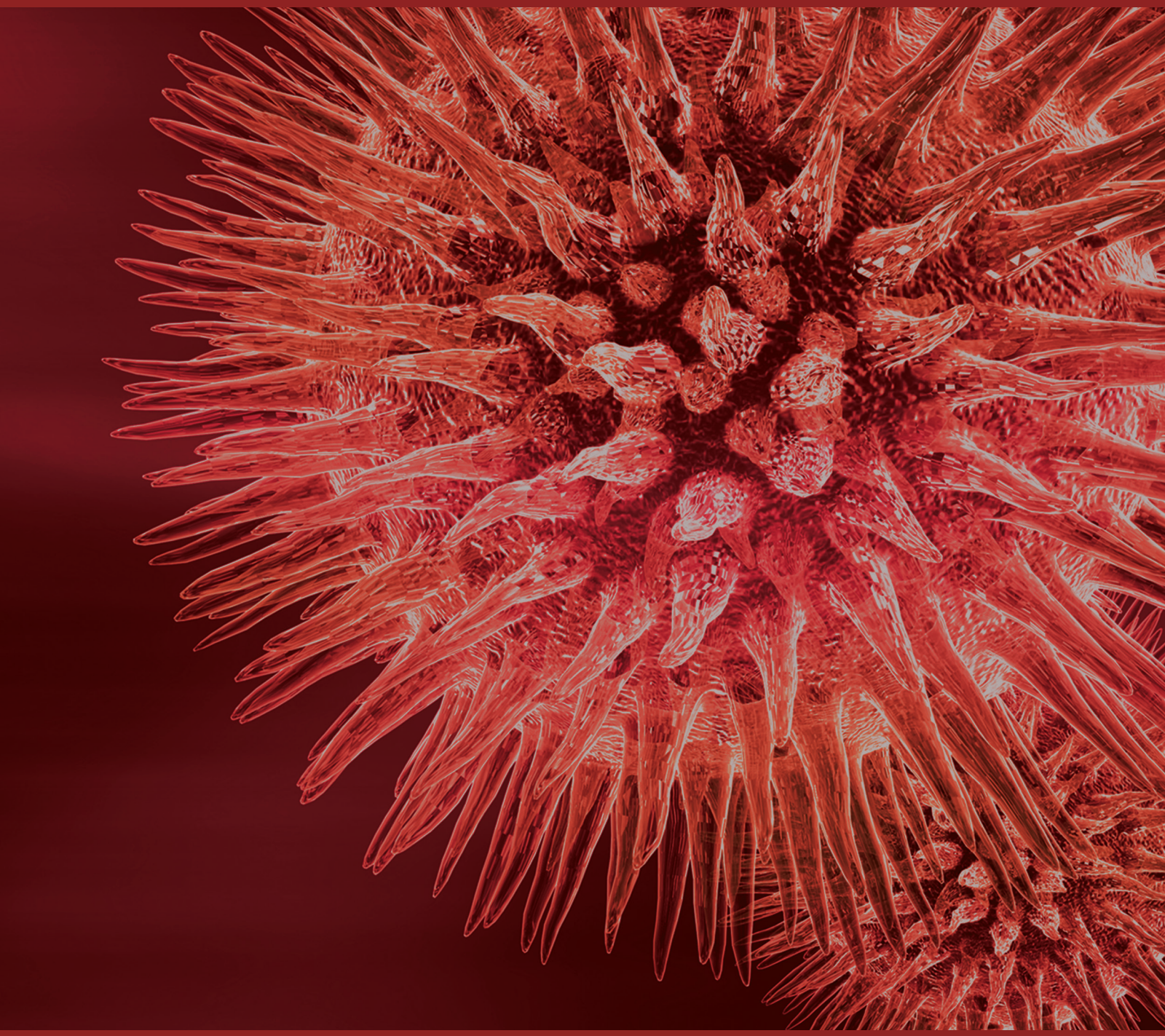
BioMed Research International

Screening for Complex Diseases and Personalized Health Care

Guest Editors: Stefania Boccia, Paolo Boffetta, and Paolo Villari



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Editorial

Screening for Complex Diseases and Personalized Health Care

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The extraordinary accomplishment of decoding the human genome, together with the technological advancement of the later part of the 20th century, has strong implications for the health care, as it provides the opportunity to transform its delivery health from a disease-focused model to one that is personalized, predictive, proactive, precise, and patient-centred. Personalized health care can be broadly defined as a customization of the medical provision that accommodates individual differences in all stages in the process, from prevention, to diagnosis and treatment, to posttreatment follow-up. Despite the fast moving human genome discoveries in a wide proportion of diseases having large public health impact, however, the promise of personalized healthcare has far lagged behind due the complexity involved [1]. In addition, besides the pharmacogenomics field, the evidence on the extent to which genomic information has provided measurable population health benefits and actionable intelligence to citizens is still limited.

This special issue includes five contributions spanning from genetic association studies to the awareness of citizens on cervical cancer risk factors and screening programs and physician knowledge on genetic tests for diagnosis of hereditary cancer.

The first set of papers is represented by three genetic association studies on chronic diseases. The paper of F. Coppedè et al. reported on the absence of association between the common c.80G>A polymorphism (rs1051266) of the gene coding for the reduced folate carrier (*RFC*-1 ge) and Alzheimer's disease in an Italian case-control studies. Similarly, despite the potential for a biological significance of *TNFA* −308G>A polymorphism on the risk of obesity, M. Barchitta et al. reported the absence of gene-Mediterranean diet interaction on the risk of overweight/obesity among Italian women. A poor adherence to the Mediterranean diet, however, was associated with educational level (less common among those less educated) and younger ages. Lastly, the paper of S. Boccia et al. investigated the effect of *CYP*, *GST*, and *SULT* polymorphisms and their interaction with smoking on the risk of hepatocellular carcinoma. Results show that *CYP2E1*5B* and *CYP2E1*6* polymorphisms have a favorable effect on the development of HCC, while polymorphisms of *GSTT1* and *SULT1A1* might play role in increasing the susceptibility among smokers.

The paper of C. D. Vito et al. investigated the knowledge of cervical cancer risk factors and the predictors of adherence to cervical cancer screening in relation to mass media campaigns in a large study conducted in Italy. Results show that higher educational level, being not married, and living in urban areas were the main independent characteristics associated with a higher level of knowledge of cervical cancer etiology. During the campaign period women had the Pap test more frequently as a consequence of the mass media campaign, strengthening the evidence of the usefulness of media campaigns via local television to foster cervical screening compliance.

Lastly, the paper of N. Panic et al. assessed the knowledge and attitudes on genetic tests for breast and colorectal cancer of young medical residents of postgraduates schools. The study represents a follow-up of a larger study of a sample of Italian physicians [2]. Results show that knowledge on hereditary breast cancer genetic tests was high; for colorectal cancer it was largely insufficient. Knowledge on tests was

higher among residents who attended course on cancer genetic testing during graduate training. More than 70% asked for the additional training on the genetic tests for cancer during the specialization school.

Stefania Boccia
Paolo Boffetta
Paolo Villari

References

[1] S. Boccia, "Why is personalized medicine relevant to public health?" *European Journal of Public Health*, vol. 24, no. 3, pp. 349–350, 2014.

[2] C. Marzuillo, C. de Vito, S. Boccia et al., "Knowledge, attitudes and behavior of physicians regarding predictive genetic tests for breast and colorectal cancer," *Preventive Medicine*, vol. 57, no. 5, pp. 477–482, 2013.

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Research Article

The Effect of *CYP*, *GST*, and *SULT* Polymorphisms and Their Interaction with Smoking on the Risk of Hepatocellular Carcinoma

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Aim. The aim of our study was to assess whether selected single nucleotide polymorphisms of *CYP1A1* and *2E1*, *GSTM1*, *GSTT1*, and *SULT1A1* influence susceptibility towards HCC, considering their interaction with cigarette smoking. *Methods*. We recruited HCC cases and controls among patients admitted to the hospital "Agostino Gemelli," from January 2005 until July 2010. Odds ratios (OR) of HCC were derived from unconditional multiple logistic regression. Gene-gene and gene-smoking interaction were quantified by computing the attributable proportion (AP) due to biological interaction. *Results*. The presence of any *CYP2E1* 5B* variant allele (OR: 0.23; 95% CI: 0.06-0.71) and *CYP2E1* 6* variant allele (OR: 0.08; 95% CI: 0.01–0.33) was inversely related to HCC. There was a borderline increased risk among carriers of combined *CYP1A1* 2A* and *SULT1A1* variant alleles (OR: 1.67; 95% CI: 0.97–3.24). A significant biological interaction was observed between *GSTT1* and smoking (AP = 0.48; 95% CI: 0.001–0.815), with an OR of 3.13 (95% CI: 1.69–5.82), and borderline significant interaction was observed for *SULT1A1* and smoking (AP = 0.36; 95% CI: −0.021–0.747), with an OR of 3.05 (95% CI: 1.73–5.40). *Conclusion*. *CYP2E1* 5B* and *CYP2E1* 6* polymorphisms have a favourable effect on the development of HCC, while polymorphisms of *GSTT1* and *SULT1A1* might play role in increasing the susceptibility among smokers.

1. Introduction

Hepatocellular carcinoma (HCC) is currently the sixth most common cancer and the third cause of cancer deaths worldwide [1]. Its prognosis remains poor, with a 5-year survival rate less than 20% in Europe [2]. Risk factors for HCC include infection with hepatitis B (HBV) and hepatitis C viruses (HCV), history of diabetes mellitus, nonalcoholic fatty liver disease and cirrhosis, heavy alcohol consumption, and cigarette smoking [3–5]. Coffee consumption appears to have a favorable effect [6]. Further, genetic factors appear to modulate the individual susceptibility as the siblings of HCC

individuals are more prone to develop the HCC even in the absence of HBV infection [7]. So far, several single nucleotide polymorphisms (SNPs) have been investigated in association with HCC, with contradictory results [8].

As the liver is the main metabolic organ, the SNPs related to genes encoding carcinogen-metabolizing enzymes represent key target candidates for association analyses. Cytochrome P-450 (*CYP*) is a superfamily of monooxygenases responsible for phase I enzyme reactions, preparing substrates for phase II conjugation reactions, but they can also lead to the metabolic activation of toxic or carcinogenic compounds [9]. The glutathione S-transferases (*GSTs*) are a gene superfamily coding phase II enzymes that detoxify free radicals in tobacco smoke, products of oxidative stress, and polycyclic aromatic hydrocarbons [10]. Sulfotransferase (*SULT*) catalyzes sulfonate conjugation to detoxify the procarcinogens to metabolites, which are easily eliminated from the body [11]. Polymorphisms in these genes, their combination, and interaction with environmental factors have the potential to lead to increased susceptibility to HCC. While some studies investigated the effect of *GST* and *CYP* genes on HCC, as well as their combination and interaction with smoking [12, 13], little information is available on the effect of *SULT1A1*.

The aim of our study was to assess whether the selected SNPs of *CYP1A1* and *2E1*, *GSTM1, GSTT1,* and *SULT1A1* genes influence individual susceptibility to HCC, also considering their combination and interaction with cigarette smoking.

2. Methods

2.1. Study Population. Study participants were recruited among patients admitted to the teaching hospital "Agostino Gemelli" of the Università Cattolica del Sacro Cuore (Rome, Italy) from January 2005 until July 2010, and eligibility was restricted to white individuals born in Italy. Cases were 221 patients with HCC recruited among subjects referred to the Outpatient Liver Unit of the hospital. The diagnosis of HCC was performed according the AASLD guidelines [14]. The control group included 290 patients from the same hospital with a broad range of diagnoses, enrolled during the same time period. In closer detail, around 50% of the controls were outpatients, and the remaining were patients undergoing surgical interventions (laparoscopic cholecystectomy, appendicitis, and inguinal hernia) or admitted for a wide spectrum of other nonneoplastic conditions (elderly patients on physical-therapy rehabilitation after stroke or orthopedic injuries). Written informed consent was obtained from all study subjects. The study was conducted according to the Declaration of Helsinki and was approved by the Ethical Committee of Università Cattolica del Sacro Cuore.

HCC cases and controls were interviewed by trained physicians using a structured questionnaire to collect information on demographics, medical history, and lifestyle habits including smoking history. Questions about lifestyle habits focused on the time period ending one year prior to diagnosis for cases and on the year prior to the interview date for controls.

Table 1: Distribution of 221 cases of hepatocellular carcinoma (HCC) and 290 controls according to selected factors.

	HCC cases ($N = 221$)		Controls ($N = 290$)	
	N	(%)	N	(%)
Age (years)				
<60	43	(19.5)	105	(36.2)
60–69	80	(36.2)	78	(26.9)
≥70	98	(44.3)	107	(36.9)
Sex				
Male	160	(72.4)	176	(60.7)
Female	61	(27.6)	114	(39.3)
Smoking[a]				
Never	85	(39.2)	173	(59.7)
Ever	133	(60.8)	117	(40.3)
$P^b < 0.001$				
Hepatitis[a,c]				
No	64	(29.0)	282	(97.9)
Yes	157	(71.0)	6	(2.1)
$P^b < 0.001$				

[a]The sum does not add up to the total because of some missing values.
[b]P values from χ^2 test.
[c]Hepatitis was defined as history of hepatitis B and/or C.

2.2. Genotyping Methods. DNA was extracted from the peripheral blood lymphocytes of each participating subject. *GSTM1* and *GSTT1* null alleles were identified using a multiplex-polymerase chain reaction- (PCR-) based method [15]. The polymorphic site at nucleotide 638 in exon 7 (Arg213His (**2* allele), rs9282861) of the *SULT1A1* gene was genotyped by PCR-restriction fragment length polymorphisms (RFLP) analysis as described by Coughtrie et al. [16]. *CYP1A1* 3′-flanking region *Msp*I polymorphism (*CYP1A1*2A* allele, rs4646903), *CYP2E1 Pst*I/*Rsa*I polymorphism (*CYP2E1*5B* allele, rs3813867 (*Pst*I)), and *CYP2E1 Dra*I (**5A* or **6* alleles, rs6413432) were also determined by PCR-RFLP analyses. Quality control for each genotyping was performed in each experiment, and 10% of the total samples were randomly selected and reanalyzed with 100% concordance. All laboratory procedures were carried out blindly to case-control status.

2.3. Statistical Analysis. Hardy-Weinberg equilibrium (HWE) was tested for the control SNPs. Odds ratios (OR) of HCC and corresponding 95% confidence intervals (CI) according to analyzed polymorphisms were derived from unconditional multiple logistic regression models [17] using dominant model for carriers of the mutated allele, including terms for age and sex. When cell sizes were small (<5), exact logistic regression was used [18].

We also examined the possible confounding effect of smoking, alcohol, and chronic infection with HBV and/or HCV. However, models including these covariates yielded very similar results; thus, given the small numbers in some strata, only the age- and sex-adjusted estimates were presented.

Table 2: Distribution of cases and controls, odds ratios[a] (OR), and 95% confidence intervals (CI) for hepatocellular carcinoma (HCC) according to selected polymorphisms.

	HCC cases (N = 221)		Controls (N = 290)		OR (95% CI)
	N	(%)	N	(%)	
CYP1A1 2A*					
wt/wt	165	(74.7)	226	(77.9)	1[b]
wt/mt and mt/mt	56	(25.3)	64	(22.7)	1.21 (0.80–1.84)
CYP2E1 5B*					
*c*1/*c*1	217	(98.2)	270	(93.1)	1[b]
*c*1/*c*2 and *c*2/*c*2	4	(1.8)	20	(6.9)	0.23 (0.06–0.71)
CYP2E1 6*[c]					
wt/wt	204	(99.0)	261	(90.0)	1[d]
wt/mt and mt/mt	2	(1.0)	29	(10.0)	0.08 (0.01–0.33)
GSTM1[c]					
Present	96	(47.8)	139	(48.1)	1[b]
Null	105	(52.2)	150	(51.9)	0.99 (0.68–1.43)
GSTT1[c]					
Present	141	(70.1)	220	(76.1)	1[b]
Null	60	(29.9)	69	(23.9)	1.35 (0.89–2.05)
SULT1A1					
wt/wt	132	(59.7)	180	(62.1)	1[b]
wt/mt and mt/mt	89	(40.3)	110	(37.9)	1.22 (0.84–1.77)

[a]Adjusted for age and sex.
[b]Reference category.
[c]The sum does not add up to the total because of some missing values.
[d]Calculated from exact logistic regression analysis.
wt: wild-type allele.
mt: variant-type allele.

Gene-gene interaction analysis was conducted, using as a reference group the homozygous wild-type individuals for both genes, while for gene-environment interaction analyses, the reference group was wild-type homozygotes not exposed to the environmental risk factor. Biological interaction between two genes was estimated using departure from additivity of effects as the criterion of interaction, as proposed by Rothman [19]. To quantify the amount of interaction, the attributable proportion (AP) due to interaction was calculated together with its 95% CI as described by Andersson et al. [20]. The AP due to interaction is the proportion of individuals among those exposed to the two interacting factors that is attributable to the interaction per se and it is equal to 0 in the absence of a biological interaction [19]. In order to test for more than multiplicative effect among two genes, the likelihood ratio test was used.

The paper has been written according to the STREGA guidelines [21].

3. Results

The demographics, clinical features and lifestyle habits of 221 HCC cases and 290 controls are reported in Table 1. Ever smokers were more common among cases, as well as infection with HBV and/or HCV (Table 1). The genotype frequencies were in HWE ($P > 0.05$).

Table 2 reports the distribution of the polymorphisms considered among HCC patients and controls. The carriers of *c2* variant allele of *CYP2E1* 5B* polymorphisms were less common in cases (1.8%) than in controls (6.9%) corresponding to an OR of 0.23 (95% CI: 0.06–0.71). Similarly, the frequency of the variant allele of *CYP2E1* 6* polymorphism was also less common among cases (1.0%) than controls (10%), with an OR of 0.08 (95% CI: 0.01–0.33) (Table 2). The selected polymorphisms of *CYP1A1*, *GSTM1*, *GSTT1*, and *SULT1A1* did not significantly influence susceptibility to HCC.

The gene-gene and gene-smoking interaction results are reported in Tables 3 and 4. We observed a borderline increased risk for HCC among carriers of combined *CYP1A1* 2A* and *SULT1A1* variant alleles as compared to the double wild-type homozygotes (OR = 1.67; 95% CI: 0.97–3.24) (Table 3). A significant interaction was reported between *GSTT1* and smoking (AP = 0.48; 95% CI: 0.001–0.815), with an OR of 3.13 (95% CI: 1.69–5.82) for *GSTT1* null genotype carriers who were smokers (Table 4). A borderline significant interaction was also observed for *SULT1A1* and smoking (AP = 0.36; 95% CI: −0.021–0.747), with an OR of 3.05 (95 CI: 1.73–5.40) for those *SULT1A1* variant allele carriers who were smokers (Table 4).

Table 3: Effect of the genes-gene interaction on the development of hepatocellular carcinoma.

