Does familial breast cancer and thymoma suggest a cancer syndrome? A family perspective

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Abstract

Concurrence of breast cancer or thymoma with other malignancies in individual families is often observed, but the familial concurrence of breast cancer and thymoma has not yet been reported. Herein we reported a family encompassing five breast/ovarian cancer patients and two thymoma patients. Whole genome linkage analysis detected no haplotype co-segregating with both types of the tumors. In all patients with breast/ovarian cancer, genetic analysis revealed a clinically untested variant c.5141T>G in exon 18 of the BRCA1 gene, which could be a cancer-causing variant based on the functional study of Lee et al. (2010) and our current pedigree analysis. In the two thymoma patients in our family, targeted sequencing of RAD51L1 and BMP2 genes in and near the translocation site of chromosome 14 and 20 previously reported in two thymoma families, did not find any pathogenic mutation. In the present study, we identified a clinically unconfirmed BRCA1 variant segregating with breast/ovarian cancer patients in an individual family, suggesting it to be clinically functional. Our evidence, however, did not support the notion that the concurrent appearance of breast cancer and thymoma in our family represents a familial cancer syndrome caused by the same genetic disorder.

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1. Introduction

Concurrence of breast cancer or thymoma with other malignancies in individual families has been described (Purtilo et al., 1981; Offit et al., 2003; Tehrani et al., 2010). Breast cancer, the most common malignancy in women, often co-occurs with other malignancies such as ovarian, pancreatic, brain cancer or sarcoma in familial cancer syndromes. Some of the syndromes have been defined as autosomal dominant disorders, such as hereditary breast and ovarian cancer, Li-Fraumeni, Lynch, Peutz-Jeghers, and Ataxia-Telangiectasia syndrome caused by mutations in the BRCA1/BRCA2, TP53/STK11, MSH2/MLH1/MSH6, PTEN, STK11 and ATM genes, respectively (Malkin et al., 1990; de Jong et al., 2002; Waite and Eng, 2002; Lynch and de la Chapelle, 2003; Garber and Offit, 2005).

Thymoma is the most common neoplasm in the anterosuperior mediastinum. Familial occurrence of thymoma is rare with a prevalence of 1 to 4 per million (Nicodeme et al., 2005). The cause of familial thymoma is largely unknown. Deminatti et al. (1994) and Nicodeme et al. (2005) reported a familial thymoma with a translocation between chromosome 14q and chromosome 20p (Deminatti et al., 1994; Nicodeme et al., 2005). Multiple studies of thymoma patients have shown an increased risk of different malignancies such as colorectal adenocarcinoma, lung cancer, hematologic malignancies and autoimmune diseases in family members (Levy et al., 1996; Yen et al., 2011). Some researchers suggest that thymoma associated with other malignancies in the family herald a hereditary cancer syndrome (Tehrani et al., 2010). Thus far, familial occurrence of breast cancer and thymoma has not yet been reported.

Here we report a family with both breast/ovarian cancer and thymoma patients. The malignancies are inherited in an autosomal dominant pattern (Fig. 1). The types of malignancies did not match the known familial cancer syndromes. We assume a new cancer syndrome caused by a common genetic defect and perform a whole genome linkage analysis, expecting to identify the potential genetic disorder that causes a cancer syndrome of breast/ovarian cancer and thymoma.
2. Materials and methods

2.1. Pedigree and subjects

We studied a large three-generation Chinese family with breast/ovarian cancer and thymoma recruited from the Department of Cardiothoracic Surgery, Medical School Affiliated Drum Tower Hospital, Nanjing University, consisting of 38 family members, 33 of whom are living, including five subjects diagnosed with breast/ovarian cancer and two subjects with thymoma (Fig. 1). The proband was referred to the department for the diagnosis of thymoma. The pedigree showed that the malignancies were inherited in an autosomal dominant pattern with incomplete penetrance. Blood samples were obtained from affected and unaffected family members for genetic analysis. Additional information including the type of cancer, current age, age of diagnosis and treatments of the patient was also obtained (Table 1). A questionnaire on the environmental exposure was completed in the studied family, including living environment, chemical carcinogens (insecticides, herbicides and painting dyes), infectious agent (Epstein Barr virus, cytomegalovirus) and radiation exposure. As a control group, 100 unrelated healthy individuals without known malignancies were also recruited.

