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Breast Cancer RESEARCH

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An original phylogenetic approach identified mitochondrial haplogroup T1a1 as inversely associated with breast cancer risk in *BRCA2* mutation carriers

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Abstract

Introduction: Individuals carrying pathogenic mutations in the *BRCA1* and *BRCA2* genes have a high lifetime risk of breast cancer. *BRCA1* and *BRCA2* are involved in DNA double-strand break repair, DNA alterations that can be caused by exposure to reactive oxygen species, a main source of which are mitochondria. Mitochondrial genome variations affect electron transport chain efficiency and reactive oxygen species production. Individuals with different mitochondrial haplogroups differ in their metabolism and sensitivity to oxidative stress. Variability in mitochondrial genetic background can alter reactive oxygen species production, leading to cancer risk. In the present study, we tested the hypothesis that mitochondrial haplogroups modify breast cancer risk in *BRCA1/2* mutation carriers.

Methods: We genotyped 22,214 (11,421 affected, 10,793 unaffected) mutation carriers belonging to the Consortium of Investigators of Modifiers of *BRCA1/2* for 129 mitochondrial polymorphisms using the iCOGS array. Haplogroup inference and association detection were performed using a phylogenetic approach. ALTree was applied to explore the reference mitochondrial evolutionary tree and detect subclades enriched in affected or unaffected individuals.

Results: We discovered that subclade T1a1 was depleted in affected *BRCA2* mutation carriers compared with the rest of clade T (hazard ratio (HR) = 0.55; 95% confidence interval (CI), 0.34 to 0.88; $P = 0.01$). Compared with the most frequent haplogroup in the general population (that is, H and T clades), the T1a1 haplogroup has a HR of 0.62 (95% CI, 0.40 to 0.95; $P = 0.03$). We also identified three potential susceptibility loci, including G13708A/rs28359178, which has demonstrated an inverse association with familial breast cancer risk.

Conclusions: This study illustrates how original approaches such as the phylogeny-based method we used can empower classical molecular epidemiological studies aimed at identifying association or risk modification effects.

Introduction

Breast cancer is a multifactorial disease with genetic, lifestyle and environmental susceptibility factors. Approximately 15% to 20% of the familial aggregation of breast cancer is accounted for by mutations in high-penetrance susceptibility genes [1-3], such as *BRCA1* and *BRCA2*. Pathogenic mutations in *BRCA1* and *BRCA2* confer lifetime breast cancer risk of 60% to 85% [4,5] and 40% to 85% [4,5], respectively. Other genomic variations (for example, in genes encoding proteins interacting with *BRCA1* and *BRCA2*) have been identified as modifiers of breast cancer risk and increase or decrease the risk initially conferred by *BRCA1* or *BRCA2* mutation [6].

BRCA1 and *BRCA2* are involved in DNA repair mechanisms, including double-strand break (DSB) repair by homologous recombination [7,8]. DSBs are considered to be among the most deleterious forms of DNA damage because the integrity of both DNA strands is compromised simultaneously. These breaks can lead to genomic instability resulting in translocations, deletions, duplications or mutations when not correctly repaired [9]. Reactive oxygen species (ROS) are one of the main causes of DSBs, along with exposure to ionizing radiation, various chemical agents and ultraviolet light [10].

ROS are naturally occurring chemical derivatives of metabolism. Elevated levels of ROS and downregulation of ROS scavengers and/or antioxidant enzymes can lead to oxidative stress, which is associated with a number of human diseases, including various cancers [11]. The electron transport chain process, which takes place in the mitochondria, generates the majority of ROS in human cells. Variations in the mitochondrial genome have been shown to be associated with metabolic phenotypes and

oxidative stress markers [12]. Mitochondrial dysfunction recently was shown to promote breast cancer cell migration and invasion through the accumulation of a transcription factor, hypoxia-inducible factor 1α, via increased production of ROS [13].