	Cases : controls	OR[a] (95% CI)	P[b]	AP (95% CI)
GSTM1 × *CYP1A1* 2A*				
Present/wt homozygote	74 : 113	1[c]		
Null/wt homozygote	78 : 112	1.05 (0.69–1.61)		
Present/any mt	22 : 26	1.32 (0.69–2.53)		
Null/any mt	27 : 38	1.04 (0.58–1.88)	0.521	nc
GSTM1 × *GSTT1*				
Present/present	66 : 103	1[c]		
Null/present	75 : 117	0.97 (0.63–1.50)		
Present/null	30 : 36	1.28 (0.71–2.30)		
Null/null	30 : 33	1.39 (0.77–2.53)	0.773	0.109 (−0.603; 0.820)
GSTM1 × *SULT1A1*				
Present/present	53 : 84	1[c]		
Null/present	67 : 96	1.09 (0.68–1.76)		
Present/null	43 : 55	1.40 (0.81–2.41)		
Null/null	38 : 54	1.22 (0.70–2.13)	0.566	nc
GSTT1 × *CYP1A1* 2A*				
Present/wt homozygote	108 : 169	1[c]		
Null/wt homozygote	44 : 56	1.20 (0.74–1.93)		
Present/any mt	33 : 51	0.98 (0.58–1.63)		
Null/any mt	16 : 13	2.00 (0.91–4.41)	0.289	0.414 (−0.149; 0.976)
GSTT1 × *SULT1A1*				
Present/wt/wt	81 : 138	1[c]		
Null/wt/wt	39 : 42	1.59 (0.94–2.70)		
Present/any mt	60 : 82	1.40 (0.90–2.20)		
Null/any mt	21 : 27	1.48 (0.77–2.84)	0.347	nc
SULT1A1 × *CYP1A1* 2A*				
wt homozygote/wt homozygote	99 : 138	1[c]		
mt carrier/wt homozygote	66 : 88	1.14 (0.75–1.74)		
wt homozygote/mt carrier	33 : 62	1.09 (0.64–1.85)		
mt carrier/mt carrier	23 : 22	1.67 (0.97–3.24)	0.493	0.269 (−0.330; 0.867)

OR: odds ratio; CI: confidence interval; AP: attributable proportion; nc: not calculable.
[a]Adjusted for age and sex.
[b]P from test for multiplicative interaction.
[c]Reference category.
wt: wild-type allele.
mt: variant-type allele.

4. Discussion

Our study identified *CYP2E1* 5B* and *CYP2E1* 6* variant alleles associated with a reduced risk of HCC. There was a borderline increased risk for HCC among carriers of combined *SULT1A1* and *CYP1A1* 2A* variant alleles. The polymorphisms in *GSTT1* and *SULT1A1* are associated with increased susceptibility to smoking-related HCC.

CYP2E1 can activate N-nitrosamines and benzene contained in cigarette smoke [22] and is involved in alcohol-mediated generation of oxidative stress [23]. The expression of *CYP2E1* correlates with the generation of hydroxyethyl radicals and lipid peroxidation products [23]. The variant *CYP2E1* 5B* and *CYP2E1* 6* alleles are associated with increased transcription of *CYP2E1* [24] that leads to development of HCC by promoting carcinogenesis. No significant association between *CYP2E1* 6* and HCC was reported so far [25, 26], while contradictory results were reported for the *CYP2E1* 5B* variant allele [27–35]. A recent meta-analysis did not find *CYP2E1* 5B* c2 allele to be associated with HCC [36], also after stratifying among Asians and white. Studies conducted among white individuals, however, were few [26, 30, 35] and included limited numbers of cases.

The favorable effect of both *CYP2E1* polymorphisms on HCC development is not consistent with the biological premises implying a promoting role of a high activity enzyme. However, the only study previously conducted in an Italian population [35] on *CYP2E1* 5B* c2 allele and HCC did report a similar association, indicating a favorable role of the variant allele against HCC which deserves further investigation.

Our results suggest that up to 48% and 36% of HCC cases among smokers carrying, respectively, variant *GSTT1*

Table 4: Effect of the gene-smoking interaction on the development of hepatocellular carcinoma.

	Cases : controls	OR[a] (95% CI)	P[b]	AP (95% CI)
CYP1A1 2A* × smoking[c]				
wt homozygote/no	65 : 136	1[d]		
wt homozygote/yes	98 : 90	2.16 (1.29–3.36)		
mt carrier/no	20 : 37	1.08 (0.58–2.03)		
mt carrier/yes	35 : 27	2.81 (1.51–5.23)	0.680	0.201 (−0.328; 0.730)
GSTM1 × smoking[c]				
Present/no	31 : 91	1[d]		
Present/yes	62 : 48	4.01 (2.20–7.28)		
Null/no	48 : 81	1.82 (1.05–3.18)		
Null/yes	57 : 69	2.34 (1.32–4.23)	0.004	nc
GSTT1 × smoking[c]				
Present/no	59 : 129	1[d]		
Present/yes	81 : 91	1.86 (1.17–2.97)		
Null/no	20 : 43	0.99 (0.53–1.86)		
Null/yes	38 : 26	3.13 (1.69–5.82)	0.230	0.480 (0.001; 0.815)
SULT1A1 × smoking[c]				
wt homozygote/no	52 : 103	1[d]		
wt homozygote/yes	78 : 77	1.93 (1.19–3.14)		
mt carrier/no	33 : 70	1.01 (0.59–1.75)		
mt carrier/yes	55 : 40	3.05 (1.73–5.40)	0.250	0.363 (−0.021; 0.747)

OR: odds ratio; CI: confidence interval; AP: attributable proportion; nc: not calculable.
[a]Adjusted for age and sex.
[b]P from test for multiplicative interaction.
[c]The sum does not add up to the total because of some missing values.
[d]Reference category.
wt: wild-type allele.
mt: variant-type allele.

and *SULT1A1* alleles occurred because of gene-smoking interaction. However, we could not further stratify these two results according to quantity of smoking, as numbers were low. Smoking is recognized as a risk factor for HCC [3–5] and, together with HBV and HCV, one of the major risk factors in Europe [37], and enzymes coded by *GSTT1* and *SULT1A1* have their role in the metabolism of tobacco carcinogens. There is therefore a biological ground for possible synergic carcinogenic effect.

There was a borderline synergic effect of *SULT1A1* and *CYP1A1* 2A* polymorphisms in HCC carcinogenesis. The effect of *SULT1A1* polymorphism on HCC development has not been investigated so far. It has been reported, however, that an enzyme coded by *SULT1A1* variant allele has twofold lower catalytic activity in detoxifying the procarcinogens [38]. The biological significance of variant *CYP1A1* 2A* variant allele is uncertain, but *CYP1A1* 2A* has been reported to increase susceptibility to several cancer types, including lung, breast, and cervical cancer [39–41]. There is therefore a rationale for a synergic effect of these two SNPs in HCC carcinogenesis.

In our study, there was no significant association between HCC and alcohol. As most of the cases have hepatitis, this could lead them to stop drinking. Consequently we were unable to perform gene-alcohol interaction analysis. Secondly, we cannot exclude a selection bias. However, since the observed frequencies of the variant alleles of *CYP1A1* 2A*, as well as the null genotypes of *GSTM1* and *GSTT1*, were in line with those previously reported in the Italian population, a major impact of such bias is unlikely [35, 42]. Thirdly, our study was underpowered to perform gene-gene and gene-interaction analysis.

In conclusion, we report that *CYP2E1* 5B* and *CYP2E1* 6* polymorphisms have a favorable effect on the development of HCC, while the polymorphisms in *GSTT1* and *SULT1A1* may increase HCC susceptibility among smokers.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] International Agency on Research of Cancer, 2012, http://globocan.iarc.fr/Default.aspx.

[2] M. op den Winkel, D. Nagel, J. Sappl et al., "Prognosis of patients with hepatocellular carcinoma. validation and ranking of established staging-systems in a large Western HCC-Cohort," *PLoS ONE*, vol. 7, no. 10, Article ID e45066, 2012.

[3] S. Chuang, C. L. Vecchia, and P. Boffetta, "Liver cancer: descriptive epidemiology and risk factors other than HBV and HCV infection," *Cancer Letters*, vol. 286, no. 1, pp. 9–14, 2009.

[4] S. Franceschi, M. Montella, J. Polesel et al., "Hepatitis viruses, alcohol, and tobacco in the etiology of hepatocellular carcinoma in Italy," *Cancer Epidemiology Biomarkers and Prevention*, vol. 15, no. 4, pp. 683–689, 2006.

[5] F. Turati, R. Talamini, C. Pelucchi et al., "Metabolic syndrome and hepatocellular carcinoma risk," *British Journal of Cancer*, vol. 108, no. 1, pp. 222–228, 2013.

[6] F. Bravi, C. Bosetti, A. Tavani, S. Gallus, and C. La Vecchia, "Coffee reduces risk for hepatocellular carcinoma: an updated meta-analysis," *Clinical Gastroenterology and Hepatology*, vol. 11, no. 11, pp. 1413–1421, 2013.

[7] Y. Gao, Q. Jiang, X. Zhou et al., "HBV infection and familial aggregation of liver cancer: an analysis of case-control family study," *Cancer Causes and Control*, vol. 15, no. 8, pp. 845–850, 2004.

[8] F. Jin, W. J. Xiong, J. C. Jing, Z. Feng, L. S. Qu, and X. Z. Shen, "Evaluation of the association studies of single nucleotide polymorphisms and hepatocellular carcinoma: a systematic review," *Journal of Cancer Research and Clinical Oncology*, vol. 137, no. 7, pp. 1095–1104, 2011.

[9] J. A. Hasler, "Pharmacogenetics of cytochromes P450," *Molecular Aspects of Medicine*, vol. 20, no. 1-2, pp. 12–137, 1999.

[10] J. D. Hayes and D. J. Pulford, "The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance," *Critical Reviews in Biochemistry and Molecular Biology*, vol. 30, no. 6, pp. 445–600, 1995.

[11] N. Gamage, A. Barnett, N. Hempel et al., "Human sulfotransferases and their role in chemical metabolism," *Toxicological Sciences*, vol. 90, no. 1, pp. 5–22, 2006.

[12] K. Song, J. Yi, X. Shen, and Y. Cai, "Genetic polymorphisms of glutathione S- transferase genes GSTM1, GSTT1 and risk of hepatocellular carcinoma," *PLoS ONE*, vol. 7, no. 11, Article ID e48924, 2012.

[13] L. Yu, L. Sun, Y. Jiang, B. Lu, D. Sun, and L. Zhu, "Interactions between CYP1A1 polymorphisms and cigarette smoking are associated with the risk of hepatocellular carcinoma: evidence from epidemiological studies," *Molecular Biology Reports*, vol. 39, no. 6, pp. 6641–6646, 2012.

[14] J. Bruix and M. Sherman, "Management of *Hepatocellular carcinoma*," *Hepatology*, vol. 42, no. 5, pp. 1208–1236, 2005.

[15] M. Arand, R. Mühlbauer, J. Hengstler et al., "A multiplex polymerase chain reaction protocol for the simultaneous analysis of the glutathione S-transferase *GSTM1* and *GSTT1* polymorphisms," *Analytical Biochemistry*, vol. 236, no. 1, pp. 184–186, 1996.

[16] M. W. Coughtrie, R. A. Gilissen, B. Shek et al., "Phenol sulphotransferase SULT1A1 polymorphism: molecular diagnosis and allele frequencies in Caucasian and African populations," *Biochemical Journal*, vol. 337, no. 1, pp. 45–49, 1999.

[17] N. E. Breslow and N. E. Day, *Statistical Methods in Cancer Research, Vol. 1. The Analysis of Case-Control Studies*, IARC, Lyon, France, 1980.

[18] K. F. Hirji, C. R. Mehta, and N. R. Patel, "Exact inference for matched case-control studies," *Biometrics. Journal of the Biometric Society*, vol. 44, no. 3, pp. 803–814, 1988.

[19] K. J. Rothman, "Measuring interactions," in *Epidemiology: An Introduction*, K. J. Rothman, Ed., pp. 168–190, Oxford University Press, New York, NY, USA, 2002.

[20] T. Andersson, L. Alfredsson, H. Källberg, S. Zdravkovic, and A. Ahlbom, "Calculating measures of biological interaction," *European Journal of Epidemiology*, vol. 20, no. 7, pp. 575–579, 2005.

[21] J. Little, J. P. T. Higgins, J. P. A. Ioannidis et al., "Strengthening the reporting of genetic association studies (STREGA): an extension of the STROBE statement," *Italian Journal of Public Health*, vol. 6, no. 3, pp. 238–255, 2009.

[22] F. P. Guengerich, D.-H. Kim, and M. Iwasaki, "Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects," *Chemical Research in Toxicology*, vol. 4, no. 2, pp. 168–179, 1991.

[23] E. Albano, P. Clot, M. Morimoto, A. Tomasi, M. Ingelman-Sundberg, and S. W. French, "Role of cytochrome P4502E1-dependent formation of hydroxyethyl free radical in the development of liver damage in rats intragastrically fed with ethanol," *Hepatology*, vol. 23, no. 1, pp. 155–163, 1996.

[24] S.-M. Wang, A.-P. Zhu, D. Li, Z. Wang, P. Zhang, and G. Zhang, "Frequencies of genotypes and alleles of the functional SNPs in *CYP2C19* and *CYP2E1* in mainland chinese kazakh, uygur and han populations," *Journal of Human Genetics*, vol. 54, no. 6, pp. 372–375, 2009.

[25] M.-W. Yu, A. Gladek-Yarborough, S. Chiamprasert et al., "Cytochrome P450 2E1 and glutathione S-transferase M1 polymorphisms and susceptibility to hepatocellular carcinoma," *Gastroenterology*, vol. 109, no. 4, pp. 1266–1273, 1995.

[26] N. A. Wong, F. Rae, K. J. Simpson, G. D. Murray, and D. J. Harrison, "Genetic polymorphisms of cytochrome p4502E1 and susceptibility to alcoholic liver disease and hepatocellular carcinoma in a white population: a study and literature review, including meta-analysis," *Journal of Clinical Pathology—Molecular Pathology*, vol. 53, no. 2, pp. 88–93, 2000.

[27] M. Munaka, K. Kohshi, T. Kawamoto et al., "Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma," *Journal of Cancer Research and Clinical Oncology*, vol. 129, no. 6, pp. 355–360, 2003.

[28] T. Koide, T. Ohno, X. E. Huang et al., "HBV/HCV infection, alcohol, tobacco and genetic polymorphisms for hepatocellular carcinoma in Nagoya, Japan," *Asian Pacific Journal of Cancer Prevention*, vol. 1, no. 3, pp. 237–243, 2000.

[29] X. P. Ye, T. Peng, T. W. Liu et al., "The effect of interaction between alcohol drinking and polymorphisms of cytochrome P450 2E1 on the susceptibility of Hepatocellular carcinoma in Guangxi region," *Journal of Guangxi Medical University*, vol. 25, no. 4, pp. 493–495, 2008 (Chinese).

[30] J. M. Ladero, J. A. Agúndez, A. Rodríguez-Lescure, M. Diaz-Rubio, and J. Benítez, "RsaI polymorphism at the cytochrome P4502E1 locus and risk of hepatocellular carcinoma," *Gut*, vol. 39, no. 2, pp. 330–333, 1996.

[31] T. Imaizumi, Y. Higaki, M. Hara et al., "Interaction between cytochrome P450 1A2 genetic polymorphism and cigarette smoking on the risk of hepatocellular carcinoma in a Japanese population," *Carcinogenesis*, vol. 30, no. 10, pp. 1729–1734, 2009.

[32] N. A. Wong, F. Rae, K. J. Simpson, G. D. Murray, and D. J. Harrison, "Genetic polymorphisms of cytochrome p4502E1 and susceptibility to alcoholic liver disease and hepatocellular carcinoma in a white population: a study and literature review, including meta-analysis," *Molecular Pathology*, vol. 53, no. 2, pp. 88–93, 2000.

[33] S. Z. Yu, X. E. Huang, T. Koide et al., "Hepatitis B and C viruses infection, lifestyle and genetic polymorphisms as risk factors for hepatocellular carcinoma in Haimen, China," *Japanese Journal of Cancer Research*, vol. 93, no. 12, pp. 1287–1292, 2002.

[34] H. S. Lee, J. H. Yoon, S. Kamimura et al., "Lack of association of cytochrome P450 2E1 genetic polymorphisms with the risk of human hepatocellular carcinoma," *International Journal of Cancer*, vol. 71, no. 5, pp. 737–740, 1997.

[35] L. Silvestri, L. Sonzogni, A. De Silvestri et al., "CYP enzyme polymorphisms and susceptibility to HCV-related chronic liver disease and liver cancer," *International Journal of Cancer*, vol. 104, no. 3, pp. 310–317, 2003.

[36] C. Liu, H. Wang, C. Pan, J. Shen, and Y. Liang, "CYP2E1 PstI/RsaI polymorphism and interaction with alcohol consumption in hepatocellular carcinoma susceptibility: evidence from 1,661 cases and 2,317 controls," *Tumour Biology*, vol. 33, no. 4, pp. 979–984, 2012.

[37] D. Trichopoulos, C. Bamia, P. Lagiou et al., "Hepatocellular carcinoma risk factors and disease burden in a European cohort: a nested case-control study," *Journal of the National Cancer Institute*, vol. 103, no. 22, pp. 1686–1695, 2011.

[38] S. Nowell, C. B. Ambrosone, S. Ozawa et al., "Relationship of phenol sulfotransferase activity (SULT1A1) genotype to sulfotransferase phenotype in platelet cytosol," *Pharmacogenetics*, vol. 10, no. 9, pp. 789–797, 2000.

[39] T. Juárez-Cedillo, M. Vallejo, J. M. Fragoso et al., "The risk of developing cervical cancer in Mexican women is associated to CYP1A1 MspI polymorphism," *European Journal of Cancer*, vol. 43, no. 10, pp. 1590–1595, 2007.

[40] A. Shin, D. Kang, J. Y. Choi et al., "Cytochrome P450 1A1 (CYP1A1) polymorphisms and breast cancer risk in Korean women," *Experimental and Molecular Medicine*, vol. 39, no. 3, pp. 361–366, 2007.

[41] P. Zhan, Q. Wang, Q. Qian, S. Wei, and L. Yu, "CYP1A1 MspI and exon7 gene polymorphisms and lung cancer risk: an updated meta-analysis and review," *Journal of Experimental and Clinical Cancer Research*, vol. 30, no. 1, article 99, 2011.

[42] L. Covolo, U. Gelatti, R. Talamini et al., "Alcohol dehydrogenase 3, glutathione S-transferase M1 and T1 polymorphisms, alcohol consumption and hepatocellular carcinoma (Italy)," *Cancer Causes and Control*, vol. 16, no. 7, pp. 831–838, 2005.

