Relationship between *MUTYH*, *OGG1* and *BRCA1* mutations and mRNA expression in breast and ovarian cancer predisposition

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Abstract. The aetiology of breast and ovarian cancer (BC/OC) is multi-factorial. At present, the involvement of base excision repair (BER) glycosylases (MUTYH and OGG1) in BC/OC predisposition is controversial. The present study investigated whether germline mutation status and mRNA expression of two BER genes, *MUTHY* and *OGG1*, were correlated with *BRCA1* in 59 patients with BC/OC and 50 matched population controls. In addition, to evaluate the relationship between *MUTYH*, *OGG1* and *BRCA1*, their possible mutual modulation and correlation among mutational spectrum, gene expression and demographic characteristics were evaluated. The results identified 18 *MUTYH* and *OGG1* variants, of which 4 were novel (2 *MUTYH* and 2 *OGG1*) in 44 of the 59 patients. In addition, two pathogenic mutations were identified: *OGG1* p.Arg46Gln, detected in a patient with BC and a family history of cancer, and *MUTYH* p.Val234Gly in a patient with OC, also with a family history of cancer. A significant reduced transcript expression in *MUTYH* was observed (P=0.033) in cases, and in association with the presence of rare variants in the same gene (P=0.030). A significant correlation in the expression of the two BER genes was observed in cases (P=0.004), whereas OGG1 and BRCA1 was significantly correlated in cases (P=0.001) compared with controls (P=0.010). The results of the present study indicated that the relationship among mutational spectrum, gene expression and demographic characteristics may improve the genetic diagnosis and primary prevention of at-risk individuals belonging to families with reduced mRNA expression, regardless of mutation presence.

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Abbreviations: APC, adenomatous polyposis coli; BC, breast cancer; BC/OC, breast and/or ovarian cancer; BER, base excition repair; BOC, breast and ovarian cancer; CRC, colorectal carcinoma; DHPLC, denaturing high performance liquid chromatography; HGVS, Human Genome Variations Society; IQR, interquartile range; LOH, loss of heterozygosity; MAF, minor allele frequency; MAP, MUTYH-associated polyposis; MUTYH, mutY DNA glycosylase; OC, ovarian cancer; OGG1, 8-oxoguanine DNA glycosylase; PARP, poly-(ADP-ribose) polymerase; PBMD, peripheral blood mononuclear cells; RT-qPCR, reverse transcription-quantitative PCR; ROS, reactive oxygen species; VUS, variant of unknown clinical significance

Key words: breast cancer, ovarian cancer, prevention, mutation, gene expression, BRCA1, MUTYH, OGG1, base excision repair, oxidative stress

Introduction

Breast cancer (BC) is the second most common cancer worldwide (1), and the first cause of cancer death among women in across all age groups (1). The etiology of BC is multifactorial, and both endogenous and environmental factors are implicated in its pathogenesis (2).

The risk of BC and/or ovarian cancer (OC) is increased in carriers of deleterious mutations in *BRCA1* and *BRCA2*, these two high penetrance genes encode for proteins involved in DNA damage response and repair (3). Among endogenous factors, oxidative stress (OS) produces potentially mutagenic reactive oxygen species (ROS) and can play an important role in breast carcinogenesis (4); since breast tissue can be particularly exposed to OS due to estrogen metabolism and hormonal status (5-9). Considering that DNA damage may contribute to breast cancer development, an efficient repair of oxidative lesions is expected to protect mammary cells from neoplastic transformation. Among the genes involved in the response and repair of DNA damage, BRCA1 has been shown to have a decisive role, since it induces the gene expression of the antioxidant response and thus protecting cells from OS (10). In particular, BRCA1 stimulates the activity of key base excision repair (BER) enzymes, including 8-oxoguanine DNA glycosylase (OGG1), primarily by increasing

