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ORIGINAL PAPER

The rate of the recurrent *MSH6* mutations in Ashkenazi Jewish breast cancer patients

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Abstract

Background Whether breast cancer (BC) should be considered within the spectrum of tumors in Lynch syndrome (LS) is unsettled. Recently, *MSH6* and *PMS2* germline mutations have reportedly been associated with an increased BC risk and with hereditary breast and ovarian cancer (HBOC) phenotype. We assessed the rates of the recurring Ashkenazi Jewish (AJ) mutations in the *MSH6* gene (c.3984_3987dupGTCA and c.3959_3962delCAAG) in AJ cases with seemingly sporadic BC or HBOC phenotype, who were negative for the founder AJ *BRCA1/2* mutations.

Methods All AJ individuals, affected with BC ≤ 70 years and/or ovarian cancer at any age who were counseled, genotyped and tested negative for the *BRCA1/2* founder mutations between January 2010 and February 2018 at the Oncogenetics unit, Sheba Medical Center, were genotyped for the AJ mutations in *MSH6*.

Results Of 1016 genotyped participants (815 BC cases, 132 ovarian cancer cases, and 69 with more than one cancer), five carriers (0.49%) of the recurring AJ mutations in *MSH6* were identified. All had BC, and two had personal history of additional cancers (pancreatic, endometrial, colorectal). The rate of *MSH6* mutations was 0.93% (4/429) when considering only cases with a personal or first-degree relative with LS-related cancer, and 0.17% (1/587) of cases with second-degree relative or no family history of LS-related cancers ($p = 0.087$).

Conclusions Our data suggest the spectrum of genotyped mutations in AJ BC patients with a personal or family history of LS-related cancers should be expanded. These data should be validated in other populations with a similar phenotype.

Keywords Breast cancer · *MSH6* · Lynch syndrome · Ashkenazi Jews

Introduction

An association of breast cancer (BC) with Lynch syndrome (LS) and to what extent BC should be considered as an integral part of the spectrum of LS-associated malignancies has been debated for years, with conflicting and inconsistent data [1]. As the clinical application of multigene panel testing became more popular, a few studies suggested that mutations in two of the LS genes, *MSH6* and *PMS2* may also confer an increased risk for BC. Roberts et al. reported that standard incidence ratios (SIRs) of BC were 2.11 for *MSH6* mutation carriers and 2.92 for *PMS2* mutation carriers, whereas no increased risk was observed for *MLH1* (SIR = 0.87) or *MSH2* (SIR = 1.22) [2]. Data from the Danish Lynch syndrome cohort [3], as well as from the German and Dutch national Lynch syndrome registries [4] reported SIR for BC of 1.9. Additionally, it was reported that a proportion of *MSH6* and *PMS2* mutation carriers may present with a hereditary breast and ovarian cancer syndrome (HBOC)

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phenotype: Espenschied and coworkers found that 22.2% of mismatch repair (MMR) mutation carriers ($n = 528$) met HBOC testing criteria and not LS criteria [5]. These observations have led the authors to recommending genotyping these two genes in cases with HBOC phenotype [2, 5].

Although relatively rare in the general population, in certain ethnic groups, such as Ashkenazi Jews (AJ), recurring founder mutations in cancer susceptibility genes are described. Notably, three mutations in *BRCA1* (c.68_69delAG and c.5266dupC) and *BRCA2* (c.5946delT) genes, account for the majority of HBOC cases in this population [6–8] and are also detected in up to 35% of consecutive ovarian cancer (OC) cases 12% of BC cases and 2.5% of the general cancer free population [6, 9, 10]. Similarly, recurring mutations in AJ families with LS were reported: *MSH6* c.3984_3987dupGTCA and c.3959_3962delCAAG and *MSH2* c.1906G > C; A636P [11, 12]. Raskin et al. assessed the rate of the two *MSH6* mutations in 2685 colorectal cancer (CRC) cases, 337 endometrial cancer (EC) cases and 3310 healthy controls of AJ descent from population-based and hospital-based case–control studies. *MSH6**c.3984_3987dupGTCA was detected in 8/2685 CRC cases, 2/337 EC cases, and 1/3310 controls, consistent with a high risk of CRC (odds ratio (OR) 9.9) and a very high risk of EC (OR 19.6). The second mutation—*MSH6**c.3959_3962delCAAG was identified in 3/2685 CRC cases, 2/337 EC cases and none of the controls [11].