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Research Article

Survey on Knowledge, Attitudes, and Training Needs of Italian Residents on Genetic Tests for Hereditary Breast and Colorectal Cancer

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Objectives. The aim of the study was to assess knowledge and attitudes of medical residents working in Università Cattolica del Sacro Cuore, Rome, Italy, on genetic tests for breast and colorectal cancer. *Methods.* We distributed self-administered questionnaire to the residents. Logistic regression models were used to evaluate the determinants of knowledge and attitudes towards the tests. *Results.* Of 754 residents, 364 filled in questionnaire. Around 70% and 20% answered correctly >80% of questions on breast and colorectal cancer tests, respectively. Knowledge on tests for breast cancer was higher among residents who attended course on cancer genetic testing during graduate training (odds ratio (OR): 1.72; 95% confidence interval (CI): 1.05–2.82) and inversely associated with male gender (OR: 0.55; 95% CI: 0.35–0.87). As for colorectal cancer, residents were more knowledgeable if they attended courses on cancer genetic testing (OR: 2.08; 95% CI: 1.07–4.03) or postgraduate training courses in epidemiology and evidence-based medicine (OR: 1.95; 95% CI: 1.03–3.69). More than 70% asked for the additional training on the genetic tests for cancer during the specialization school. *Conclusion.* The knowledge of Italian residents on genetic tests for colorectal cancer appears to be insufficient. There is a need for additional training in this field.

1. Introduction

Since the first global look at the content of the human genetic code, published more than a decade ago [1], it became clear that the implementation of genomic medicine has the potential of bringing clinically important advances, to rival those of any other major discovery in the history of medicine [2]. In this context, doctors have been envisaged as the key players in properly incorporating emerging DNA technologies in the health care system [3, 4], leading to calls for enhanced genomic education for healthcare professionals. Nevertheless, several studies conducted in USA, Europe, and Canada showed unsatisfactory level of clinicians' ability to use genetic tests in clinical care [3–5].

Recent research showed that providing genetic educational outreach to doctors has a positive impact in improving their competency and confidence in the use of genetic testing to guide prevention or treatment decisions [6–8]. Although the importance of genomic education for health care professionals has been recognized even before completion of the Human Genome Project [9], many physicians do not feel to have a proper training and knowledge [3, 4].

Genetic tests for breast and colorectal cancer, if used appropriately, have been demonstrated to be efficacious and cost-effective [10]. However, Marzuillo et al. reported a significant lack of knowledge on *BRCA1/2* and *APC* tests among Italian medical doctors [5], irrespective of their specialty. Along similar lines, we conducted survey on Italian doctors attending postgraduate medical schools in order to examine the level of education they had received on genetic tests for breast and colorectal cancer during their recent medical training.

More specifically, our objective was to assess knowledge, attitudes, and educational needs of Italian residents related to the use of genetic tests for breast and colorectal cancer, particularly the *BRCA1/2* and *APC* tests, irrespective of the field of specialization.

2. Methods

A self-administered anonymous questionnaire was distributed in 2011 to all the residents enrolled at the Gemelli Teaching Hospital of the Università Cattolica del Sacro Cuore in Rome, Italy. The total number of eligible participants was 754, and the number of surveyed postgraduate schools was 43.

A similar questionnaire was previously used and validated in a study conducted on a sample of Italian medical specialists [5]. It comprises questions designed to assess (i) demographic and professional characteristics; (ii) knowledge and attitudes towards genetic tests for hereditary breast and colorectal cancer; (iii) self-assessed level of knowledge and training needs.

Knowledge of genetic tests for breast and colorectal cancer was investigated with three questions, each using a three-point options Likert scale ("agree," "uncertain," and "disagree"). Additional four multiple-choice questions were designed to evaluate the residents' knowledge of prevalence of hereditary breast cancer and inherited forms of colorectal cancer and of penetrance of *BRCA1/BRCA2* and *APC* mutations (two each).

Attitudes towards genetic tests for breast and colorectal cancers were assessed with seven different questions, also using a Likert scale evaluation.

The final set of questions required the residents to assess their own perceived level of knowledge according to a four-answer format ("inadequate," "sufficient," "good," and "excellent"), and training needs ("yes/no" answer).

2.1. Statistical Analysis. A descriptive analysis was conducted to report demographic, social, and professional characteristics of responding residents. For the questions on knowledge and attitudes of residents towards genetic testing for breast and colorectal cancer, we calculated the proportions of correct answers (plus 95% confidence intervals (CI)). We considered residents who gave 80% correct answers for each form of hereditary cancer as knowledgeable. A general positive attitude towards predicative genetic testing was defined as the presence of a positive attitude in at least 70% of the questions assessing the attitude. Variables associated (P value of <0.20) with a positive outcome (satisfactory knowledge/attitude) from the univariate analysis were included in the multivariate conditional logistic regression model. A backward elimination procedure was used for the multivariate analysis. Data were analyzed using Stata software (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

3. Results

Of the initial number of 754 eligible for inclusion, 364 residents responded (overall response rate 48.3%).

The demographic and professional characteristics of responding residents are reported in Table 1. Among respondents, 61.2% (222/364) were female; the age mode was of 28-29 years (151/364, 41.6%) and 63.1% (224/364) have had their specialization associated with clinical activity.

Majority of included residents (67.3%, 245/364) answered correctly at least 80% of questions on genetic tests for breast cancer, prevalence of hereditary forms, and penetrance of *BRCA* mutations. Residents knowledgeable on genetic tests for colorectal cancer, prevalence of hereditary forms, and penetrance of *APC* mutations were 21.2% (77/364). Table 2 reports the correct answer rates in relation to each particular question. The knowledge on test for breast cancer was higher among residents who reported having attended a specific cancer course on genetic testing during their graduate training (OR: 1.72; 95% CI: 1.05–2.82) (Table 3). Male gender was inversely associated with knowledge on tests for breast cancer (OR: 0.55; 95% CI: 0.35–0.87) (Table 3). Residents who attended a specific cancer course on genetic testing during the graduate training (OR: 2.08; 95% CI: 1.07–4.03) as those who took postgraduate training courses in epidemiology and evidence-based medicine (EBM) (OR: 1.95; 95% CI: 1.03–3.69) showed higher knowledge on test for colorectal cancer (Table 3).

Residents' attitudes towards genetic testing for breast and colorectal cancer are reported in Table 4. A total of 45.6% showed positive attitude in at least 70% of questions. Less than half appeared to comply with principles of efficacy (attitude number 2, 48.3%) and cost-effectiveness (attitude number 3, 46.3%) in this field (Table 4). When we analyzed predictors of positive attitude towards genetic testing; only personal family history of breast and/or colorectal cancer (OR: 1.74; 95% CI: 1.11–2.71) appeared to be significantly associated.

Table 5 reports the self-estimated level of knowledge on genetic tests for breast and colorectal cancer and training needs of participants. More than half of our sample (178/364, 50.6%) described their knowledge as poor and 84.6% (296/364) declared that they did not feel qualified to prescribe genetic tests (Table 5). Conversely, 87.8% (309/364) of them declared that is was important for them to increase their knowledge in this field (Table 5). The majority of residents felt that more training time should be allotted to genetic testing during medical studies (289/364, 82.3%) or specialization school (263/364, 74.7%) or through specific postgraduate courses (289/229, 65.4%).

Table 1: The demographic and professional characteristics of responding residents ($n = 364$).

Variables	n	%
Gender		
Male	141	38.8%
Female	222	61.2%
Age (years)		
<28	78	21.5%
28-29	151	41.6%
30-31	84	23.1%
≥32	50	13.8%
Professional area[i]		
Medicine	147	40.4%
Surgery	47	12.9%
Others	170	46.7%
Clinical activity		
No	131	36.9%
Yes	224	63.1%
Exposure to cancer genetic testing during graduate training		
No	95	26.7%
Yes	261	73.3%
Postgraduate training courses in epidemiology and EBM		
No	292	83.0%
Yes	60	17.1%
English language knowledge		
Very low	9	2.5%
Low	38	10.6%
Intermediate	130	36.3%
Good	153	42.7%
Excellent	28	7.8%
Hours per week dedicated to continuing medical education		
<1	48	13.5%
1–5	212	59.4%
6–10	73	20.5%
>10	24	6.7%
Patient request of cancer genetic tests in the previous year*		
No	162	74.0%
Yes	57	26.0%
Personal or family history of breast cancer		
No	292	81.8%
Yes	65	18.2%
Personal or family history of colorectal cancer		
No	285	80.1%
Yes	71	19.9%
Promotional material about breast cancer received in the previous year		
No	311	86.9%
Yes	47	13.1%
Promotional material about colorectal cancer received in the previous year		
No	325	91.0%
Yes	32	9.0%

EBM: evidence based medicine.
[i]List of specializations according to each area is available in Supplementary Material S1 available online at http://dx.doi.org/10.1155/2014/418416.
*The number of responders was 219 as only physicians with clinical activity were included.

Table 2: Knowledge of residents (n = 364) on genetic tests for breast and colorectal cancer, prevalence of hereditary forms, and penetrance of *BRCA1/2* and *APC* mutations.

	Number of responders to the question	% of correct answers	CI 95%
BRCA1/2			
Genetic tests for BRCA1/BRCA2 mutations are able to identify patients at high risk to develop breast cancer (**agree**, uncertain, disagree)	357	93.3	90.2–95.6
The percentage of breast cancer cases associated with mutations in BRCA1/BRCA2 is **1–10%**, 15–35%, >35%	354	42.9	37.7–48.3
The absolute risk of developing breast cancer in presence of BRCA1/BRCA2 mutations is <10%, **40–80%**, 100%	356	80.3	75.8–84.3
Women with breast cancer and strong family history should perform BRCA1/BRCA2 testing (**agree**, uncertain, disagree)	356	78.7	74.0–82.8
Scientific evidence recommend for BRCA1/BRCA2 positive women clinical and instrumental surveillance starting from the age of 25 (**agree**, uncertain, disagree)	358	84.4	80.2–88.0
APC			
Genetic tests for APC mutations are able to identify patients who will develop colorectal carcinoma (**agree**, uncertain, disagree)	355	77.7	73.1–82.0
The percentage of colon cancer cases associated with APC mutations is **<5%**, 10–25%, >40%	352	31.8	27.0–37.0
The absolute risk of developing colorectal cancer in presence of APC mutations is <10%, 40–80%, **100%**	351	27.9	23.3–32.9
APC testing is recommended for 10–12 years old children with a first degree relative with known APC mutation (**agree**, uncertain, disagree)	357	57.4	52.1–62.6
Scientific evidence recommend for APC positive individuals periodic colonoscopy starting from the age of 10–15 (**agree**, uncertain, disagree)	356	55.9	50.6–61.1

Correct answers are in bold.

4. Discussion

Our study reports unsatisfactory level of residents' knowledge on genetic tests for colorectal cancer and on prevalence of hereditary forms and penetrance of *APC* mutations. We identified female gender and attendance to cancer genetic testing courses during graduate training to be predictors of a better knowledge of genetic tests for breast cancer. An attendance to cancer genetic testing courses during the graduate training and postgraduate training courses in epidemiology end EBM were associated with better knowledge on genetic tests on colorectal cancer. Although the vast majority of participants recognized the important role of genetic tests in prevention, as well as the need for evidence-based guidelines, complex prevention strategies, and genetic counseling, the principles of efficacy and cost-effectiveness appear to be not so widely accepted. The self-assessment revealed that participants are not satisfied with their own knowledge of genetic test and that they do not feel qualified to prescribe them. However, the need for training in this field during graduate and postgraduate studies was clearly recognized.

Some surveys already reported lack of knowledge among medical doctors on genetic tests for cancer [5, 11–20]. Nevertheless, most of these were conducted among specialists, while only one referred to residents [18]. Younger age [20] and recent graduation from medical school [21], as well as being in medical practice less than 10 years [11], have been previously reported as predictors of better knowledge on genetic tests and increased confidence in using them in everyday practice. Marzuillo et al. [5] previously reported insufficient knowledge on genetic test on breast and colorectal cancer among Italian specialists. Although our study showed relatively more satisfactory results in relation to tests for breast cancer, knowledge on genetic tests on colorectal cancer among residents in our study was also fairly low, indicating the need for further improvement in specialists formatting process. Finally, our results clearly pointed the need for additional education in field of genomics as exposure to genetic test during graduate training as well as postgraduate training courses in epidemiology and EBM were associated with higher knowledge on genetic test for breast and colorectal cancer.

Attitude of medical doctors is crucial for the dissemination and implementation of new medical technologies. Although residents in our study have shown high rates of some individual positive attitudes towards genetic testing, only a minority showed positive attitude in all issues. Furthermore, the majority of residents do not recognize the importance of the principles of efficacy and cost-effectiveness in genetic testing. Similar results were obtained from the survey on Italian specialists, who also did not show cost-conscious behavior regarding genetic tests [5]. This could lead to introducing of genetic test in clinical practice for commercial purposes only. Having that in mind, specific educational programs and trainings are needed in order to promote more cost-conscious behavior of physicians.

We found that family history of breast and/or colorectal cancer was a significant predictor of positive attitude towards

Table 3: Sociodemographic and professional characteristics associated with knowledge on genetic testing for breast cancer (*BRCA1/BRCA2* mutations) and colorectal cancer (*APC* mutations).

	Breast cancer				Colorectal cancer			
	OR	95% CI	OR adj*	95% CI	OR	95% CI	OR adj$^{\gamma}$	95% CI
Gender								
Female	1.00		1.00		1.00		1.00	
Male	0.53	0.34–0.83	0.55	0.35–0.87	1.24	0.74–2.06	1.36	0.80–2.31
Age								
<28	1.00		1.00		1.00		1.00	
28-29	0.72	0.39–1.32	0.66	0.35–1.24	0.78	0.42–1.43	0.74	0.39–1.41
30-31	0.70	0.36–1.37	0.62	0.31–1.26	0.32	0.14–0.73	0.25	0.10–0.61
≥32	0.71	0.33–1.54	0.68	0.30–1.56	0.39	0.15–0.99	0.33	0.12–0.92
Personal or family history of breast or colon cancer								
No	1.00		1.00		1.00		1.00	
Yes	1.40	0.77–2.57	1.21	0.65–2.25	0.98	0.52–1.86	0.91	0.47–1.77
Professional area~								
Medicine	1.00		1.00		1.00		1.00	
Surgery	0.60	0.31–1.17	0.80	0.39–1.64	1.10	0.50–2.40	1.24	0.55–2.80
Others	1.16	0.72–1.87	1.20	0.74–1.96	0.90	0.52–1.55	0.88	0.50–1.55
Clinical activity								
No	1.00		1.00		1.00		1.00	
Yes	0.94	0.59–1.50	0.97	0.60–1.57	0.93	0.55–1.58	1.02	0.59–1.76
Exposure to cancer genetic testing during graduate training								
No	1.00		1.00		1.00		1.00	
Yes	1.73	1.06–2.82	1.72	1.05–2.82	2.01	1.05–3.84	2.08	1.07–4.03
Postgraduate training courses in epidemiology and EBM								
No	1.00		1.00		1.00		1.00	
Yes	0.90	0.50–1.61	0.88	048–1.60	1.85	1.01–3.45	1.95	1.03–3.69
Patient request of cancer genetic tests in the previous year[i]								
No	1.00		1.00		1.00		1.00	
Yes	2.15	1.05–4.38	1.84	0.89–3.83	0.90	0.42–1.92	0.84	0.38–1.84
Hours per week dedicated to continuing medical education								
<1	1.00		1.00		1.00		1.00	
1–5	1.45	0.76–2.75	1.39	0.71–2.73	1.31	0.57–3.00	0.98	0.41–2.30
6–10	2.03	0.93–4.41	2.11	0.93–4.77	1.64	0.65–4.14	1.25	0.48–3.29
>10	1.73	0.61–4.96	1.84	0.62–5.43	1.32	0.38–4.56	0.74	0.19–2.91
Promotional material about breast or colon cancer received in the previous year								
No	1.00		1.00		1.00		1.00	
Yes	1.29	0.65–2.56	1.12	0.56–2.25	0.67	0.25–1.82	0.41	0.14–1.25

OR: odds ratio; CI: confidence interval; EBM: evidence based medicine.
*OR adjusted by professional area, exposure to cancer genetic testing during graduate training.
$^{\gamma}$OR adjusted by gender, postgraduate training courses about epidemiology and EBM.
~List of specializations according to each area is available in Supplementary file S1.
[i]Included physicians with clinical activity.

Table 4: Attitudes of residents (n = 364) towards genetic testing for breast and colorectal cancer.

	Number of responders to the question	% of correct answers	CI 95%
(1) Genetic tests for breast cancer and colorectal cancer increase the chances of prevention opportunities (**agree**, uncertain, disagree)	355	85.1	80.9–88.6
(2) Genetic tests that able to identify an increased risk of developing breast or colorectal cancer should be performed even if there are no preventive and/or curative interventions of proven efficacy (agree, uncertain, **disagree**)	352	48.3	43.0–53.6
(3) Genetic tests for breast cancer or colorectal cancer should be performed only if economical evaluations show cost effectiveness ratios favorable compared to alternative health interventions (**agree**, uncertain, disagree)	354	46.3	41.0–51.7
(4) Authoritative and evidence-based guidelines are needed for the appropriate use of genetic tests for breast cancer and colorectal cancer (**agree**, uncertain, disagree)	355	92.4	89.1–94.9
(5) Genetic tests for breast and colorectal cancer should be performed without genetic counseling informing patients of the benefits and risks of the tests (agree, uncertain, **disagree**)	355	76.9	72.2–81.2
(6) Genetic tests for breast and colorectal cancer can contribute efficaciously to health promotion and cancer prevention only if included in wider strategies taking into account the other available health interventions (**agree**, uncertain, disagree)	354	83.1	78.7–86.8
(7) The implementation of genetic tests for breast and colorectal cancer, being a medical matter, should not take into account ethical, legal and social implications (agree, uncertain, **disagree**)	356	75.3	70.4–80.0

Correct answers bolded.

Table 5: Self-estimated level of residents' knowledge and training needs on genetic tests for breast and colorectal cancer (n = 364).

		n	%
How would you rate your level of knowledge on the appropriate use of genetics tests for cancer in clinical practice?	Poor	178	50.6
	Fair	143	40.6
	Good	29	8.2
	Excellent	2	0.6
		Yes	No
How important do you think it is to increase your knowledge about the use of genetics tests for cancer in clinical practice?		309 (87.8%)	43 (12.2%)
Do you find yourself qualified enough to prescribe genetic tests for cancer?		54 (15.4%)	296 (84.6%)
Should more time be dedicated to learning on genetic test during the medical studies?		289 (82.3%)	62 (17.7%)
Should more time be dedicated to learning on genetic test during the medical specialization?		263 (74.7%)	89 (25.3%)
Is there a need for specific postgraduate course on use of genetic testing for cancer?		229 (65.4%)	121 (34.6%)

genetic testing. This is to be expected, as personal experiences represent a major influence in determining individual attitudes, and those with positive family cancer history were personally motivated to find out more on genetic tests.

Residents in our study have deemed their knowledge of genetic tests for breast and colorectal cancer insufficient. Insufficient level of knowledge on genetic test has been previously self-reported among medical doctors several times [5, 15]. Our residents also reported a high level of interest in additional training in this field. As earlier studies also reported readiness of physicians to attend additional courses on genetic testing [5, 11, 15, 18], an organized approach to genomics education is needed in order to make the best use of available genetic testing resources.

Our study has some limitations. Firstly, we have conducted our research among residents working in the same hospital, so the results may not be reflecting the knowledge and attitudes of the Italian residents' population. Secondly, our nonresponse rate was relatively high; thus we do not have data on age and gender structure of nonresponders. Although it is not likely that age could differ between responders and nonresponders, as most of the residents belong to the same age group, the difference in gender structure may be an issue as we recognized gender to be factor for knowledge in some specific fields. Nevertheless, our study is, to our knowledge, first in Europe reflecting the knowledge and attitudes of residents on genetic tests and can be valuable in assessing knowledge, attitudes, and educational needs of young doctors in training on a wider scale.

