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EPIDEMIOLOGY

MDM2 SNP309 accelerates breast and ovarian carcinogenesis in BRCA1 and BRCA2 carriers of Jewish–Ashkenazi descent

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Abstract A functional single nucleotide polymorphism in the promoter of the MDM2 gene, SNP309 (T>G), was recently found to accelerate tumorigenesis in early onset cancer cases. The SNP309 G-allele, introduces an SP1 site in the MDM2 promoter, resulting in enhanced MDM2 expression and activity. Thus, the G-allele of MDM2 SNP309 may represent a cancer predisposing allele. In this report, we assessed the role of SNP309 as a modifier of mutant BRCA1/BRCA2 alleles in inherited breast and ovarian cancer cases among Ashkenazi–Jewish (AJ) women. We genotyped several subsets of AJ women: 138 healthy women, 140 affected BRCA1/2 mutation carriers, 120 asymptomatic BRCA1/2 mutation carriers and 187 sporadic breast cancer patients. The frequency of GG genotype of SNP309 was similar among the different groups. Interestingly, we found almost three times higher frequency of the GG genotype among BRCA1/2 carriers diagnosed with breast and/or ovarian cancer at or under the age of 51 years compared with carriers diagnosed with cancer above the age of 51 years (allele frequency, $P = 0.019$). The GG genotype was significantly associated with breast and ovarian cancer risk among BRCA1/2 carriers diagnosed before 51 years of age (OR, 3.93; 95% CI, 1.41–10.90, $P = 0.009$). No significant difference in frequency of the GG genotype was observed between early and late onset non-carrier cancer patients and no association with risk, OR, 1.30; 95% CI 0.69–2.47, $P = 0.419$). These data suggest that MDM2 SNP309 acts as a modifier of mutant BRCA1/2 mutant alleles in AJ and may accelerate breast and ovarian carcinogenesis in genetically predisposed individuals.

Keywords MDM2 snp309 · BRCA1 · BRCA2 · Breast and ovarian cancers · Cancer risk · Jewish–Ashkenazi population

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Introduction

Germline mutations in the *BRCA1* (MIM #113705) and *BRCA2* (MIM #600185) genes account for a substantial portion of familial breast and ovarian cancer cases. *BRCA1* and *BRCA2*-associated tumors are characteristic in their early age of onset with aggressive profile [1]. The cumulative lifetime risk of *BRCA1/BRCA2* carriers for developing cancer is clearly elevated above the average risk population: up to 80% of developing breast cancer [2] and up to 54% of developing ovarian cancer [2]. In addition to incomplete penetrance, the age of onset of first malignancy varies even among carriers of identical mutations [3]. Thus, additional genetic or environmental factors may play a role in modifying breast and ovarian risk in *BRCA1/BRCA2* mutation carriers' tumorigenesis and account for the differences.

One of the goals of individualized medicine is to more accurately predict individual's risk for being diagnosed with cancer and identify means to favorably alter that risk by tailoring early detection and prevention schemes. Defining the precise modifier genes that affect cancer risk in *BRCA1/ BRCA2* carriers has obvious clinical implications To date, except for the ovarian cancer region within *BRCA2* (OCCR), the penetrance of the different mutations within the *BRCA1/2* genes has not been documented. It is conceivable that variations in additional genes will affect the penetrance of *BRCA1* and *BRCA2*. Several examples have been reported: a polymorphism in the 5′ UTR of the *RAD51* gene (135 GC, rs1801320) increases the risk for breast cancer, among *BRCA1/2* carriers [4–6]. The length of the polyglutamine repeat in the *AIB1* gene is associated with younger age at breast cancer diagnosis among Ashkenazi-Jewish and non Jewish *BRCA1* carriers [7, 8] and recently conflicting results were reported on the additional risk conferred by the functional snp in the *AURKA* gene, F31I, to *BRCA1/2* carriers. A small study found an increased breast cancer risk in *BRCA1/2* carriers [9], while a larger study did not detect any association with a modified risk of breast cancer in *BRCA1* and *BRCA2* carriers [10].

The *BRCA1* and *BRCA2* genes encode for large multifunctional proteins involved in DNA damage repair, cell cycle regulation and overall maintenance of genome stability [11]. Phenotypic similarities as embryonic lethality and gross chromosomal rearrangements between *Brca1*- and *Brca2*-deficient murine models, imply that the two gene products function in a common pathway necessary for maintenance of genomic stability [12, 13].