Human mitochondrial DNA (mtDNA) has undergone a large number of mutations that have segregated during evolution. Those changes are now used to define mitochondrial haplogroups. Some of these changes slightly modify metabolic performance and energy production; thus, not all haplogroups have identical metabolic capacities [14]. It has been hypothesized that the geographic distribution of mitochondrial haplogroups results from selection of metabolic capacities driven mainly by adaptation to climate and nutrition [15,16].

Mitochondrial haplogroups have been associated with diverse multifactorial diseases, such as Alzheimer's disease [17], hypertrophic cardiomyopathy [18], retinal diseases [19] or age-related macular degeneration [20]. Variations in mtDNA have also been linked to several types of cancer, such as gastric cancer [21] or renal cell carcinoma [22]. Interestingly, variations in mtDNA have been linked to several types of female cancers, including endometrial [23], ovarian [24] and breast cancer [25,26]. A recent study underlined the possibility that mtDNA might be involved in the pathogenic and molecular mechanisms of familial breast cancer [27].

The Collaborative Oncological Gene-environment Study [28] (COGS) is a European project designed to improve understanding of genetic susceptibility to breast, ovarian and prostate cancer. This project involves several consortia: the Breast Cancer Association Consortium (BCAC) [29], the Ovarian Cancer Association Consortium [30], the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) [31] and the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA) [32]. CIMBA is a collaborative group of researchers working on genetic modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers. As part of the COGS project, more than 200,000 single-nucleotide polymorphisms (SNPs) were genotyped for *BRCA1* and *BRCA2* female mutation carriers on the iCOGS chip, including 129 mitochondrial polymorphisms. The iCOGS chip is a custom Illumina™ Infinium genotyping array (Illumina, San Diego, CA, USA) designed to test, in a cost-effective manner, genetic variants related to breast, ovarian and prostate cancers.

In this study, we explored mitochondrial haplogroups as potential modifiers of breast cancer risk in women carrying pathogenic *BRCA1* or *BRCA2* mutations. Our study includes females diagnosed with breast cancer and unaffected carriers belonging to CIMBA. We used an original analytic phylogenetics-based approach implemented in a homemade algorithm and in the program ALTree [33,34] to infer haplogroups and to detect associations between haplogroups and breast cancer risk.

Methods

Ethics statement

A signed informed written consent form was obtained from all participants. All contributing studies involved in CIMBA received approvals from the institutional review committees at their host institutions. Ethical committees that approved access to the data analyzed in this study are listed in Additional file 1.

BRCA1 and *BRCA2* mutation carriers

Final analyses included 7,432 breast cancer cases and 7,104 unaffected *BRCA1* mutation carriers, as well as 3,989 invasive breast cancer and 3,689 unaffected *BRCA2* mutation carriers, all belonging to CIMBA. Supplementary specifications regarding inclusion profiles and studies belonging to CIMBA are available in the reports by Couch *et al.* [35] and Gaudet *et al.* [36]. All analyses were conducted separately on CIMBA *BRCA1* and *BRCA2* mutation carriers (abbreviated *pop1* and *pop2*, respectively). Eligible female carriers were aged 18 years or older and had a pathogenic mutation in *BRCA1* and/or *BRCA2*. Women with both *BRCA1* and *BRCA2* mutations were included in downstream analyses. Data were available for year of birth, age at study recruitment, age at cancer diagnosis, *BRCA1* and *BRCA2* mutation description and self-reported ethnicity. Women with ovarian cancer history were not excluded from analyses, and they represented 15% and 7% of *BRCA1* and *BRCA2* mutation carriers, respectively. Information regarding mastectomy was incomplete and was therefore not used as an inclusion or exclusion parameter.