The *MSH2* c.1906G > C; A636P mutation was found in 0.44% of 686 consecutive CRC in AJ individuals, and in none of 1588 AJ controls [13]. In the same study, this mutation was not found in the series of 271 AJ BC probands who had a family history of either CRC or OC [13].

Our group previously evaluated the rates of the predominant *MSH2* and *MSH6* mutations in Jewish individuals with familial and sporadic gastric and pancreatic cancer [14] and in consecutive cases of EC [15]. While none of 143 gastric and pancreatic cases harbored any of the recurring mutations in either gene, 1.8% and 1.4% of the 217 AJ EC cases harbored the *MSH6**c.3984_3987dupGTCA, and *MSH2**A636P mutations, respectively [15].

To the best of our knowledge, no studies evaluated the rate of the recurring *MSH6* mutations in AJ BC patients. In the light of recent studies suggesting that *MSH6* and *PMS2* genes are moderate-penetrance BC susceptibility genes and taking advantage of the limited spectrum of germline mutations in the relevant genes, this study aimed to assess the rate of the predominant AJ mutations in the *MSH6* gene in AJ patients with BC and/or OC, who tested negative for the recurring AJ *BRCA1/2* mutations.

Patients and methods

Individuals who were counseled at the Oncogenetics unit, Sheba Medical Center from January 2010 to February 2018, who reported that all four grandparents were of Ashkenazi decent, who were diagnosed with BC as their primary diagnosis under age 70 and/or have had OC at any age, formed the basis of recruitment. All cases underwent genotyping for *BRCA1* and *BRCA2* founder mutations as previously described [16], and those who tested negative were eligible for the current study. Personal and family data of cancer diagnoses including type, number, and ages at cancer diagnosis were obtained from all participants. The study was approved by the Sheba institutional review board and all participants provided an informed consent.

Peripheral blood leukocyte DNA was extracted using the PureGene kit (Gentra Inc., Minneapolis, MN), following the manufacturer's recommended protocol.

Only the two recurring AJ mutations in MSH6 were genotyped: c.3984_3987dupGTCA (rs267608121) and c.3959_3962delCAAG (rs267608120). Genomic DNA from each subject was PCR-amplified using the following primer sequences: F-5′-AAGCTATGGCTTTAATGC AGCAAG-3′ R-5′TCATAGTGCATCATCCCTTCCC-3′, as previously described [14]. The forward primer was labeled with FAM. About 1 µl of each PCR product was mixed with 0.5 µl of the GeneScan 500 LIZ® Size Standard (Applied Biosystem), and 12 µl of formamide. Samples were loaded on The ABI PRISM 3100 Genetic Analyzer (Applied Biosystem). The raw data were analyzed using the GeneMapper Software to obtain the allele size in base pairs. Each run was accompanied with running a known mutation carrier. Abnormal fragments (i.e., fragments that displayed more than one allele) were directly sequenced to confirm the existence of the mutation.

The observed mutation prevalence was compared between the subgroups in the cohort and with that previously described by Raskin et al. [11] using a single-sample *Z* test.

Results

Participants' characteristics

Overall, 1,065 individuals were identified as eligible for participation. However, 49 could not be genotyped because of technical failure (low quality or insufficient amount of DNA). Thus, 1,016 individuals (1,013 women and 3 men) were included in the analysis. Of these, 815 (80.2%) were diagnosed with BC, median age at diagnosis

55 years, (range 24–70); 132 (13%) were diagnosed with OC, median age at diagnosis 65 years, (range 26–91). Additional 69 patients were diagnosed with more than one cancer (Table 1).

Of participants, 694 (68.3%) had personal or family history of cancers diagnosed at any age that are clearly LS-associated (colon, endometrium, stomach, ovary, small bowel, brain, pancreas and bile ducts) [17]. Of these, 429 had a personal history or at least one first-degree relative (FDR) with LS-associated malignancies, and the rest ($n=265$) had second (SDR)/third-degree (TDR) relatives with LS-associated malignancies. Three hundred and twenty-two patients (31.7%) had no family history of LS-associated cancers. Additional proband and family relevant characteristics are shown in Table 1.

