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ORIGINAL ARTICLE

Breast cancer-associated gene 1/2 mutations are not associated with risk of exudative age-related macular degeneration in Ashkenazi Jews

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Abstract

Objective: The objective of the study was to determine whether breast cancer-associated gene (BRCA1/2) mutations are associated with wet age-related macular degeneration (AMD), together with other risk factors of older age and impaired DNA repair. In addition, role of *RAD51B* in the BRCA pathway was also investigated.
Design: This was a prospective, hospital-based study.
Participants: Ninety-four patients (26 male/26 female Ashkenazi Jews and 24 male/18 female non-Ashkenazi Jews) with a clinical diagnosis of wet AMD.
Methods: Genomic DNA was extracted and tested for mutations in BRCA1 (c.5382insC and 185delAG) and BRCA2 (c.6174delT). *RAD51B* analysis for rs17105278, rs4902566, and rs8017304 was performed using single-nucleotide polymorphisms (SNP) genotyping analysis. The results were validated by direct sequencing.
Results: No BRCA1 mutations were found in the Ashkenazi or non-Ashkenazi Jews, and only one Ashkenazi Jew (1.92%) carried the BRCA2 mutation. In the *RAD51B* SNP analysis, the Ashkenazi Jews showed 1.75-fold higher frequency in rs17105278 and 1.43-fold lower frequency of rs4902566 SNP than the non-Ashkenazi group, and no difference in rs8017304.
Conclusions: Ashkenazi Jews with wet ARMD did not show a higher rate of BRCA1/2 mutations than the general population (<2.5%), suggesting that BRCA1/2 plays no role in wet AMD in this ethnic group. No difference was found between Ashkenazi and non-Ashkenazi Jews suffering from wet AMD patients. The differences in the SNP variations of *RAD51* need to be further explored.

Introduction

Age-related macular degeneration (AMD) is the third leading cause of blindness worldwide.[1] There are two major forms of AMD, dry (drusen and geographic atrophy) and wet (exudative). Specifically, wet AMD is associated with the presence of exudates in the extracellular space between the neural retina and the retinal pigment epithelium (RPE), leading to structural changes in the RPE and severe loss of central vision due to neovascularization from the choroid into the retina.[2]

Genetic factors are the primary contributors to disease risk, although environmental and behavioral factors also play important pathophysiologic roles.[3] Since the pioneer study of Klein *et al.*[4] which identified complement factor H as a major AMD susceptibility gene, extensive investigations have confirmed 19 additional genetic risk loci.[1] The exact role of single-nucleotide polymorphisms (SNP) in the progression of AMD or in predicting the response to treatment is not yet clearly understood.

Although the etiology of AMD remains unclear, oxidative stress is believed to contribute to the pathogenesis and its role in generating cellular damage in RPE cells and choriocapillaries is well documented.[5] At high levels, reactive oxygen species can lead to impaired physiological function through cellular damage of DNA and other macromolecules. DNA damage to nuclear DNA normally activates a number of mechanisms aimed at ensuring that DNA replication passes through DNA lesions and repairing the gaps. Defects in this response cause replication fork stalling and genetic instability and are associated with many diseases, such as cancer, atherosclerosis, and diabetes. The role of DNA damage/DNA repair in AMD pathogenesis was recently confirmed.[6,7]

The protein encoded by RAD51B is a member of the RAD51 protein family and is essential for DNA repair mechanisms. RAD51 plays a key role in homologous recombination of DNA during double-strand break repair in vertebrate cells, and inactivation of RAD51 results in the accumulation of chromosomal breaks in mitotic cells and inhibits the completion of even a single cell cycle. The polymerization of RAD51 at damage sites is strictly regulated by a number of accessory factors, including breast cancer-associated gene 1 (BRCA1) and BRCA2.

DNA damage response relies in error-prone translation synthesis and error-free template switch mechanisms. Error-prone recovery mechanism fills the gap by extending the 3′-end past the damaged template, using specialized DNA polymerases that are able to incorporate a nucleotide opposite the lesion, while error-free recovery mechanism uses the information of the sister chromatid to bypass damage.