Genotyping and quality filtering

Genotyping was conducted using the iCOGS custom Illumina Infinium array. Data from this array are available to the scientific community upon request. Please see [37] for more information. Genotypes were called using Illumina's proprietary GenCall algorithm. Genotyping and quality filtering were described previously [35,36]. Initially, 129 mitochondrial SNPs were genotyped for both *BRCA1* and *BRCA2* mutation carriers. SNPs fulfilling the following criteria were excluded from downstream analyses: monoallelic SNPs (minor allele frequency = 0), SNPs with more than 5% data missing, annotated as triallelic, or having probes cross-matching with the nuclear genome. Heterozygous genotypes were removed from analyses, and we further filtered out SNPs having more than 5% of heterozygous calls to limit the potential for heteroplasmy affecting our results. We also did not retain SNPs representing private mutations. These mutations are rare, often restricted to a few families, and not sufficiently prevalent in the general population to be included in the reference

mitochondrial evolutionary tree (see below). This last step of filtration yielded 93 and 92 SNPs for the *pop1* and *pop2* analyses, respectively (see Additional file 2). Only individuals with fully defined haplotypes (that is, non-missing genotypes for the 93 and 92 SNPs selected for *pop1* and *pop2*, respectively) were included in downstream analyses (14,536 and 7,678 individuals, respectively).

Mitochondrial genome evolution and haplogroup definition

Analyses were based on the theoretical reconstructed phylogenetic tree of the mitochondrial genome (mtTree) known as PhyloTree [38] (v.15). The mtTree is rooted by the Reconstructed Sapiens Reference Sequence (RSRS). RSRS has been identified as the most likely candidate to root the mtTree by refining human mitochondrial phylogeny by parsimony [39]. Each haplogroup in mtTree is defined by the set of mtDNA SNPs that have segregated in RSRS until today in the mitochondrial genome. Each haplogroup is fully characterized by the 16,569-bp sequence resulting from the application of all the substitutions that are encoded by the corresponding SNPs in the RSRS sequence.

Haplogroups imputation

The phylogenetic approach used to infer haplogroups is described in Figure 1. Mitochondrial genome sequences can be reconstructed at each node of mtTree, given the