In conclusion, knowledge of Italian residents on genetic tests for colorectal cancer appears to be insufficient. There is a need for additional training in field of genetic tests during graduate and postgraduate studies as well as during specializations. The principles of efficacy and cost-effectiveness in genetic testing are not fully accepted among residents. Specific educational programs are needed in order to promote more cost-conscious behavior.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] International Human Genome Sequencing Consortium, "Initial sequencing and analysis of the human genome," *Nature*, vol. 409, pp. 860–921, 2001.

[2] E. S. Lander, "Initial impact of the sequencing of the human genome," *Nature*, vol. 470, no. 7333, pp. 187–197, 2011.

[3] "Physicians as guardians of genetic knowledge," *The Lancet*, vol. 377, no. 9770, p. 967, 2011.

[4] W. G. Feero and E. D. Green, "Genomics education for health care professionals in the 21st century," *The Journal of the American Medical Association*, vol. 306, no. 9, pp. 989–990, 2011.

[5] C. Marzuillo, C. de Vito, S. Boccia et al., "Knowledge, attitudes and behavior of physicians regarding predictive genetic tests for breast and colorectal cancer," *Preventive Medicine*, vol. 57, no. 5, pp. 477–482, 2013.

[6] J. Bethea, N. Qureshi, N. Drury, and P. Guilbert, "The impact of genetic outreach education and support to primary care on practitioner's confidence and competence in dealing with familial cancers," *Community Genetics*, vol. 11, no. 5, pp. 289–294, 2008.

[7] J. C. Carroll, A. L. Rideout, B. J. Wilson et al., "Genetic education for primary care providers: improving attitudes, knowledge, and confidence," *Canadian Family Physician*, vol. 55, no. 12, pp. e92–e99, 2009.

[8] N. Drury, J. Bethea, P. Guilbert, and N. Qureshi, "Genetics support to primary care practitioners—a demonstration project," *Journal of Genetic Counseling*, vol. 16, no. 5, pp. 583–591, 2007.

[9] F. S. Collins, "Preparing health professionals for the genetic revolution," *The Journal of the American Medical Association*, vol. 278, no. 15, pp. 1285–1286, 1997.

[10] F. Becker, C. G. van El, D. Ibarreta et al., "Genetic testing and common disorders in a public health framework: how to assess relevance and possibilities. background document to the ESHG recommendations on genetic testing and common disorders," *European Journal of Human Genetics*, vol. 19, supplement 1, pp. S6–S44, 2011.

[11] R. T. Acton, N. M. Burst, L. Casebeer et al., "Knowledge, attitudes, and behaviors of Alabama's primary care physicians regarding cancer genetics," *Academic Medicine*, vol. 75, no. 8, pp. 850–852, 2000.

[12] S. Batra, H. Valdimarsdottir, M. McGovern, S. Itzkowitz, and K. Brown, "Awareness of genetic testing for colorectal cancer predisposition among specialists in gastroenterology," *The American Journal of Gastroenterology*, vol. 97, no. 3, pp. 729–733, 2002.

[13] C. A. Bellcross, K. Kolor, K. A. B. Goddard, R. J. Coates, M. Reyes, and M. J. Khoury, "Awareness and utilization of BRCA1/2 testing among U.S. primary care physicians," *The American Journal of Preventive Medicine*, vol. 40, no. 1, pp. 61–66, 2011.

[14] M. Escher and A.-P. Sappino, "Primary care physicians' knowledge and attitudes towards genetic testing for breast-ovarian cancer predisposition," *Annals of Oncology*, vol. 11, no. 9, pp. 1131–1135, 2000.

[15] R. Klitzman, W. Chung, K. Marder et al., "Attitudes and practices among internists concerning genetic testing," *Journal of Genetic Counseling*, vol. 22, no. 1, pp. 90–100, 2013.

[16] I. Nippert, H. J. Harris, C. Julian-Reynier et al., "Confidence of primary care physicians in their ability to carry out basic medical genetic tasks—a European survey in five countries—part 1," *Journal of Community Genetics*, vol. 2, no. 1, pp. 1–11, 2011.

[17] G. Pichert, D. Dietrich, P. Moosmann, M. Zwahlen, R. A. Stahel, and A.-P. Sappino, "Swiss primary care physicians' knowledge, attitudes and perception towards genetic testing for hereditary breast cancer," *Familial Cancer*, vol. 2, no. 3-4, pp. 153–158, 2003.

[18] K. J. Ready, M. S. Daniels, C. C. Sun, S. K. Peterson, H. Northrup, and K. H. Lu, "Obstetrics/gynecology residents' knowledge of hereditary breast and ovarian cancer and lynch syndrome," *Journal of Cancer Education*, vol. 25, no. 3, pp. 401–404, 2010.

[19] L. Wideroff, S. T. Vadaparampil, M. H. Greene, S. Taplin, L. Olson, and A. N. Freedman, "Hereditary breast/ovarian and colorectal cancer genetics knowledge in a national sample of US physicians," *Journal of Medical Genetics*, vol. 42, no. 10, pp. 749–755, 2005.

[20] L. Wilkins-Haug, L. D. Hill, M. L. Power, G. B. Holzman, and J. Schulkin, "Gynecologists' training, knowledge, and experiences in genetics: a survey," *Obstetrics and Gynecology*, vol. 95, no. 3, pp. 421–424, 2000.

[21] K. J. Hofman, E. S. Tambor, G. A. Chase, G. Geller, R. R. Faden, and N. A. Holtzman, "Physicians' knowledge of genetics and genetic tests," *Academic Medicine*, vol. 68, no. 8, pp. 625–632, 1993.

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Research Article

Tumor Necrosis Factor-Alpha −308 G>A Polymorphism, Adherence to Mediterranean Diet, and Risk of Overweight/Obesity in Young Women

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The present study was conducted in order to (i) characterize the adherence to the Mediterranean diet (MD) pattern and fatty acids (FAs) intakes and (ii) explore interactions between *TNFA* −308 G>A polymorphism and adherence to MD and FAs intakes, respectively, on overweight/obesity risk. From 2010 to 2013, 380 healthy women were enrolled, and MD score (MDS) and FAs intakes were evaluated by a Food Frequencies Questionnaire in relation to nutritional status. *TNFA* −308 G/A polymorphism was characterized using PCR-RFLP. A total of 32.6% of women were overweight or obese. Lower mean MDS values were more observed in the younger age group than in the older age group (3.60 versus 4.45). The risk of being overweight/obese was 3.5-fold increased due to poor adherence to MD and was about twofold increased in less educated women. Furthermore, younger age was associated with poor adherence to MD. No evidence for an independent effect of the polymorphism on overweight/obesity risk was found. There was no evidence of biological interaction from the gene-diet interaction analyses. Young women, less educated and with poor adherence to MD, are a target group for the nutritional interventions that aimed to control the obesity risk, thus improving the adherence to MD and particularly the intake of unsaturated FAs.

1. Introduction

Obesity is a multifactorial disorder, reflecting complex interactions of genes and environment, as lifestyle [1], associated with a high risk of chronic diseases such as diabetes, cardiovascular disease, and certain cancers [2]. Obesity constitutes a major public health problem that, in current years, evolved into a worldwide epidemic [3]. A recent study conducted in the Diogenes (Diet, Obesity, and Genes Dietary Study in European countries) cohort [4] shows an increase in obesity prevalence since the 1990s and predicts a further increase in European populations of about 30% in 2015.

The Mediterranean diet (MD) has long been reported to be the optimal diet for preventing noncommunicable diseases and preserving good health. A meta-analysis confirms the significant and consistent protection provided by adherence to the MD in relation to the occurrence and mortality of major chronic degenerative diseases [5, 6].

Independently of energy and macronutrient quantity intakes, a better adherence to the MD is associated with lower obesity risk [7, 8]. As such, research interest over the past years has been focused on estimating adherence to the whole MD rather than analyzing the individual components of the dietary pattern in order to consider important interactions between components of the diet.

Lifestyle factors, including dietary components, such as fatty acids (FAs), interact with genetic variants to regulate the development and progression of obesity and its comorbidities, and these interactions may explain differences observed across populations [9].

A number of candidate genes have been implicated in the pathogenesis of obesity in humans, and screenings of those candidate regions as well as genome-wide scans have helped to identify single nucleotide polymorphisms (SNPs) that increase the risk of overweight or of obesity [10].

Obesity is a chronic low-grade inflammatory state, and elevated levels of tumor necrosis factor-alpha (TNF-α), a proinflammatory cytokine secreted in adipocytes, have been implicated in the development of obesity and insulin resistance; in fact, expression and circulating levels are increased with obesity and decreased with weight loss [11]. Several SNPs have been identified in the promoter region of *TNFA* gene. A meta-analysis by Sookoian et al. [12] described the impact of the best characterized *TNFA* polymorphism (−308 G>A; rs1800629) on the components of the metabolic syndrome and concluded that the −308 A variant was positively associated with obesity. The *TNFA* −308 A allele has been associated with obesity, obesity-related insulin resistance, and altered serum lipid concentrations in some Caucasian populations [13, 14], but not all [15, 16]. In addition, in some populations, the *TNFA* −308 G>A polymorphism changes the relationship between FAs intake and the risk of obesity [9, 17], but this interaction was not observed in other populations [18]. Furthermore, researches using *in vitro* and *in vivo* mouse models have shown that *TNFA* expression is differentially regulated by FAs, and these results appear to translate to human [19]. Although the molecular mechanisms by which FAs regulate adipokine production remain unclear, one proposed link between dietary FA and inflammation may be via the toll-like receptor 4 (TLR4) pathway [20]. TLR4 is expressed in adipose tissue and has been shown to be activated by SFA, inducing inflammatory cytokine production and signalling. This results in a localized inflammation in adipose tissue that propagates an overall systemic inflammation [21, 22].

The main aim of the present study was to assess the risk of overweight/obesity in a Sicilian population of healthy women and to define control strategies and targets. Particularly, the specific purposes were (i) to characterize adherence to MD pattern and FAs intakes and (ii) to explore interactions between *TNFA* −308 G>A polymorphism and adherence to MD pattern and FAs intakes, respectively, on overweight/obesity risk.

2. Materials and Methods

2.1. Study Population and Dietary Intake. During a three-year period, from 2010 to 2013, a total of 380 consecutive healthy women, referred to the Laboratory of the S. Bambino Hospital, Catania, Sicily, Italy, were prospectively enrolled in the present cross-sectional study. All women gave their informed consent to participate in the study. The study protocol was approved by the ethics committee of the involved institution and was performed according to the Declaration of Helsinki.

Data were collected by trained epidemiologists using a structured questionnaire to obtain information on demographic and lifestyle data, including smoking habits and obstetrical history.

Furthermore, education level was collected and divided into three categories: ≤8 (low), >8 and ≤13 (medium), and >13 (high) years of studies. Employment status was also recorded, and women were classified as employed, unemployed, student, and housewife. Body mass index (BMI) as kg/m^2 was based on criteria from the World Health Organization [23]. Prepregnancy BMI was based on self-reported prepregnancy weight.

Adherence to MD and FAs intakes, during the past month, was estimated by a semiquantitative 153-item Food Frequencies Questionnaire (FFQ), previously validated [24–26]. For each of the food items, women were asked to report their frequency of consumption and portion size. The table of alimentary composition of the US Department of Agriculture, which had been modified to accommodate the particularities of the Italian diet, was used to determine FAs intakes. FAs intakes were evaluated both as average intakes in grams per day (g/day) and as percentage of energy (%E) using the "nutrient density" method [27]. Total daily energy intake was calculated as Kcal of energy provided by macronutrients (total proteins, carbohydrates, and lipids) and alcohol.

Adherence to the MD pattern was assessed using the Mediterranean diet score (MDS) [28]. Women were classified as with greater adherence to MD if MDS was >90th percentile of MDS distribution (i.e., >6) and as with poor adherence to MD (i.e., ≤90th percentile: ≤6).

2.2. Analysis of TNFA −308 G>A Polymorphism. Fasting venous blood samples were collected from each enrolled woman in EDTA-containing tubes, and aliquots were stored at −80°C until analysis.

Genomic DNAs were extracted from whole blood using the Illustra blood genomic Prep Mini Spin Kit (GE Healthcare) according to the manufacturer's protocol and stored at −20°C. Subjects were genotyped for the *TNFA* −308 G>A polymorphism using the PCR-RFLP method, as described previously [29]. Electrophoresis of the digested PCR products was performed on a 5% NuSieve agarose gel (Lonza, ME, USA). Gels were stained with GelRed (Biotium, Inc., Hayward, CA, USA) in order to visualize the DNA fragments.

2.3. Statistical Analyses. Statistical analyses were performed using the SPSS software (Version 14.0, SPSS, Chicago, IL). The χ^2 test was used for the statistical comparison of proportions. Continuous variables were tested using Student's t-test and one-way ANOVA. Genotype frequencies were calculated by determining the percentage of individuals carrying the different genotypes. In the analysis, the number of homozygote wild-type individuals (GG) was compared to the number of heterozygotes and homozygote mutant individuals (AG/AA). Furthermore, to ascertain if population sample was in Hardy-Weinberg equilibrium for the polymorphisms, a χ^2 test was performed for the overall population.

Statistical significance of relationship between adherence to MD, overweight/obesity, and risk factors was determined using the χ^2 test, and the strength of associations was estimated by calculating the odds ratios (ORs) and 95%

confidence intervals (95% CIs). Statistical significance was established at a P value of 0.05.

Gene-environment interaction analyses were conducted in order to evaluate the potential interaction between *TNFA* −308 G>A polymorphism and adherence to MD pattern or FAs intakes, respectively, on overweight/obesity risk, using as a reference group the homozygous wild-type (GG) women who had not been exposed to dietary factors (i.e., those with MDS > 6 or with FAs intakes above the 75th percentile of the unsaturated FAs intakes distribution or below or equal to the 75th percentile of the saturated FAs intakes distribution, resp.). Furthermore, biological interaction analyses, using departure from additivity, were performed using the synergy index proposed by Rothman et al. [29, 30] with adjusted ORs and their 95% CIs measured from logistic regression analysis.

3. Results

3.1. Population Characteristics and Distribution of TNFA −308 G>A Polymorphism. During the study period, a total of 380 women (mean age 28.7 years) of a southern European Mediterranean population were enrolled. The main characteristics of the women are shown in Table 1. Particularly, a total of 32.6% of women were overweight or obese.

The distribution of *TNFA* −308 G>A polymorphism is shown in Table 1: the most frequent genotype was the wild-type GG (80.5%). The G allele frequency was 89.6%. Genotype frequencies follow the Hardy-Weinberg equilibrium expectations ($P = 0.27$).

3.2. Dietary Assessment. Mean energy and FAs intakes (g/day) of underweight/normal weight women and of those who are overweight/obese are reported in Table 2. Except for unsaturated/saturated FA ratio, the mean values of FAs intakes (unsaturated and saturated FAs) and the mean energy intake were statistically significantly higher in underweight/normal weight women than in overweight/obese women. However, considering the mean FAs intakes as daily %E, differences between the two groups were not statistically significant (data not shown).

According to MDS (Table 1), women reported a poor adherence to MD (median value of MDS equal to 4), and only 8.2% of women were classified as with greater adherence to MD (MDS > 90th percentile of MDS distribution, i.e., >6).

A significantly higher proportion of women with poor adherence to MD (34.4%) were more overweight/obese than those with greater adherence (12.9%; $P = 0.015$). Therefore, the risk of being overweight/obese due to poor adherence to MD was 3.5-fold increased (OR: 3.54, 95% CI: 1.21–10.34).

Following the percentile distribution, the population was divided into four age groups, and mean MDS values were compared between groups. A significant increase of mean MDS values was observed from 3.60 in the age group of 13–22 years to 4.04 in the age group of 23–33 years, to 4.24 in the age group of 34–41 years, and to 4.45 in the age group of 42–85 years (one-way ANOVA, $P = 0.004$). Therefore, the risk of poor adherence to MD was 2.2-fold increased in younger women (i.e., age ≤ 27 years median value).

Table 1: Characteristics of study participants ($N = 380$).

	N (%)
Education (years of schooling)	
≤8 (low)	202 (53.3)
≤13 (medium)	126 (33.2)
>13 (high)	51 (13.5)
Employment status	
Employed	120 (31.9)
Unemployed	33 (8.8)
Student	46 (12.2)
Housewife	177 (47.1)
BMI	
Underweight	33 (8.7)
Normal weight	223 (58.7)
Overweight	78 (20.5)
Obese	46 (12.1)
Smoking	
Current smokers	90 (23.7)
Nonsmokers	244 (64.4)
Former smokers	45 (11.9)
Pregnancy status (yes)	203 (53.4)
MDS	
0–3 (≤25th percentile)	140 (36.8)
4–6 (>25th percentile–≤90th percentile)	209 (55.0)
7–9 (>90th percentile)	31 (8.2)
TNFA −308 G>A genotypes	
GG	306 (80.5)
GA	69 (18.2)
AA	5 (1.3)

BMI: body mass index; MDS: Mediterranean diet score.

Education was positively associated with adherence to MD; that is, less-educated women showed a lower adherence to MD, although this association was not statistically significant (data not shown).

A significant association between education and overweight/obesity was observed: 39.6% of women in lower (<=8), 26.1% in medium (>8 and ≤13), and 21.6% in highly (>13 years of school) educated groups were overweight or obese (P for trend = 0.003). The risk of being overweight/obese due to low-medium education was 1.94-fold increased (OR: 1.94, 95% CI: 1.25–3.02). Considering employment status, no statistically significant association was shown with adherence to MD; instead, 40.7% of housewives, 30.0% of the employed, 21.2% of the unemployed, and 19.6% of the students were overweight or obese (P for trend = 0.042).

Comparisons of mean energy and FAs intakes (g/day) between women with greater adherence to MD and those with poor adherence to MD are reported in Table 3. Except for some unsaturated FAs, that is, arachidonic acid and docosahexaenoic acid, for total saturated FA and palmitic acid, the mean FAs intakes and the mean energy intake

Table 2: Fatty acids and energy intakes in underweight/normal weight and overweight/obese women[a].

Nutrient intake (g/day)	Underweight/normal weight Mean ± SD	Overweight/obese Mean ± SD	P value[b]
Linoleic acid	17.07 ± 12.09	12.66 ± 8.05	<**0.001**
Arachidonic acid	0.18 ± 0.28	0.13 ± 0.06	**0.012**
γ-Linolenic acid	1.60 ± 1.10	1.20 ± 0.54	<**0.001**
α-Linolenic acid	1.60 ± 1.11	1.21 ± 0.53	<**0.001**
Eicosapentaenoic acid	0.13 ± 0.23	0.08 ± 0.07	**0.011**
Docosahexaenoic acid	0.30 ± 0.67	0.20 ± 0.14	**0.031**
Polyunsaturated fatty acids	18.43 ± 10.10	14.50 ± 9.10	<**0.001**
Monounsaturated fatty acids	55.25 ± 25.24	47.41 ± 23.88	**0.004**
Total unsaturated fatty acids	73.68 ± 33.27	61.91 ± 31.62	**0.001**
Unsaturated/saturated fatty acids ratio	2.34 ± 0.69	2.34 ± 0.66	0.990
Saturated fatty acids	33.65 ± 18.13	27.33 ± 13.65	<**0.001**
Palmitic acid	19.35 ± 13.18	15.01 ± 7.12	<**0.001**
Energy intake (Kcal/day)	2272.0	1811.1	<**0.001**

[a]Statistically significant P values ($P < 0.05$) are indicated in bold font.
[b]Student's t-test for the comparison of means between underweight/normal weight women and overweight/obese women (two-sided P values).