The tumor suppressor p53 has a pivotal role in cells decision to live or die. *p53* deficiency eliminates the cell cycle arrest and the ability of cells to undergo apoptosis in response to DNA damage. Thus, p53 deficiency renders cells hypersensitive to DNA damage, and allow for cell survival and accumulation of genomic instability [14]. Thus, cooperation between the BRCA1/2 and p53 pathways in tumor suppression has been suggested [13]. Mutations in the *p53* gene are highly frequent in breast cancer as in many types of cancers. It has been noted that *BRCA1*-tumors have higher frequency of *p53* mutations than sporadic breast cancer [15, 16]. Moreover, deficiencies in both pathways seem to enhance tumor development. Mutant p53 rescued the embryonic lethality of *Brca1* as well as *Brca2* knockout mice [12] and in a different model, p53 deficiency accelerated mammary tumor formation in *Brca1* conditional mutant mice [17]. Thus, it is plausible that the p53 pathway modulate BRCA1-related tumorigenesis.

MDM2 is a negative regulator of p53 and attenuates the p53 tumor suppression pathway [18]. Recently, Bond et al. [19] reported that the G-allele of SNP309 at the promoter of the MDM2 gene, introduces an SP1 binding site, which results in increased MDM2 transcript and protein [19]. The original report as well as subsequent reports described that the presence of the GG genotype influences the age of diagnosis and tumor formation in Li-Fraumeni syndrome and in variety of additional cancers including soft tissue sarcoma, endometrial cancer, gastric cancer, renal cell carcinoma and hepatocellular cancer [20–23]. Conflicting results were published in regard to risk association of SNP309 with breast cancer. While some studies found no association between SNP309 and cancer risk, others reported earlier age of onset among the GG genotype carriers [24–27].

Taken together, the present study aims to investigate whether SNP309 modulates the age at diagnosis of breast and ovarian tumors in *BRCA1* and *BRCA2* mutation carriers as well as in non-carrier breast cancer patients among AJ women. The population in the study is unique in that the *BRCA1/BRCA2* mutation spectrum is limited to three predominant mutations: 185delAG and 5382insC in the *BRCA1* gene and 6174delT in the *BRCA2* gene. Moreover, the haplotypes associated with these mutations are limited [28–30] and recently we reported that the MDM2 haplotype associated with GG genotype among AJ show little variability [31].

Materials and methods

Study population

All study participants were of Ashkenazi–Jewish (AJ) origin consulted and tested at the Oncogenetics unit or at the Genetic Institute of the Sheba Medical Center in Tel-Hashomer, Israel. Three populations were studied: (1) *BRCA1/2* mutation carriers, (2) non-carriers breast cancer patients and (3) control group without cancer. The *BRCA1/2* carriers group ($n = 260$) is further divided into two subgroups of women who developed breast and/or ovarian cancer ($n = 140$) and unaffected women ($n = 120$). Carriers who underwent preventive surgeries were removed from the study. The distribution of mutations within the carrier groups is as follow: 183 women are carriers of mutations in the *BRCA1* gene (140 carriers of 185delAG, 42 carriers of 5382insC) and 74 women are carriers of the mutation 6174delT in the *BRCA2* gene. Of the 140 *BRCA1/2* carrier cases: 93 were affected with breast cancer, 33 with ovarian cancer and 14 had both cancers.

A group of consecutive non-carrier breast cancer patients ($n = 187$) were identified through the Oncology Department of the Sheba Medical Center between the years 2001–2004. Patients were genotyped for the presence of

predominant *BRCA1/2* mutations at the Shaare Tsedek Medical Center in Jerusalem, Israel and all were found to be non-mutation carriers.

The Control group ($n = 138$) consisted of cancer-free females who tested at the Genetic institute of the Sheba Medical Center for parental diagnostic tests. The median age of this group was significantly younger than the other two groups and cancer expectancy is considered as in the general Jewish–Ashkenazi population. All participants signed an informed consent approved by the Institutional Review Board and by the Israeli National Genetic Review Board. Characteristics of patients and controls in the different study groups are shown in Table 1.