transcription of BER enzymes (10). The BER pathway is responsible for the repair of many DNA nucleobases modified, indeed its action is essential for the maintenance of genetic integrity and stability (11) and in playing a crucial role in repair of DNA damage induced by ROS (12). BER glycosylases may provide a genome surveillance mechanism and may act as molecular sensors that induce apoptosis in response to extensive DNA damage through interaction of complex pathways (13-15). A deficiency and/or inactivity of the BER DNA glycosylase enzymes can induce deleterious outcomes in the cells driving the onset of various tumors (11). In particular, OGG1 (MIM 601982) and MUTYH (MIM 604933) remove DNA oxidative purine lesions and seem involved in the regulation of cell-cycle progression and cell division under OS (16,17). In fact, low activity and/or reduced expression of MUTYH and OGG1 enzymes may result in DNA repair impairment and failure to induce apoptosis in response to oxidative damage, resulting in survival of cells with oncogenic mutations (12,18,19). In previous studies the contribution to BC risk due to *MUTYH* seems to be limited or not relevant, although in most researches only specific mutations of *MUTYH* were considered, which had previously been characterized in families with gastrointestinal polyposis (20-22). Also the *OGG1* contribution to BC risk has been significantly associated with the presence of some polymorphic nucleotide markers (SNPs) (21,23-25). However, these associations may have been influenced by the characteristics of the population examined or by the specificity of each germinal variant considered (21-24). To date, the contribution to BC or OC predisposition due to the reduced expression of these genes has not yet been considered in humans. Some doubts remain about the genetic susceptibility related to low penetrance genes and their expression, which could contribute to the onset of BC and/or OC (BC/OC) in people belonging to families with tumor phenotypes other than BC/OC (e.g. pancreas, thyroid and colon) or in patients with early onset of cancer and without family history (26). The patients with these characteristics and without germline mutations in high-penetrance BRCA1/2 genes might be a good model to clarify some aspects that contribute to the multifactorial etiology of BC and OC.

In light of the above, it can be assumed that genetic variability related to low expression in enzymes that protect cellular DNA from oxidative damage, causing genetic instability, may favor the onset of BC and OC.

In the present study we investigated *MUTYH*, *OGG1* germline mutations and mRNA expression levels, in the peripheral blood mononuclear cell (PBMC) from patients with and without mutations and compared the gene expression with control individuals. In addition, to evaluate the relationship of these BER glycosylases (MUTYH, OGG1) and BRCA1 pathway, we investigated the possible mutual modulation. This exploratory study on correlation among mutational spectrum, gene expression and demographic characteristics, could improve the genetic diagnosis performing predictive testing of at-risk individuals belonging to families with reduced mRNA expression regardless of the mutation presence. The identification of carriers with reduced mRNA expression may be useful for improving clinical management of patients.

Materials and methods

Patients and nucleic acid preparation. The study was conducted on a series of BC/OC unrelated Italian patients previously analyzed for BRCA1/2 as a public health service between 2000 and 2006 from Medical Genetic Service of University 'G. d'Annunzio' of Chieti (27,28). The study was performed after completion of the standardized routine diagnostic investigations. Familiar pedigrees of the cases were not updated during the course of the study and only the original pedigrees were considered. DNA samples were obtained from 59 patients and RNA from 51. We also analysed 120 consecutive population healthy blood donors. This population was used to assess the frequency of rare variants in gDNA analyses. RNA from 50 age and sex matched control individuals was employed to analyze gene expression and 16 of these women reported positive family history for cancer. All patients and control individuals provided written informed consent and the study was approved by the Ethics Committee of the University 'G. d'Annunzio' of Chieti.

Nucleic acid extraction from peripheral blood mononuclear cells (PBMCs) and synthesis of complementary DNA (cDNA) from 1.5 μg of total RNA were performed as previously described (29).

Sequence variants analysis. The coding sequence and intron-exon borders of *MUTYH* (GeneID: 4595; MIM 604933; Gene Bank accession number: NM _12222.1) and *OGG1 (*GeneID: 4968; MIM 601982; Gene Bank accession number: NM_016821) were analyzed in patients and controls by denaturing high performance liquid chromatography (DHPLC) after PCR amplification (Wave 1100, Transgenomic Inc.), followed by direct sequencing of samples showing unique profiles.

Primer sequences for *MUTYH* genes were based on those reported previously (29), whereas, primers for *OGG1* are listed in supplementary Table SI.

To estimate the frequency of novel mutations we examined 240 chromosomes from control individuals, from the same geographical area, with no personal history of BC/OC and colorectal cancer (CC). All mutations were confirmed by sequencing of independent PCRs. The nomenclature of sequence variants follows the guidelines proposed by the Human Genome Variation Society (HGVS).