Table 3: Fatty acids and energy intakes between women with greater adherence to MD and women with lower adherence to MD[a].

Nutrient intake (g/day)	MDS ≤ 6 Mean ± SD	MDS > 6 Mean ± SD	P value[b]
Linoleic acid	15.24 ± 10.94	20.05 ± 12.39	**0.021**
Arachidonic acid	0.16 ± 0.24	0.17 ± 0.08	0.830
γ-Linolenic acid	1.44 ± 0.98	1.80 ± 0.75	**0.017**
α-Linolenic acid	1.44 ± 0.99	1.80 ± 0.67	**0.008**
Eicosapentaenoic acid	0.11 ± 0.20	0.16 ± 0.10	**0.021**
Docosahexaenoic acid	0.26 ± 0.58	0.34 ± 0.16	0.081
Polyunsaturated fatty acids	16.69 ± 9.53	22.35 ± 12.85	**0.002**
Monounsaturated fatty acids	51.36 ± 24.27	67.64 ± 28.95	<**0.001**
Total unsaturated fatty acids	68.05 ± 31.95	89.99 ± 39.90	<**0.001**
Unsaturated/saturated fatty acids ratio	2.32 ± 0.68	2.67 ± 0.62	**0.006**
Saturated fatty acids	31.26 ± 16.93	35.26 ± 18.18	0.210
Palmitic acid	17.74 ± 11.88	20.16 ± 9.65	0.197
Energy intake (Kcal/day)	2065.7 ± 897.5	2750 ± 1006.7	<**0.001**

[a]Statistically significant P values ($P < 0.05$) are indicated in bold font.
[b]Student's t-test for the comparison of means between MDS ≤ 6 and MDS > 6 (two-sided P values).

were statistically significantly higher in women with greater adherence to MD than in women with poor adherence. Furthermore, considering the mean FAs intakes as daily %E, only the mean saturated FAs intakes were statistically significantly higher in women with poor adherence to MD than in the others (data not shown).

3.3. Gene-Environment Interactions. A total of 37.0% of carriers of the *TNFA* −308 A allele (AA or GA genotypes) and a total of 31.6% of carriers of the *TNFA* −308 GG genotype were overweight/obese, and this difference was not statistically significant ($P = 0.383$).

Results of the interaction analysis between *TNFA* −308 G>A genotypes and FAs intakes in relation to obesity risk are reported in Tables 4 and 5. From our analysis, there was no evidence of gene-FA intakes interaction and of gene-MD adherence (data not shown).

4. Discussion

The present study was conducted in a Mediterranean population with a poor adherence to MD (median value of MDS equal to 4) and a high prevalence of overweight and obesity (32.6%), as recently reported among Italian and Sicilian adult women (33.8%) [26, 31]. Notably, in our population, the risk of being overweight/obese was 3.5-fold increased due to poor adherence to MD and was about twofold increased in less

Table 4: *TNFA* −308 G>A polymorphism and unsaturated fatty acids intake interactions on overweight/obesity risk[a,b].

Fatty acids	A[c] OR (95% CI)	B[d] OR (95% CI)	A + B[e] OR (95% CI)	Synergy index
Linoleic acid	2.68 (0.83–8.66)	3.61 (1.85–7.45)	4.27 (1.78–10.26)	0.76 (0.28–2.07)
Arachidonic acid	4.41 (1.53–12.72)	2.41 (1.25–4.64)	2.18 (0.94–5.05)	0.25 (0.06–1.00)
γ-Linolenic acid	3.06 (0.93–10.08)	3.47 (1.72–6.97)	3.80 (1.63–8.84)	0.62 (0.22–1.76)
α-Linolenic acid	3.98 (1.24–12.78)	4.20 (2.00–8.82)	4.40 (1.81–10.70)	0.55 (0.21–1.46)
Eicosapentaenoic acid	4.44 (1.43–13.73)	2.66 (1.35–5.23)	2.56 (1.10–5.94)	0.31 (0.09–1.10)
Docosahexaenoic acid	2.50 (0.85–7.32)	2.34 (1.21–4.54)	2.75 (1.19–6.34)	0.62 (0.18–2.12)
Polyunsaturated fatty acids	2.94 (0.90–9.56)	3.31 (1.64–6.68)	3.68 (1.57–8.63)	0.63 (0.22–1.84)
Monounsaturated fatty acids	2.58 (0.84–7.92)	2.18 (1.14–4.17)	2.48 (1.10–5.57)	0.54 (0.14–2.02)
Total unsaturated fatty acids	2.36 (0.77–7.20)	1.96 (1.03–3.72)	2.27 (1.02–5.07)	0.55 (0.13–2.29)
Unsaturated/saturated fatty acids ratio	0.86 (0.29–2.58)	0.71 (0.40–1.26)	1.13 (0.53–2.41)	—

[a]OR adjusted for age, adherence to MD, and education.
[b]Reference category: individuals carrying the homozygous wild-type genotype GG who had not been exposed to environmental factor.
[c]A: risk of developing overweight/obesity in carriers of *TNFA* −308 A allele (individuals carrying the homozygous mutated genotype or the heterozygous genotype AA or AG, who had not been exposed to environmental factor-FAs intakes above the 75th percentile of the unsaturated FAs intakes distribution).
[d]B: risk of developing overweight/obesity in women exposed to environmental factor only (individuals carrying the homozygous wild-type genotype GG, who had been exposed to environmental factor-FAs intakes below or equal to the 75th percentile of the unsaturated FAs intakes distribution).
[e]A + B: risk of developing overweight/obesity in women exposed to both A and B (individuals carrying the homozygous mutated genotype or the heterozygous genotype AA or AG, who had been exposed to environmental factor-FAs intakes below or equal to the 75th percentile of the unsaturated FAs intakes distribution).

Table 5: *TNFA* −308 G>A polymorphism and saturated fatty acids intake interactions on overweight/obesity risk[a,b].

Fatty acids	A[c] OR (95% CI)	B[d] OR (95% CI)	A + B[e] OR (95% CI)	Synergy index
Palmitic acid	0.94 (0.50–1.76)	0.26 (0.13–0.53)	1.34 (0.49–3.71)	—
Saturated fatty acids	1.11 (0.59–2.10)	0.29 (0.15–0.59)	0.90 (0.33–2.43)	—

[a]OR adjusted for age, adherence to MD, and education.
[b]Reference category: individuals carrying the homozygous wild-type genotype GG, who had not been exposed to environmental factor.
[c]A: risk of developing overweight/obesity in carriers of *TNFA* −308 A allele (individuals carrying the homozygous mutated genotype or the heterozygous genotype AA or AG, who had not been exposed to environmental factor-FAs intakes below the 75th percentile of the saturated FAs intakes distribution).
[d]B: risk of developing overweight/obesity in women exposed to environmental factor only (individuals carrying the homozygous wild-type genotype GG, who had been exposed to environmental factor-FAs intakes above the 75th percentile of the saturated FAs intakes distribution).
[e]A + B: risk of developing overweight/obesity in women exposed to both A and B (individuals carrying the homozygous mutated genotype or the heterozygous genotype AA or AG, who had been exposed to environmental factor-FAs intakes above the 75th percentile of the saturated FAs intakes distribution).

educated women. Furthermore, younger age was associated with poor adherence to MD, as shown in a recent study [32].

However, although the relationship between the MD and overweight/obesity is complex and important methodological differences (such as the methodology used to construct MD indices) and limitations in the studies make it difficult to compare results, the evidence points towards a possible role of the MD in preventing overweight/obesity and in protecting against weight gain, and, additionally, physiological mechanisms can explain this protective effect [33].

In recent years, southern European countries, which used to follow a traditional MD, have also been adopting a more Western-style diet, and a dramatic change in the sources of fat intake in the general population has been observed. This change mainly consists in replacing polyunsaturated or monounsaturated FAs, which have been considered as healthy lipids because they reduce the incidence of cardiovascular disease, with saturated FAs, recognized risk factors for cardiovascular disease [33].

However, in our study, women with greater adherence to MD consume significantly more unsaturated FAs (g/day) and less saturated FAs (daily %E) than women with poor adherence. Furthermore, unsaturated and saturated FAs intakes (g/day) were higher in underweight/normal weight women than in overweight/obese women, but, considering the mean FAs intakes as daily %E, differences between the two groups were not statistically significant (data not shown).

Some studies report that the MD pattern may be protective against the development of obesity through its high-fiber content and low energy density [27], but other studies have speculated that the high-fat content, particularly from olive oil, of the MD may promote excess energy intake and weight gain, and this may explain the high prevalence of overweight and obesity in Mediterranean countries [34]. In our population, mean energy intake was higher in women with greater adherence to MD than in women with poor adherence, and a similar association was previously reported [8, 35]. Nevertheless, it may be possible that this association

is methodologically driven given that energy intake was not corrected for when constructing the MDS. Furthermore, mean energy intake was higher in underweight/normal weight women than in overweight/obese women, confirming that the root physiological cause of obesity is energy imbalance as a consequence of low physical activity and/or high energy intake, and several lifestyle factors may influence whether or not a person can maintain energy balance over the long term [7, 35, 36]. In fact, some studies support the theory that the problem of obesity in Mediterranean countries is likely to be related to limited physical activity in conjunction with excessive positive energy balance brought about by the westernization of their diet [37].

Lifestyle changes are the most important determinants of the rapid rise in the prevalence of obesity worldwide, and genetic factors are likely to modify the susceptibility to these changes [35]. The current lack of understanding of the numerous gene-gene and gene-environment interactions in obesity poses one of the major obstacles for the development of effective, preventive, and therapeutic intervention strategies [17]. It has been described that the A allele of the *TNFA* −308 G>A polymorphism produces a twofold increase of *TNFA* transcription and subsequent increase in TNF-α production [38]. Furthermore, the polymorphism has been strongly associated with increased risk of different outcome in women such as spontaneous preterm birth [39].

Despite the fact that many studies show independent associations between the *TNFA* SNPs and obesity, only few studies have investigated diet-gene interactions. German Caucasian men and women with the *TNFA* −308 A allele, who were in the highest tertile for intake of linoleic acid and arachidonic acid (%E), showed an increased obesity risk [40]. More recently, Joffe and colleagues reported that the odds of obesity for black South-African women with the *TNFA* −308 A allele increased with total dietary fat intake (%E) [41]; however, this interaction was not observed in white South-African women [18].

Despite the biological plausibility of *TNFA* −308 G>A polymorphism as risk modifiers of obesity, in the present study no evidence for an independent effect of the polymorphism on overweight/obesity risk was found. Also, there was no evidence of biological interaction from the gene-diet interaction analyses. These results are in keeping with previous findings in other populations [15, 16, 18], confirming the role of dietary factors, such as the FAs intake and the adherence to MD, in obesity risk irrespective of *TNFA* −308 genotype. Additional SNPs within the *TNFA* gene, as well as SNPs in other genes involved in inflammation, may also be involved, and these should be investigated. Finally, other dietary factors, lifestyle, and environmental factors may modulate these associations and contribute to the different results observed.

5. Conclusions

A number of epidemiological studies have shown that greater adherence to the traditional MD is associated with a significant reduction in total mortality and death due to coronary heart disease and to cancer [37], could reduce overall cancer risk [2], and could provide a consistent protection for the occurrence of major chronic degenerative diseases [5]. Even though most of these chronic conditions are also associated with obesity, the link between MD and obesity is not clear.

The present research has certain limitations that need to be taken into account when considering the study and its contributions. Its cross-sectional design could limit the inference on the time sequence of the association between MD and nutritional status. In addition, selection bias, recall bias, and confounding might be present; indeed, women's diet can be especially difficult to assess, as women tend to underreport their intakes more often than men and are more likely to do so if they are overweight or obese [42], and this phenomenon will bias diet-disease relationships. Further, in our study, physical activity was not determined. Furthermore, although our FFQ is validated, it may contain measurement errors. Additionally, our research was limited to the assessment of only one SNP from a single gene, and it is known that a number of other candidate genes have been implicated in the pathogenesis of obesity.

In conclusion, our study identifies young women, less educated, and with poor adherence to MD as a cause for concern and a target group for nutritional interventions that aimed to control the obesity risk, thus improving the adherence to MD and particularly the intake of unsaturated FAs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] A. Newell, A. Zlot, K. Silvey, and K. Arail, "Addressing the obesity epidemic: a genomics perspective," *Preventing Chronic Disease*, vol. 4, no. 2, p. A31, 2007.

[2] E. Couto, P. Boffetta, P. Lagiou et al., "Mediterranean dietary pattern and cancer risk in the EPIC cohort," *British Journal of Cancer*, vol. 104, no. 9, pp. 1493–1499, 2011.

[3] World Health Organization, "Obesity: preventing and managing the global epidemic," Report of a WHO Consultation. World Health Organization Technical Report Series 894, 2000.

[4] A. von Ruesten, A. Steffen, A. Floegel et al., "Trend in obesity prevalence in European adult cohort populations during follow-up since 1996 and their predictions to 2015," *PLoS ONE*, vol. 6, no. 11, Article ID e27455, 2011.

[5] F. Sofi, R. Abbate, G. F. Gensini, and A. Casini, "Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis," *The American Journal of Clinical Nutrition*, vol. 92, no. 5, pp. 1189–1196, 2010.

[6] I. Lourida, M. Soni, J. Thompson-Coon et al., "Mediterranean diet, cognitive function, and dementia: a systematic review," *Epidemiology*, vol. 24, no. 4, pp. 479–489, 2013.

[7] D. Mozaffarian, T. Hao, E. B. Rimm, W. C. Willett, and F. B. Hu, "Changes in diet and lifestyle and long-term weight gain in women and men," *The New England Journal of Medicine*, vol. 364, no. 25, pp. 2392–2404, 2011.

[8] M. A. Martìnez-Gonzàlez, A. Garcìa-Arellano, E. Toledo et al., "A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial," *PLoS ONE*, vol. 7, Article ID 43134, 2012.

[9] Y. T. Joffe, M. Collins, and J. H. Goedecke, "The relationship between dietary fatty acids and inflammatory genes on the obese phenotype and serum lipids," *Nutrients*, vol. 5, no. 5, pp. 1672–1705, 2013.

[10] A. Peeters, S. Beckers, A. Verrijken, I. Mertens, L. Van Gaal, and W. Van Hul, "Possible role for ENPP1 polymorphism in obesity but not for INSIG2 and PLIN variants," *Endocrine*, vol. 36, no. 1, pp. 103–109, 2009.

[11] E. Maury and S. M. Brichard, "Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome," *Molecular and Cellular Endocrinology*, vol. 314, pp. 1–16, 2010.

[12] S. C. Sookoian, C. González, and C. J. Pirola, "Meta-analysis on the G-308A tumor necrosis factor α gene variant and phenotypes associated with the metabolic syndrome," *Obesity Research*, vol. 13, no. 12, pp. 2122–2131, 2005.

[13] B. Fontaine-Bisson, T. M. S. Wolever, J.-L. Chiasson et al., "Genetic polymorphisms of tumor necrosis factor-α modify the association between dietary polyunsaturated fatty acids and fasting HDL-cholesterol and apo A-I concentrations," *The American Journal of Clinical Nutrition*, vol. 86, no. 3, pp. 768–774, 2007.

[14] E. Brand, U. Schorr, I. Kunz et al., "Tumor necrosis factor-α -308 G/A polymorphism in obese Caucasians," *International Journal of Obesity and Related Metabolic Disorders*, vol. 25, no. 4, pp. 581–585, 2001.

[15] J.-Y. Um, J.-H. Park, and H.-M. Kim, "Gene polymorphisms in tumor necrosis factor locus and waist-hip ratio in obese Koreans," *Clinica Chimica Acta*, vol. 338, no. 1-2, pp. 117–122, 2003.

[16] S. Romeo, F. Sentinelli, F. Capici et al., "The G-308A variant of the Tumor Necrosis Factor-α (TNF-α) gene is not associated with obesity, insulin resistance and body fat distribution," *BMC Medical Genetics*, vol. 2, article 10, 2001.

[17] C. Stryjecki and D. M. Mutch, "Fatty acid-gene interactions, adipokines and obesity," *European Journal of Clinical Nutrition*, vol. 65, no. 3, pp. 285–297, 2011.

[18] Y. T. Joffe, L. Van Der Merwe, M. Collins et al., "The -308 G/A polymorphism of the tumour necrosis factor-α gene modifies the association between saturated fat intake and serum total cholesterol levels in white South African women," *Genes and Nutrition*, vol. 6, no. 4, pp. 353–359, 2011.

[19] S. Endres, R. Ghorbani, V. E. Kelley et al., "The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells," *The New England Journal of Medicine*, vol. 320, no. 5, pp. 265–271, 1989.

[20] M. B. Fessler, L. L. Rudel, and J. M. Brown, "Toll-like receptor signaling links dietary fatty acids to the metabolic syndrome," *Current Opinion in Lipidology*, vol. 20, no. 5, pp. 379–385, 2009.

[21] H. Shi, M. V. Kokoeva, K. Inouye, I. Tzameli, H. Yin, and J. S. Flier, "TLR4 links innate immunity and fatty acid-induced insulin resistance," *Journal of Clinical Investigation*, vol. 116, no. 11, pp. 3015–3025, 2006.

[22] O. Poulain-Godefroy, O. Le Bacquer, P. Plancq et al., "Inflammatory role of toll-like receptors in human and murine adipose tissue," *Mediators of Inflammation*, vol. 2010, Article ID 823486, 9 pages, 2010.

[23] World Health Organization, "Physical status: the use and interpretation of anthropometry," Report of a WHO Expert Committee. World Health Organization Technical Report Series 854, 1995.

[24] A. Agodi, M. Barchitta, G. Valenti, R. Marzagalli, V. Frontini, and A. E. Marchese, "Increase in the prevalence of the MTHFR 677 TT polymorphism in women born since 1959: potential implications for folate requirements," *European Journal of Clinical Nutrition*, vol. 65, no. 12, pp. 1302–1308, 2011.

[25] A. Agodi, M. Barchitta, G. Valenti et al., "Folate dietary intake and blood biomarkers reveal high risk groups in a Mediterranean population of healthy women of childbearing potential," *Annals of Nutrition and Metabolism*, vol. 63, pp. 179–185, 2013.

[26] A. Agodi, M. Barchitta, A. Quattrocchi, A. E. Marchese, and P. Boffetta, "Folate deficiency is not associated with increased mitochondrial genomic instability: results from dietary intake and lymphocytic mtDNA, 4977-bp deletion in healthy young women in Italy," *Mutagenesis*, vol. 29, no. 2, pp. 101–106, 2014.

