Haplotype Analysis of BRCA2 8765delAG Mutation Carriers in French Canadian and Yemenite Jewish Hereditary Breast Cancer Families

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BRCA2 · French Canadian · Yemenite Jew · Haplotype · Breast cancer

Abstract
The BRCA2 8765delAG mutation was previously reported in hereditary breast cancer families of French Canadian and Yemenite Jewish descent. Haplotype analysis, using six microsatellite markers that span BRCA2 and two intragenic polymorphisms, was performed on 8765delAG mutation carriers to determine if there was evidence that the mutations were identical by descent. The alleles of the microsatellite markers most closely flanking BRCA2 (D13S1697 and D13S1701) were found to be identical in state in all the mutation carriers. However, the disease-associated allele of one of the intragenic markers differed between the Yemenite Jews and French Canadian families, indicating that the 8765delAG mutation has independent origins in these two geographically and ethnically distinct populations.
regard to family history of breast or ovarian cancer [11]. It was the second most common mutation found in 11 out of 97 French Canadian hereditary breast and ovarian cancer families (at least 3 cases of breast or ovarian cancer, and 2 affected individuals were related to the index case as third-degree relatives or closer) [8]. The proportion of French Canadian women who carried 8765delAG and were ascertained without regard to family history of cancer was 2 out of 113 women with ovarian cancer [9] and 2 out of 128 women with breast cancer [Foulkes, W.D. and Tonin, P.T., unpubl. data]. Haplotype analysis using two or five microsatellite markers flanking \( BRCA2 \) suggested that 8765delAG was a founder mutation in each of the populations [10, 11]. Here, we extend the haplotype analysis to include additional markers, and compare the segregating \( BRCA2 \) haplotype in families from these two ethnically and geographically distinct populations.

Seventeen Canadian families of French descent and two Yemenite Jewish families harboring the \( BRCA2 \) 8765delAG mutation were genotyped for six microsatellite markers flanking \( BRCA2 \) and two intragenic single-nucleotide polymorphisms (SNPs). Six of the 17 French Canadian families have not been reported previously and were recruited from the Hereditary Cancer Clinics of McGill University, the Breast Clinic of the Centre de recherche-Centre Hospitalier de l’Université de Montréal (CR-CHUM) (Hôtel Dieu) and the Gynecology and Oncology Clinic of CR-CHUM (Hôpital Notre-Dame), Mon-
Table 2. Allele frequencies in French Canadian carriers (C) and noncarriers (NC) of 8765delAG

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<th>Allele</th>
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The figures in bold type show the most common allele among carriers and noncarriers of 8765delAG. Allele frequencies are not available for Yemenite noncarriers of 8765delAG. Figures in parentheses denote the number of subjects.

tréal. Mutation carriers and, where available, additional family members were genotyped to determine phase as previously described [8]. We also genotyped two SNPs, IVS8+56C>T and IVS16-14T>C, located in introns 8 and 16 of BRCA2, respectively, by DNA sequencing and allele-specific oligonucleotide hybridization (details available from authors). These SNPs were previously described as sequence variants at nucleotides 909+56 and 8034–14, respectively [12]. The disease-associated haplotype was deduced by inspection of segregating genotypes in the families.

The most common haplotype that segregated with 8765delAG in the French Canadian breast cancer families was 4-3-11-2-C-C-4-9 (table 1). In two families (SO28 and HD86), the disease-associated haplotype was inferred to be recombinant for one of the most distal flanking markers, D13S260 or D13S171. Where phasing was not possible, the disease-associated allele was present. Thus, we infer that the disease-associated haplotype (3-11-2-C-C-4), comprised of four microsatellite markers and two intragenic SNPs, is identical in state in the French Canadian families. In order to estimate marker allele frequencies in the general French Canadian population, we genotyped French Canadian women who were affected with breast cancer, but were not carriers of 8765delAG. Due to limited amounts of DNA, we were not able to genotype a sufficiently large number of samples for the same set of markers in order to estimate haplotype frequencies that take into account linkage disequilibrium. However, the most common allele for five of the eight markers in this sample of ‘normal’ chromosomes was not the allele associated with 8765delAG (table 2). Under the assumption of linkage equilibrium, the six-marker haplotype is expected to occur less than 1% of the time. This suggests that 8765delAG is carried on an uncommon haplotype in the French Canadian population and provides support for the hypothesis that alleles harboring 8765delAG are identical by descent in this population. The birthplaces of the grandparents of the mutation carriers were not concentrated in any particular region of Quebec; this is consistent with the families not being closely related. Identity by descent for mutations that are relatively common in the French Canadian population, but rare elsewhere, is expected, since the contemporary population was founded by about 8,500 French migrants who settled in the St. Lawrence valley between 1608 and 1760 [13]. About 3,525 founders who settled in New France before 1680 have been estimated to account for approximately two...
thirds of the present-day French Canadian gene pool [13, 14]. The 8765delAG mutation is probably not common in France, as it was not found among the BRCA2 mutations identified in 16 French breast cancer families [15]. Thus, it is likely that 8765delAG was introduced by one founder, or descendants of one founder, prior to 1680.

Haplotype analysis of three Yemenite Jewish families from Israel using the most distal flanking markers, D13S260 and D13S171, showed that the 8765delAG carriers shared the same alleles [11]. The analysis of additional markers in family BC10 revealed that the disease-associated haplotype was 7-3-2-2-T-C-4-6 (table 1). We were unable to establish the disease-associated haplotype for family BC149; however, the genotypes of the carriers in the two Yemenite families are consistent with 8765delAG being identical by descent.

The BRCA2 8765delAG mutation is a deletion of 2 bp from the sequence AGAGAG. This sequence of three repeats, compared to a longer stretch of repeats, is likely to have a lower mutation rate due to slipped-strand mispairing during DNA replication [16]. However, the presence of three DNA sequence-symmetric elements that include part of the AGAGAG sequence may increase the chance of a deletion of AG. Small deletions in BRCA1 and other genes have been shown to be associated with symmetric elements [17, 18].

Our analysis provides evidence that 8765delAG arose independently in the ancestors of the French Canadian and Yemenite Jewish families. Although we cannot rule out an ancient common origin for 8765delAG, we think it less likely, since two recombination events, including an intragenic recombination between the SNPs (or other events), need to have occurred to support the hypothesis of a common origin. During the preparation of this paper, 8765delAG was reported as a founder mutation in hereditary breast cancer families in Sardinia [19]. A comparison of haplotypes from the French Canadian and Sardinian mutation carriers revealed that 8765delAG is associated with different haplotypes in these two populations (data not shown). In conclusion, 8765delAG appears to have arisen independently at least three times, but within the individual populations, it is likely to be identical by descent.

Acknowledgments

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