ClinVar-NCBI database (30) was employed to evaluate expected clinical significance of sequence variants. This tool aggregates information about genomic variation and its relationship to human health (31). Furthermore, novel molecular alterations potentially causative of disease were tested by MutPred2 (http://mutpred.mutdb.org) that predict pathogenicity of amino acid substitutions (32).

Reverse transcription-quantitative PCR (RT-qPCR). The levels of *BRCA1*, *MUTYH* and *OGG1* mRNA expression in peripheral blood mononuclear cells were investigated by TaqMan RT-qPCR analysis using StepOne™ 2.0 (Applied Biosystems). mRNA amounts of the target genes (*BRCA1*, *MUTYH*, *OGG1*, #Hs01556193_m1, #Hs01014856_m1, #Hs00213454_m1, respectively, Applied Biosystems) were normalized to the endogenous housekeeping gene *GUSB*

(#Hs99999908_m1, Applied Biosystems) and analyzed as previously reported (29).

Statistical analysis. Continuous variables were summarized as mean and standard deviation (SD), or median and interquartile range (IQR). Categorical variables were summarized as frequency and percentages. The Student's t-test for unpaired data was performed to test the difference between the means of age at sampling in different groups. The Mann-Whitney U tests performed to evaluate differences in gene expression levels among groups (51 cases and 43 controls without cancer family history). To assess for a possible correlation between the three genes, Spearman's ϱ correlation coefficient was evaluated. All P-values are two-sided and a P-value of <0.05 was considered significant. All analyses were performed using SPSS (version 20) software.

Results

Germline mutational analysis. Coding regions and flanking introns of *MUTYH* and *OGG1* were analyzed for germline mutations in 59 cases (51-BC; 7-OC; 1-BOC). Forty-seven referred cancer family history, of these 29 showed direct transmission of BC/OC, and 12 were early onset BC/OC without family history. Cases carrying *BRCA1* deleterious mutations and neutral missense variants with minor allele frequency (MAF) <0.05 were previously published (27,28) and listed in Table SII.

Overall, germline mutational analysis identified 11 variants in *MUTYH* and 7 in *OGG1* in 44 patients, including 4 novel variants: 2 of *MUTYH* (p.Val234Gly and p.Val390Leu) and 2 of *OGG1* (p.Gln128Gln and p.Ala223Thr) (Table I).

In particular, *MUTYH* analysis identified the following variants: 6 missense (p.Pro18Leu, p.Val22Met, p.Gly25Asp, p.Val234Gly, p.Gln338His, p.Val390Leu), 4 located in the untranslated region (c.36+11C>T, c.157+30A>G, c.504+35A>G, c.1477-40C>G) and 1 synonymous (p.Thr477Thr) (Table I). The p.Val234Gly novel variant was predicted to be deleterious (MutPred2 score: 0.798; cut-off: 0.611) and occurred in an OC affected patient (B48) referring family history for this tumor (Table II).

Notably, 2 missense (Pro18Leu and Gly25Asp) mutations and 1 intronic variant (c.36+11C>T) were identified in the same case (B66), a patient affected by breast and thyroid cancer, who referred family history for BC and colon cancer (CC) (Table II). The *MUTYH* frequent coding SNP rs3219489 (p.Gln338His) was detected in 19 of the 59 patients (32%).

Analysis of *OGG1* identified the following variants (Table I): 1 novel (p.Ala223Thr) missense, not predicted to be deleterious (MutPred2 score: 0.095; cut-off: 0.61), 1 novel synonymous (p.Gln128Gln), 1 missense (p.Arg46Gln) previously reported as deleterious in ClinVar-NCBI, 3 missense previously reported as VUS in ClinVar-NCBI (p.Ala85Thr; Gly300Glu; Gly308Glu) and 1 frequent coding SNP rs.1052133 (p.Ser326Cys) of uncertain significance. The *OGG1* deleterious variant p.Arg46Gln, located in a highly conserved region, was previously demonstrated to cause splicing donor inactivation (33). This pathogenic variant was found in case B58 diagnosed with breast cancer. She referred family history for BC (sister) and leukaemia (brother) (Table II). Unfortunately, we could not verify segregation of this *OGG1* variant in OC affected siblings since DNA samples from these patients were not available. The three OC cases carrying novel variants reported family history of this tumor (Table II).

