Research article

Mutation analysis of the AATF gene in breast cancer families

Maria Haanpää1,2, Mervi Reiman1,2, Jenni Nikkilä1,2, Hannele Erkko1,2, Katri Pylkäs1,2 and Robert Winqvist*1,2

Address: 1Laboratory of Cancer Genetics, Oulu University Hospital, P.O. Box 22, FIN-90221 Oulu, Finland and 2Department of Clinical Genetics and Biocenter, University of Oulu, P.O. Box 5000, FIN-90014 University of Oulu

Email: Maria Haanpää - maappi@mail.student.oulu.fi; Mervi Reiman - mervirei@mail.student.oulu.fi; Jenni Nikkilä - jenni.nikkila@oulu.fi; Hannele Erkko - hannele.erkko@oulu.fi; Katri Pylkäs - katri.pylkas@oulu.fi; Robert Winqvist* - robert.winqvist@oulu.fi

* Corresponding author

Abstract

Background: About 5-10% of breast cancer is due to inherited disease predisposition. Many previously identified susceptibility factors are involved in the maintenance of genomic integrity. AATF plays an important role in the regulation of gene transcription and cell proliferation. It induces apoptosis by associating with p53. The checkpoint kinases ATM/ATR and CHEK2 interact with and phosphorylate AATF, enhancing its accumulation and stability. Based on its biological function, and direct interaction with several known breast cancer risk factors, AATF is a good candidate gene for being involved in heritable cancer susceptibility.

Methods: Here we have screened the entire coding region of AATF in affected index cases from 121 Finnish cancer families for germline defects, using conformation sensitive gel electrophoresis and direct sequencing.

Results: Altogether seven different sequence changes were observed, one missense variant and six intronic ones. Based on the in silico analyses of these sequence alterations, as well as their occurrence in cases and controls, none of them, however, were predicted to be pathogenic.

Conclusions: To our knowledge, this is the first study reporting the mutation screening of the AATF gene in familial breast cancer cases. No evidence for the association with breast cancer was observed.

Background

In most Western populations, about one in ten women develop breast cancer [1]. Approximately 5-10% of these cases are considered to be familial [2]. Mutations in two major high penetrance genes BRCA1 and BRCA2 are well known, but they seem to be responsible for less than 20% of heritable disease predisposition [3,4]. Only a small number of the familial cases are explained by mutations in other known cancer susceptibility genes, such as TP53, PTEN, ATM, CHEK2, NBS1, RAD50, BRIP1 and PALB2 [5,6]. The identification of additional genes involved in breast cancer predisposition is complicated by genetic heterogeneity. The remaining cases could be the result of a few additional, yet unidentified, high penetrance mutations, but the polygenic model may provide a more plausible explanation [7]. Recent genome-wide association studies have identified a few common low penetrance breast cancer susceptibility alleles. Together these loci are, however, estimated to account less than 4% of the familial risk of breast cancer in European populations [1]. As most
of the known breast cancer susceptibility genes are involved in DNA damage response pathways, other genes involved in these essential and highly complex and multi-layered processes represent excellent candidates for identifying further cancer predisposing alleles.

AATF (apoptosis antagonizing transcription factor, also know as CHE1) was originally characterized as an interacting protein for RNA polymerase II. The AATF gene is located at chromosome 17q11.2-q12. It encodes a phosphoprotein containing 558 amino acids [8] and consists of 12 exons. The protein is highly conserved among eukaryotic species during evolution [9]. AATF does not display homology to any previously described protein. It contains a leucine zipper motif, several phosphorylation sites for different kinases, a nuclear localization signal motif, three nuclear receptor LXXLL binding motifs and several four nucleotide repeats (Figure 1) [9,10]. The functions of AATF are essential during the early stages of embryogenesis and cell proliferation [11,12]. One significant function of AATF is to promote cellular transcription, acting as an adaptor that links specific transcription factors to the general transcription apparatus. Furthermore, due to its interaction with various important components of the cell survival machinery, AATF has been found to play an important role in DNA damage response, cell-cycle checkpoint control, apoptosis and also in chromatin remodeling [13]. Interestingly, AATF operates in a dualistic way, showing both inhibitory and stimulatory roles in regard to cell-cycle progression and cell proliferation [10].