MSH6 mutation rates

Overall, five carriers of mutations in *MSH6* gene were identified—four with c.3984_3987dupGTCA, and one with c.3959_3962delCAAG. All five were diagnosed with BC between ages 40–53, two had additional personal history of other cancers, two had at least one FDR with LS-related cancers, and one had no family history of LS-related cancers (Table 2). The overall rate of harboring a *MSH6* mutation for the entire group was 0.49% (5/1016). In the subset of patients with personal or FDR history of LS-related cancers the rate was 0.93% (4/429), and 0.17% (1/587) in patients with second-degree relative or no family history of LS-related cancers ($p=0.087$). Excluding OC with or without additional cancer cases ($n=139$), there were 5/877 (0.57%) *MSH6* mutation carriers. The rates in these subsets were significantly higher than the rates reported in the general population (1/3310)[11] ($p=0.00016$).

Neither mutation was identified in any of the OC patients or patients with SDR/TDR with LS-related cancers.

Within the studied population, 18 patients chose to perform multigene panel testing, in one of them *MSH6* c.3743_3744insT; p.Tyr1249Leufs*26 was identified (Table 2).

Table 1 Characteristics of patients tested for *MSH6* AJ founder mutations

Characteristic	Patients, No.
Diagnosis	
All patients	1,016
Breast cancer only	815
Age at first diagnosis	
<40	61
40–60	450
60–70	304
Ovarian cancer only	132
Multiple cancers	69
Breast and ovarian	8
Breast and GI/uterine ca	36
Breast and another non-GI cancer[a]	18
Ovarian and other cancers (GI and non-GI)[a]	7
Family history	
FDR with LS-related cancer	429
SDR/TDR with LS-related cancer	265
No family history of LS-related ca	322

GI gastrointestinal, *LS* Lynch syndrome, *FDR* first-degree relative, *SDR/TDR* second/third degree relative

[a]Cancers of kidney, melanoma, lymphoma, sarcoma, thyroid, cervix

Discussion

In this study, the rate of the recurring AJ *MSH6* mutations in BC patients was 0.57%—significantly higher than rate of 1/3310 (0.03%) (OR 18.87, 95% CI 2.2–161.7, $p=0.0074$) in historical healthy population controls [11]. This can only be compared to a small number of studies with different designs. In the study by Tung and coworkers [18], multigene panel testing of 1781 non-Jewish BC patients detected two patients with *MSH6* mutations and four additional patients with *PMS2* mutations, cumulatively comprising 0.34% of the cohort. In a study from Singapore on 220 patients of Asian ancestry, with a personal and/or familial cancer history suggestive of hereditary BC, three patients (1.4%) with *MSH6* mutation were reported [19]. In a large cohort of AJ women with a primary diagnosis of invasive BC from the New York Breast Cancer Study (NYBCS), *MSH6* gene was not included in the BROCA panel of 23 breast cancer predisposition genes, and thus the rate of mutations in this gene in a similarly recruited population of AJ could not be evaluated [20].

In line with previous reports [5, 21], *MSH6* mutation carriers in the current study did not have "classical" LS family phenotype [17], with relatively old ages of colon and endometrial cancer in affected individuals in the families, and some of them presenting with a predominantly HBOC syndrome phenotype (Table 2). These findings raise the question of whether the formal clinically accepted and applied recommendations for early detection of LS-associated cancers [17] are appropriate in BC patients of Ashkenazi origin with *MSH6* mutations.

Whether early detection of BC should be added to the recommendations for LS patients, there are limited data in the literature. In the Danish LS cohort, BC showed peak incidence rates in the 50–69-year age group [3]. As the BC risk increase in *MSH6* mutation carriers is at most double [2–4], and most countries offer average risk population screening for BC at age 50 [22], current data are insufficient to justify

Table 2 Characteristics of patients identified with *MSH6* mutations

Case	Age at BC diagnosis	Additional cancers	Follow-up	Family history (age at diagnosis)
1	51 ER-positive local recurrence at 54, 59	Pancreas 67		Mother EC (61)—obligatory carrier (father tested negative) Daughter GBM (15)
2	56 ILC ER-positive local recurrence after radiotherapy completion		Metastatic disease at 57 Genomic testing of liver met showed MSS and low TMB	Mother EC (55) Maternal grandmother BC (45) Maternal grandfather CRC (60) Parents not tested
3	65			Brother prostate and CRC (71), his daughter ovary (50) Brother CRC (78), his daughter BC (40). None tested
4	40	EC 47 CRC 60		Father CRC (60) Paternal aunt EC (60) Paternal uncle leukemia (27)
5	53			Brother "liver" cancer (53) Father leukemia (70)
6[a]	63 ER-positive Oncotype high RS = 32			Brother CRC (57) Nephew melanoma (39) Mother BC (63) Paternal cousin BC (36) Paternal grandmother esophagus (77)

BC breast cancer, *ER* estrogen receptor, *ILC* infiltrating lobular carcinoma, *GBM* glioblastoma multiforme, *EC* endometrial cancer, *CRC* colorectal cancer, *MSS* microsatellite stable, *RS* recurrence score, *TMB* tumor mutational burden

[a]A patient with a non-founder *MSH6* c.3743_3744insT; p.Tyr1249Leufs*26 mutation

additional preventive measures or earlier age screening by MRI for *MSH6* carriers. Yet, this decision should be individualized according to personal and family history and discussed with the patient.

