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ARTICLE

Pediatrics

Influence of genetic variants in *FADS2* and *ELOVL2* genes on BMI and PUFAs homeostasis in children and adolescents with obesity

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Abstract

Background Several studies identified genetic variants in *FADS* and *ELOVL2* genes associated with obesity-related conditions, such as alterations in blood lipid parameters and insulin homeostasis. The aim of this cross-sectional study was to determine whether *FADS* and *ELOVL2* genetic variants were associated with obesity and adiposity, besides dyslipidaemia and insulin resistance, in a large sample of obese children and adolescents.

Materials and methods One thousand six hundred and forty-nine obese children underwent physical examination, anthropometry, fasting blood tests measuring plasma glucose, lipid and liver profile. Two genetic variants were genotyped: rs2236212 in *ELOVL2* gene and rs1535 in *FADS2*, for the gene cluster FADS. In a subgroup of obese children ($n = 105$), erythrocyte fatty acid composition was measured. Generalized linear models were used to assess association between genotypes and variables.

Results A positive association between zBMI and the minor allele of rs2236212 ($p = 0.028$), the major allele of rs1535 ($p = 0.046$) and the genetic score ($p = 0.008$), created by summing up both risk alleles, were found. The estimation of enzymatic activity revealed that minor alleles were associated significantly with a reduction of the enzymatic activity of elongase and desaturase ($p = 0.048$ and $p = 0.0001$, respectively).

Discussion and conclusions Common variants in the *FADS2* and *ELOVL2* genes were associated with BMI in a large population of obese Italian children. These SNPs were associated with alterations in LC-PUFAs homeostasis, not accompanied by modifications of plasma lipids or HOMA-IR. These findings provide additional support to the genetics accounting for BMI interindividual variability and the molecular basis of obesity.

Introduction

Childhood and adolescent obesity is one of the most serious public health challenges of the twenty-first century[1]. Obesity has become a global epidemic since the prevalence of overweight and obesity rose by 47.1% in children over the last three decades[1]. Overweight and obesity are major risk factors for several chronic diseases, including cardiovascular disease (CVD), type 2 diabetes, dyslipidaemia, atherosclerosis, hypertension, and cancer[2,3]. To date, most prevention programs based on diet and exercise, as well as pharmacological treatments demonstrated only modest effects[4]. Therefore, a better understanding of the underlying causes of obesity may be useful in developing more effective prediction, prevention, and treatment[5].

Genetics accounts for 40–75% of body mass index (BMI) interindividual variability[6]. Indeed, a significant number of single nucleotide polymorphisms (SNPs) associated with BMI have been identified by genome-wide association studies (GWAS) and recent studies suggested that they may explain up to 30–40% of BMI variance[7]. Therefore, further investigations are needed for expanding our understanding of the genetic predisposition to obesity.

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Long-chain polyunsaturated fatty acids (LC-PUFAs) have been recently related to the pathogenesis of obesity by affecting energy metabolism, cellular membrane integrity, signaling and transcriptional regulation[8]. Adequate consumption of n-3 LC-PUFAs is required to ensure many physiological conditions providing healthy cardiovascular functions and anti-inflammatory activities[9,10]. High levels of n-3 LC-PUFAs have been associated with a lower risk of multiple diseases, including CVD and metabolic syndrome[11] and with better profiles of body fat and fat distribution[3,12]. In contrast, an excessive dietary intake of n-6 LC-PUFAs, resulting in an increase of the omega-6/omega-3 ratio, was associated with obesity and obesity-related complications[13].

In addition to the variability of dietary intake, the quantity and quality of LC-PUFAs are also finely regulated by endogenous production. The biosynthesis of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), or arachidonic acid (AA) from C18 polyunsaturated fatty acids (linoleic and alpha-linoleic acids) requires the concerted activities of two classes of enzymes, the fatty acyl desaturase and elongase and the n-3 and n-6 PUFAs compete for these enzymes. Evidence emerged for considerable inter-individual variation in the capacity of processing dietary LC-PUFAs via the desaturation/elongation pathways[9,14,15]. In particular, desaturases enzymes play an important role in modulating fatty acids composition and their estimated activity has been associated to obesity and related metabolic disorders both in adults and children[16–19].

In the past decade, GWAS have identified genetic variations in the desaturase *FADS1* and *FADS2* genes and in the elongase *ELOVL2* gene associated with variation in the pattern of circulating levels of LC- PUFAs, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), total cholesterol (TC), and triglycerides (TG) levels[11,20–22]. Subsequent studies confirmed the association between SNPs in these genes and obesity-related conditions such as alterations in blood lipid parameters[23,24]. Moreover, other studies demonstrated that the gene cluster FADS was associated with fasting glucose, homeostasis model assessment for insulin resistance, and insulin secretory capacity[25,26].