AATF is also a nuclear receptor co-activator and regulates the physiological effect of p53. The p53 protein plays a critical role in the cellular response to DNA damage and other stresses by inhibiting proliferation or by inducing apoptosis [14]. Upon DNA damage, AATF is phosphorylated by ATM and CHEK2, consequently increasing its stability and accumulation to the cell nucleus, but also enhancing p53 expression and G2-M arrest [10,15]. Although AATF induces apoptosis by associating with p53, it also has an antagonistic role in several cell types, acting as an inhibitor of apoptosis [10]. Its function as transcription factor or as co-activator has not yet been fully worked out. However, there seem to be different modes by which AATF cooperates with other transcription factors [16]. Based on all these observations it is reasonable to speculate that AATF may be a component of the checkpoint anticancer barrier that protects cells from DNA damage or oncogenic stress. Furthermore, AATF has been found to be down-regulated in several colon carcinomas and is involved in growth arrest through induction of p21 [17].

Based on its biological function, we wanted to determine whether AATF germline mutations are involved in hereditary susceptibility to breast cancer. We have, therefore, screened the entire coding sequence and exon-intron boundaries of the gene in Finnish cancer families. AATF sequence alterations have not previously been studied in relation to breast cancer predisposition.

Methods

Cases and controls
Breast and breast-ovarian cancer families (N = 121) originating in northern Finland were selected for the screening of possible germline mutations in AATF. Inclusion criteria for the 70 (58%) families classified as high-risk ones were the following: 1) three or more cases of breast, or breast and ovarian cancer in first- or second-degree relatives, or 2) two cases of breast, or breast and ovarian cancer in first- or second-degree relatives, of which at least one with early disease onset (≤ 35 years), bilateral disease or multiple primary tumors, including breast cancer in the same individual. Most of the high-risk families had three or more cases. The remaining 51 (42%) families with moderate disease susceptibility displayed two cases of breast cancer
in first- or second-degree relatives. All high-risk families were previously screened for germline mutations in known or potential susceptibility genes BRCA1, BRCA2, CHEK2, TP53, RAD50, RAP80 or PALB2 [6,18-22], and disease associated alterations in these genes were seen in altogether 17 of the families. These mutation-positive families were included in the current study because we did not want to rule out potential genetic modifier effects. The frequency of all observed germline variants were determined in control samples (N ≥307) obtained from anonymous cancer-free female Finnish Red-Cross blood donors originating in the same geographical region as the studied families. All patients had given their informed consent for obtaining pedigree data and blood specimens for the study of cancer susceptibility gene mutations. An approval to perform the study was obtained from the Ethical Board of the Northern Ostrobothnia Health Care District and the Finnish Ministry of Social Affairs and Health.

**DNA isolation and mutation analysis**

DNA was extracted from blood lymphocytes using the standard phenol-chloroform method or the Purgene D-50 K purification kit (Genta, Minneapolis, USA). The entire coding region and exon-intron boundaries of the *AATF* gene were screened for germline mutations by conformation sensitive gel electrophoresis (CSGE) [23]. Samples with band shifts were reamplified and the sequencing analysis was performed on a Li-Cor IR2 4200-S DNA Analysis system (Li-Cor Inc., Lincoln, USA) using the SequiTherm EXEL TM II DNA Sequencing Kit-LC (Epicentre Technologies, Madison, USA). Oligonucleotides for CSGE and sequencing were designed using the Primer3 software based on sequence information obtained from public databases (Genomic sequence NC_000017.9, mRNA NM_012138.3). Primers and PCR conditions for mutation screening and sequencing are available upon request.

**Statistical and bioinformatic methods**

Carrier frequencies were compared by using Pearson’s χ² or Fisher’s exact test with SPSS version 16.0 for Windows (SPSS Inc., Chicago, USA). All P-values were two-sided. The missense alteration c.739G>T (p.Ala247Ser) was tested for possible pathogenicity by using PolyPhen software. ESEfinder software was applied to determine if the exonic variant was located in an ESE (exonic splicing enhancer) sequence and might, therefore, affect the ESE function. All alterations were also checked for potential splicing effects with NNSplice software.

**Results**

The study of 121 breast or breast-ovarian cancer families revealed altogether seven different germline changes in the *AATF* gene (Table 1). Only one of the observed changes was exonic. This novel alteration resulted in an Ala247Ser amino acid substitution in the protein product. All the other seen variants were intronic. In order to evaluate possible pathogenicity of the observed changes, their frequencies were compared between cases and healthy control individuals. Assessment of the consequences of the observed changes was also done by using PolyPhen, ESEfinder and NNSplice software.