[27] W. C. Willett, G. R. Howe, and L. H. Kushi, "Adjustment for total energy intake in epidemiologic studies," *The American Journal of Clinical Nutrition*, vol. 65, no. 4, supplement, pp. 1220S–1228S, 1997.

[28] A. Trichopoulou, T. Costacou, C. Bamia, and D. Trichopoulos, "Adherence to a Mediterranean diet and survival in a Greek population," *The New England Journal of Medicine*, vol. 348, pp. 2599–2608, 2003.

[29] A. Agodi, M. Barchitta, G. Valenti et al., "Role of the TNFA -308G>A polymorphism in the genetic susceptibility to acne vulgaris in a Sicilian population," *Annali di Igiene*, vol. 24, no. 5, pp. 351–357, 2012.

[30] K. J. Rothman, "The estimation of synergy or antagonism," *The American Journal of Epidemiology*, vol. 103, no. 5, pp. 506–511, 1976.

[31] S. Gallus, A. Odone, A. Lugo et al., "Overweight and obesity prevalence and determinants in Italy: an update to 2010," *European Journal of Nutrition*, vol. 52, no. 2, pp. 677–685, 2013.

[32] R. Olmedo-Requena, J. G. Fernández, C. A. Prieto, J. M. Moreno, A. Bueno-Cavanillas, and J. J. Jiménez-Moleón, "Factors associated with a low adherence to a Mediterranean diet pattern in healthy Spanish women before pregnancy," *Public Health Nutrition*, vol. 18, pp. 1–9, 2013.

[33] G. Buckland, A. Bach, and L. Serra-Majem, "Obesity and the Mediterranean diet: a systematic review of observational and intervention studies," *Obesity Reviews*, vol. 9, no. 6, pp. 582–593, 2008.

[34] A. Ferro-Luzzi, W. P. T. James, and A. Kafatos, "The high-fat Greek diet: a recipe for all?" *European Journal of Clinical Nutrition*, vol. 56, no. 9, pp. 796–809, 2002.

[35] D. Romaguera, T. Norat, T. Mouw et al., "Adherence to the Mediterranean diet is associated with lower abdominal adiposity in European men and women," *Journal of Nutrition*, vol. 139, no. 9, pp. 1728–1737, 2009.

[36] C. Lassale, L. Fezeu, V. A. Andreeva et al., "Association between dietary scores and 13-year weight change and obesity risk in a French prospective cohort," *International Journal of Obesity*, vol. 36, no. 11, pp. 1455–1462, 2012.

[37] A. Trichopoulou, A. Naska, P. Orfanos, and D. Trichopoulos, "Mediterranean diet in relation to body mass index and waist-to-hip ratio: The Greek European Prospective Investigation into Cancer and Nutrition Study," *The American Journal of Clinical Nutrition*, vol. 82, no. 5, pp. 935–940, 2005.

[38] A. G. Wilson, J. A. Symons, T. L. Mcdowell, H. O. Mcdevitt, and G. W. Duff, "Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 7, pp. 3195–3199, 1997.

[39] S. A. M. Engel, H. C. Erichsen, D. A. Savitz, J. Thorp, S. J. Chanock, and A. F. Olshan, "Risk of spontaneous preterm birth is associated with common proinflammatory cytokine polymorphisms," *Epidemiology*, vol. 16, no. 4, pp. 469–477, 2005.

[40] A. Nieters, N. Becker, and J. Linseisen, "Polymorphisms in candidate obesity genes and their interaction with dietary intake of n-6 polyunsaturated fatty acids affect obesity risk in a subsample of the EPIC-Heidelberg cohort," *European Journal of Nutrition*, vol. 41, no. 5, pp. 210–221, 2002.

[41] Y. T. Joffe, L. Van Der Merwe, M. Carstens et al., "Tumor necrosis factor-α gene -308 G/A polymorphism modulates the relationship between dietary fat intake, serum lipids, and obesity risk in black south African women," *Journal of Nutrition*, vol. 140, no. 5, pp. 901–907, 2010.

[42] A. S. Olafsdottir, I. Thorsdottir, I. Gunnarsdottir, H. Thorgeirsdottir, and L. Steingrimsdottir, "Comparison of women's diet assessed by FFQs and 24-hour recalls with and without underreporters: Associations with biomarkers," *Annals of Nutrition and Metabolism*, vol. 50, no. 5, pp. 450–460, 2006.

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Research Article

A Large Cross-Sectional Survey Investigating the Knowledge of Cervical Cancer Risk Aetiology and the Predictors of the Adherence to Cervical Cancer Screening Related to Mass Media Campaign

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Objectives. The aims of this study were to compare the characteristics of women who got a Pap-test during the mass media campaign, carried out in an Italian region by broadcasts advertising, and two years later and to identify the determinants of knowledge of cervical cancer etiology and of the adherence to the mass media campaign. *Methods.* A cross-sectional survey was carried out through a self-administered questionnaire. *Results.* A total of 8570 randomly selected women were surveyed, 823 of these had a Pap-test during the mass media campaign period and 7747 two years later. Higher educational level, being not married, and living in urban areas were the main independent characteristics associated with a higher level of knowledge of cervical cancer etiology, although a previous treatment following a Pap smear abnormality was the strongest predictor (OR = 2.88; 95% CI: 2.43–3.41). During the campaign period women had the Pap-test more frequently as a consequence of the mass media campaign (OR = 8.28; 95% CI; 5.51–12.45). *Conclusions.* Mass media campaign is a useful tool to foster cervical screening compliance; however, its short-term effect suggests repeating it regularly.

1. Introduction

Cancer is the third most common malignancy among females, with estimated 529,000 new cases and 275,000 deaths worldwide in 2008 [1]. According to the most recent data of the Italian Network of Cancer Registries (AIRTUM), cervical cancer accounts for 1.6% of all newly diagnosed cancers among Italian women [2]. The vast majority of cervical cancer cases are attributable to human papilloma virus (HPV), which is a sexually transmitted disease [3, 4]. The latency period between initial HPV exposure and the development of cervical cancer may be months to years. Although rapid progression is possible, average time from initial infection to manifestation of invasive cervical cancer is estimated at up to 15 years [5], thus providing a large window of action for Papanicolaou (Pap-test) screening and treatment of cervical cancer precursors.

Cervical cancer incidence and mortality rates have dropped dramatically during the 20th century in developed countries due to implementation of screening programs. According to AIRTUM data [6], the implementation of organized nationwide cervical screening (CS) programme in Italy since 1996 contributed to the decreasing mortality trends for cervical cancer in the past decade [7]. In Italy CS includes personal invitations for a Pap-test sent to women aged 25–64 years every three years by ordinary mail and a

monitoring system and quality assurance of the program [8]. According to the National Centre for Screening Monitoring, 121 active programs were in place in Italy in 2007 [8], with a target population of almost 12 million women. By looking at the actual extension of CS as the ratio between the number of women regularly invited each year and the entire target group in case of full implementation, some differences emerged between Centre (above 70%), North (above 55%), and South (about 43%) of Italy, suggesting that many women are actually underscreened [9]. As regard to the screening compliance, a clear North to South gradient is observed, with the highest percentage of women who actually have the Pap-test in the northern regions (60.1%) and the lowest in the southern regions (41.7%), probably due to the more recent introduction of screening programs in southern regions (The National Centre for Screening Monitoring. Tenth report 2012. Available at: http://www.osservatorionazionalescreening.it/sites/default/files/allegati/EPv36i6s1.pdf).

Abruzzo is an Italian southern region with a population of more than 1,300 million inhabitants. Although a fully active regional CS programme has been implemented since 1999, with an actual compliance estimated around 5 67.5% (screening activity in Abruzzo region (available at: http://sanitab.regione.abruzzo.it/screening/resoconto+statistico/regione+abruzzo+al+31+dicembre+2009.pdf), to counteract the low compliance the Regional Health Authority and the CS Committee designed and conducted in 2006 a media campaign to encourage women to undergo a Pap-test. To counteract the low compliance the Regional Health Authority and the CS Committee designed and conducted in 2006 a media campaign to encourage women to undergo a Pap-test. Even though mass media campaigns have been shown to be effective in improving screening participation [10, 11], woman compliance is strongly influenced by the level of knowledge on the risk factors and the general attitude toward CS procedure [12–14]. Based on these observations, the objectives of our study were (i) to describe the characteristics of woman who got the Pap-test during the mass media campaign and two years later when the campaign was ceased; (ii) to identify the predictors of knowledge of cervical cancer aetiology; and (iii) to identify the predictors of the adherence to the mass media campaign in the Abruzzo Region.

2. Methods

2.1. Mass Media Campaign Development and Characteristics. The Abruzzo Regional Health Authority and the CS Committee designed a mass media campaign on local television stations by developing three different advertisements on: (1) Pap-test safety: it can be performed even and during pregnancy; (2) Pap-test effectiveness: it can detect early cancer signs; and (3) HPV testing: in addition to Pap-test women can be tested to detect HPV infection. The time period of the televised marketing message lasted about three months (from January to March 2006).

2.2. Survey Population and Data Collection. To compare the characteristics of the women who got a Pap-test during the media campaign on CS programme (Group 1) with those who got a Pap-test two years later (Group 2), we conducted a cross-sectional survey on two representative samples of women, aged from 25 to 64 years. A structured questionnaire was administered to the first sample of women through a telephone interview, while a face-to-face interview using the same questionnaire was conducted on the second sample of women. Both telephone and face-to-face interviews were carried out by the same team of specifically trained nurses, in order to minimize the risk of response bias. The source population was the entire female population of Abruzzo region aged from 25 to 64 years. Sampling for Group 1 was performed using a list-assisted method with valid phone numbers appended with random numbers using the list of women who had access to consulting rooms and other public facilities of Abruzzo region to get the Pap-test during the year 2006, and on the basis of the lists of the local health authorities of the women who got the Pap-test during the period April 2008–March 2009 for Group 2. Both samples were stratified according to the age population distribution and residence area to enrich the 15% of the potentially screenable population.

2.3. Survey Tool: Questionnaire Measures. A structured questionnaire was designed to collect women's sociodemographic characteristics and to assess the extent of their knowledge about CS as well as their compliance to the media campaign on CS programme. The questionnaire was focused on the women's own assessment of their knowledge of the CS and its frequency, previous Pap-test, the healthcare facility involved in Pap-test delivery, and possible surgical treatment after a positive result as well as knowledge regarding HPV infection and cervical cancer. In addition age, residence area, educational level, marital status, professional occupation, sexual activity, age at first intercourse, and contraception were collected. Finally, the reason to get the Pap-test was answered using a multiple-choice single answer question to assess if the respondents got the pap test to prevent cervical cancer, in response of the invitation letter or of the mass media campaign, after suggestion of the general practitioner/gynaecologist or after suggestion of a friend.

2.4. Statistical Analysis. A descriptive analysis and a univariate analysis were carried out to compare the main characteristics of the investigated groups. Two different models of stepwise logistic regression with backward elimination were performed on the overall sample of woman to identify: (i) predictors of knowledge of cervical cancer aetiology and (ii) predictors of having a Pap-test as a consequence of the mass media campaign. In the first model, women who knew that cervical cancer is caused by sexual transmission were compared with all others; in the second model, women who got the Pap-test in response to the mass media campaign were evaluated against all others. Age, residence area, educational level, marital status, previous treatment after Pap-test, and professional occupation were initially tested in all models as predictor variables. Pap-test as a consequence of the mass media campaign and knowledge about cervical cancer aetiology were also considered as main independent variables in models one and two, respectively.

Multiple logistic regression models were built as suggested by Hosmer and Lemeshow [15]. Each variable was examined by univariate analysis using the appropriate statistic test (Student t-test and chi-squared test) and included in the model when the P value was lower than 0.25. Subsequently, multivariate logistic regression with backward elimination of any variable that did not contribute to the model according to the likelihood ratio test (significance level set at $P = 0.05$) was performed. Variables whose exclusion altered the coefficient of the remaining ones were kept in the model. Interaction terms were tested using a cut-off of 0.15 significance level. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. The statistical analyses were performed using Stata version 8.0 (College Station, Texas, Stata Corporation, 2003).

3. Results

A total of 8570 women were interviewed. Eight-hundred twenty-three subjects had a Pap-test during the mass media campaign period and 7747 two years later. The women who got a Pap-test during the campaign period (first group) were slightly younger than those who got a Pap-test two years after the campaign period (second group). The mean age was 42.5 years (±10.9) in the first group and 43.9 years (±10.7) in the second group ($P < 0.001$). Most of the women that got a Pap-test during the campaign period had a high school degree (45.1%) and only 8.9% had none or a primary school degree, while in the second group there were less high school degree (41.9%) and a little more women with none or primary school degree (12.7%). Almost three-fourths of the women in both groups were married, even if in the first group there were more singles (19.3% versus 16.3%) and less separated/divorced (3.9% versus 6.2%) than in the second one. The most represented occupations were housewife, employee, labourer, and teacher in both groups (Table 1).

Notably, a higher percentage of women in the first group than in the second one became aware of the screening program through the mass media campaign and got the Pap-test in response to it (11.9% versus 0.9%). Around 14.3% and 27.9% of the responders were aware that cervical cancer is attributable to sexual transmission in the first and second groups, respectively. Only 11.4% of women in the first group and 9.0% in the second group were at the first Pap-test (Table 2).

Globally, 26.6% of women knew that cervical cancer is attributable to sexual transmission. The multiple logistic regression analysis showed that knowledge about cervical cancer aetiology increases with the educational level (OR = 1.97; 95% CI: 1.83–2.12), if the women are single, widow, or divorced (OR = 1.30; 95% CI: 1.12–1.52) and if they live in urban areas in both groups (OR = 1.21; 95% CI: 1.11–1.32). A previous therapeutic treatment following a Pap smear abnormality is the strongest predictor of the adequate knowledge (OR = 2.88; 95% CI: 2.43–3.41) (Table 3).

Globally, 1.7% of woman stated that they got the Pap-test as a consequence of the mass media campaign. The results of the multiple logistic regression analysis showed that this phenomenon was higher during the mass media campaign period (OR = 8.28; 95% CI 5.51–12.45), among younger women (OR = 0.97; 95% CI: 0.95–0.99), those resident in rural areas (OR = 1.52; 95% CI: 1.06–2.18), and among those with lower knowledge of cervical cancer aetiology (OR = 0.45; 95% CI: 0.24–0.84) (Table 4).

4. Discussion

A meta-analysis of media health campaigns reported that campaigns promoting mammography and cervical cancer screening program reported that almost 4% of women positively change their behavior in response to a television campaign [10]. Little is currently known on the effectiveness of mass media campaign to promote Pap-test screening programs in Italy, especially in southern regions. This large cross-sectional study was specifically designed to identify the characteristics of women resident in a southern region of Italy who adhered to a local television campaign on the importance of having a Pap-test to prevent cervical cancer. The results of our study show that women adhering to the CS program as a result of the media campaign are more likely to be young, to be rural resident, and to have a poor knowledge of cervical cancer etiology. The effectiveness of the campaign to encourage women to do Pap smear analysis, however, decreased over time. These results are quite consistent with those of a recent paper [16] that reported a statistically significant 18% increase in the number of Pap-tests performed during a television campaign in Australia compared to the same period in previous years. Nevertheless, a decline to background levels was observed approximately three weeks immediately after the television campaign [16].

The results of the multivariate analysis showed that mass media campaign is a good tool to increase CS participation of women with a high risk of unscreening or underscreening due to a low level of knowledge, since poor knowledge on the risk factors for cervical cancer has been recognized as one of the most important predictors that reduces participation in CS [17]. The level of knowledge of women on cervical cancer aetiology in this study was quite low, as around 26.6% of the respondents declared to know that cervical cancer is caused by a sexually transmitted infection. A previous population-based survey of women aged 18 to 75 living in the USA found that ~40% of women had heard of HPV, but less than half of those knew that it caused cervical cancer [18]. A lower percentage of knowledge was detected among German women aged 25 to 75, since only 3.2% of them knew that infection with HPV virus is a risk factor for cervical cancer [19]. Moreover, consistently with other surveys, we found that women with adequate knowledge are more likely to have a higher level of education and to have been previously experienced with an abnormal Pap smear and positive HPV results [18].

The results of the multivariate analysis also showed that the mass media campaign was effective on rural resident women. This finding appears very important and is worthy of comment. A previous research carried out in the USA suggested that rural residents are less likely to receive timely cancer screening test, thus causing cancer diagnosis at more

Table 1: Selected demographic and professional characteristics of the women who got the Pap-test during the campaign period (Group 1) and two years later (Group 2).

Characteristics	Group 1		Group 2		*P* value
	Mean	SD	Mean	SD	
Age, years	42.5	10.9	43.9	10.7	<0.001
	N = 823	%	*N* = 7747	%	
Place of residence					
Urban	450	54.68	4079	52.65	0.268
Rural	373	45.32	3668	47.35	
Educational level					
None/primary school	65	8.89	978	12.71	0.009
Middle school	223	30.51	2184	28.39	
High school	330	45.14	3223	41.90	
Academic degree	113	15.46	1307	16.99	
Missing	*55*	*0.71*	*92*	*11.18*	
Marital status					
Single	135	19.29	1239	16.30	0.025
Married	518	74.00	5662	74.49	
Separated/divorced	27	3.86	471	6.20	
Widow	20	2.86	229	3.01	
Missing	*123*	*14.94*	*146*	*1.88*	
Professional occupation					
Trader	27	3.76	299	3.90	<0.001
Laborer	101	14.05	1063	13.88	
Employee	94	13.07	1240	16.19	
Corporate entrepreneur	10	1.39	88	1.15	
Agricultural entrepreneur	8	1.11	134	1.75	
Freelancer	8	1.11	293	3.82	
Teacher	43	5.98	548	7.15	
Housewife	237	32.96	2645	34.53	
Retired	31	4.31	384	5.01	
Craftsman	1	0.14	92	1.20	
Caregiver	27	3.76	239	3.12	
Waitress	14	1.95	145	1.89	
Shop assistant	10	1.39	78	1.02	
Sanitary	16	2.23	71	0.93	
Student	9	1.25	47	0.61	
Unemployed	28	3.89	162	2.11	
Other	55	7.65	132	1.73	
Missing	*105*	*12.76*	*86*	*1.11*	

advanced stages [20]. This result was also reported in a case-control study carried out in Italy to evaluate the compliance to the colorectal cancer screening [21]. A possible explanation of this finding is the presence of logistical barriers and the distance from the test provider that often may limit the use of preventive care units. Subjects who live close to the provider are often more likely to comply [22], while the others perceive the prevention activity such as a screening test as a time-consuming activity.

This study has some potential limitations. Firstly, the research questions were investigated by a cross-sectional study design. As well known, such design precludes determination of causal relationships between different factors and outcomes. Secondly, information was gathered by telephone and face-to-face direct interview questionnaire, so misclassification of the covariates collected cannot be ruled out. Interviewers, however, were professionally trained nurses thus the risk of social desirability response bias should be low.

5. Conclusions

In conclusion, mass media campaign can enhance healthy behaviours and can track the diffusion of adequate knowledge. However, the short-term effects suggest that the media campaign should be repeated regularly to steadily increment the compliance to cervical cancer screening program.

Table 2: Knowledge of cervical cancer aetiology and related screening procedure and motivation to get the Pap-test among women who got the Pap-test during the campaign period (Group 1) and two years later (Group 2).