Additional *MUTYH* and *OGG1* variants considered neutral in ClinVar-NCBI are reported in Table I. The frequency of novel mutations and rare variants (MAF <0.01) of *MUTYH* and *OGG1* detected in patients was analyzed in 120 population controls (63 males and 57 females) from the same geographical area (mean age, 46.1±10.4 years) but none of them were detected (expected frequency CI 95%, 0-1.57%) (Table I).

Gene expression analysis. Gene expression by qRT-PCR was analyzed in 51 patients (mean age, 48.60±12.88) and in 50 age and sex matched control individuals (mean age, 48.11±7.35). Demographic and clinical characteristics of patients undergoing gene expression analysis are shown in Table III.

Forty-seven patients reported BC and 4 patients OC, 28 (54.9%) had a first-degree family member with BC/OC. Twenty-six patients (51%) at the time of sampling were in menopause and 13 (25.5%) were smokers (Table III).

We evaluated the association among *BRCA1*, *MUTYH* and *OGG1* mRNA expression levels, variant carrier status (MAF<0.05) and clinical characteristics.

The presence of *MUTYH* variants resulted associated with reduced transcript expression of the same gene [0.34 (0.29-0.59)] in carriers *vs*. [0.66 (0.46-2.38)] non-carriers (P=0.030). Incidentally, the most frequent SNPs rs3219489 (p.Gln338His) in *MUTYH* and rs1052133 in *OGG1* (p.Ser326Cys) with a Frequency in our population of 0.32 and 0.39 respectively, did not affect mRNA expression (data not shown) according to previous studies (33,34). The rare variants of *BRCA1*, reported in Table SII, seemed not affect significantly its own expression.

BC family history (either direct or indirect) did not influence the expression of the three genes; while the direct family history of cancer, other than BC, was significantly associated with the increased expression of *OGG1* [from 0.81 (0.27-2.90) to 3.58 (0.83-5.11)] (P=0.030). Considering the time lag from diagnosis to blood sampling, no significant association was observed between gene expression levels and this period of time.

Correlation of MUTYH, OGG1 and BRCA1 genes expression. The Mann-Whitney U tests was performed to compare differences in gene expression levels between groups of controls without family history of cancer (n=43) and cases (n=51). BER genes showed lower expression levels in cases [0.58 (0.32-1.72); 0.93 (0.48-4.36)] than controls without cancer family history [1.04 (0.50-1.88); 1.91 (0.82-3.09)]; in particular, this difference resulted significant for *MUTYH* (P=0.035). *BRCA1* showed a very low expression and it increased values in controls without cancer family history respect to cases similarly to the other two genes (Table IV). We correlated *MUTYH*, *OGG1* and *BRCA1* genes expression by Spearman's test (Table V).

The results indicated that *OGG1* and *BRCA1* gene expression positively correlated both in cases (P=0.001) and controls without family history (P=0.011).

Table I. *MUTYH* and *OGG1* germline variants identified in 59 patients with breast and ovarian cancer.

A, *MUTYH*

Nucleotide change(s)	Effect	SNP	Clinical significance[a]	Frequency n (%)		Word population MAF[b]	Mutpred value
				Cases (n=59)	Controls (n=120)		
c.36+11C>T	-	rs2275602	VUS	1 (1.7)	0 (0)	0.01	-
c.53C>T	Pro18Leu	rs79777494	VUS	1 (1.7)	0 (0)	<0.01	-
c.64G>A	Val22Met	rs3219484	N	2 (3.4)	-	0.02	-
c.74G>A	Gly25Asp	rs75321043	VUS	1 (1.7)	0 (0)	<0.01	-
c.157+30A>G	-	rs3219485	N	2 (3.4)	-	0.01	-
c.504+35A>G	-	rs3219487	N	8 (13.6)	-	0.06	-
c.701T>A	Val234Gly	-	D/Novel	1 (1.7)	0 (0)	-	0.798
c.1014 G>C	Gln338His	rs3219489	N	19 (32)	-	0.31	-
c.1171 G>T	Val390Leu	-	Novel	1 (1.7)	0 (0)	-	0.335
c.1431G>C	Thr477Thr	rs74318065	N	1 (1.7)	0 (0)	0.01	-
c.1477-40C>G	-	rs3219493	N	5 (8.5)	-	0.06	-