Genotyping

Genomic DNA was extracted using the PUREGENE DNA isolation kit (Gentra Systems) according to manufacturer instructions. SNP 309 (rs2279744) was described previously [19]. SNP genotyping was performed in 384-well microplates with a high-throughput system of chip-based mass spectrometry (MALDI-TOF) using the MassARRAY by Sequenom™ Inc. (La-Jolla, CA) as described previously [31]. For determination of allele-specific primer products, PCR primers were tagged with 5′-ACGTTG-GATG-3′ at the 5′ end to avoid interference with the mass spectra. The primer sequences were as follows: forward: 5′-AGTTCAGGGTAAAGGTCACG-3′ and reverse: 5′-TCACACTAGTGACCCGACAG-3′. Amplification of 2.5 ng of cDNA was performed in 2.75 mM $MgCl_2$, 200 μM dNTP, 0.1 U HotStart *Taq* DNA polymerase (Qiagen) and 1 pmol of each forward and reverse PCR primers in 5 μl total volume. PCR conditions: 95°C 15 min, followed by 45 cycles of 95°C for 30 s, 56°C for 1 min, then 72°C for 1:30 min, with an extension at 72°C for 7 min. Products were then treated with 0.04 U shrimp alkaline phosphatase, SAP (Sequenom) followed by extension cycle, to which 1.2 μM final concentration of extension primer 5′-GGGCTGCGGGGCCGCT-3′ and 0.6 U of ThermoSequenase (Sequenom) were added to a total reaction of 9 μl with the termination mixture. The extension conditions include a 94°C 2 min with 75 cycles of the following: 94°C for 5 s, 52°C for 5 s and 72°C for 5 s. Quality control and quality assurance were provided by randomly including non-DNA containing wells in the chip as well as re-genotyping about 10% of samples on different chips. Reproducibly was determined as 97%.

Statistical analysis

Difference in allele frequencies between younger and older *BRCA1/2* carrier's cancer patients and non-carrier cancer patients were calculated by χ^2-square analysis. To evaluate specific genotype associated risk, age-adjusted odds ratio (OR) with corresponding 95% confidence intervals (95% CI) and P values were calculated. All statistical analyses were done using SPSS software.

Results

The genotype distribution of the rs2279744 in the entire population sample set did not deviate from the Hardy–Weinberg equilibrium ($P = 0.34$). However, the frequency of the G allele in the AJ population is significantly different from the frequency originally published for mixed North American population [19]. While the G allele is the minor allele in the North American population with frequency of 0.34, in the Jewish–Ashkenazi population, the G allele is the major allele with frequency of 0.54. The distribution of genotypes among the populations of healthy individuals ($n = 138$), breast cancer cases ($n = 187$) and BRCA1/2 mutation carriers ($n = 260$) is shown in Table 2. In all the groups the genotype distribution did not deviate from

Table 1 Characteristics of case and control subjects

Variable	Healthy controls ($n = 138$)	Breast cancer cases ($n = 187$)	BRCA1/2 healthy carriers ($n = 120$)	BRCA1/2 carriers with breast/ovarian cancer ($n = 140$)
Date of birth				
(Mean year ± SD)	1968 ± 7.05	1948 ± 11.4	1957 ± 10.6	1949 ± 11.3
Range	1948–1979	1922–1976	1914–1976	1920–1971
Current age				
Mean	37.2	57.5	49	57
Median	35	58	48.5	56
Age of detection				
Mean	Not applicable	54.3	Not applicable	45.9
Median		54		44.5

Hardy–Weinberg equilibrium (Table 2) and we did not find any significant differences in genotype or allele distribution among the different study groups.

Next, we determined the frequency of the *MDM2* SNP309 within the *BRCA1/2* carriers cohort, stratified by type of mutation and disease occurrence (Table 3). As expected, the frequency of the three common mutations in *BRCA1* and *BRCA2* genes: 185delAG, 5382insC and 6174delT is equal among cancer patients and healthy carriers (Table 3). The *MDM2* SNP309 genotype distribution among the *BRCA1* cancer patients and healthy carriers did not vary significantly. However, we noticed that the GG genotype is two times more prevalent in healthy *BRCA2* carriers than in cancer-diagnosed *BRCA2* carriers. (Table 3).

