02

The cutoff points for the criteria of central obesity (waist circumference >88 cm in female and >102 in male), hypertension (systolic BP >130 mmHg or diastolic BP >85 mmHg or specific treatment), hypertriglyceridemia (triglycerides >150 mg/dl or specific treatment) or hyperglycemia (fasting plasma glucose >110 mg/dl or drug treatment for elevated blood glucose). ^{#,§} $p < 0.05$ among three genotypes with ANOVA test and Bonferroni post-hoc test. [#](AA vs. GA), [§](AA vs. GG). * $p < 0.05$ between genotypes in a recessive model (GG+GA vs. AA).

As we previously mentioned, the *FUT2* gene codes the enzyme alpha-1,2-L-fucosyltransferase2. This gene conditions the secretion of H antigens in the intestinal epithelial¹⁹. Non-secretors have lower diversity of intestinal microbiota⁹. Moreover, some observational studies^{20,21} have demonstrated a relationship between serum vitamin B12 levels and elevated body mass index and adverse metabolic profiles, such as insulin resistance and cardiovascular disorders. Allin et al¹⁴ reported contradictory results for a causal role of low serum B12 levels and obesity. In this context, perhaps some genetic variants in *FUT2* gene are playing an unknown effect. In this previous interesting investigation¹⁴, the authors hypothesize that genetic variant may have pleiotropic effects. For example, the G-allele of *FUT2* genetic variant rs602662 has been associated with an increase of BMI and a decrease of serum vitamin B12.

According to literature, homozygous carriers (AA) are non-secretors and they could theoretically modulate the composition of gut microbiota. For example, these subjects have been reported to be resistant to colonization and infection with Norovirus or *Helicobacter Pylori*^{22,23}. Possibly, the lack of blood group antigens on the intestinal mucosa may inhibit the infectious potential of some pathogenic bacteria. Moreover, some investigations^{24,25} have found a link between microbiota and the human obesity. For example, it has been reported²⁶ that germ-free mice are leaner than normal mice and that transplantation of germ-free mice with microbiota resulted in an increased total body fat. In our present study, the

low weight and fat mass observed among AA obese subjects of *FUT2* rs602662 may be explained by the lack of expression of these blood group antigens in the gastrointestinal tract, and a later decreased cross talk with commensal microbes. Secondly, this decrease in fat mass would generate a decrease in insulin resistance and free fatty acids levels into the bloodstream. These facts would avoid the elevation of triglyceride levels and a decrease in HDL-cholesterol. All these parameters are components of patients with metabolic syndrome. The association between *FUT2* gene variants and anthropometric parameters has also been described²⁷ in patients undergoing bariatric surgery. In a study²⁷ about bariatric surgery, GG carriers of the *FUT2* gene rs601338 showed a correlation between waist-hip ratio and propionate concentrations and some microbes in the gut. These high concentrations of propionate among GG carriers may be used as a source of energy for the patients. This positive energy balance may be related to waist circumference as an indicator of visceral fat mass. In our present study, there was a significant decrease in total fat and visceral fat determined by waist circumference and waist to hip ratio in obese patients with AA genotype, regardless of dietary intake, which was similar in both groups. However, we did not determine the microbiota or the production of short-chain fatty acids. We hypothesized an increased production of short-chain fatty acids and their use as a source of energy with a secondary increase of fat mass. This increase in the amount of fat

Table V. Relation between AA genotype and metabolic syndrome.

	Odds Ratio	CI 95%	p-value
Crude odds ratio	0.31	0.13-0.75	0.01
Adjusted odds ratio by dietary fatty acid intakes, gender, BMI and age	0.28	0.11-0.71	0.01

mass could produce insulin resistance. A second hypothesis is that short-chain fatty acids can stimulate weight gain and adipogenesis by binding to *FFAR3* and *FFAR2* (free fatty acid receptor)²⁸.

Limitations

Several limitations should be considered when evaluating our findings. Firstly, the study has been designed for Caucasian obese subjects aged 26-54 years, so the data are not generalizable to the entire population. Secondly, the microbiota determination in our patients was absent. Thirdly, the study was designed as a cross sectional study, therefore we cannot provide causality. Finally, circulating levels of vitamin B12 have not been determined, in order to study the effect of this genetic variant on its levels and potential interactions.

Conclusions

This study can confirm that the AA genotype of *FUT2* rs602662 is associated with lower BMI and a better metabolic profile than GG+GA genotypes. This fact produces a lower percentage of metabolic syndrome in non-G allele carriers. Further investigations are needed to confirm this association, especially because the GG+GA carriers have a higher cardiovascular risk and would also need more intensive treatment for their obesity and metabolic syndrome.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contributions

Daniel de Luis designed the study, realized statistical analysis and wrote the article. He contributed to the conceptualization, analysis of data for the work, and revised it critically for important intellectual content.

Olatz Izaola realized anthropometric evaluation and control of dietary intake. She contributed to the acquisition of data for the work and revised it critically for important intellectual content.

David Primo realized biochemical evaluation, genotype and wrote the article. He contributed to the analysis of data for the work, methodology, to write the article and revised it critically for important intellectual content.

All authors approved the version to be published and accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of

the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by our local Ethical Committee (HCUVA Clínico Universitario Valladolid Committee, No. 06/2016).

Informed Consent

Informed consent was obtained from all individual participants included in the study.

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