2.2. Ethics statement

The study was approved by the Ethical Committee of Medical School Affiliated Drum Tower Hospital, Nanjing University. Informed consent was obtained from all individual participants included in the study.

2.3. DNA isolation

Genomic DNA was extracted from peripheral blood samples of collected family members and controls using a Qiagen DNA Mini blood Kit (Qiagen, Hilden, Germany) according to manufacturer instruction. DNA purity and concentration were assessed by the NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Florida, USA) and DNA quality was assessed by agarose gel electrophoresis.

2.4. Linkage and haplotype analysis

Whole genome linkage analysis was performed with 400 microsatellite markers from a commercially available set ABI PRISM Linkage Mapping Set V2.5 MD10 (Applied Biosystems, Foster City, CA). The markers were amplified by polymerase chain reaction (PCR). Markers were genotyped in all family members obtained, and linkage analysis was performed with ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and the length of each allele was determined by the GeneMapper software (Applied Biosystems, Foster City, CA). Two-point log of the odds (LOD) scores were calculated by computer program MLINK (Cottingham et al., 1993). To minimize the number of crossovers in the family, haplotypes were constructed and assigned.

2.5. BRCA1 mutation screening by Sanger sequencing

Mutation analysis was performed by direct DNA sequencing of all coding exons and all intron-exon boundaries including at least 50

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**Table 1**

Clinical data of the affected family members.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Gender</th>
<th>Tumor type</th>
<th>Age, years</th>
<th>Age at diagnosis, years</th>
<th>Treatments received</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-4</td>
<td>Female</td>
<td>Breast cancer</td>
<td>84</td>
<td>40</td>
<td>Right breast resected at diagnosis; underwent radiotherapy and chemotherapy; uterine resected</td>
</tr>
<tr>
<td>II-8</td>
<td>Female</td>
<td>Ovarian carcinoma</td>
<td>55</td>
<td>36</td>
<td>Ovarian resected at diagnosis; left breast resected</td>
</tr>
<tr>
<td>II-10</td>
<td>Female</td>
<td>Breast cancer in situ</td>
<td>52</td>
<td>45</td>
<td>Ovarian resected at diagnosis</td>
</tr>
<tr>
<td>II-16</td>
<td>Female</td>
<td>Ovarian carcinoma in situ</td>
<td>61</td>
<td>41</td>
<td>Right breast resected at diagnosis; underwent radiotherapy and chemotherapy; left breast were resected at age 43</td>
</tr>
<tr>
<td>II-19</td>
<td>Female</td>
<td>Thymoma</td>
<td>51</td>
<td>40</td>
<td>Right breast resected at diagnosis; underwent radiotherapy and chemotherapy; underwent ovarian resection</td>
</tr>
<tr>
<td>III-7</td>
<td>Female</td>
<td>Thymoma</td>
<td>44</td>
<td>30</td>
<td>Underwent radiotherapy and chemotherapy</td>
</tr>
<tr>
<td>III-11</td>
<td>Male</td>
<td>Thymoma</td>
<td>25</td>
<td>19</td>
<td>Underwent radiotherapy and chemotherapy</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Pedigree of the three-generation family. Patients with breast/ovarian cancer are shown by black symbols. Patients with thymoma are shown by grey symbols. Numbers shown below each individual indicate the genotypes of the microsatellite markers in chromosomal order. The black bars represent the mutation associated haplotype. Arrow indicates the proband. BC: breast cancer, UCC: uterine cervix carcinoma, OC: ovarian carcinoma.
intonic nucleotides of BRCA1. The primers were designed using the online software Primer3. The amplification reaction mixture (50 μl) was subjected to denaturation at 95 °C for 2 min followed by 30 cycles at 94 °C for 1 min, annealing temperature 60 °C for 1 min, 72 °C for 1 min and by a final extension at 72 °C for 15 min. Bi-directional sequencing was performed using BigDye Terminator v3.1 Cycle Sequencing Kit, version 3.1 (Applied Biosystems, Foster, CA, USA) and analyzed on an ABI Prism 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Sequencing results were compared with the reference DNA sequences derived from GenBank (accession number: BRCA1: NM_007294.3) and then reviewed manually. Altered nucleotides were confirmed by double-strand sequencing. The protein sequences of other species were obtained from NCBI GenBank and conservation analysis was performed by ClustalX software (Larkin et al., 2007). Missense variants identified, if any, were subjected to bioinformatics tool PolyPhen (Adzhubei et al., 2010) and SIFT (Kumar et al., 2009) to evaluate their possible impact on protein structure and function.