The current study is limited to patients of AJ descent from a single institution tested for only two *MSH6* recurring AJ mutations after exclusion of founder mutations in *BRCA1/2*. Based on the paucity of reports in the literature on individuals who carry both *BRCA* and *MSH6* mutations, as well as the experience in the high risk *BRCA* mutation carrier clinic at the same center (Friedman, unpublished observation), this co-occurrence of mutations is rare, and their actual rate could not be assessed in the present study by design. In addition, one of the patients in our cohort was found to carry a non-founder mutation in *MSH6* gene, suggesting that additional patients could have been missed by this limited testing, thus leaving the precise burden of BC due to *MSH6* mutations still undefined.

Since most family members of our patients who had cancer diagnoses died or did not undergo genetic counseling in our unit, and thus could not be genotyped for these mutations, co-segregation analysis was not performed.

An additional limitation of this study is that tumor tissue of the *MSH6* mutation carriers was available in only one patient, and hence further analyses to assess the involvement of this gene in BC tumorigenesis, by either exhibiting microsatellite instability (MSI) and/or somatic loss of MSH6 protein expression could not be performed. Yet, Win et al. [1], in their review report that only ~ 50% of BC in carriers of mismatch repair (MMR) gene mutations exhibit somatic MMR-deficiency. So, the true relationship between MMR-deficient breast tumors and inherited predisposition to BC remains undefined, since it is not clear whether MMR gene mutations indeed predispose to the development of BC, or if MMR deficiency is a phenotype arising somatically within BC that was caused by another factor (as a passenger). Somatic whole-genome sequencing of large cohort of BC identified 11/640 tumors as MMR deficient based on mutational signature profiling, with only two of these 11 clearly labeled as LS related (since germline mutations in MMR genes were noted in these cases) [23]. Thus, somatic MMR-deficiency is rare in BC, and although FDA granted accelerated approval to pembrolizumab for patients with unresectable or metastatic MSI-H or MMR-deficient solid tumors [24], its efficacy in MMR-deficient BC or in BC patients harboring mutations in LS genes remains to be proven.

In conclusion, ~ 1% of AJ BC patients who do not carry any of the predominant AJ *BRCA1/2* mutations and have a personal or family history in FDR of LS-associated malignancies, carry one of the AJ founder *MSH6* mutations. This finding, if validated in a larger set of tested individuals, combined with data on results derived from multigene panel in non-Jewish populations [5], may provide a basis for

considering adding *MSH6* to BC multigene panel testing, and offering testing for this mutation to all AJ HBOC cases.