To determine whether the GG genotype is associated with early onset breast/ovarian cancer in *BRCA1/2* carriers, we compared the frequency of the MDM2 SNP309 genotype in symptomatic *BRCA1/2* carriers diagnosed with cancer at or before age 51 old with the frequencies of the SNP309 genotype in women diagnosed with cancer older than 51 years. Age 51 years was selected as our cut-off because it is considered as the average age of menopause and was shown previously to be the age discriminator in B-cell lymphomas, sarcomas and invasive ductal breast carcinoma [19]. Seventy percent (98/140) of breast and ovarian cancer cases in the *BRCA1/2* carriers group were diagnosed before age of 51 years, while only 33.7% (63/187) of sporadic breast cancer cases were diagnosed before that age. These expected results reflect the genetic predisposition and the predicted early age at diagnosis of cancer among *BRCA1/2* carriers.

We found that the frequency of the GG genotype is as much as 2.9 times higher in the group of *BRCA1/2* carriers diagnosed with cancer at or before 51 years (35.5%), than in the group of symptomatic *BRCA1/2* carriers with late onset breast cancer, older than 51 years old (11.9%) ($P = 0.019$) (Fig. 1a). Among the early onset cancer patients 35 were GG (35.5%) 47 were GT (49%) and 16 were TT (16.3%), while among cancer patients diagnosed after the age of 51 years old 5 were GG (11.9%) 27 were GT (64.3%) and 10 were TT (23.8%). The overall OR for young *BRCA1/2* patients with the GG genotype was 3.93 (95% CI 1.41–10.90, $P = 0.009$).

A similar analysis was carried out for breast cancer patients who are non-mutation carriers. No differences in genotype frequency and distribution by age at diagnosis using 51 years as a cut-off were noted in these cases: 63 of sporadic breast cancer patients who were diagnosed by age 51 years and 124 patients were diagnosed after that age. Among the early onset cancer patients 23 were GG (36%) 28 were GT (44%) and 12 were TT (19%). Similar distribution was observed in cancer patients diagnosed

Table 2 Distribution of MDM2 SNP309 in cases and controls

	Healthy controls (n = 138)			BRCA1 and BRCA2 carriers (n = 260)						Breast cancer cases (n = 187)		
				BRCA1/2 cancer cases (n = 140)			BRCA1/2 healthy carriers (n = 120)					
Deviation from HWE	No, P = 0.85			No, P = 0.29			No, P = 0.73			No, P = 0.06		
Genotype	GG	GT	TT	GG	GT	TT	GG	GT	TT	GG	GT	TT
Individuals (%)	40 (29)	68 (49)	30 (22)	39 (28)	74 (53.6)	26 (17)	34 (28.3)	58 (48.3)	28 (23.3)	61 (33)	77 (41)	49 (26)
Allele frequency (G;T)	(0.54; 0.46)			(0.55;0.45) (P = 0.803)			(0.53;0.48) (P = 0.798)			(0.53;0.47) (P = 0.916)		

Table 3 MDM2 SNP309 genotype among BRCA1 and BRCA2 carriers

BRCA1/2	*BRCA1/2* carriers cancer patients (n = 140*)			Healthy *BRCA1/2* carriers (n = 120**)		
	MDM2					
	GG (%)	GT (%)	TT (%)	GG (%)	GT (%)	TT (%)
185delAG	25 (33)	39 (52)	11 (15)	19 (30)	30 (48)	14 (22)
5382insC	9 (36)	9 (36)	7 (28)	6 (35)	7 (41)	4 (24)
6174delT	4 (11)	25 (69)	7 (20)	8 (22)	19 (52)	9 (23)

*One women carried two mutations: one in BRCA1 (185delAG) and one in BRCA2 (6174delT)