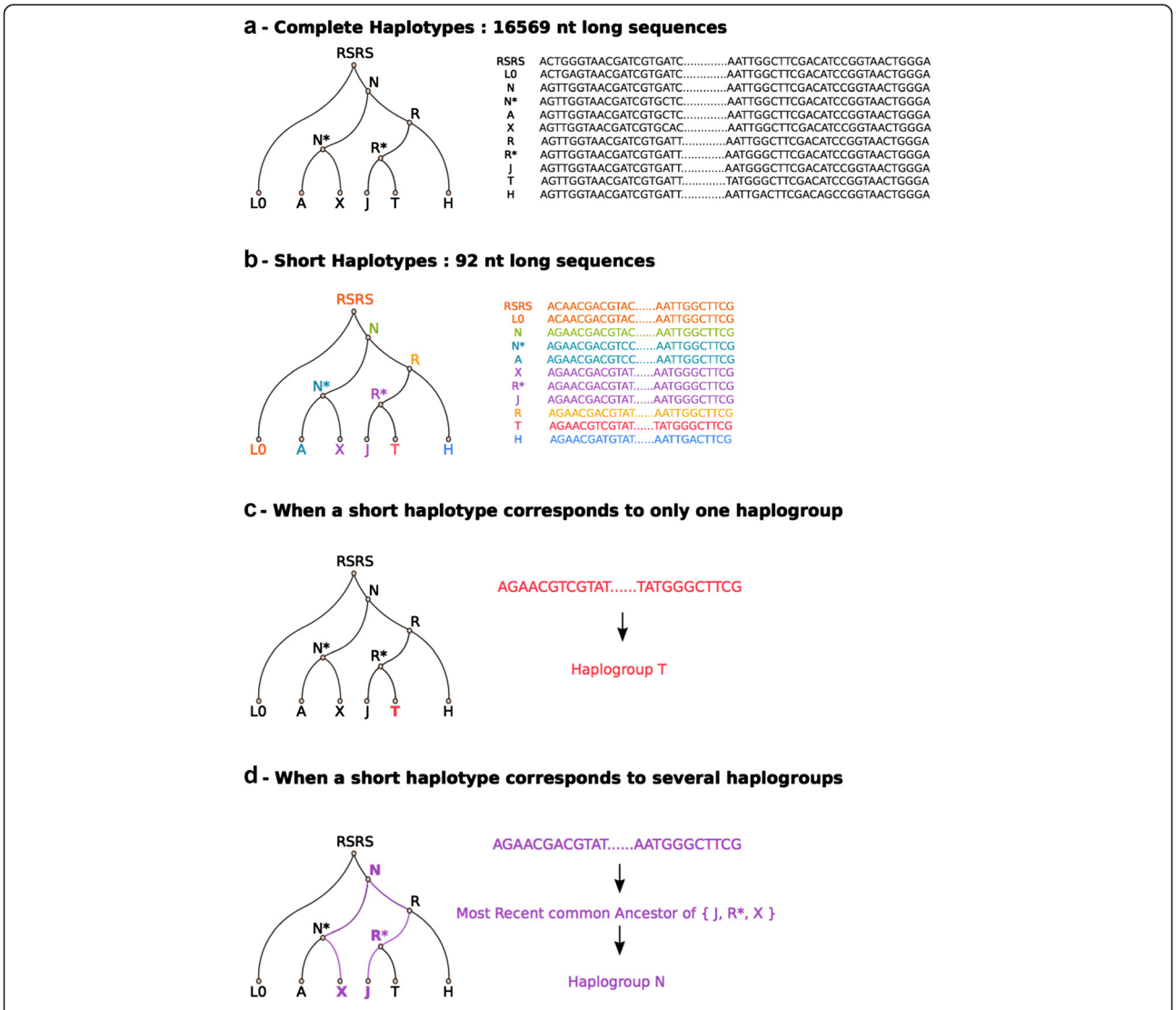


Figure 1 Simplified representation of the phylogenic method used to infer haplogroups. **(a)** Full-length haplotypic sequences are reconstructed at each node of the reference tree. **(b)** Haplotypes are then restricted to available loci. Sequences of the same color are identical. **(c)** Unique short haplotypes are matched directly with the corresponding haplogroup. **(d)** Sequences that match with several haplogroups are associated with their most recent common ancestor haplogroup. RSRS, Reconstructed Sapiens Reference Sequence.

substitutions that have segregated in RSRS. Each haplogroup therefore has a corresponding full-length mitochondrial sequence. However, the full-length mitochondrial sequence is not available in the data, because the iCOGS platform captured only 93 and 92 SNPs for *pop1* and *pop2*, respectively. Thus, for each of the 7,864 nodes of the phylogenetic tree, the corresponding short haplotype (that is, the full-length sequence restricted to available loci) was defined. Some of the short haplotypes are unique, and they can be matched with their corresponding haplogroup directly. However, most of the time, given the small number of SNPs analyzed, several haplogroups correspond to the same short haplotype. Consequently, a unique haplogroup cannot confidently be assigned to each short haplotype. Therefore, each short haplotype was assigned the most recent common ancestor of all the haplogroups that share the same short haplotype. Once this matching was done, short haplotypes were reconstructed in the same way for each individual in our dataset and were assigned the corresponding haplogroup. The accuracy of the method used was assessed by application to a set of 630 mtDNA sequences of known European and Caucasian haplogroups (see Additional file 3).

Association detection

This phylogenetic approach is based on the identification of subclades in the reference phylogenetic tree of the mitochondrial genome differentially enriched for cases and unaffected controls compared with neighboring subclades. We used ALTree [33,34] to perform association testing. ALTree—standing for Association detection and Localization of susceptibility sites using haplotype phylogenetic Trees—is an algorithm used to perform nested homogeneity tests to compare distributions of affected and unaffected individuals in the different clades of a given phylogenetic tree. The objective is to detect if some clades of a phylogenetic tree are more or less enriched in affected or unaffected individuals compared with the rest of the tree. There are as many tests performed as there are levels in the phylogenetic tree. The *P*-value at each level of the tree is obtained by a permutation procedure in which 1,000 permutations are performed. Individual labels ("affected" or "unaffected") are permutated 1,000 times to see to what extent the observed distribution of affected or unaffected is different from a random distribution. A procedure to correct for multiple testing adapted to nested tests [40] is implemented in ALTree. The objective of ALTree is to detect an enrichment difference at the level of the whole tree. To conserve computational time and resources, only the most significant *P*-value obtained for all tests performed on one tree is corrected.