B, *OGG1*

Nucleotide change(s)	Effect	SNP	Clinical significance[a]	Frequency n (%)		Word population MAF[b]	Mutpred value
				Cases (n=59)	Controls (n=120)		
c.137 G>A	Arg46Gln	rs104893751	D	1 (1.7)	0 (0)	<0.01	-
c.253G>A	Ala85Thr	rs17050550	VUS	1 (1.7)	0 (0)	<0.01	-
c.384 G>A	Gln128Gln	-	Novel	1 (1.7)	0 (0)	-	-
c.667G>A	Ala223Thr	-	Novel	1 (1.7)	0 (0)	-	0.095
c.899G>A	Gly300Glu	rs548981683	VUS	1 (1.7)	0 (0)	<0.01	-
c.923 G>A	Gly308Glu	rs113561019	VUS	1 (1.7)	0 (0)	<0.01	-
c.977 C>G	Ser326Cys	rs1052133	N	23 (39)	-	0.30	-

[a]Results based on the ClinVar-NCBI database. [b]Results based on Ensembl genome browser 9. The pathogenicity of novel missense mutations was predicted using MutPred2 with a cut-off value of 0.61. D, deleterious; N, most likely neutral; VUS, variant of unknown clinical significance; SNP, single polymorphic nucleotide; MAF, minor allele frequency.

Interestingly, *MUTYH* and *OGG1* gene expression did not shown any significant correlation in controls but a positive and significant correlation is reported in cases (Rho=0.406, P=0.004).

In summary, we observed a significant and positive correlation between gene expression of *OGG1* and *BRCA1* both in cases and controls while BER genes showed a significant correlation only in cases.

Discussion

In this study we aimed to evaluate the involvement of two BER glycosylases (OGG1 and MUTYH) in the predisposition to breast and OC. We also investigated the correlation among *BRCA1* and these BER genes expression, in the contest of the BC and OC predisposition. In this regard we evaluated a retrospective series, previously selected for genetic analysis, of the main breast and OC predisposing genes: *BRCA1* and *BRCA2* (27,28).

Since BRCA1 is involved in oxidative stress regulation and BER after oxidative damage (35), we evaluated germline mutations status and gene expression of the *MUTHY* and *OGG1* associated to clinical characteristics of 59 BC/OC cases and to *BRCA1* gene expression.

In this series we identified: 4 novel variants and one of them (in *MUTYH*) is predicted deleterious; one known deleterious mutation in *OGG1*; 6 VUS (3 in *MUTYH* and 3 in *OGG1*) (Table I). The *OGG1* deleterious mutation, c.137 G>A, causing a substitution from basic to acidic amino acid (p.Arg46Gln), has never been reported in BC/OC patients. While, it was previously described as deleterious germline variant in non-polyposis hereditary colorectal cancer (HNPCC) with stable microsatellites (MSS) (33,36). In our study we found this mutation in a case (B58) that referred

Table II. Clinical and molecular characteristics of cases carrying rare *MUTYH* and *OGG1* variants.

Case code	Age range at diagnosis (years)	*MUTYH* variant	*OGG1* variant	Cancer type	Direct BC/OC transmission	Family history
B12	30-35	-	Gly197Glu	BC	No	-
B17	25-30	-	Gly300Glu	BC	No	-
B28	40-45	-	**Gln128Gln**	BC	No	-
B48	40-45	**Val234Gly**[b]	-	OC	No	OC
B58	40-45	-	Arg46Gln[a]	BC	Yes	BC, Leu
B62	30-35	**Val390Leu**	-	OC	Yes	OC
B66	55-60	c.36 + 11 C>T Pro18Leu Gly25Asp	-	BC/TC	No	BC, CC
B75	45-50	-	Ala85Thr	BC	No	BC, OC
B105[c]	45-50	-	**Ala223Thr**	BC/OC	Yes	OC

[a]Pathogenic variants. [b]Putative pathogenic variants predicted by MutPred2 software; [c]BRCA1 mutation carrier (Glu1373Ter). Novel variants are in bold. BC, breast cancer; OC, ovarian cancer; TC, thyroid cancer; CC, colon cancer; Leu, leukemia.