Characteristics	Group 1		Group 2		*P* value
	N = 823	%	*N* = 7747	%	
Reason to get the Pap-test					
To prevent cervical cancer	258	35.88	2902	41.80	
Invitation letter	176	24.48	1942	27.97	
Mass media campaign	86	11.95	63	0.91	
General practitioner/gynaecologist suggestion	103	14.33	1139	16.41	<0.001
Friend suggestion	27	3.76	85	1.22	
Other	69	9.60	811	11.69	
Missing	*104*	*12.63*	*805*	*10.39*	
Knowledge of cervical cancer aetiology					
Yes, sexual mode	104	14.31	1994	27.87	
Yes, unknown way	321	44.15	2502	34.97	<0.001
No	302	41.54	2659	37.16	
Missing	*96*	*11.66*	*592*	*7.64*	
Previous Pap-test					
No	94	11.42	695	9.00	
Yes	729	88.58	7023	91.00	0.023
Missing	—	—	*29*	*0.37*	

Table 3: Results of the logistic regression analysis to identify predictors of knowledge of cervical cancer aetiology in the entire sample.

Variable	OR	95% CI	*P*value
Education degree* (*none/elementary school = 0; middle school = 1; high school = 2; university = 3*)	1.97	1.83–2.12	<0.001
Previous treatment after Pap-test (*no = 0; yes = 1*)	2.88	2.43–3.41	<0.001
Marital status (*married = 0; single/divorced/widow = 1*)	1.30	1.12–1.52	0.001
Place of residence (*rural = 0; urban = 1*)	1.21	1.11–1.32	<0.001

*Variable modelled as ordinal, since linearity was assessed.

Table 4: Results of the logistic regression model to identify the characteristics of the women who got the Pap-test as a consequence of the mass media campaign in the entire sample.

Variable	OR	95% CI	*P* value
Age (*continue*)	0.97	0.95–0.99	0.001
Place of residence (*urban = 0; rural = 1*)	1.52	1.06–2.18	0.024
Group source (0 = Pap-test after 1 year; 1 = Pap-test during the mass media campaign)	8.28	5.51–12.45	<0.001
Knowledge of cervical cancer causes (*no = 0; yes = 1*)	0.45	0.24–0.84	0.012

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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References

[1] J. Ferlay, H.-R. Shin, F. Bray, D. Forman, C. Mathers, and D. M. Parkin, "Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008," *International Journal of Cancer*, vol. 127, no. 12, pp. 2893–2917, 2010.

[2] AIRTUM Working Group, "Italian cancer figures, report 2009: cancer trend (1998–2005)," *Epidemiologia e Prevenzione*, vol. 33, no. 4-5, supplement 1, pp. 1–168, 2009.

[3] J. G. Baseman and L. A. Koutsky, "The epidemiology of human papillomavirus infections," *Journal of Clinical Virology*, vol. 32, supplement, pp. S16–S24, 2005.

[4] C. Clavel, M. Masure, J.-P. Bory et al., "Human papillomavirus testing in primary screening for the detection of high-grade cervical lesions: a study of 7932 women," *British Journal of Cancer*, vol. 84, no. 12, pp. 1616–1623, 2001.

[5] C. J. L. M. Meijer, P. J. F. Snijders, and A. J. C. van den Brule, "Screening for cervical cancer: should we test for infection with high-risk HPV?" *Canadian Medical Association Journal*, vol. 163, no. 5, pp. 535–538, 2000.

[6] M. Ferraroni, C. La Vecchia, R. Pagano, E. Negri, F. Parazzini, and A. Decarli, "Pattern of cervical screening utilization in Italy," *Tumori*, vol. 75, no. 5, pp. 420–422, 1989.

[7] AIRTUM Working Group, "Italian cancer figures—report 2009. Incidence and mortality: comparisons," *Epidemiologia e Prevenzione*, vol. 33, supplement 1, pp. 106–118, 2009.

[8] G. Ronco, P. Giubilato, C. Naldoni et al., "Extension of organised cervical cancer screening programmes in Italy and their process indicators: 2007 activity," *Epidemiologia e Prevenzione*, vol. 33, no. 3, supplement 2, pp. 41–56, 2009.

[9] http://www.osservatorionazionalescreening.it/sites/default/files/allegati/EPv36i6s1.pdf.

[10] A. C. Marcus and L. A. Crane, "A review of cervical cancer screening intervention research: implications for public health programs and future research," *Preventive Medicine*, vol. 27, no. 1, pp. 13–31, 1998.

[11] L. B. Snyder, M. A. Hamilton, E. W. Mitchell, J. Kiwanuka-Tondo, F. Fleming-Milici, and D. Proctor, "A meta-analysis of the effect of mediated health communication campaigns on behavior change in the United States," *Journal of Health Communication*, vol. 9, supplement 1, pp. 71–96, 2004.

[12] G. Grangé, D. Malvy, F. Lançon, A. F. Gaudin, and A. El Hasnaoui, "Factors associated with regular cervical cancer screening," *International Journal of Gynecology & Obstetrics*, vol. 102, no. 1, pp. 28–33, 2008.

[13] D. Seidel, N. Becker, S. Rohrmann, K. Nimptsch, and J. Linseisen, "Socio-demographic characteristics of participation in the opportunistic German cervical cancer screening programme: results from the EPIC-Heidelberg cohort," *Journal of Cancer Research and Clinical Oncology*, vol. 135, no. 4, pp. 533–541, 2009.

[14] M. Pavia, G. Ricciardi, A. Bianco, P. Pantisano, E. Langiano, and I. F. Angelillo, "Breast and cervical cancer screening: knowledge, attitudes and behavior among schoolteachers in Italy," *European Journal of Epidemiology*, vol. 15, no. 4, pp. 307–311, 1999.

[15] D. W. Hosmer and S. Lemeshow, *Applied Logistic Regression*, John Wiley & Sons, New York, NY, USA, 2000.

[16] R. Mullins, M. Wakefield, and K. Broun, "Encouraging the right women to attend for cervical cancer screening: results from a targeted television campaign in Victoria, Australia," *Health Education Research*, vol. 23, no. 3, pp. 477–486, 2008.

[17] F. Fylan, "Screening for cervical cancer: a review of women's attitudes, knowledge, and behaviour," *British Journal of General Practice*, vol. 48, no. 433, pp. 1509–1514, 1998.

[18] J. A. Tiro, H. I. Meissner, S. Kobrin, and V. Chollette, "What do women in the U.S. know about human papillomavirus and cervical cancer?" *Cancer Epidemiology Biomarkers and Prevention*, vol. 16, no. 2, pp. 288–294, 2007.

[19] S. J. Klug, M. Hetzer, and M. Blettner, "Screening for breast and cervical cancer in a large German city: participation, motivation and knowledge of risk factors," *European Journal of Public Health*, vol. 15, no. 1, pp. 70–77, 2005.

[20] M. M. Casey, K. Thiede Call, and J. M. Klingner, "Are rural residents less likely to obtain recommended preventive healthcare services?" *American Journal of Preventive Medicine*, vol. 21, no. 3, pp. 182–188, 2001.

[21] P. G. Rossi, A. Federici, F. Bartolozzi, S. Farchi, P. Borgia, and G. Guasticchi, "Understanding non-compliance to colorectal cancer screening: a case control study, nested in a randomised trial [ISRCTN83029072]," *BMC Public Health*, vol. 5, article 139, 2005.

[22] S. W. Vernon, "Participation in colorectal cancer screening: a review," *Journal of the National Cancer Institute*, vol. 89, no. 19, pp. 1406–1422, 1997.

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Research Article

A Pilot Study Evaluating the Contribution of *SLC19A1* (*RFC-1*) 80G>A Polymorphism to Alzheimer's Disease in Italian Caucasians

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Alzheimer's disease (AD) is the most common neurodegenerative disorder and the primary form of dementia in the elderly. Polymorphisms of genes involved in folate metabolism have been frequently suggested as risk factors for sporadic AD. A common c.80G>A polymorphism (rs1051266) in the gene coding for the reduced folate carrier (*SLC19A1* gene, commonly known as *RFC-1* gene) was investigated as AD risk factor in Asian populations, yielding conflicting results. We screened a Caucasian population of Italian origin composed of 192 sporadic AD patients and 186 healthy matched controls, for the presence of the *RFC-1* c.80G>A polymorphism, and searched for correlation with circulating levels of folate, homocysteine, and vitamin B12. No difference in the distribution of allele and genotype frequencies was observed between AD patients and controls. No correlation was observed among the genotypes generated by the *RFC-1* c.80G>A polymorphism and circulating levels of folate, homocysteine, and vitamin B12 either in the whole cohort of subjects or after stratification into clinical subtypes. Present results do not support a role for the *RFC-1* c.80G>A polymorphism as independent risk factor for sporadic AD in Italian Caucasians.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder and the primary form of dementia in the elderly, clinically characterized by a progressive neurodegeneration in selected brain regions, including the temporal and parietal lobes and restricted regions within the frontal cortex and the cingulate gyrus [1]. The term "dementia" describes a set of symptoms, which include loss of memory, mood changes, and problems with communication and reasoning. Indeed, AD leads to memory loss accompanied by changes of behaviour and personality severe enough to affect daily life. The disease symptoms get worse over time and available treatments may only help in keeping patients from getting worse for a limited period. It is estimated that there are over 36 million people living with dementia in the world, and projections estimate that the number of affected individuals will increase quickly in the next decades following the worldwide increase in life expectancy. Therefore, there is particular interest in searching for early detectable biomarkers allowing us to better characterize those individuals at increased risk to develop AD [1].

Homocysteine (hcy), folates, and related B-vitamins participate in one-carbon metabolism, a pathway required for

Table 1: Demographic characteristics of the study population and data on plasma total homocysteine and serum folate and vitamin B12 levels.

	n	Males n (%)	Females n (%)	Age (years, mean ± SD)	t-hcy[a] (μmol/L, mean ± SEM)	Folate[a] (ng/mL, mean ± SEM)	Vitamin B12[a] (pg/mL, mean ± SEM)
AD	192	65 (33.8%)	127 (66.2%)	76.4 ± 7.3	21.2 ± 1.7	7.1 ± 0.8[b]	407.8 ± 25.2
Controls	186	72 (38.7%)	114 (61.3%)	73.5 ± 6.4	14.6 ± 0.7	8.2 ± 1.0	437.9 ± 30.9

[a]Available from 104 AD and 64 controls.
[b]Significant difference versus controls (P value obtained with analysis of covariance using log transformed data and corrected for age and gender).

DNA synthesis and methylation reactions [2]. Both prospective and retrospective studies suggest that impairments of one-carbon metabolism leading to increased hcy levels might contribute to Alzheimer's disease (AD), and genetic polymorphisms of metabolic enzymes have been suspected to contribute to those impairments as well as to sporadic AD risk [2–16].

The reduced folate carrier (RFC-1) participates in the uptake of folate cofactors from the blood [17], and a common c.80G>A polymorphism (rs1051266) in the gene coding for RFC-1 (*SLC19A1* gene: solute carrier family 19 member 1, commonly known as *RFC-1* gene) was hypothesized to have a functional role in folate transport [18]. Subsequent studies gave conflicting results, and the contribution of this polymorphism to circulating folate or hcy levels is still a matter of debate [15, 19–21]. In 2009, Bi and coworkers observed association of both the *RFC-1* 80G allele and the GG genotype with increased risk of late-onset AD in Han Chinese individuals [15]. However, no significant effect of the *RFC-1* 80G>A polymorphism on plasma folate and hcy levels was detected [15]. A more recent study performed in Indian subjects failed to find association of the *RFC-1* 80G>A polymorphism with risk of AD or vascular dementia, and no association of the polymorphism with serum folate levels was detected [21]. Moreover, others failed to observe association of rs1051266 with cognitive status, folate, and hcy levels in Caucasian Parkinson's disease (PD) patients [22].

At best of our knowledge, except for the above two conflicting studies in Asian populations [15, 21], there is no other available case-control genetic association study evaluating the possible contribution of rs1051266 to AD risk. Therefore, we performed the present pilot study to address the contribution of the *RFC-1* 80G>A polymorphism to AD risk in a cohort of Caucasian sporadic AD patients and healthy matched controls and searched for correlation between rs1051266 and circulating levels of folate, hcy, and vitamin B12.

2. Materials and Methods

2.1. Study Population. DNA samples from 192 sporadic AD patients and 186 matched controls were collected at the Department of Neurosciences, University of Pisa, and at the Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence (Table 1). The AD patients were clinically evaluated according to the published guidelines and the AD diagnosis fulfilled the Diagnostic and Statistical Manual of Mental Disorders criteria (DSM-IV) [23, 24]. According to disease age at onset and absence of a family history of dementia all the AD subjects were assumed to be sporadic late-onset (>65 years) cases. The apolipoprotein E (*APOE*) genotype was known for 30 AD patients and 40 controls, and *APOE ε4* (+) carriers were higher in AD patients than in controls (47% versus 27%). As normal controls we recruited healthy volunteer subjects without relationship with the AD patients. Controls were selected among people ageing more than 65 years (i.e., people at risk to develop late onset AD) and were matched to AD patients for age (±3 years) and gender (Table 1), as well as for ethnicity and geographic origin (all individuals were Caucasians from northern Tuscany and neighbouring areas). Family history of dementia was ascertained, excluding all the subjects with even one relative who developed AD or other dementias. All the control subjects were evaluated in order to exclude the presence of cognitive impairment (MMSE score over 26). Each subject gave an informed and written consent for genotype analysis. The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Pisa University Hospital (Protocol number 3618/2012).

2.2. Genotyping. Genomic DNA was isolated from whole blood by means of the QIAamp Blood Mini Kit (Qiagen, Milan, Italy) following the manufacturer's instructions. The genotyping protocol for the *RFC-1* 80G>A polymorphism was adapted from Bi et al. [15]: a 230-bp product was amplified using 1.25 units of Taq DNA polymerase (Invitrogen, Milan, Italy), 10 pmol of the forward primer (5′-AGCGTCACCTTCGTCCC-3′) and the reverse primer (5′-TCCCGCGTGAAGTTCTTG-3′), 0.15 mM of each dNTP, 1.5 mM $MgCl_2$, and 30 ng of genomic DNA in a total volume of 25 μL. PCR conditions consisted of an initial denaturation step of 5 minutes at 95°C, followed by 40 cycles of 30 s at 95°C, 45 s at 62°C, and 45 s at 72°C and a final extension of 10 minutes at 72°C. The PCR products were digested with *Cfo I* (SIGMA, Milan, Italy) and resulted in three fragments of 125-bp, 68-bp, and 37-bp in the presence of the 80G allele, while the 80A allele produced two fragments of 162- and 68-bp. Digestion products were visualized after electrophoresis on a 3% agarose gel containing ethidium bromide. Internal control samples, whose genotypes had been previously assessed, were always included and analyzed on each gel.

2.3. Biochemical Analyses. Peripheral blood samples for the evaluation of folate, total homocysteine (t-hcy), and vitamin B12 levels were collected from 104 AD patients and 64 healthy controls. Plasma was immediately separated and stored in freezer at −80°C. All the analyses were performed with standard protocols at the diagnostic laboratory of

Table 2: Distribution of genotypes and allele frequencies of the *RFC-1* 80G>A polymorphism in Alzheimer's disease and control individuals.

Genotypes/alleles	AD patients $n = 192$ (%)	Controls $n = 186$ (%)	Crude OR (95% CI)	P value	Adjusted OR[a] (95% CI)	P value
Genotypes						
GG	53 (27.6)	49 (26.3)	1.00[b]	—	1.00[b]	—
GA	102 (53.1)	98 (52.7)	0.96 (0.59–1.55)	0.87	0.97 (0.58–1.62)	0.89
AA	37 (19.3)	39 (21.0)	0.88 (0.48–1.59)	0.66	0.94 (0.50–1.78)	0.86
AA + GA versus GG	139 (72.4)	137 (73.7)	0.94 (0.60–1.48)	0.78	0.96 (0.59–1.57)	0.89
Alleles						
Allele G	0.54	0.53	1.00[b]	—		—
Allele A	0.46	0.47	0.94 (0.71–1.25)	0.69	0.98 (0.72–1.32)	0.88

[a]Adjusted for age and gender.
[b]Reference value for OR.

the University Hospital of Pisa, as previously described by us [5]. In order to minimize the effect of polymedication in our cohort of subjects, individuals taking medicaments or supplements known or suspected to interfere with one-carbon metabolism were not enrolled for the study.

2.4. Statistical Analyses. To verify that allele frequencies were in Hardy-Weinberg equilibrium and to assess differences in allele distributions between groups, we used the Chi-square (χ^2) analysis. The differences in genotype frequencies were analyzed by 2×2 contingency tables using χ^2 analysis. Logistic regression analyses were used to examine the associations between the study polymorphism and AD risk by estimating odds ratios (ORs) and 95% confidence intervals (CIs) with and without adjustment for age and gender. All individual values were analyzed with the MedCalc 12.5 statistical package for Windows. The statistical package QUANTO 1.2.4.exe was used to evaluate the statistical power of the study. Given a case-control cohort of almost 190 subjects each and a minor allele frequency ranging from 0.45 to 0.49 from present and previous studies in Caucasians [25, 26], the study had 80% power to detect ORs of 1.5 or higher under the additive model.

Analysis of covariance (ANCOVA) was used to evaluate differences in mean plasma t-hcy, folate, and vitamin B12 levels among groups, including age at sampling and gender as covariates. ANCOVA was also used to correlate biochemical data with the possible genotypes generated by the studied polymorphism, including age and gender as covariates. Since data on t-hcy, folates, and vitamin B12 had a skewed distribution, logarithm transformation of all values was done before analysis. Analyses were performed with the Statgraphics Centurion XVI.1 software package for Windows.

3. Results

3.1. RFC-1 Allele and Genotype Frequencies among Groups. Table 2 shows the distribution of *RFC-1* 80G>A genotype and allele frequencies in AD patients and controls. Genotype distributions in controls conformed to Hardy-Weinberg expectations ($P = 0.44$). The frequencies of the *RFC-1* 80A minor allele in AD patients and controls were 0.46 and 0.47, respectively ($P = 0.69$). Also the distribution of genotype frequencies was similar and not significantly different between AD and control subjects (Table 2). As stated in the Materials and Methods section the *APOE* genotype was known only for a small subgroup of AD and control individuals. However, no significant difference in *RFC-1* 80G>A allele frequencies was observed between *APOE* ε4 (+) AD patients and *APOE* ε4 (+) controls ($P = 0.37$. Not shown).

3.2. Folate, t-hcy, and Vitamin B12 Levels among Groups. Data on circulating t-hcy, folate, and vitamin B12 levels were available from 104 AD and 64 control individuals (Table 1). Analysis of variance revealed that mean t-hcy levels were higher in AD patients than in controls ($P = 0.002$), but after inclusion of age at sampling and gender as covariates in the analysis the difference between AD and control subjects was not statistically significant ($P = 0.14$), whilst a strong effect of age at sampling on increasing t-hcy levels was observed ($P < 0.001$). A significant difference was observed concerning serum folate levels between AD and control subjects ($P = 0.01$) that remained significant after correcting for age at sampling and gender ($P = 0.04$). Also increasing age at sampling showed a significant contribution to reducing serum folate levels in our population ($P = 0.03$). No difference in mean vitamin B12 levels was observed between AD and controls ($P = 0.31$ without correction and $P = 0.47$ after correction for age and gender). No significant effect of age and gender on mean vitamin B12 levels was observed.