Despite the strict relationship between lipid homeostasis and glucose tolerance and obesity, few studies analyzed the association between genetic variants at the desaturation/elongation pathways and obesity and adiposity in human adults[9,27], none in children. Considering overlapping genetic background between obesity and dyslipidaemia and glucose intolerance, the aim of our study was to determine whether *FADS2* and *ELOVL2* SNPs were associated with a higher degree of obesity and indices of adiposity, besides dyslipidaemia and insulin resistance, in a large sample of obese children and adolescents.

Methods

Subjects

The study population included 1649, 10.98 ± 2.62 years old overweight and obese (zBMI 3.35 ± 0.69) children and adolescents. Three hundred and forty-three subjects were enrolled at the Pediatric Diabetes and Metabolic Disorders Unit, University Hospital, Verona (Italy); 43 were part of a cross-sectional school-based study that involved children from primary schools of Verona South District, as previously described[28]; 62 children were recruited from the "Pediatric Obesity Outpatients Unit" of the University Hospital of Verona and of the "Local Health Unit n. 20" of Verona[19] and 1201 individuals were recruited at their first visit at the obesity outpatient clinic of the Pediatrics Section of the Luigi Vanvitelli University of Naples (Italy).

Inclusion criteria: European ethnicity, obesity according to sex and age-specific BMI cutoff for obesity, according to WHO growth references[29].

Exclusion criteria: genetic or endocrine causes of obesity, diabetes[30], either malignancy, preterm or postterm birth, associated chronic diseases, or chronic pharmacological therapies. The protocol was approved by the Institutional Ethics Committee of Verona (Italy). Informed consent was obtained from the children and their parents.

Physical examination

At recruitment, physical examination was performed according to standard procedures, as previously described[19,28,31]. Weight was measured to the nearest 0.5 kg on standard physician's beam scales, with the child wearing only underwear and no shoes. Height was measured to the nearest 0.5 cm on a stadiometer without shoes, with the child's heels, buttocks, shoulders, and head against the vertical wall with a line of sight aligned horizontally. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. BMI values were standardized using age- and sex-specific median, standard deviation (SD), and power of the Box-Cox transformation (least mean square method) based on WHO growth references[29]. Waist circumference was measured to the nearest 0.5 cm while the subjects were standing, after gently exhaling, as the minimal circumference measurable on the horizontal plane between the lowest portion of the rib cage and the iliac crest[32]. Waist-to-height ratio (WHtR), an index of body fat distribution, was calculated as previously described[33]. The pubertal stage was assessed according to Tanner criteria[34]. Subjects were categorized in prepubertal (Tanner stage 1), pubertal (Tanner stage 2–4), and post-pubertal (Tanner stage 5). Systolic blood pressure (SBP)

and diastolic blood pressure (DBP) were recorded three times on the right arm in mmHg using a manual sphygmomanometer; for analysis, three blood pressure measurements were averaged[35].

Biochemical measurements

Within 15 days from the recruitment visit, all patients underwent fasting blood tests for measuring plasma glucose, serum insulin concentration, lipid profile, and liver enzymes. Plasma glucose was measured with the glucose oxidase method (Accu-Chek Inform II, Roche, Swaziland). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum TG, TC, and HDL-c were measured by standard methods. LDL-c was calculated using the Friedewald's equation.

Erythrocyte and whole-blood collection

In the group of 62 children of "Pediatric Obesity Outpatients Unit," fatty acids from erythrocytes membrane were measured. EDTA blood tubes were collected and centrifuged. Plasma and buffy coat were isolated, and erythrocytes frozen at −80 °C until analysis.

In 43 children of the school-based study, fatty acids were measured on drop of whole blood that was collected directly to a filter paper (Ahlstrom 226, PerkinElmer, Greenville, SC) that was previously pretreated with an antioxidant cocktail (Oxystop, OmegaQuant Analytics, LLC, Sioux Falls, SD) to protect unsaturated FAs from oxidation. After collection, cards were stored in a resealable plastic bag.

Fatty acids measurement

The collected samples, erythrocytes for the 62, and whole-blood sample for the 43 children, were delivered to Omegametrix GmbH (Martinsried, Germany), and erythrocyte and plasmatic phospholipid fatty acids composition were analyzed using the HS-Omega-3 Index® methodology as previously described[36,37]. In particular, fatty acid methyl esters were generated by acid transesterification with boron trifluoride for 10 min at 100 °C. Were then extracted into hexane after the addition of water, analyzed by gas chromatography (GC2010 GasChromatograph, Shimadzu Corporation, Columbia, MD) equipped with a SP2560, 100-m column (Supelco, Bellefonte, PA). Fatty acids were then identified by comparison with a standard mixture of fatty acids (GLC 727, Nucheck Prep, Elysian, MN). Fatty acid levels are expressed as a weight percent of total blood fatty acids composition. The stability of FAs collected and stored in this manner has been previously evaluated and no sample degradation was detected[38].