Table III. Demographic characteristics of cases employed for gene expression analysis (n=51).

Variable	Value
Age at diagnosis, years (mean±SD)	45.2±11.9
Age at sampling, years (mean±SD)	48.6±12.9
Menopause, n (%)	26 (51.0)
Cigarette smoking, n (%)	13 (25.5)
Type of cancer, n (%)	
BC	47 (92.2)
OC	4 (7.8)
Family history n (%)	
BC/OC	
Direct	28 (54.9)
Indirect	10 (19.6)
Other cancers	
Direct	16 (31.4)
Indirect	5 (9.8)

BC, breast cancer; OC, ovarian cancer.

BC (age range, 40-45 years), BC direct transmission, bilateral ovariectomy and a brother died for leukemia.

It is interesting to note that the three novel missense mutations were found in three out of eight OC/BOC patients with OC family history (Table II); furthermore, one of these patients (B105), carrying a truncating *BRCA1* mutation (E1373X), was also affected by BC. It is possible that, in this case, two defects simultaneously contributed to the onset and progression of the tumor in the breast tissue, although the truncating *BRCA1* mutation doesn't affect gene expression levels in PBMCs, but its expression value falls within the BRCA1 median of the cases. The precise mechanisms that govern mutant allele penetrance depend on many factors, including personal and/or reproductive history, mutation location, and actually undefined genetic factors ('modifier genes') (26).

The BC, as extra-colic manifestation, occurred in 18% of female patients affected by MUTYH-associated polyposis (MAP) (20). To support these evidences, the *MUTYH* knock-out mice are prone to develop mammary tumors (37). We did not observe *MUTYH* mutations related to MAP, while we found 2 missenses and 1 untranslated variant (p.Pro18Leu, p.Gly25Asp, c.36+11C>T) in a patient (B66) affected by BC/TC, with family history for BC and polyposis (Table II). The Pro18Leu and Gly25Asp missense mutations were previously reported as occurring in the same *MUTYH* allele (38).

The presence of *MUTYH* rare variants resulted associated with reduced transcript expression of the same gene in carriers vs. non carriers (P=0.030), whereas the most frequent SNPs rs3219489 in *MUTYH* and rs1052133 in *OGG1* did not affect mRNA expression. From this study a new assumption emerges that in BC/OC subjects, the *MUTYH* rare variants exert a gene pressure on the reduction of *MUTYH* expression, as already reported in MAP cases carrying *MUTYH* mutations (33).

The time lag between diagnosis of cancer and sampling, menopausal status, and cigarette smoking did not influence the median expression of *BRCA1* and *MUTYH* whereas *OGG1* expression displayed a significant rise (P=0.030) in the cases presenting a direct family history for tumor different than BC.

It is shown that OGG1 is involved in the acute and systemic inflammatory response that may favor carcinogenesis (39-41). *OGG1* expression showed an increase in post-menopausal women suggesting that the physiological menopause-related decrease of estrogens may increase *OGG1* expression in PBMC; this relation was already found in other female tissues (42).

These data, obtained from the mRNA analysis on PBMC, are very interesting and deserves further studies also on the MUTYH role in the sphere of immune functions, since the loss of this gene appears to be associated with immunosuppression

Table IV. Comparison of median gene expression between cases and controls without a family history of cancer.

Variable	Cases (n=51)	Controls without a family history of cancer (n=43)	Mann-Whitney P-value
Age at sampling, mean±SD	48.60±12.88	50.09±7.49	0.159[a]
MUTYH, median (IQR)	0.58 (0.32-1.72)	1.04 (0.50-1.88)	0.035
OGG1, median (IQR)	0.93 (0.48-4.36)	1.91 (0.82-3.09)	0.358
BRCA1, median (IQR)	0.09 (0.03-0.32)	0.14 (0.07-0.21)	0.297

[a]Student's t test for unpaired data. IQR, interquartile range.

Table V. Correlation among *MUTYH, OGG1* and *BRCA1* gene expression.