2.6. Target enrichment sequencing of RAD51L1 and BMP2 and the next-generation sequencing

All coding exons, intron-exon boundaries including 50 intronic nucleotides and 5′ UTR (untranslated region), 3′ UTR of RAD51L1 and the whole gene sequences of BMP2 were targeted for enrichment. Capture design was done using the Agilent eArray. Genomic DNA was obtained from the two thymoma patients. The target regions were captured using the Agilent Haloplex Target Enrichment System Kits for Illumina Sequencing (Custom Panel Tier 1, ILM, 46 reactions; Agilent Technologies, Inc. Santa Clare, CA) following Agilent protocols. Differentially indexed samples were pooled before 2 × 300 bp paired-end sequencing on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). Sequence reads were aligned, analyzed and annotated with Agilent software Surecall v2.1.1.13. Whenever a mutation was identified, validation was performed by Sanger sequencing.

3. Results

According to obtained data from questionnaire, none of the family members were exposed to radiation or environmental pollution or had access to carcinogens or infectious agents before diagnosis. Whole genome linkage analysis was performed and no haplotype co-segregating with breast/ovarian cancer and thymoma was found in the family, suggesting that the thymoma observed in this family may not be caused by the same gene as for the breast/ovarian cancer patients in the family. Interestingly, in patients with breast/ovarian cancer in the family, evidence of linkage was observed on chromosome 17q21. Haplotype analysis defined the region to ~5.3 cM in D17S1299, D17S932 and D17S1868 shared by all affected breast/ovarian cancer patients in the family (Fig. 1). Located between D17S1299 and D17S1868 is the BRCA1 gene, which is responsible for hereditary breast and ovarian cancer syndrome. We subsequently performed sequencing analysis of the BRCA1 gene in order to identify any potential genetic mutation.

The 24 exons of the BRCA1 and partial flanking intronic sequences were sequenced for all the family members. Our sequencing analysis revealed a c.5141 T>G transversion in exon 18 of the BRCA1 gene, which converted the codon GTT for valine at position 1714 to GGT, a codon for glycine (Fig. 2a). Family members diagnosed with breast/ovarian cancer (1-4, II-8, II-10, II-16, II-19) were all heterozygous for the variant. This variant was not detected in the two thymoma patients. Other unaffected family members, including 7 males (II-1, II-3, II-5, II-11, II-17, II-1, III-3) and 1 female (III-2, 32 years old) also shared this heterozygous variant. None of the 100 healthy control individuals with the same ethnic background were found to carry this genetic variant. This variant is recorded as rs80357243 in the National Center for Biotechnology Information (NCBI) human SNP database (dbSNP) and Breast Cancer Information Core (BIC) database with uncertain clinical significance. This variant was not identified in the NHLBI Exome Sequencing Project (ESP) database.

According to PolyPhen and SIFT, this variant is predicted to be “probably damaging” and “damaging” with a score of 0.993 and 0, respectively. Cross-species multiple alignment of BRCA1 protein sequences around the mutation site showed the evolutionary conservation of the altered amino acid p.Val11714. (c) A schematic presentation of the BRCA1 protein with its functional domains and the mutation site.

To discover any potential genetic defect in the thymoma patients, next-generation sequencing was performed in the targeted regions of RAD51L1 and BMP2, which were previously reported to be in or near the breakpoints of chromosome translocation between chromosome 14q and chromosome 20p in two thymoma families (Deminatti et al., 1994; Nicodeme et al., 2005), but no pathogenic mutation was identified. Taken together, our findings did not support the notion that the concurrent appearance of breast/ovarian cancer and thymoma in our family represent a familial cancer syndrome.