3.3. Correlation between RFC-1 Genotypes and Biochemical Data. Table 3 shows the correlation between *RFC-1* 80 (GG, GA, AA, and GA+AA) genotypes and circulating levels of t-hcy, folate, and vitamin B12. Analyses were performed in the whole cohort of subjects (AD + controls) and in AD and control individuals separately. No significant difference was observed for each of the studied biochemical markers among different *RFC-1* genotypes (Table 3).

Table 3: Correlation between *RFC-1* 80G>A genotypes and biochemical data.

	RFC-1 GG	*RFC-1* GA	*RFC-1* AA	*RFC-1* GA + AA	*P* value[a]
Total (n = 168)	39	95	34	129	
Folate (ng/mL, mean ± SEM)	8.4 ± 1.2	7.9 ± 0.8	5.8 ± 1.3	7.3 ± 0.7	0.51
t-hcy (μmol/L, mean ± SEM)	15.6 ± 2.2	19.2 ± 1.4	19.1 ± 2.2	19.1 ± 1.2	0.55
Vit. B12 (pg/mL, mean ± SEM)	462.1 ± 39.3	421.7 ± 24.9	366.1 ± 41.6	406.9 ± 21.4	0.63
AD (n = 104)	26	60	18	78	
Folate (ng/mL, mean ± SEM)	8.1 ± 1.5	7.4 ± 1.0	4.9 ± 1.8	6.8 ± 0.9	0.65
t-hcy (μmol/L, mean ± SEM)	16.1 ± 3.5	22.6 ± 2.2	22.1 ± 4.1	22.5 ± 2.0	0.50
Vit. B12 (pg/mL, mean ± SEM)	467.4 ± 47.9	398.7 ± 31.6	351.7 ± 57.5	387.7 ± 27.6	0.70
Controls (n = 64)	13	35	16	51	
Folate (ng/mL, mean ± SEM)	8.3 ± 2.2	8.5 ± 1.3	7.1 ± 1.9	8.1 ± 1.1	0.75
t-hcy (μmol/L, mean ± SEM)	13.2 ± 1.4	14.3 ± 0.8	16.6 ± 1.2	15.0 ± 0.7	0.17
Vit. B12 (pg/mL, mean ± SEM)	407.9 ± 70.7	457.8 ± 40.8	404.2 ± 60.9	441 ± 33.7	0.76

[a] *P* value obtained with analysis of covariance using log transformed data and corrected for age and gender.

4. Discussion

At best of our knowledge the present is the first case-control study performed in Caucasians and aimed at addressing the contribution of the *RFC-1* 80G>A polymorphism to late-onset AD risk. The study revealed no significant difference in *RFC-1* allele or genotype frequencies between late-onset AD patients and healthy matched controls, both results being very similar between the two groups (Table 2). In addition, no significant effect of the studied polymorphism on circulating levels of folate, vitamin B12, or t-hcy was observed (Table 3).

In their original report, Bi and coworkers included 275 late-onset AD patients and 271 age-matched controls observing an additive effect for the G allele and odds ratios (ORs) ranging from 1.4 to 1.6 for genotype comparisons. The present study had enough power to detect similar ORs under an additive genetic model or at least to detect some trends toward an association. However, both allele and genotype frequencies were closely similar between AD patients and controls; the ORs for genotype comparison were close to 1.0 and the respective *P* values did not even suggest trends for association. Therefore, rs1051266 is unlikely to represent an independent risk factor for sporadic AD in our population, at least with a similar effect size as previously reported in Han Chinese individuals [15]. Furthermore, present results are in agreement with those of Mansoori and coworkers who screened 80 AD patients, 50 patients with vascular dementia, and 120 healthy control subjects from India, observing an increased risk of dementia in subjects with low serum folate values but no association of rs1051266 with circulating folate levels and risk of AD or vascular dementia [21]. In addition, Białecka and coworkers [22] screened 248 PD individuals and 254 matched controls from Poland, searching for correlation between rs1051266 and risk of dementia in Parkinson's disease. The authors observed that both age and plasma hcy levels were risk factors for dementia in PD but failed to find association of the *RFC-1* 80G>A polymorphism with cognitive decline or plasma hcy levels [22]. Similarly, Kumudini and coworkers [27] recently screened a cohort of 151 Indian PD patients and 416 healthy controls, observing increased plasma hcy levels in PD patients than in controls but no association of the *RFC-1* 80G>A polymorphism with either PD risk or plasma hcy levels [27].

Taken overall, both the present and the four previous studies performed in individuals with different forms of dementia or neurodegeneration [15, 21, 22, 27] failed to find association of the *RFC-1* 80G>A polymorphism with circulating folate, hcy, or vitamin B12 levels, and only one study [15] suggests association with dementia of Alzheimer's type.

Several factors could account for the above conflicting results, including differences in allele frequency, dietary habits, environmental and geographic factors, and the presence or absence of other genetic variants. For example, the frequency of the alleles generated by the *RFC-1* 80G>A polymorphism varies among different populations, with the *RFC-1* G allele often reported to be the major allele in certain populations [18, 21, 25] and the minor allele in others [15, 28]. Dietary regimens rich in folate, such as the Mediterranean diet, could mask the effect of certain polymorphisms, as it happens for the *MTHFR* 677C>T one, the most studied polymorphism of the folate pathway, which is associated with increased risk of sporadic AD in Asians but not in Caucasians [16]. It was also suggested that geographic factors, such as the latitude, could interfere with ultraviolet B solar radiation and promote, in less pigmented skins, intravascular folate photolysis, thereby affecting circulating folate levels and folate metabolism [29]. In this regard, a recent literature meta-analysis reported a significant effect of the *MTHFR* 677C>T polymorphism on pregnancy outcome only in subtropical regions [29], and it is also of interest that the *RFC-1* 80G>A polymorphism was associated with increased chromosome damage in blood cells of healthy Australian individuals but not in those of healthy Italian ones [30, 31]. Moreover, the presence/absence of other polymorphisms of the pathway could mask or potentiate the effect of a single one [26]. Altogether those factors can account for a different weight of each genetic polymorphism on a given disease among different populations, and also the *APOE* ε4 variant, which is the most known and replicated risk factor for sporadic

AD, seems to confer different relative risks in different ethnic groups [32].

Interestingly, we observed reduced serum folate levels in AD patients with respect to controls, and this is in agreement with several recent reports suggesting that reduced serum folate levels are a valuable biomarker of AD in aged individuals and might be linked to increased atrophy of both cortical and subcortical regions [21, 26, 33, 34]. However, as discussed above, neither the present nor the previous studies observed association of the *RFC-1* 80G>A polymorphism with serum folate levels in individuals affected by different forms of dementia or neurodegeneration [15, 21, 22, 27].

A limitation of the present study is that patients were selected retrospectively among prevalent AD cases followed up at our neurological clinics. The cognitive decline leading to AD usually starts several years before the onset of dementia, a condition which is referred to as mild cognitive impairment (MCI) [35]. The analysis of individuals with MCI would help to better clarify factors linked to the earliest phases of the disease than the analysis of late onset AD cases [35], and a similar study is highly desired in order to clarify the contribution of both present and other polymorphisms of the folate metabolic pathway to the earliest phases of the neurodegenerative process leading to AD. MCI patients should, however, be followed up over a period of time in order to discriminate those that will develop dementia of AD type from other forms of dementia. Indeed, the question of whether or not impairments of the folate metabolic pathway are cause or consequence of the neurodegenerative process in AD is still open in the literature [8–10]. In order to minimize factors, such as polymedications, that could interfere with the measured values of folate, hcy, and vitamin B12, we have not included in the present study patients or controls taking drugs or vitamin supplements known to alter those metabolites. In addition, in order to minimize the effect of geographic factors, both cases and controls had the same geographical origin and were residents of Pisa, Florence, and neighbouring areas at the time of enrolment for the study. Another limitation that we should acknowledge is that we had no opportunity to measure folate, hcy, and vitamin B12 in the whole cohort of subjects but only in a subgroup of them. As a consequence, data shown in Table 3 should be taken with caution, and replication in a larger cohort of Italian elderly subjects is warranted prior to exclude a role for the studied polymorphism on circulating folate, hcy, and vitamin B12 levels. In this regard, a large similar study, performed in over 1.000 elderly English subjects (mean age 77.9 years), revealed no association of the *RFC-1* 80G>A polymorphism with circulating folate or hcy levels [36], supporting present and previous observations in patients with neurodegenerative diseases and their matched controls [15, 21, 22, 27].

In conclusion, the present pilot study does not support a role for the *RFC-1* 80G>A polymorphism as independent risk factor for sporadic AD in Italian Caucasians. Despite that the group of patients and controls was relatively small, both allele and genotype frequencies results were so similar between AD and control samples that not even a trend for association was detected. Furthermore, no functional contribution of the studied polymorphism on circulating levels of folate, t-hcy, and vitamin B12 was observed. However, a large prospective study is warranted to confirm the results of the present pilot study and exclude a role for this polymorphism in the onset of dementia of Alzheimer's type in Italian Caucasians.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] A. L. Sosa-Ortiz, I. Acosta-Castillo, and M. J. Prince, "Epidemiology of dementias and Alzheimer's disease," *Archives of Medical Research*, vol. 43, no. 8, pp. 600–608, 2012.

[2] F. Coppedè, "One-carbon metabolism and Alzheimer's disease: focus on Epigenetics," *Current Genomics*, vol. 11, no. 4, pp. 246–260, 2010.

[3] B. Regland, L. Abrahamsson, C. G. Gottfries, and E. Magnus, "Vitamin B12 analogues, homocysteine, methylmalonic acid, and transcobalamins in the study of vitamin B12 deficiency in primary degenerative dementia," *Dementia*, vol. 1, no. 5, pp. 272–277, 1990.

[4] P. Quadri, C. Fragiacomo, R. Pezzati et al., "Homocysteine, folate, and vitamin B-12 in mild cognitive impairment, Alzheimer disease, and vascular dementia," *The American Journal of Clinical Nutrition*, vol. 80, no. 1, pp. 114–122, 2004.

[5] N. G. Faux, K. A. Ellis, L. Porter et al., "Homocysteine, vitamin B12, and folic acid levels in Alzheimer's disease, mild cognitive impairment, and healthy elderly: Baseline characteristics in subjects of the australian imaging biomarker lifestyle study," *Journal of Alzheimer's Disease*, vol. 27, no. 4, pp. 909–922, 2011.

[6] F. Coppedè, P. Tannorella, I. Pezzini et al., "Folate, homocysteine, vitamin B12, and polymorphisms of genes participating in one-carbon metabolism in late-onset Alzheimer's disease patients and healthy controls," *Antioxidants and Redox Signaling*, vol. 17, no. 2, pp. 195–204, 2012.

[7] D. S. Wald, A. Kasturiratne, and M. Simmonds, "Serum homocysteine and dementia: Meta-analysis of eight cohort studies including 8669 participants," *Alzheimer's and Dementia*, vol. 7, no. 4, pp. 412–417, 2011.

[8] J.-M. Zhuo, H. Wang, and D. Praticò, "Is hyperhomocysteinemia an Alzheimer's disease (AD) risk factor, an AD marker, or neither?" *Trends in Pharmacological Sciences*, vol. 32, no. 9, pp. 562–571, 2011.

[9] F. van Dam and W. A. van Gool, "Hyperhomocysteinemia and Alzheimer's disease: a systematic review," *Archives of Gerontology and Geriatrics*, vol. 48, no. 3, pp. 425–430, 2009.

[10] M. Hinterberger and P. Fischer, "Folate and alzheimer: when time matters," *Journal of Neural Transmission*, vol. 120, no. 1, pp. 211–224, 2013.

[11] E. Moore, A. Mander, D. Ames, R. Carne, K. Sanders, and D. Watters, "Cognitive impairment and vitamin B12: a review," *International Psychogeriatrics*, vol. 24, no. 4, pp. 541–556, 2012.

[12] F. Cacciapuoti, "Lowering homocysteine levels with folic acid and B-vitamins do not reduce early atherosclerosis, but could interfere with cognitive decline and Alzheimer's disease," *Journal of Thrombosis and Thrombolysis*, vol. 36, no. 3, pp. 258–262, 2013.

[13] A. C. Naj, G. W. Beecham, E. R. Martin et al., "Dementia revealed: novel chromosome 6 locus for Late-onset alzheimer disease provides genetic evidence for folate-pathway abnormalities," *PLoS Genetics*, vol. 6, no. 9, Article ID e1001130, 2010.

[14] Y. Hua, H. Zhao, Y. Kong, and M. Ye, "Association between the *MTHFR* gene and Alzheimer's disease: a meta-analysis," *International Journal of Neuroscience*, vol. 121, no. 8, pp. 462–471, 2011.

[15] X. H. Bi, H. L. Zhao, Z. X. Zhang, and J. W. Zhang, "Association of *RFC1* A80G and *MTHFR* C677T polymorphisms with Alzheimer's disease," *Neurobiology of Aging*, vol. 30, no. 10, pp. 1601–1607, 2009.

[16] M. Kageyama, M. Hiraoka, and Y. Kagawa, "Relationship between genetic polymorphism, serum folate and homocysteine in Alzheimer's disease," *Asia-Pacific Journal of Public Health*, vol. 20, pp. 111–117, 2008.

[17] R. Zhao, L. H. Matherly, and I. D. Goldman, "Membrane transporters and folate homeostasis: intestinal absorption and transport into systemic compartments and tissues," *Expert Reviews in Molecular Medicine*, vol. 11, article e4, 2009.

[18] A. Chango, N. Emery-Fillon, G. P. De Courcy et al., "A polymorphism (80G>A) in the reduced folate carrier gene and its associations with folate status and homocysteinemia," *Molecular Genetics and Metabolism*, vol. 70, no. 4, pp. 310–315, 2000.

[19] V. Eklöf, B. Van Guelpen, J. Hultdin, I. Johansson, G. Hallmans, and R. Palmqvist, "The reduced folate carrier (*RFC1*) 80G>A and folate hydrolase 1 (*FOLH1*) 1561C>T polymorphisms and the risk of colorectal cancer: a nested case-referent study," *Scandinavian Journal of Clinical and Laboratory Investigation*, vol. 68, no. 5, pp. 393–401, 2008.

[20] A. Stanisławska-Sachadyn, L. E. Mitchell, J. V. Woodside et al., "The reduced folate carrier (*SLCI9A1*) c.80G>A polymorphism is associated with red cell folate concentrations among women," *Annals of Human Genetics*, vol. 73, no. 5, pp. 484–491, 2009.

[21] N. Mansoori, M. Tripathi, R. Alam et al., "Serum folic acid and *RFC* A80G polymorphism in Alzheimer's disease and Vascular Dementia," *The American Journal of Alzheimer's Disease & Other Dementias*, vol. 29, no. 1, pp. 38–44, 2014.

[22] M. Białecka, M. Kurzawski, A. Roszmann et al., "Association of *COMT, MTHFR*, and *SLC19A1* (*RFC-1*) polymorphisms with homocysteine blood levels and cognitive impairment in Parkinson's disease," *Pharmacogenetics and Genomics*, vol. 22, no. 10, pp. 716–724, 2012.

[23] The Dementia Study Group of the Italian Neurological Society, "Guidelines for the diagnosis of dementia and Alzheimer's disease," *Neurological Sciences*, vol. 21, pp. 187–194, 2000.

[24] American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders*, American Psychiatric Association, Washington, DC, USA, 4th edition, 1994.

[25] F. Coppedè, G. Marini, S. Bargagna et al., "Folate gene polymorphisms and the risk of Down syndrome pregnancies in young Italian women," *The American Journal of Medical Genetics*, vol. 140, no. 10, pp. 1083–1091, 2006.

[26] F. Coppedè, E. Grossi, M. Buscema, and L. Migliore, "Application of artificial neural networks to investigate one-carbon metabolism in Alzheimer's disease and healthy matched individuals," *PLoS ONE*, vol. 8, no. 8, Article ID e74012, 2013.

[27] N. Kumudini, A. Uma, S. M. Naushad, R. Mridula, R. Borgohain, and V. K. Kutala, "Association of seven functional polymorphisms of one-carbon metabolic pathway with total plasma homocysteine levels and susceptibility to Parkinson's disease among South Indians," *Neuroscience Letters*, vol. 568, pp. 1–5, 2014.

[28] S. S. Wang, C. Wang, F. Y. Qiao, J. J. Lv, and L. Feng, "Polymorphisms in genes RFC-1/CBS as maternal risk factors for Down syndrome in China," *Archives of Gynecology and Obstetrics*, vol. 288, no. 2, pp. 273–277, 2013.

[29] M. A. Costa-Lima, M. R. Amorim, and I. M. Orioli, "Association of methylenetetrahydrofolate reductase gene 677C>T polymorphism and Down syndrome," *Molecular Biology Reports*, vol. 40, no. 3, pp. 2115–2125, 2013.

[30] V. Dhillon, P. Thomas, and M. Fenech, "Effect of common polymorphisms in folate uptake and metabolism genes on frequency of micronucleated lymphocytes in a South Australian cohort," *Mutation Research*, vol. 665, no. 1-2, pp. 1–6, 2009.

[31] F. Coppedè, R. Colognato, A. Bonelli et al., "Polymorphisms in folate and homocysteine metabolizing genes and chromosome damage in mothers of Down syndrome children," *The American Journal of Medical Genetics A*, vol. 143, no. 17, pp. 2006–2015, 2007.

[32] C. Reitz, G. Jun, A. Naj et al., "Variants in the ATP-binding cassette transporter (*ABCA7*), apolipoprotein e ε4, and the risk of late-onset Alzheimer disease in African Americans," *Journal of the American Medical Association*, vol. 309, no. 14, pp. 1483–1492, 2013.

[33] M. Gallucci, A. Zanardo, M. Bendini, F. Di Paola, P. Boldrini, and E. Grossi, "Serum folate, homocysteine, brain Atrophy, and auto-CM system: the treviso dementia (TREDEM) Study," *Journal of Alzheimer's Disease*, vol. 38, no. 3, pp. 581–587, 2014.

[34] M. Hinterberger, S. Zehetmayer, S. Jungwirth et al., "High cortisol and low folate are the only routine blood tests predicting probable alzheimer's disease after age 75 - Results of the vienna transdanube aging study," *Journal of the American Geriatrics Society*, vol. 61, no. 4, pp. 648–651, 2013.

[35] Z. Hu, L. Wu, J. Jia, and Y. Han, "Advances in longitudinal studies of amnestic mild cognitive impairment and Alzheimer's disease based on multi-modal MRI techniques," *Neuroscience Bulletin*, vol. 30, no. 2, pp. 198–206, 2014.

[36] A. M. Devlin, R. Clarke, J. Birks, J. G. Evans, and C. H. Halsted, "Interactions among polymorphisms in folate-metabolizing genes and serum total homocysteine concentrations in a healthy elderly population," *The American Journal of Clinical Nutrition*, vol. 83, no. 3, pp. 708–713, 2006.