Estimation of D6D and D5D desaturase activity

Delta-5 (D5D) and delta-6 (D6D)-desaturases activity was estimated by computing the ratio of product to precursor of individual FA as follows: D6D desaturase (D6D) = C18:3n-6 (gamma-linolenic acid, GLA)/C18:2n-6 (linolenic acid) and D5D desaturase (D5D) = C20:4n-6 (AA)/C20:3n-6 (dihomo-gamma-linolenic acid, DGLA)[39–42]. Activity of elongase II was estimated as follows: C22:5n3 (docosapentaenoic acid, DPA)/C20:5n3 (EPA)[43].

Variant selection

Genomic DNA was extracted from peripheral blood leukocytes using salting-out procedures or by DNeasy Blood & Tissue Kits (Qiagen) or isolated from a buccal swab through PureLink™ Genomic DNA Mini Kit (Invitrogen). DNA was purified (QIAamp DNA Mini Kit, Qiagen) according to manufacturer's protocol. Genotyping was carried out by a predesigned TaqMan probe (Applied Biosystem, California, USA), according to the manufacturers' protocol. Polymorphism genotyping was performed using 7900 HT Real Time PCR (Applied Biosystem, California, USA). About 100 subjects were, instead, genotyped using Infinium Global Screening Array beadchip Array (GSA 24v2, Illumina, Inc.) according to manufacturer's protocol. As quality control, 45 DNA were genotyped twice and 100% of concordance was detected.

We genotyped two polymorphisms in which the minor allele was known to be associated to a reduced enzymatic activity of their own coded proteins[9,11]: rs2236212, a downstream transcript variant of the *ELOVL2* gene, and rs1535, an intronic variant within *FADS2* gene. *FADS2* gene belongs to a cluster of fatty acid desaturases composed by *FADS1*, *FADS2*, and *FADS3* genes, located on chromosome 11 in a region with a high linkage disequilibrium (LD). Therefore, we selected the rs1535 as a tag-SNP of the whole *FADS* cluster region, since among the surrounding SNPs it showed an r^2 comprised between 0.74 and 1[11].

The distributions of the genotypes were compatible with Hardy–Weinberg equilibrium (all $p > 0.439$).

To estimate the synergic contribution of the two genetic variants under study, an additive genetic score was also created summing up the number of risk alleles in each patient ((G) allele of rs2236212 in *ELOVL2* gene and the (A) allele of rs1535 in *FADS2* gene).

Statistical analysis

Patients' baseline characteristics are reported as mean ± SD. Kolmogorov–Smirnov test was used to assess the normal distribution of variables. Skewed variables were transformed (natural log-transformed, or square root

Table 1 Physical and biochemical features of the total sample.

Variables	Male	Female	Total
n (Male/female)	843	806	1649
Age (years)	11.2 (2.5)	10.8 (2.8)*	11.0 (2.6)
Body height (m)	1.51 (0.14)	1.47 (0.14)*	1.49 (0.14)
Body weight (kg)	73.4 (22.5)	68.9 (21.5)*	71.2 (22.1)
BMI (kg/m^2)	31.2 (5.3)	31.1 (5.4)	31.2 (5.4)
BMI SDS	3.45 (0.75)	3.25 (0.61)*	3.35 (0.69)
Waist circumference (cm), $n = 1313$	93.1 (12.5)	87.9 (11.5)*	90.5 (12.5)
WHtR, $n = 1313$	0.62 (0.06)	0.60 (0.06)*	0.61 (0.06)
Puberty status, $n = 1163$	502/68/9	500/46/38*	1 002/114/47
SBP (mmHg), $n = 1241$	115 (14)	112 (15)*	113 (14)
DBP (mmHg), $n = 1241$	68 (10)	66 (11)**	66 (11)
Triglycerides (mg/dL), $n = 1555$	98.8 (51.9)	97.1 (51.6)	98.0 (51.8)
Total cholesterol (mg/dL), $n = 1581$	161.3 (33.5)	158.9 (32.5)	160.1 (33.0)
HDL cholesterol (mg/dL), $n = 1474$	46.6 (11.5)	46.2 (11.6)	46.4 (11.5)
LDL cholesterol (mg/dL), $n = 1390$	94.2 (26.1)	92.9 (27.5)	93.6 (26.8)
ALT, $n = 1499$	32.3 (22.5)	25.7 (15.6)*	29.1 (19.7)
AST, $n = 1446$	25.5 (10.2)	22.8 (12.9)*	24.1 (11.7)
HOMA-IR, $n = 1475$	4.62 (4.02)	5.01 (4.26)	4.81 (1.15)

Sample size, $n = 1649$, unless otherwise indicated. Data expressed as means (SD) or number and percentage, *n* (%). Puberty status defined as prepuberty, puberty and postpuberty status. Differences between male and female were tested by *t*-test or chi-squared test.