The p.Ala247Ser alteration was observed in 1.7% (2/121) of the patients and 1.3% (4/317) of the controls (P = 0.7). These two amino acids display very different characteristics. Alanine is a small hydrophobic and aliphatic amino acid, whereas serine is a polaric and hydrophilic residue. Based on the analysis using PolyPhen software the effect of this change, however, was predicted to be neutral. Neither had any influence on splicing nor ESE functions indicated.

All six intronic alterations observed were single nucleotide changes, four of which were novel ones, whereas two had already been described earlier in the internet-based sequence variation database [http://www.ncbi.nlm.nih.gov/projects/SNP/](http://www.ncbi.nlm.nih.gov/projects/SNP/). Most of the alterations were present in cases and controls at similar frequencies. However, one of the variants, c.832+17C>T, was only found among cases 0.8% (1/123) but not in controls (0/317). Furthermore, one alteration, c.1619+29A>C,
occurred more frequently among cases 2.5% (3/121) than in controls 0.3% (1/324), but nevertheless the difference was not statistically significant (P = 0.066). According to the analysis using NNSplice software, none of the intronic changes observed had any effect on splicing.

Discussion
The aim of our study was to determine the relationship between breast cancer susceptibility and potential alterations in the AATF candidate gene, which plays an important role in the maintenance of genomic integrity and cell-cycle checkpoint control [10]. Because of its influence on vital cellular functions it was considered possible that mutations in the AATF gene might contribute to hereditary disposition to breast cancer.

In the current study, the whole coding region of the AATF gene was systematically screened for mutations in 121 breast cancer families. We found several sequence variants in the AATF gene: one exonic and six intronic ones. The observed exonic change c.739G>T (p.Ala247Ser) was a novel one, but it located outside the functionally important domains. Furthermore, none of the intronic changes seemed to affect consensus splicing sequences. All observed variants displayed similar allele frequencies in cases and controls. Consequently, none of the observed alterations seemed to associate with an increased cancer risk. The absence of deleterious germline mutations in the AATF gene could indicate conserved and essential function of the protein in cell cycle control and DNA damage response. However, a small study like this cannot exclude the contribution of rare mutations in AATF that might predispose to breast cancer, but based on our findings, they unlikely make any sizeable contribution to cancer predisposition.

Conclusions
The observed AATF gene alterations lacked association with breast cancer risk and therefore mutations in this gene are likely not to play a significant role in hereditary predisposition to this malignancy. To our knowledge, this is the first investigation reporting the mutation screening of the AATF gene in familial breast cancer cases.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MH, MR and JN designed the oligonucleotide primers, and MH and MR conducted the laboratory work. MH carried out the in silico as well as statistical analysis of the obtained genetic data and drafted the manuscript. RW conceived the study, and participated in its design and coordination together with KP. All authors contributed to the preparing of the manuscript and also read and approved the final manuscript.

Acknowledgements
We wish to thank Dr. Aki Mustonen, Dr. Jaakko Ignatius, and nurses Kari Mononen and Outi Kajula for their help in sample and data collection and also in patient contacts, Helmi Konola and Meeri Elina Otsukka for technical assistance. We thank all the patients and their family members for volunteering to participate in these studies, as well as the Finnish Red Cross Blood Service for help with collection of population control blood samples. This study was financially supported by the Sigrid Jusélius Foundation, the Academy of Finland, the Orion-Farms Research Foundation, the Northern Ostrobothnia Fund of the Finnish Cultural Foundation, the University of Oulu, and the Oulu University Hospital.