Handling genetic dependency

ALTree is used to perform homogeneity tests to detect differences in enrichment or depletion of affected or unaffected individuals between clades in the phylogenetic tree. This kind of test can be performed only on independent data. However, because some individuals in the CIMBA dataset belong to the same family, we constructed datasets with genetically independent data by randomly selecting one individual from among all those belonging to the same family and sharing the same short haplotype. To take into account the full variability of our data, we resampled 1,000 times. The results of the analysis pipeline are obtained for each resampling independently and then averaged over the 1,000 resamplings to obtain final results.

Character reconstruction at ancestral nodes

Before the ALTree localization algorithm was launched, ancestral sequences were reconstructed at each internal tree node; that is, short haplotypes were inferred with maximum likelihood at all nodes that were not leaves. We used the software PAML [41] to perform the reconstruction at ancestral nodes using a maximum likelihood method. The phylogeny model used was the general time-reversible model (either GTR or REV).

Localization of susceptibility sites

ALTree also includes an algorithm used to identify which sites are the most likely ones to be involved in the association detected. For each short haplotype observed, the ALTree add-on *altree-add-S* adds to the short haplotype sequence a supplementary character called *S*, which represents the disease status associated with this short haplotype. Are individuals carrying this short haplotype more often affected or unaffected? *S* is calculated based on the affected and unaffected counts, the relative proportion of affected and unaffected in the whole dataset, and sensibility parameter ε. ε was set to its default value, which is 1. After *S* character computation, haplotypes including character *S* are reconstructed at ancestral nodes. Susceptibility site localization is achieved with ALTree by computing a correlated evolution index calculated between each change of each site and the changes of the character *S* in the two possible directions of change. The sites whose evolution are the most correlated with the character *S* are the most likely susceptibility sites.

Selected subclades

The analyses were carried out on the full evolutionary tree. However, the more haplogroups there are at each level, the less statistical power homogeneity tests have. Therefore, analyses were also applied to subclades extracted from the tree. Subclades were defined using counts of individuals in each haplogroup of the clade to maximize statistical power. The chosen subclades

and corresponding affected and unaffected counts are presented in Table 2.

Statistical analysis

We quantified the effect associated with enrichment discovered by applying ALTree by building a weighted Cox regression in which the outcome variable is the status (affected or non-affected) and the explicative variable is the inferred haplogroup. Analyses were stratified by country. Data were restricted to the clades of interest. The uncertainty in haplogroup inference was not taken into account in the model. The weighting method used takes into account breast cancer incidence rate as a function of age [42] and the gene containing the observed pathogenic mutation (that is, *BRCA1* or *BRCA2*). Familial dependency was handled by using a robust sandwich estimate of variance (R package *survival*, *cluster()* function).

Results

Haplogroup imputation

In Additional file 4, absolute and relative frequencies are recapitulated for each haplogroup imputed in *BRCA1* and *BRCA2* mutation carriers. For *BRCA1* mutation carriers, we reconstructed 489 distinct short haplotypes of 93 loci from the genotypes data. Only 162 of those 489 short haplotypes matched theoretical haplotypes reconstructed in the reference mitochondrial evolutionary tree. These 162 haplotypes represented 13,315 of 14,536 individuals. Thus, 91.6% of *BRCA1* mutation carriers were successfully assigned a haplogroup. For *BRCA2* mutation carriers, we reconstructed 350 distinct short haplotypes of 92 loci from our genotype data. Only 139 of those 350 short haplotypes matched theoretical haplotypes reconstructed in the reference mitochondrial evolutionary tree. These 139 haplotypes represented 6,996 of 7,678 individuals. Thus, 91.1% of *BRCA2* mutation carriers were successfully assigned a haplogroup. Because more *BRCA1* than *BRCA2* mutation carriers were genotyped (14,536 vs. 7,678 individuals), we logically observed more distinct haplotypes in *pop1* than in *pop2* (489 vs. 350 haplotypes).