SD standard deviations, *BMI* body mass index, *WHtR* waist-to-height ratio, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *ALT* alanine transaminase, *AST* aspartate transaminase.

*$p < 0.001$; **$p < 0.05$.

transformed, if and as needed) to correct for non-Gaussian distribution. The *t*-test and the chi-squared test, for categorical variables, were used to test differences in clinical and biochemical characteristics according to sex. Generalized linear models, adjusted for age, sex, zBMI, and puberty status were applied to test the associations between clinical, anthropometric and metabolic variables, and SNPs or genetic score. The presence of a significant interaction between the two SNPs with zBMI was also tested in this regression model. Covariates included in multivariable regression models were selected as potential confounding factors based on their biological plausibility. The association between genotypes and fatty acids measurements were assessed by ANOVA test. A *p* value < 0.05 was considered as statistically significant. Our convenience sample of 1384 children was 80% powerful to detect, with a 5% alpha error, a Cohen *f* effect size (i.e., SD of group means/common SD) of 0.083, by comparison of means across three groups (e.g., three genotypes). This is below the conventional threshold of 0.10, corresponding to a small effect size. The power calculation was performed with G-power (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower.html). All analyses were performed using SPSS v.22.0 (SPSS, Chicago IL).

Phenoscanner V2 tool

PhenoScanner V2 online tool (http://www.phenoscanner.medschl.cam.ac.uk/) is a curated database of publicly available results from large-scale genetic association studies in humans[44]. We consulted this database to control the genotype-phenotype associations found in our study.

Results

One thousand six hundred and forty-nine children and adolescents (843 males, 806 females) with a mean age of 11.0 ± 2.6 years and a mean zBMI of 3.35 ± 0.69 were recruited. Anthropometric and metabolic characteristics of the study sample are shown in Table 1. Compared to girls, boys were older and with higher body weight, zBMI, waist, WHtR, SBP and DBP, ALT, and AST (all $p <$ 0.017). BMI, lipid profile, and HOMA-IR did not differ across sex.

Regression analysis adjusted for sex, age, and Tanner stage, revealed a positive association between zBMI and the minor allele (C) of rs2236212 in ELOVL2 gene ($p = 0.028$, $\beta = 0.053$ [0.006–0.097]) (Table 2). After the same adjustment, analysis showed that carriers of (G) allele of rs1535 in FADS2 gene had lower zBMI and BMI compared to heterozygotes and (AA) homozygotes ($p = 0.046$, $\beta = -0.057$ [−0.117 to −0.001]; $p = 0.025$, $\beta = -0.524$ [−0.981 to −0.067], respectively) (Table 3). By consulting phenoscanner V2 online tool, we found a significant negative association between the rs1535 minor allele and BMI in 35,669 children ($p = 0.000437$, $\beta = -0.028$)[45].

Since these two SNPs were independently associated with zBMI in regression analysis (data not shown), we performed a genetic score. This latter highlighted an addictive action of the two SNPs on zBMI ($p = 0.008$, $\beta = 0.076$ [0.014–0.094]) (Fig. 1).

Genotypes and estimated enzymatic activity

The measurement of fatty acids and the estimation of the enzymatic activity in a subgroup of obese children (n = 105) revealed that minor allele (C) of rs2236212 associated significantly with a reduction of the enzymatic activity of elongase 2 and consensual lower concentration of the

Table 2 Physical and biochemical features according to rs2236212 (*ELOVL2*) genotypes.

Variables	GG	CG	CC	*p* value
n (Male/female)	269/240	415/412	159/154	0.556
Age (years)	10.9 (2.6)	11.1 (2.6)	10.9 (2.8)	0.986
Body height (m)	1.49 (0.14)	1.49 (0.14)	1.49 (0.15)	0.691
Body weight (kg)	71.4 (22.1)	70.5 (22.2)	72.6 (22.0)	0.573
BMI (kg/m^2)	31.1 (5.4)	30.9 (5.4)	32.0 (5.2)	0.259
zBMI	3.36 (0.70)	3.29 (0.65)	3.50 (0.76)	**0.028**
Waist circumference (cm), $n = 1313$	91.0 (12.1)	89.9 (12.5)	91.0 (13.1)	0.104
WHtR, $n = 1313$	0.61 (0.06)	0.61 (0.06)	0.62 (0.07)	0.372
Puberty status, $n = 1163$	319/33/15	488/58/24	195/23/8	0.872
SBP (mmHg), $n = 1241$	113 (14)	114 (15)	114 (16)	0.798
DBP (mmHg), $n = 1241$	68 (10)	66 (11)	66 (12)	0.057
Triglycerides (mg/dL), $n = 1\ 555$	98.7 (50.0)	98.7 (53.6)	94.9 (49.8)	0.113
Total cholesterol (mg/dL), $n = 1581$	159.5 (32.7)	161.9 (34.0)	156.5 (30.7)	0.448
HDL cholesterol (mg/dL), $n = 1474$	46.0 (10.2)	46.8 (12.5)	45.9 (11.0)	0.145
LDL cholesterol (mg/dL), $n = 1390$	92.7 (24.5)	95.1 (28.6)	91.3 (25.1)	0.257
ALT	28.3 (18.0)	29.6 (20.3)	28.9 (20.6)	0.808
AST	24.1 (15.1)	24.1 (9.7)	24.2 (10.4)	0.824
HOMA-IR	4.66 (3.72)	4.84 (4.17)	4.95 (4.69)	0.300