References
1. Stratton MR, Rahman N: The emerging landscape of breast cancer susceptibility. Nat Genet 2008, 40:17-22.
2. Honrado E, Benitez J, Palacios J: The molecular pathology of hereditary breast cancer: Genetic testing and therapeutic implications. Mod Pathol 2005, 18:1305-1320.
3. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. BRCAI breast cancer study group. Br J Cancer 2000, 83:1301-1308.
4. Petro J, Collins N, Barfoot R, Seal S, Warren W, Rahman N, Easton DF, Evans C, Deacon J, Stratton MR: Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. J Natl Cancer Inst 1999, 91:943-949.
5. Turnbull C, Rahman N: Genetic predisposition to breast cancer: Past, present, and future. Annu Rev Genomics Hum Genet 2008, 9:321-345.
6. Erikko H, Xia B, Nikkilä J, Schleutker J, Syrjakoski K, Mannermaa A, Kallioniemi A, Pykälä K, Karppeinen SM, Rapakko K, Miron A, Sheng Q, Li G, Mattila H, Bell DW: A recurrent mutation in PALB2 in Finnish cancer families. Nature 2007, 446:316-319.
7. Houlston RS, Petro J: The future of association studies of common cancers. Hum Genet 2003, 112:434-435.
8. Fanciulli M, Bruno T, Di Padova M, De Angelis R, Iezzi S, Iacobini C, Floridi A, Passananti C: Identification of a novel partner of RNA polymerase II subunit II, che-1, which interacts with and affects the growth suppression function of rb. FASEB J 2000, 14:904-912.
9. Lindfors K, Halttunen T, Huozari P, Nupponen N, Vihinen M, Visakorpi T, Mäki M, Kainulainen H: Identification of novel transcription factor-like gene from human intestinal cells. Biochem Biophys Res Commun 2000, 276:660-666.
10. Passananti C, Floridi A, Fanciulli M: Che-1/AATF, a multivalent adaptor connecting transcriptional regulation, checkpoint control, and apoptosis. Biochem Cell Biol 2007, 85:477-483.
11. Bruno T, De Angelis R, De Nicola F, Barbato C, Di Padova M, Corbi N, Libri V, Benassi B, Mattei E, Chersi A, Doddu S, Floridi A, Passananti C, Fanciulli M: Che-1 affects cell growth by interfering with the recruitment of HDAC1 by rb. Curr Biol 2002, 12:387-399.
12. Thomas T, Voss AK, Peters P, Gruss P: The murine gene, traube, is essential for the growth of preimplantation embryos. Dev Biol 2000, 227:324-342.
13. Kaul D, Mehrtra A: Functional characterization of AATF transcriptional activity in human leukemic cells. Mol Cell Biochem 2007, 297:215-220.
14. Bruno T, De Nicola F, Iezzi S, Lecis D, D’Angelo C, Di Padova M, Corbi N, Dimiziani L, Zannini L, Jekimovs C, Scarsella M, Porrello A, Chersi A, CrescenzI M, Leonetti C: Che-1 phosphorylation by ATM/ATR and Chk2 kinases activates p53 transcription and the G2/M checkpoint. Cancer Cell 2006, 10:473-486.
15. Passananti C, Fanciulli M: The anti-apoptotic factor Che-1/AATF links transcriptional regulation, cell cycle control, and DNA damage response. Cell Division 2007, 2:21.
17. Di Padova M, Bruno T, De Nicola F, Iezzi S, D'Angelo C, Gallo R, Nicosia D, Corbi N, Biroccio A, Floridi A, Passananti C, Fanciulli M: Che-1 arrests human colon carcinoma cell proliferation by displacing HDAC1 from the p21WAF1/CIP1 promoter. J Biol Chem 2003, 278:36496-36504.

18. Rapakko K, Allinen M, Syrjäkoski K, Vahteristo P, Huusko P, Vähäkangas K, Eerola H, Kainu T, Kallioniemi OP, Nevanlinna H, Winqvist R, Germine TP: S3 alterations in finnish breast cancer families are rare and occur at conserved mutation-prone sites. Br J Cancer 2001, 84:116-119.

19. Huusko P, Pääkkönen K, Launonen V, Poyhonen M, Blanco G, Kauppila A, Puistola U, Kiviniemi H, Kujala M, Leisti J, Winqvist R: Evidence of founder mutations in finnish BRCA1 and BRCA2 families. Am J Hum Genet 1998, 62:1544-1548.

20. Heikkinen K, Rapakko K, Karpipinen SM, Erkkko H, Knuutila S, Lundán T, Mannermäe A, Barresen-Dale AL, Borg A, Barkardottir RB, Pettrini J, Winqvist R: RAD50 and NBS1 are breast cancer susceptibility genes associated with genomic instability. Carcinogenesis 2006, 27:1593-1599.

21. Allinen M, Huusko P, Mäntyniemi S, Launonen V, Winqvist R: Mutation analysis of the CHK2 gene in families with hereditary breast cancer. Br J Cancer 2001, 85:209-212.

22. Nikkilä J, Coleman KA, Morrissey D, Pylkäs K, Erkkko H, Messick TE, Karpipinen SM, Amelina A, Winqvist R, Greenberg RA: Familial breast cancer screening reveals an alteration in the RAP80 UIM domain that impairs DNA damage response function. Oncogene 2009, 28:1843-1852.

23. Körkkö J, Annunen S, Pihlajamäki T, Prockop DJ, Ala-Kokko L: Conformation sensitive gel electrophoresis for simple and accurate detection of mutations: Comparison with denaturing gradient gel electrophoresis and nucleotide sequencing. Proc Natl Acad Sci USA 1998, 95:1681-1685.

Pre-publication history
The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2407/9/457/prepub