	OGG1		*BRCA1*	
Group	Rho di Spearman	P-value	Rho di Spearman	P-value
Cases	0.406	0.004		
MUTYH			0.186	0.200
OGG1			0.471	0.001
Controls without a family history of cancer	0.036	0.840		
MUTYH			0.164	0.361
OGG1			0.445	0.011

and impairment of the inflammatory response (43). We observed that the expression of *MUTYH* and *OGG1* showed significant correlation only in PBMC from cases (P=0.004) (Table V). This correlation has been verified in cell lines derived from various tissues, stressed with hydrogen peroxide (our laboratory data not shown), confirming that alterations in the redox balance and BER function are involved in the promotion and progression of cancer (12), although inter-individual differences in the oxidative stress regulation can explain a part of the variability in cancer susceptibility.

In this study the expression of *OGG1* always correlated with that of *BRCA1* both in cases and population controls without cancer family history (P=0.001, P=0.011 (Table V). This data agrees with evidences that BRCA1 have a role in the regulation of OS (35,44) and suggests a possible crosstalk between BRCA1 and OGG1. Consistent with these findings, Saha T. and colleagues (10) in 2010 found that BRCA1 over-expression increases the enzymatic activities related to the BER pathway while its under-expression decreases them. These finding prompt the thought that BRCA1 might exert its tumor suppressive function through oxidative stress regulation. In the context of the BC and OC predisposition BRCA1 showed low expression in cases, although it increased in controls with no family history of cancer, as also observed for BER genes. This feature has not yet been adequately explored and deserves further study. The aspects that relate BRCA1 and BER molecules, in response to ROS and carcinogenesis, are also supported by the fact that the *BRCA1* Loss of Heterozygosity (LOH) induces an increase of 8-oxoG levels (35). Since, BRCA1 wild-type promotes 8-oxoG lesions repair via transcriptional regulation of BER. This mechanism was exploited in the therapeutic block of PARP in patients harboring BRCA1 mutations (35).

Wild-type BRCA1 expression suppresses basal and H_2O_2-induced ROS production in breast and ovarian cell models (10,44). Endogenous factors may play a role as well salient in promoting the effects of oxidative stress on breast and ovarian carcinogenesis. Indeed, some studies have shown a significantly higher level of oxidative DNA damage in normal breast tissues derived from cancer patients (45). Non-physiological OS can be decisive in the pathogenesis of cancer, in fact, it is known to induce phenotypic modifications of cancer cells through cross-talk with the surrounding stroma (46). For these reasons, individuals predisposed to BC/OC can undergo a high rate of mutations oxidative stress-related due to the deficiency on systems to repair DNA damage. Furthermore, basic metabolic changes could produce an increase in potentially reactive oxygen species, in tissues like breast and ovary, which already have a physiological exposure to oxidative stress due to specific hormonal metabolism (9) and or inflammation (39). The roles of BER go beyond maintaining DNA integrity, as they are also implicated in the metabolism regulation (47).

This study highlights that germline *MUTYH*/*OGG1* transcript levels may reflect physiological and pathological changes that induce a different status in patients and in controls with and without cancer family history. This is the first study that evaluated germline mutations and expression of *MUTYH* and *OGG1* genes in BC/OC patients in relationship to *BRCA1*, investigating their reciprocal modulation. From this exploratory study emerged interesting and significant correlations among these three genes related to cancer predisposition.

Relationship among mutational spectrum, gene expression and demographic characteristics, could improve the genetic diagnosis performing predictive testing of at-risk individuals belonging to families with reduced mRNA expression regardless of presence of mutation. Finally, an accurate evaluation of the reduced expression of *MUTYH* and *OGG1* genes in PBMCs could represent a useful way to monitor primary prevention and clinical management of cancer-free patients.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

CM designed the present study, performed mutation screening, RT-qPCR analyses and wrote the manuscript. MDN performed statistical calculations. SV prepared samples and performed genetic analysis. AC and MCC critically reviewed the manuscript. LS, EC, PDG and PB collected and evaluated the clinical data. GMA designed and coordinated the present study, reviewed all genetic and clinical data and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All participants provided their written informed consent after verbal counselling. The study was approved by the Ethics Committee of the University 'G. d'Annunzio' of Chieti.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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