**One women carried two mutations in BRCA1 (185delAG + 5382insC)

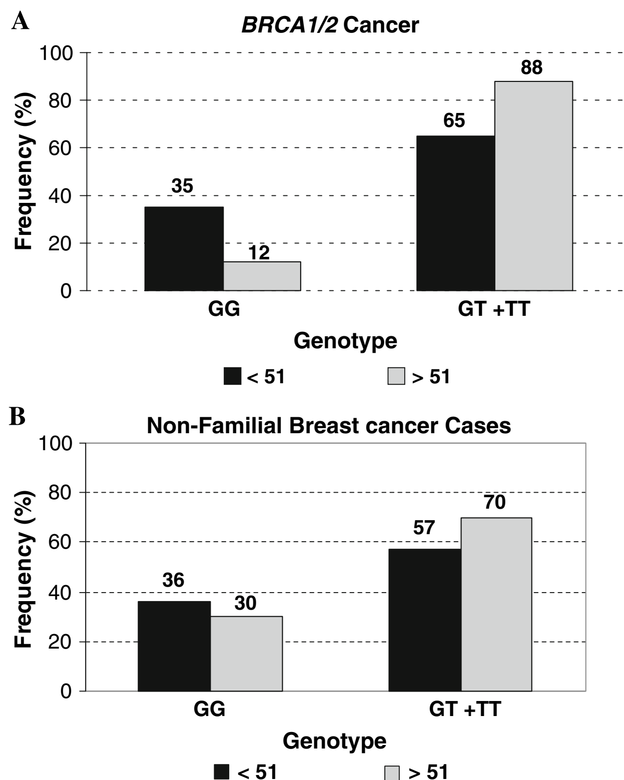

Fig. 1 MDM2 SNP309 genotype frequencies in early and late onset of *BRCA1/2* and non-familial cancer cases. (**a**) MDM2 SNP309 genotype frequencies in early and late onset of *BRCA1/2* cancer cases. Frequencies of SNP309 genotypes were calculated in *BRCA1* and *BRCA2* carriers according to age at diagnosis of breast and ovarian cancers before (dark bar) and after (light bar) age of 51 years old representing early and late onset. Chi-square analysis of genotypes indicate significant differences in allelic distribution in early and late onset cases ($P = 0.0083$). (**b**) MDM2 SNP309 genotype frequencies in early and late onset of non-familial breast cancer cases. Frequencies of SNP309 genotypes were calculated in consecutive breast cancer patients that do not carry the three predominant mutations in the Jewish–Ashkenazi population. Frequencies were calculated according to age at diagnosis of breast cancer before (dark bar) and after (light bar) age of 51 years old representing early and late onset. Chi-square analyses of genotypes indicate no significant differences in allelic distribution in early and late onset cases ($P = 0.127$)

after the age of 51 years old where 38 were GG (31%) 49 were GT (40%) and 37 were TT (29%) (Fig. 1b). The overall OR for young breast cancer patients without *BRCA1/2* mutations with the GG genotype was 1.30 (95% CI 0.69–2.47, $P = 0.419$).

To determine whether the effect of SNP309 on age at diagnosis is associated with breast cancer or ovarian cancer, we analyzed the SNP309 genotype in carriers according to their cancer type and age at diagnosis. Patients who were diagnosed with both breast and ovarian cancer were analyzed according to age at detection of the first malignancy. The numbers for ovarian cancer were too small ($n = 35$) and hence this analysis was omitted (data not shown). We found that the GG genotype is 2.8 times more frequent in early onset breast cancer patients among *BRCA1/2* carriers than in older *BRCA1/2* carriers' breast cancer patients ($P = 0.02$). The overall OR for young *BRCA1/2* breast cancer patients with the GG genotype was 3.83 (95% CI 1.05–13.94, $P = 0.024$) (Fig. 2a).

Additional analysis for assessing the age-at diagnosis effect of the MDM2 SNP309 on breast cancer by gene (*BRCA1* Vs. *BRCA2*) was carried out. This analysis showed that the GG genotype was more prevalent in early onset *BRCA1* carriers compared with late-onset cases ($P = 0.04$). Among early onset cases *BRCA1* carriers, 30 were GG (40.5%) 33 were GT (44.5%) 11 were TT (15%) while 5 were GG (18%) 16 were GT (57%) and 7 were TT (25%) among late onset Fig. 2b). The overall OR for young *BRCA1* cancer patients with the GG genotype was 3.07 (95% CI 1.05–8.96, $P = 0.04$).

The number of *BRCA2*–related cancer cases in our cohort is small ($n = 37$) thus, a larger cohort of *BRCA2* tumors is required to determine whether MDM2 SNP309 modifies the risk already conferred by *BRCA2*.