Sample size, $n = 1649$, unless otherwise indicated. Data expressed as means (SD) or number and percentage, *n* (%). This multivariate regression analysis model was adjusted, along with *ELOVL2* rs2236212 genotype, for the following covariates: age, gender, zBMI, and Tanner status.

SD standard deviations, *BMI* body mass index, *WHtR* waist-to-height ratio, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *ALT* alanine transaminase, *AST* aspartate transaminase.

Bold values indictates statistical significance $p < 0.05$.

Table 3 Physical and biochemical features according to rs1535 (FADS2) genotypes.

Variables	AA	AG	GG	*p* value
n (Male/female)	357/345	289/270	59/64	0.196
Age (years)	11.0 (2.6)	11.0 (2.7)	10.9 (2.5)	0.494
Body height (m)	1.49 (0.14)	1.49 (0.15)	1.48 (0.13)	0.430
Body weight (kg)	71.9 (22.2)	71.0 (23.5)	67.7 (19.3)	0.057
BMI (kg/m^2)	31.3 (5.7)	30.9 (5.4)	30.2 (4.7)	**0.025**
zBMI	3.37 (0.71)	3.34 (0.69)	3.23 (0.63)	**0.046**
Waist circumference (cm), $n = 1313$	91.3 (12.5)	90.4 (13.1)	89.1 (11.3)	0.170
WHtR, $n = 1313$	0.62 (0.06)	0.61 (0.07)	0.60 (0.06)	0.306
Puberty status, $n = 1163$	374/55/29	293/49/17	72/9/1	0.080
SBP (mmHg), $n = 1241$	115 (15)	114 (14)	114 (14)	0.894
DBP (mmHg), $n = 1241$	66 (10)	67 (11)	66 (13)	0.471
Triglycerides (mg/dL), $n = 1295$	98.2 (52.2)	99.9 (56.6)	92.6 (38.9)	0.516
Total cholesterol (mg/dL), $n = 1321$	161.4 (33.3)	160.5 (33.4)	160.4 (32.9)	0.280
HDL cholesterol (mg/dL), $n = 1221$	46.4 (10.6)	46.0 (10.3)	50.3 (18.7)	0.763
LDL cholesterol (mg/dL), $n = 1140$	94.8 (27.0)	93.8 (26.1)	89.8 (26.0)	0.092
ALT	30.3 (21.1)	29.9 (19.9)	26.3 (12.8)	0.702
AST	24.7 (14.6)	24.3 (9.1)	22.9 (5.5)	0.638
HOMA-IR	4.98 (4.80)	4.90 (4.05)	4.50 (3.26)	0.097

Sample size, $n = 1649$, unless otherwise indicated. Data expressed as means (SD) or number and percentage, *n* (%). This multivariate regression analysis model was adjusted, along with *FADS2* rs1535 genotype, for the following covariates: age, gender, zBMI, and Tanner status.

SD standard deviations, *BMI* body mass index, *WHtR* waist-to-height ratio, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *ALT* alanine transaminase, *AST* aspartate transaminase.

Bold values indictates statistical significance $p < 0.05$.

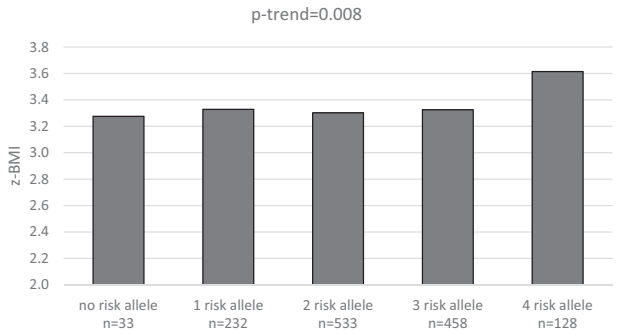

Fig. 1 *FADS2* and *ELOVL2* genetic score is associated with increased zBMI. Data are shown as mean (SEM). This multivariable linear regression model was adjusted, along with genetic score, for the following covariates: age, sex, puberty status, zBMI. SEM standard error of the mean, zBMI body mass index *z*-score.

product DPA and with an increase of the substrate EPA ($p = 0.048$, $p = 0.027$, $p = 0.006$, respectively) (Fig. 2).