4. Discussion and conclusion

In this study, we report a family with both breast/ovarian cancer and thymoma patients. According to investigations, none of the family members were exposed to radiation or environmental pollution or had access to carcinogens or infectious agents before diagnosis. The malignancies are inherited in an autosomal dominant pattern. Whole genome linkage and Sanger sequencing analysis revealed that the two malignancies were not caused by the same genetic disorder. An
untested missense variant in exon 18 of BRCA1 (c.5141 T>G) in the family confirmed co-segregation with breast/ovarian cancer. This is the first report of the variant in a breast/ovarian cancer family.

Since the cloning of BRCA1, the first major gene responsible for hereditary breast/ovarian cancer susceptibility, in 1994 (Miki et al., 1994), a great effort has been made to sequence the BRCA1 gene of women who are at an increased risk for early-onset breast/ovarian cancer or with severe family histories. This effort identified a large number of variants linked to breast/ovarian cancers (Breast Cancer Information Core, BIC). Most disease-associated mutations in BRCA1 are frameshift, nonsense, insertions, deletions, or splice site alterations, which lead to the formation of truncated BRCA1 proteins. Although a large number of rare missense BRCA1 variants uncovered in the human population have also been recorded in the BIC database, most of them cannot be readily distinguished as either disease-associated or benign polymorphisms due to lack of informative pedigree data.

BRCA1 protein includes an amino-terminal RING finger domain and two BRCT repeat domains (Fig. 2c). Cancer-associated mutations have a tendency to cluster in the RING and the BRCT region. Lee et al. (2010) studied 117 missense variations in the BRCT domain of BRCA1 including p.V1714G we detected (Fig. 2c) by a series of structural and functional assays and showed the p.V1714G variant to be functionally deleterious (Lee et al., 2010). Together with our pedigree analysis, we propose that this clinically unconfirmed variant may be a cancer-associated mutation.

Of note, all the unaffected p.V1714G carriers in the family were male except a 32-year-old female, who is at her age far from reaching the average onset age of BRCA1-related breast cancer. Although male BRCA1 mutation carriers were reported to be at an increased risk of the prostate, breast and pancreatic cancers (Liede et al., 2004; Mohamad and Afplestead, 2008; Stadler et al., 2012), we did not find these manifestations in the male p.V1714G carriers in this family. However, as women with paternally inherited BRCA1 mutation have the same risk of breast/ovarian cancer as women with maternally inherited BRCA1 mutation (Walsh et al., 2010), male BRCA1 mutation carriers should receive serious attention.

Familial occurrence of thymoma is rare. Several studies reported that thymoma may occur in siblings (Matani and Dritsas, 1973; Wick et al., 1982; Lam et al., 1993), suggesting that recessive pattern should not be ruled out. In the present study, we found thymoma in cousins. Targeted sequencing of RAD51L and BMP2, which was in and near the breakpoints of chromosome translocation between chromosome 14q and chromosome 20p in 2 two-generation thymoma families (Deminiatti et al., 1994; Nicodeme et al., 2005), detected no pathogenic mutation. However, this study is limited by the employed methods and does not rule out the potential inheritable changes undetectable by these methods, including changes in promoters, splice sites, 3 UTRs, microRNAs, long non-coding RNAs and other non-coding regulatory elements, DNA imprinting, and certain gene amplifications. Further study should consider whole genome sequencing analysis.

In this study, we identified a novel BRCA1 variant segregating with breast cancer patients in a family, suggesting a clinically functional effect. Besides, we provided evidence that such a family which presented as a familial cancer syndrome may be accidental and not caused by the same genetic disorder. Because cancer is relatively common in the general population, it is possible that multiple tumors co-occur within a family.

Conflict of interest

The authors declare that they have no conflict of interest.

Web resources

Primer3: http://bioinfo.ut.ee/primer3-0.4.0/primer3/
PolyPhen: http://genetics.bwh.harvard.edu/pph/

SIFT: http://sift.jcvi.org/
Agilent eArray: https://earray.chem.agilent.com/suredesign/index.htm
Breast Cancer Information Core, BIC: http://research.nhgri.nih.gov/bic/
NHLEI Exome Sequencing Project (ESP) database: http://evs.gs.washington.edu/EVS/

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References