The accuracy of the main haplogroup inference method used was estimated at 82% and reached 100% for haplogroups I, J, K, T, U, W and X. Given the set of SNPs we disposed of, our method has difficulty differentiating between H and V haplogroups (see Additional file 3).

Association results

For both populations of *BRCA1* or *BRCA2* mutation carriers, as well as for the full tree as for all selected subclades (see Table 1), we extracted the mean corrected *P*-values for association testing over all resamplings performed (see Table 2). The only corrected *P*-value that remained significant was that obtained for subclade T (abbreviated T*) in the population of individuals of *BRCA2* mutation carriers ($P = 0.04$).

Table 1 Counts of participants in selected subclades

Subclade	*BRCA1* mutation carriers	*BRCA2* mutation carriers
U8	1,458	863
T	1,243	651
J	1,270	630
J1	1,043	513
H	3,706	1,967
H1	582	337
U5	868	458
X1′2′3	221	103
K1a	608	364

The phylogenetic tree of subclade T (see Figure 2a) contains only three levels; thus, only three tests were performed within this clade. Raw *P*-values were examined to determine at which level of the tree ALTree detects a difference of enrichment in affected or unaffected individuals (see Table 3). Only the *P*-value associated with the test performed at the first level of the tree is significant. We looked more closely at the mean frequencies of affected and unaffected individuals in the tree at this level (see Figure 2b). In the T1a1 subclade, the mean count of affected and unaffected are 32 and 47, respectively. In the T2* subclade, we observed, on average, 217 and 148 affected and unaffected individuals, respectively, whereas in the T subclade, we observed, on average, 13 and 11 affected and unaffected individuals, respectively. The ranges observed for each of these values over the 1,000 resamplings are represented in Figure 2b. On the basis of these observations, we conclude that subclade T1a1 is depleted in affected carriers compared with the neighboring subclades T and T2.

Table 2 Mean corrected *P*-values for association testing with ALTree

Subclade	*pop1* corrected *P*-value	*pop2* corrected *P*-value
Full	0.830	0.681
U8	0.146	0.626
T	0.285	**0.040**
J	0.718	0.112
J1	0.621	0.150
H	0.747	0.930
H1	0.268	0.804
U5	0.829	0.747
X1′2 ′3	0.416	0.629
K1a	0.170	0.162

[a]*pop1*, *BRCA1* mutation carrier; *pop2*, *BRCA2* mutation carrier. Bold indicates a significant *P*-value.

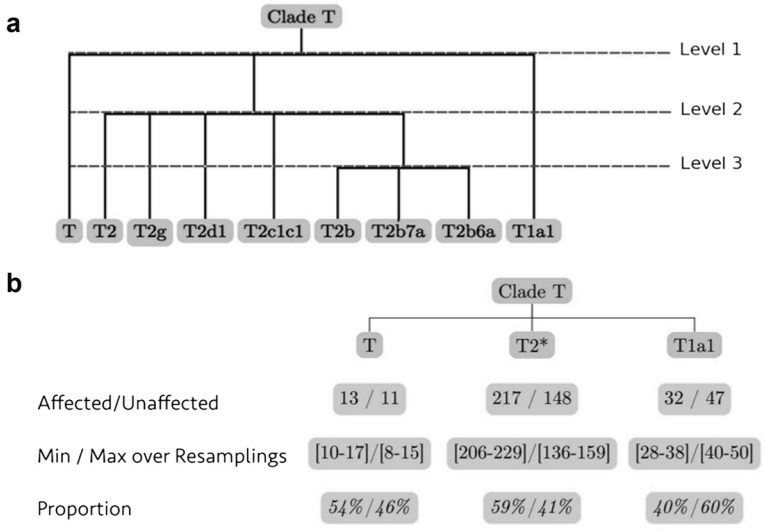


Figure 2 Phylogenetic tree of subclade T tested for association with ALTree. **(a)** Phylogenetic tree of subclade T with all observed haplogroups. A homogeneity test is performed at each level of the tree. **(b)** First level of the phylogenetic tree of subclade T. Averaged counts, ranges and proportions of affected and unaffected observed in resamplings are indicated below each subclade. T2* represents the entire T2 subclade.