The analysis performed on *FADS2* gene revealed that major allele (A) carriers of rs1535, besides being more obese, had a higher enzymatic activity of the enzyme D5D and a significantly lower concentration of DGLA, the substrate of D5D ($p = 0.0001$, $p = 1.8 \times 10^{-9}$, respectively) (Fig. 2). These differences were maintained also when the analysis was performed in the two groups using different matrices for fatty acids composition (62 obese children from the "Pediatric Obesity Outpatients Clinic" and 43 from a school-based study), separately (Supplementary Table 1). We also provided data regarding the differences in fatty acids profile among the two groups (Supplementary Table 2). No difference was found for AA neither for D6D.

As regards the analysis of fatty acid proportions and the score characterized by the presence of both risk alleles, in linear regression model adjusted for age and sex we found a significant association between the genetic risk score and the levels of D5D ($p = 0.004$, $\beta = 0.286$ [0.100–0.474]) and EPA ($p = 0.009$, $\beta = 0.252$ [0.066–0.439]). An inverse association was found between the score and the level of DGLA ($p = 0.0001$, $\beta = -0.382$ [−0.196 to −0.568]). No significant association was found between the genetic risk score and AA, DPA, or elongase 2 activity.

Discussion

To the best of our knowledge, this study is the first investigating *FADS2* and *ELOVL2* genetic variants in children and supporting an association between BMI and *FADS* cluster and *ELOVL2* genes in a large sample of obese children and adolescents. In our study, we highlighted that children carrying the (A) allele of rs1535 and the (C) allele of rs2236212 have a higher zBMI. Notably, data of a significant association between rs1535 and BMI in 35,669 children, reported by GWAS, in phenoscanner V2 database, supports our finding[45]. In support of these evidences, Stoffel et al. reported that FADS2$^{-/-}$ mice had an obesity resistance phenotype, according to our results[46]. Moreover, we firstly demonstrated that the synergic cooperation between the two risk alleles within *FADS2* and *ELOVL2* genes correlates with the severity of obesity.

Previously, only a few studies have found an association between *FADS2* genetic variants and BMI[9,27], none between BMI and *ELOVL2*. De la Garza Puentes et al. found that pregnant women who were carriers of the minor allele of SNPs within *FADS1* and *FADS2* genes were associated with a higher risk of having a BMI major or equal to 25 than homozygotes for the major allele. However, the same authors did not find any association between rs2236212 (*ELOVL2*) and BMI[27]. Kwong et al. showed a trend toward higher BMI in (AA) carriers of rs1535 (*FADS2*) in a population of patients with post-acute myocardial infarction[9].

Although pieces of evidence of an association between these SNPs and lipid profile[21,23,24] and FADS variants with insulin resistance[25,26], we did not find any association between these SNPs and plasma lipid variables (TG, LDL-c, HDL-c, TC) and HOMA-IR in our study. We didn't even find any association between SNPs and indices of adiposity and fat distribution (waist circumference and WHtR).

The main mechanism that could be involved in the association between genetic variants in desaturation and elongation pathways and BMI is an alteration of the PUFAs synthesis pathways, leading to an imbalance of the omega-6/omega-3 ratio. Indeed, there are several proposed mechanisms by which n-3 LC-PUFAs could work in reducing body weight and improving the metabolic profile and insulin sensitivity of obese subjects[47]. N-3 PUFAs might inhibit the proliferation and induce apoptosis of preadipocytes promoting their differentiation serving as ligands for peroxisome proliferator-activated receptors[48]. In addition, n-3 PUFAs have a documented role in reducing the low-grade inflammation, are key factors in the pathogenesis of metabolic derangements in obesity, via G protein-coupled receptor 120-mediated suppression of macrophage proinflammatory cytokine secretion and resolvins/protectins-mediated resolution of inflammation[49]. Therefore, n-3 PUFAs affected adipocyte lipid metabolism by modulating adipokine secretion. N-3 PUFAs promote hepatic, adipose tissue, and skeletal muscle fatty acid oxidation, suppress lipogenesis; increase secretion of adiponectin, leptin, and visfatin, shifting the balance of fatty acid metabolism toward oxidation rather than storage, facilitating the reduction of triglyceride accumulation in adipose tissue, especially in white adipose tissue[49]. Taken together,