Recently, a genome wide association study detected the association of a SNP in *RAD51B* (rs8017304 A>G) with AMD.[7] Another study revealed two novel AMD-associated SNPs in *RAD51B*, rs17105278 T>C and rs4902566 C>T that reduced RAD51B mRNA expression in cultured primary human fetal RPE.[6]

BRCA1 plays essential roles in three types of DNA repair: Homologous recombination repair, non-homologous end-joining, and nucleotide excision repair.[8] BRCA1 mediates these functions by interacting with components of the DNA repair machinery and by regulating the expression of genes involved in the DNA damage repair pathways.[8] Germline mutations of BRCA1 predispose women to breast and ovarian cancer.[9,10]

BRCA2 was originally identified as a tumor suppressor, as germline mutation of the BRCA2 gene results in a high risk of developing breast, ovarian, pancreatic, prostatic, and male breast cancer.[11-15] BRCA2 is recruited to repair DNA double-strand breaks and facilitates the assembly of RAD51 at the single-strand tail.[13]

The aforementioned findings prompted us to determine if BRCA1/2 germline mutations and RAD51B SNP variations are associated with the development of AMD. The study was conducted in wet AMD patients, Ashkenazi Jews who are known to have high rates of BRCA mutations relative to the general population and specifically to non-Ashkenazi with wet AMD.[16]

Another reason for studying this group was that it is genetically isolated, since Jews have historically married within their faith. This closed gene pool makes it easier to identify genes linked to specific diseases.

Methods

Patients

The study was approved by the National and Institutional Review Boards. Enrolled subjects included 52 Ashkenazi Jewish patients (26 males/26 females) and 42 non-Ashkenazi Jews (24 males and 18 females) with a clinical diagnosis of wet AMD attending the retina outpatient clinic of a tertiary medical center. Ashkenazi Jews are those who originated in East Europe.

All underwent a clinically comprehensive eye examination with fundus examination, spectral domain optical coherence tomography, and fundus fluorescein angiography, and all were treated with intravitreal injections of anti-vascular endothelial growth factor (VEGF) agents. An informed consent was taken from all patients before enrollment. The study conformed to the tenets of the Declaration of Helsinki.

DNA extraction

Blood samples were collected following written informed consent. Genomic DNA was extracted from peripheral blood leukocytes using iPrep™ Purification Instrument (Life technologies, Invitrogen Grand Island, NY) and iPrep™ PureLink® gDNA Blood Kit (Invitrogen), according to the manufacturer's instructions.

Genotyping

All samples were examined for known Ashkenazi mutations in BRCA1 (c.5382insC and 185delAGand) and BRCA2 (c.6174delT) using high-resolution melting analysis of the polymerase chain reaction products with the LightCycler® 480 instrument (Hoffmann-La Roche Ltd., Basel, Switzerland). Primers for genotyping are according to De Leeneer *et al.*:[17] BRCA1 185delAG :2-2F M13-TTATCTGCTCTTCGCGTTG; 2-2R M13-CTTCCCTAGTATGTAAGGTC;162fragmentlength.BRCA1 5382insC: 20F M13-CTGCTCCACTTCCATTGAAG; 20R M13-GAGATTTTTGTCAACTTGAGGG, 187 fragment length. BRCA2 6174 c.6174delT: 11-23F M13-CCTTGTGATGTTAGTTTGGAAAC; 11-23R M13-GGGATATTAAATGTTCTGGAGTAC, 286 fragment length.

RAD51B SNP assays were purchased by Applied Biosystems (Foster City, CA, USA) for rs17105278, rs4902566, and rs8017304 (C__34019695_20, C__29282952_10, and C__31753961_20), respectively.

Analysis of RNA expression from Gene Expression Omnibus (GEO) datasets

In another approach to test the possible association between BRCA1/2, *RAD51B,* and exudative AMD development, we downloaded dataset G352980 from the GEO database containing data from the study of Newman *et al.*[18] These authors conducted an expression analysis of the BRCA pathway (including BRCA1/2 and RAD51B) in RNA extracted from RPE-choroid and retinal samples isolated from 50 healthy donor eyes, 17 eyes with dry AMD, and 7 eyes with wet AMD. Statistical analysis was performed with the series matrix file which contains corrected, absolute intensity values using the limma package[19] from the Bioconductor framework. Briefly, probes were tested for differential expression using a linear model followed by moderated *t*-test for the comparisons of interest. Correction for multiple testing was done using the Benjamini–Hochberg method,[20] and genes with a corrected $P < 0.05$ were considered to be differentially expressed.