Localization results

We performed a localization analysis with ALTree. The correlated evolution index for all non-monomorphic sites observed in short haplotype sequences of subclade T are displayed in Additional file 5. The higher the correlated evolution index, the more likely it is that corresponding sites will be involved in the observed association. Three short haplotype sites numbered 44, 57 and 72 and corresponding to SNPs T988C, G11812A/rs4154217 and G13708A/rs28359178, respectively, clearly distinguish themselves, with correlation index values of 0.390, 0.324 and 0.318, respectively, whereas the correlation index values of all other sites ranged from −0.270 to −0.101. Table 4 shows the details for these three loci.

Effect quantification

The ALTree method is able to detect an association, but cannot to quantify the associated effect. We estimated the risk of breast cancer for individuals with the T1a1 haplogroup compared with individuals with another T subclade haplogroup in the population of *BRCA2* mutation carriers using a more classical statistical method, a weighted Cox regression. We found a breast cancer HR of 0.55 (95% CI, 0.34 to 0.88; $P = 0.014$). We also tested haplogroup T1a1 and compared it with other T* haplogroups and the H haplogroup (the main haplogroup in the general population), and we found a breast cancer HR of 0.62 (95% CI, 0.40 to 0.95; $P = 0.03$).

Table 3 Non-corrected *P*-values by level of phylogenetic tree for subclade T in *BRCA2* mutation carriers

Level	Degrees of freedom	Mean of non-corrected *P*-value
1	2	0.02141039
2	6	0.14355900
3	8	0.22249700

Discussion

We employed an original phylogenetic analytic method, coupled with more classical molecular epidemiologic analyses, to detect mitochondrial haplogroups differentially enriched for affected *BRCA1/2* mutation carriers. We successfully inferred haplogroups for more than 90% of individuals in our dataset. After haplogroup imputation, the ALTree method identified T1a1 in the T clade as differentially enriched in affected *BRCA2* mutation carriers, whereas no enrichment difference was found for *BRCA1* mutation carriers. The T subclade is present in 4% of African populations compared with 11% in Caucasian and Eastern European populations [43]. In our data, the T subclade represented 9.34% of *BRCA1* mutation carriers and 9.30% of *BRCA2* carriers. The ALTree method also identified three potential breast cancer susceptibility loci in mtDNA. The main goals of using the phylogenetic method we used were to improve statistical power by regrouping subclades according to genetic considerations, to limit the number of tests performed and to precisely quantify this number. ALTree identified three SNPs of interest. Whereas the association we observed could possibly be driven by a single SNP, no difference was observed between multivariate and univariate cox models including the three SNPs identified by ALTree (data not shown).

In this study, we investigated to what extent mtDNA variability modified breast cancer risk in individuals

Table 4 Description of loci identified as potential susceptibility sites by ALTree[a]

Site	SNP name	Position	Direction of change	Correlated evolution index	Major allele	Minor allele	MAF in *pop2*
44	MitoT9900C	9,899	T → C	0.390	T	C	0.016
57	rs41544217	11,812	G → A	0.324	A	G	0.071
72	rs28359178	13,708	G → A	0.318	G	A	0.111

[a]MAF, Mean allele frequency; *pop2*, *BRCA2* mutation carrier.

carrying pathogenic mutations in *BRCA1/2*. A large proportion of breast cancer heritability still remains unexplained today [44]. Different methods exist to study genomic susceptibility to a disease, such as linkage analyses (which identified the *BRCA1* and *BRCA2* susceptibility genes) or genome-wide association studies (GWASs). However, classical linkage analysis cannot be applied to the haploid mitochondrial genome. Furthermore, commercial GWAS chips available do not adequately capture the majority of mtDNA SNPs. A non-genome-wide and mtDNA-focused approach was required to explore how mtDNA variability influences breast cancer risk. Here we have shown that *BRCA2* mutation carriers with the subclade T1a1 have between 30% and 50% less risk of breast cancer than those with other clades, which, if validated, is a clinically meaningful risk reduction and may influence the choice of risk management strategies.