Compliance with ethical standards

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

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

References

1. Win AK, Lindor NM, Jenkins MA (2013) Risk of breast cancer in Lynch syndrome: a systematic review. Breast Cancer Res BCR 15:R27
2. Roberts ME, Jackson SA, Susswein LR, Zeinomar N, Ma X, Marshall ML et al (2018) *MSH6* and *PMS2* germ-line pathogenic variants implicated in Lynch syndrome are associated with breast cancer. Genet Med. https://doi.org/10.1038/gim.2017.254
3. Therkildsen C, Ladelund S, Smith-Hansen L, Lindberg LJ, Nilbert M (2017) Towards gene- and gender-based risk estimates in Lynch syndrome; age-specific incidences for 13 extra-colorectal cancer types. Br J Cancer 117(11):1702–1710
4. Engel C, Loeffler M, Steinke V, Rahner N, Holinski-Feder E, Dietmaier W et al (2012) Risks of less common cancers in proven mutation carriers with Lynch syndrome. J Clin Oncol 30:4409–4415
5. Espenschied CR, LaDuca H, Li S, McFarland R, Gau C-L, Hampel H (2017) Multigene panel testing provides a new perspective on Lynch syndrome. J Clin Oncol 35:2568–2575
6. Robson M, Dabney MK, Rosenthal G, Ludwig S, Seltzer MH, Gilewski T et al (1997) Prevalence of recurring BRCA mutations among Ashkenazi Jewish women with breast cancer. Genet Test 1:47–51
7. Roa BB, Boyd AA, Volcik K, Richards CS (1996) Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. Nat Genet 14:185–187
8. Fodor FH, Weston A, Bleiweiss IJ, McCurdy LD, Walsh MM, Tartter PI et al (1998) Frequency and carrier risk associated with common BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer patients. Am J Hum Genet 63:45–51
9. Warner E, Foulkes W, Goodwin P, Meschino W, Blondal J, Paterson C et al (1999) Prevalence and penetrance of BRCA1 and BRCA2 gene mutations in unselected Ashkenazi Jewish women with breast cancer. J Natl Cancer Inst 91:1241–1247
10. Tobias DH, Eng C, McCurdy LD, Kalir T, Mandelli J, Dottino PR et al (2000) Founder BRCA 1 and 2 mutations among a consecutive series of Ashkenazi Jewish ovarian cancer patients. Gynecol Oncol 78:148–151
11. Raskin L, Schwenter F, Freytsis M, Tischkowitz M, Wong N, Chong G et al (2011) Characterization of two Ashkenazi Jewish founder mutations in MSH6 gene causing Lynch syndrome. Clin Genet 79:512–522
12. Mukherjee B, Rennert G, Ahn J, Dishon S, Lejbkowicz F, Rennert HS et al (2011) High risk of colorectal and endometrial cancer in Ashkenazi families with the MSH2 A636P founder mutation. Gastroenterology 140:1919–1926
13. Foulkes WD, Thiffault I, Gruber SB, Horwitz M, Hamel N, Lee C et al (2002) The founder mutation MSH2*1906G→C is an important cause of hereditary nonpolyposis colorectal cancer in the Ashkenazi Jewish population. Am J Hum Genet 71:1395–1412
14. Laitman Y, Herskovitz L, Golan T, Kaufman B, Paluch SS, Friedman E (2012) The founder Ashkenazi Jewish mutations in the MSH2 and MSH6 genes in Israeli patients with gastric and pancreatic cancer. Fam Cancer 11:243–247
15. Barak F, Milgrom R, Laitman Y, Gemer O, Rabinovich A, Piura B et al (2010) The rate of the predominant Jewish mutations in the BRCA1, BRCA2, MSH2 and MSH6 genes in unselected Jewish endometrial cancer patients. Gynecol Oncol 119:511–515
16. Schayek H, De Marco L, Starinsky-Elbaz S, Rossette M, Laitman Y, Bastos-Rodrigues L et al (2016) The rate of recurrent BRCA1, BRCA2, and TP53 mutations in the general population, and unselected ovarian cancer cases, in Belo Horizonte, Brazil. Cancer Genet 209:50–52
17. Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP (2015) Milestones of Lynch syndrome: 1895–2015. Nat Rev Cancer 15:181–194
18. Tung N, Battelli C, Allen B, Kaldate R, Bhatnagar S, Bowles K et al (2015) Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer 121:25–33
19. Wong ESY, Shekar S, Met-Domestici M, Chan C, Sze M, Yap YS et al (2016) Inherited breast cancer predisposition in Asians: multigene panel testing outcomes from Singapore. NPJ Genom Med 1:15003
20. Walsh T, Mandell JB, Norquist BM, Casadei S, Gulsuner S, Lee MK et al (2017) Genetic predisposition to breast cancer due to mutations other than BRCA1 and BRCA2 founder alleles among Ashkenazi Jewish women. JAMA Oncol 3:1647–1653
21. Goldberg Y, Porat RM, Kedar I, Shochat C, Galinsky D, Hamburger T et al (2010) An Ashkenazi founder mutation in the MSH6 gene leading to HNPCC. Fam Cancer 9:141–150
22. Shah TA, Guraya SS (2017) Breast cancer screening programs: review of merits, demerits, and recent recommendations practiced across the world. J Microsc Ultrastruct 5:59–69
23. Davies H, Morganella S, Purdie CA, Jang SJ, Borgen E, Russnes H et al (2017) Whole-genome sequencing reveals breast cancers with mismatch repair deficiency. Cancer Res 77:4755
24. Research C for DE and Approved Drugs—FDA grants accelerated approval to pembrolizumab for first tissue/site agnostic indication. https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm560040.htm. Accessed 22 May 2018