Discussion

The GG genotype of the functional MDM2 SNP309 was recently found as associated with accelerated tumorigenesis

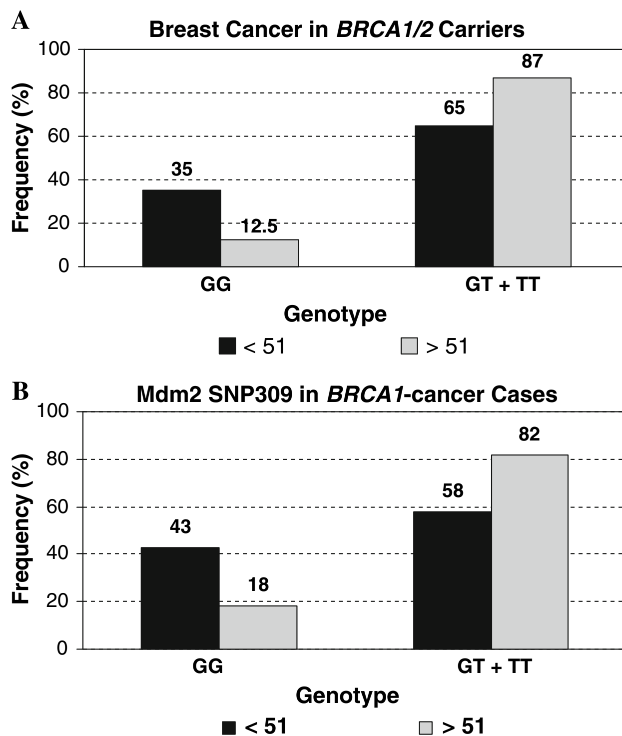

Fig. 2 The GG genotype at MDM2 SNP309 associates with early onset breast cancer in BRCA1 carriers. (**a**) MDM2 SNP309 genotype frequencies in early and late onset of *BRCA1* cancer cases. Frequencies of SNP309 genotypes were calculated in *BRCA1* carriers according to age at diagnosis of breast and ovarian cancers before (dark bar) and after (light bar) age of 51 years old representing early and late onset. Chi-square analyses of genotypes indicate significant differences in allelic distribution in early and late onset cases ($P = 0.026$). (**b**) MDM2 SNP309 genotype frequencies in early and late onset of breast cancer cases in *BRCA1/2* carriers. Frequencies of SNP309 genotypes were calculated in *BRCA1/2* carriers according to age at diagnosis of breast and ovarian cancers before (dark bars) and after (light bars) age of 51 years old representing early and late onset. Chi-square analysis of genotypes indicates significant differences in allelic distribution in early and late onset cases ($P = 0.019$)

in individuals diagnosed with the Li-Fraumeni syndrome [19]. This genotype was also associated with earlier age at diagnosis in sporadic soft-tissue sarcoma and B-cell lymphoma patients [19]. In the present study, a statistically significant difference in the frequency of the GG genotype between *BRCA1/2* mutation carriers diagnosed with cancer at or before age 51 years compared with mutation carriers diagnosed with cancer after that age. The GG genotype is three-times more frequent among younger *BRCA1/2* cancer patients than in older *BRCA1/2* cancer patients and is associated with a significant risk of developing early cancer (OR 3.93 CI 1.41–10.9 $P = 0.009$). No such differences in genotype distribution were noted among sporadic breast cancer patients. These results suggest that the GG genotype contributes to breast and ovarian cancer risk in genetically predisposed *BRCA1/2* mutation carriers, and accelerates breast and ovarian carcinogenesis. Taken together, the MDM2 SNP309 may be viewed as a modifier of penetrance of mutant *BRCA1/2* alleles.

Mutations in the *BRCA1/2* genes increase cancer risk for both breast and ovarian cancers, but incomplete penetrace suggests that additional genetic modifiers may alter the site-specific cancer risk [2]. Indeed, site-specific effects have been observed for several modifier genes such as the rare alleles of the oncogene Ha-RAS1, which increase the risk for ovarian cancer, but not breast cancer, among *BRCA1* carriers [32]. A polymorphism in the 5′ UTR of the *RAD51* gene (135 GC, rs1801320) increases the risk for breast cancer, but not for ovarian cancer, among *BRCA2* carriers [6, 33]. Our study suggests that the GG genotype at SNP309 of MDM2 increases the risk for breast cancer, predominantly in BRCA1 mutation carriers.