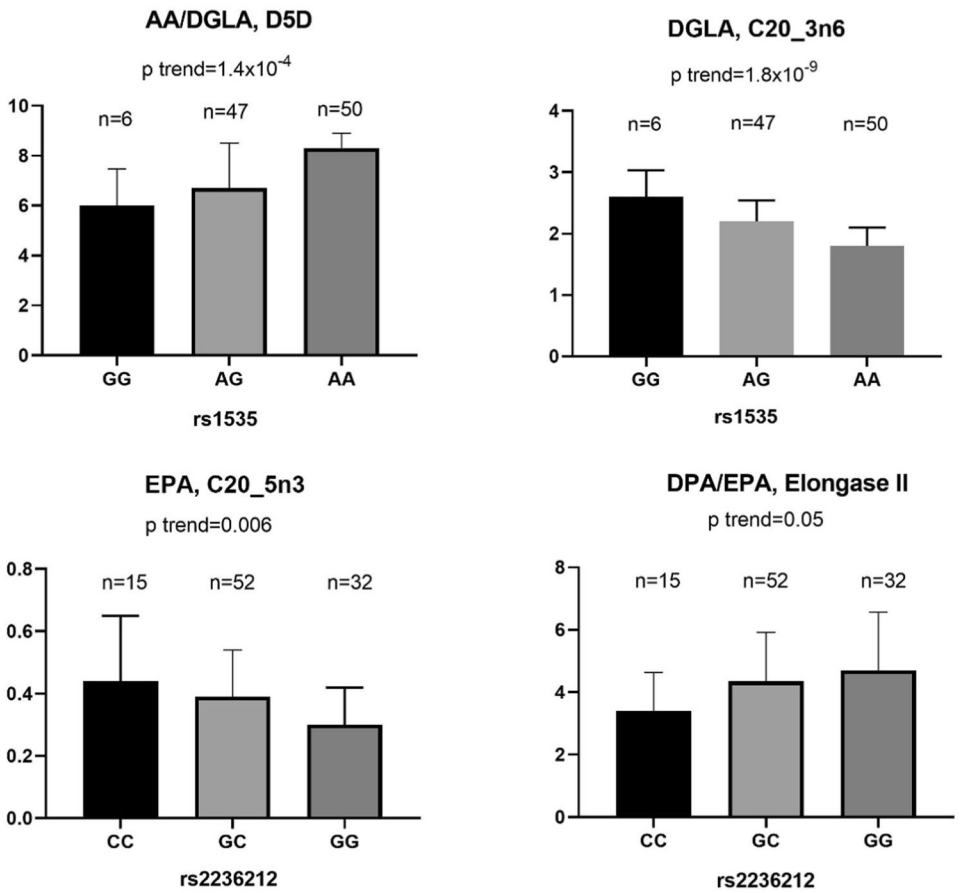

Fig. 2 Association between genotypes of rs1535 (A) and rs2236212 (C) and the enzymatic activity and the products of D5D and elongase 2. AA arachidonic acid, DGLA dihomo-gamma-linolenic acid, D5D delta-5 desaturase, EPA eicosapentaenoic acid, DPA docosapentaenoic acid.

these findings suggest that n-3 PUFAs promote adipogenesis and healthy expansion of adipose tissue, promoting a metabolically healthy phenotype[47].

Analyzing the proportions of erythrocyte and plasmatic fatty acids composition, in a subgroup of 105 children, we found a significative increase of the D5D activity in carriers of the risk allele (A) of rs1535 and a reduction of elongase 2 activity in the carriers of the risk allele (C) of rs2236212. In addition, the minor allele (C) of rs2236212 was associated significantly with a lower concentration of the product DPA and an increase of the substrate EPA of elongase 2 in the n-3 LC-PUFAs pathway. Some studies, including a meta-analysis of genome-wide associations of n-3 fatty acids comprising a total of 8866 subjects of European ancestry, found that rs2236212 minor allele was most strongly associated with increased plasma EPA and reduced DHA proportions, suggesting that a reduced elongase activity mainly affects the omega-3 pathway[11,14].

Elongase and desaturase enzymes are involved in the biosynthesis of both n-6 LC-PUFAs and n-3 LC-PUFAs. Thus, there is a competition for the enzymes between the two pathways and desaturation is the rate-limiting step[11]. By using the genetic score, characterized by the co-presence of both the two risk alleles of *FADS2* and *ELOVL2*, we noticed an additive effect involving both pathways, consisting in the increase of D5D activity and EPA levels, substrate of elongase 2. This could be interpreted as an imbalance of LC-PUFAs homeostasis toward an increase in n-6 PUFAs synthesis at the expense of n-3 PUFAs, in carriers of both risk alleles, who are the most obese patients.

About the result of rs1535, the risk allele (A) was associated with higher activity of D5D and reduced amounts of the precursor DGLA. Other authors reported associations that were not restricted to precursors or products of the specific desaturase metabolic stage (D6D in the case of *FADS2*), but included other associations of the same pathway, reflecting variants in LD with D5D in the *FADS1* gene[11,15].