Results

BRCA mutations in the study group

In the Ashkenazi Jews, conventional melting curve analysis showed that none of the patients with wet AMD carried a mutation in the BRCA1 gene. Only 1 patient (1.92%) was found to be a carrier of the BRCA2 mutation (c.6174delT).

RAD51B mutations in the study group

In the *RAD51B* SNP analysis, the Ashkenazi Jews showed 1.75-fold higher frequency in rs17105278 and 1.43-fold lower frequency of rs4902566 SNP than the non-Ashkenazi group. Similar frequencies were found for rs8017304 in both groups.

Microarray expression data of BRCA1, BRCA2, and RAD51B

Reanalysis of the data downloaded from the GEO database showed that the genes were differentially expressed (adjusted $P < 0.05$) among control, dry AMD, and wet AMD retina and choroid tissues. There was no difference in RNA expression levels of BRCA1 or BRCA2 *or RAD51B SNP's* in either retina or choroid tissue among the three groups from GEO database.[19]

Discussion

This study did not identify an association between the presence of BRCA1/2 mutation(s) and AMD in a cohort of Ashkenazi Jews as compared to the non-Ashkenazi Jewish subjects. The findings failed to prove the hypothesis that AMD is a result of impaired DNA repair in association not only with older age[21] but also with a disruption in the BRCA mechanism. The meanings of the differences in the SNP variations of *RAD51* need further exploration.

Our assumption of a possible link between BRCA1/2 and AMD was based on the dual findings of a role of mutated BRCA1 in the increase in VEGF in tumors[22] and the DNA repair functions of the BRCA1 protein[6-8] and BRCA2.[12] VEGF mediates intraocular neovascularization in various retinal diseases, including AMD,[23] and an increase in its expression in retinal cells promotes retinal endothelial cell proliferation and pathological neovascularization in AMD. However, we found no association between an increase in mutated BRCA and the development of AMD. Furthermore, analysis of our findings against global data derived from patients with AMD (dry and wet) and normal control yielded no difference in RNA expression levels of BRCA1 or BRCA2 or RAD51B among these groups.

We hypothesized that the damaged DNA repair in AMD might be induced by BRCA mutations through the increasing risk of oxidative stress with age.[24] Oxidative stress results from an imbalance between oxidants and antioxidants in favor of the oxidants and leads to damage of numerous cellular components, including lipids, proteins, and nucleic acids. It may accelerate the process of aging and underlie the pathogenesis of many aging-related diseases, including AMD.[25] BRCA1 protects cells against damage due to exogenous or endogenous reactive oxygen species by enhancing antioxidant defenses,[6,7] and a mutation in BRCA impairs this activity.[26] However, we found no mutations in BRCA linked to AMD.

We failed to show any association between wet AMD and any of the suggested mechanisms which linked DNA repair, genome instability, or aging.[27-29] Wet AMD in Ashkenazi Jews was not explained by BRCA1/2 while the RAD51B SNPs differences are yet to be explored.

Conclusions

Ashkenazi Jewish patients with wet AMD do not have a higher rate of BRCA1/2 mutations than the general population or the non-Ashkenazi Jewish patients. Age-related neovascularization of the macula is not associated with known mutations in BRCA1/2 in Ashkenazi Jews.

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Authors' Contributions

S. Weiss: Acquisition of data, analysis and interpretation of data, and drafting the article. M. Azab: Analysis and interpretation of data. A. Zahavi: interpretation of data, review the literature, revision of the article for N. Goldenberg-Cohen: Conception and design, acquisition of data, analysis and interpretation of data, and drafting the article. All authors contributed to critically revising the manuscript for important intellectual content, gave final approval, and agreed to be accountable for all aspects of work.

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