As the GG genotype of MDM2 SNP309 clearly affects age at diagnosis in breast cancer, one may speculate that hormonal changes in estrogen-signaling pathway due to menopause, account for this association [22]. Given the estrogen receptor status (ER negative) in majority of *BRCA1* associated breast cancer [34], this explanation seems unlikely. Alternatively, genomic instability accumulation may account for cancer risk conferred by the different MDM2 alleles (GG, TT or GT) in *BRCA1/2* mutation carrying women. The SNP309 G- allele generates an SP1 binding site that potentially alters the expression of the p53 pathway by up-regulating the MDM2 oncogene and down-regulating the p53 tumor suppressor gene. Loss of p53 creates a permissive environment for inappropriate proliferation and survival, impaired cell cycle checkpoint control signaling and attenuated apoptosis. Thus, loss of p53 or attenuation of the pathway leads to accumulation of genomic instability [14]. As *BRCA1* and *BRCA2* participate in the DNA damage response and specifically in DNA repair of double strand breaks (DSB) [35, 36], the combined effect of defects in both *BRCA1/2* and p53 pathway could explain the acceleration in carcinogenesis that clinically manifests as an earlier age at diagnosis. Indeed, in murine models, p53 deficiency is highly cooperative with both *Brca1* and *Brca2* in promoting tumorigenesis [13, 17].

Subsequent to the original finding by Bond et al. [19], several conflicting reports were published on the association between SNP309 and breast cancer acceleration and outcome. Reports by Wilkening et al., Petenkaya et al. and Campbell et al. [24, 37, 38] found no association between SNP309 polymorphism and breast cancer risk and outcome regardless of family history. Both Wilkening et al. [37] and Campbell et al. [38] reported no association between familial breast cancer or patients at high risk without *BRCA1/2* mutations and SNP309 while, Petenkaya et al. [24] reported no association between MDM2 SNP309 and sporadic breast cancer. Our results also indicate that there is no association between the G allele and risk of breast

cancer among sporadic breast cancer patients. However, in contrast to a study published by Copson et al. [25] that reported no association between *MDM2* SNP309 and risk in *BRCA1* carriers, we find a three time increased risk of early onset breast cancer among *BRCA1/2* carriers with a GG genotype. The discrepancy between of the two studies could stem from the fact that our population is relatively homogeneous and the mutation spectrum is limited to three predominant mutations with a strong founder effect of highly penetrant mutations (185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*). Moreover, even the haplotypes associated with *BRCA1* mutations and *MDM2* GG genotype are highly common [28–31]. Whereas, in the other studies, the population analyzed consists of male and female *BRCA1* carriers with wide spectrum of mutations and varied penetrance. Therefore, it is conceivable that the added risk of *MDM2* SNP309 is masked in that population, and analysis of risk should be carried in conjunction with haplotype analysis. Second, the G allele frequency in the Jewish Ashkenazi is higher than in mixed Caucasian population and thus, even the small added risk could have a high impact.

The fact that asymptomatic carriers are about 8 years younger than cancer-diagnosed carriers could potentially bias our results, especially as our patient's follow-up time is limited. However, the current median age of the asymptomatic group is already older than the median age at diagnosis among the cancer-diagnosed carriers. Given the similar frequencies of *BRCA1/2* mutations and *MDM2* polymorphisms between the two groups, we suggest that yet an additional modifier or modifiers play a role in altering cancer risk among carriers. The small number of *BRCA2* carriers in our cohort limited our ability to test the association between SNP309 and *BRCA2* 6174delT mutation. As the frequency of SNP309 was two times higher in asymptomatic carriers, analyzing a larger group of *BRCA2* carriers is of particular interest as it may potentially clarify whether SNP309 have an opposing effect on disease course in *BRCA2* carriers. Nevertheless, the additive risk of SNP309 to cancer-diagnosed *BRCA1/2* carriers was stronger when the two groups of patients were combined rather than assessed individually.

In conclusion, our results show that Jewish–Ashkenazi *BRCA1/2* carriers that harbor the GG genotype of SNP309 in the MDM2 intronic promoter have an increased risk of being diagnosed with early onset cancer relative to heterozygotes and TT genotype. Thus, SNP309 influences age at diagnosis among the high-risk *BRCA1* and *BRCA2* mutation carriers of Ashkenazi descent.

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