Several studies found that minor allele carriers of SNPs at the *FADS* gene cluster reduced the activity of both desaturase enzymes, also in children, with consequent alterations in the proportions of fatty acids, in line with our finding[9,11,15,21,50,51]. There is another mechanism through which an increased activity of D5D and D6D could be implicated in obesity and metabolic-related diseases, independently of impacting synthesis of PUFA end products and their downstream biologic roles. Kim et al.

demonstrated a bidirectional link between glycolysis and PUFA desaturation. The D5D and D6D activity in vitro and humans recycle NADH to NAD+, permitting ongoing glycolysis and cell growth and proliferation when aerobic respiration is impaired; a decreased cytosolic NAD +/NADH increases PUFAs synthesis, as well[52]. Genetic variants in FADS cluster genes increasing desaturase activity might cause chronic changes in the cytosolic redox status, contributing to increasing metabolic disease, obesity, and cancer[52].

The interesting result highlighted in this study is that genetic variants in *FADS2* and *ELOVL2* genes are associated to an alteration of LC-PUFAs homeostasis, not accompanied by an alteration of plasma lipids or HOMA-IR. Instead, we noted that children with risk alleles have an increased zBMI, thus *FADS* cluster genes and *ELOVL2* correlate with the severity of obesity in a population of obese children. This finding could precede the development of dyslipidaemia or glucose intolerance in these children, permitting to detect early the subjects at increased risk of obese-related metabolic complications.

Our study confirms the hypothesis that a range of SNPs in candidate genes implicated in PUFAs biosynthesis, such as *FADS* cluster and *ELOVL2*, may underpin variability in n-3 and n-6 LC-PUFAs metabolism and consequently in pathophysiological pathways contributing to obesity. We hypothesize that the presence of these polymorphisms, by altering lipid homeostasis and cytosolic redox status, may predispose children to become more obese and to develop a metabolically less healthy obesity phenotype. This may then lead to obesity-related complications such as dyslipidaemia, type 2 diabetes mellitus, and CVD. By the use of complex models built according to the great heterogeneity of genetic variants and risk factors involved in obesity, these polymorphisms, in the future, might contribute to identify early obese children with a higher likelihood of having metabolic and cardiovascular risks, in order to implement early and personalized prevention strategies.

Furthermore, several studies supported that specific genetic variants in *FADS2* and *ELOVL2* genes, altering endogenous fatty acid metabolism, are likely to contribute to variability in response to PUFAs dietary intake[9,14]. Minor allele carriers of common SNPs could particularly benefit from a high intake of specific fatty acids in maintaining high levels of plasma n-3 PUFAs, improving the metabolic profile and obesity[14].

Nevertheless, further prospective studies and long-term random controlled trials are needed to confirm the potential role of these SNPs in predicting obesity and obesity-related complications and to better understand their biological response to dietary intake or therapeutic supplementation with PUFAs.

Strengths and limitations

The study has some potential limitations: (i) ethnicity, this study was conducted in subjects with European ancestry, so that results are not directly exportable to subjects with other ethnic backgrounds; (ii) a population of obese subjects, in which some metabolic changes due to the alteration of PUFAs metabolism could be hidden by the obesity state and which instead could be better highlighted in a general population; (iii) two different matrices have been used for fatty composition dosages: red blood cell membrane fatty acids for 62 subjects and whole-blood fatty acids for the remaining 43. Whole-blood fatty acids percentage considers both plasma and erythrocytes, so it reflects both dietary and endogenous fatty acids metabolism.

This study has also some strengths: (i) first the sample size of the study was relatively large to explore associations with BMI, despite low allele frequencies and small gene-effect sizes; (ii) the sample set, including obese children and adolescents who were not taking chronic medication and have much lower obesity-associated comorbidities than obese adults. This allows to explore relationships between variables avoiding potential confounders due to co-morbidity; (iii) moreover, the SNPs studied have a minor allele frequency of sufficient prevalence to be of relevance in standard clinical practice.

Conclusion

In summary, this study identified novel common variants in the *FADS2* and *ELOVL2* genes associated with BMI in a large population of obese Italian children and adolescents using candidate genes implicated in fatty acids metabolism. These findings provide additional support to the genetics accounting for BMI interindividual variability and the molecular basis of obesity. A better understanding of the underlying genetic causes of unhealthy obesity may be useful in developing more effective prevention and treatment strategies since childhood.

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Author contributions A Maguolo, CZ, and AG researched and analyzed data and wrote the manuscript. A Maguolo, CZ, AG, AT, and A Morandi researched data and discussed the manuscript. EMDG, CF, A Morandi, and CM edited the manuscript, and provided substantial contribution to the overall discussion. CF and CM are the guarantor of

this work and, as such, had full access to all the data in the study and take responsibility for the integrity and the accuracy of the data analysis.

Compliance with ethical standards

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

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