The association we observed among *BRCA2*, but not *BRCA1*, mutation carriers may reveal a functional alteration that would be specific to mechanisms involving *BRCA2*-related breast cancer. Today, it is established that *BRCA1*- and *BRCA2*-associated breast cancers are not phenotypically identical. These two types of tumors do not harbor the same gene expression profiles or copy number alterations [45]. Breast cancer risk modifiers in *BRCA1/2* mutation carriers have already been identified [46]. However, most of them are specific from one or the other type of mutation carried [47]. It is therefore not surprising that this observation is observed in *BRCA2* mutation carriers only.

Our inability to assign haplogroups to 9% of study participants could have three main explanations. First, given the high mutation rate in the mitochondrial genome, observed combinations of mtDNA SNPs might have appeared relatively recently in the general population, and the corresponding haplotypes might not yet be incorporated into PhyloTree. Second, only one genotyping error could lead to chimeric haplotypes that do not exist, although, given the quality of our genotyping data, this is unlikely. Third, the mitochondrial reference evolutionary tree PhyloTree is based on phylogeny reconstruction by parsimony, and, for some subclades, it might be suboptimal, especially for haplogroups relying on few mitochondrial sequences, as is the case for African haplogroups [48]. In cases of uncertainty, the choice we made to assign the most recent common ancestor to the studied haplotype enabled us to improve statistical power without introducing a bias in the detected association. For the association detected between T, T1* and T2* subclades, the haplogroup inference method used did not bias the counts of affected and unaffected individuals in these subclades. More details are presented in Additional file 6. Furthermore, on the basis of the haplogroup inference with our method of 630 European and Caucasian mtDNA sequences whose haplogroup is known, we successfully assigned the correct main haplogroup and subhaplogroup of 100% of sequences belonging to T, T2* and T1a1* haplogroups.

We quantified the effect corresponding to the detected association by using a more classical approach. We built a weighted Cox regression including inferred haplogroup as an explicative variable. However, the uncertainty in haplogroup inference was not taken into account in this model. Nevertheless, based on haplogroup assignment and regrouping performed in clade T, affected and unaffected counts of individuals in this clade were not biased.

With only 129 loci genotyped over the 16,569 nucleotides composing the mitochondrial genome, we certainly did not explore the full variability of mitochondrial haplotypes. A characterization of individual mitochondrial genomes would require more complete data acquisition methods to be used, such as next-generation sequencing. However, next-generation sequencing has its own limits and challenges, because some regions of the mitochondrial genome are not easily mappable, owing to a high homology with the nuclear genome, among other factors, and important bioinformatics treatment is necessary to overcome sequencing technology biases. Finally, even for a relatively short genome of "only" 16,569 bp, mtDNA sequencing of more than 20,000 individuals would represent a major increase in cost relative to genotyping 129 SNPs.

ALTree identified T9899C, G11812A/rs41544217 and G13708A/rs28359178 as three potential susceptibility sites for the discovered association (see Additional file 7). These three SNPs are located in the coding part of genes *MT-CO3*, *MT-ND4* and *MT-ND5*, respectively. When looking at PhyloTree, T9899C seems to be involved in T1 subclade definition, whereas G13708A and A11812G are involved in T2 subclade definition. Whereas T98899C and

G11821/rs41544217 are synonymous SNPs, G10398A leads to a change of amino acid in the final protein (from alanine to threonine). These two synonymous SNPs have never been described in a disease context in the literature. G13708A is also known for being a secondary mutation for Leber's hereditary optic neuropathy (LHON) and multiple sclerosis [49]. Although the role of secondary mutations in LHON is still controversial, G13708A could be associated with impairment of the respiratory chain in this pathology. G13708A has also been described as a somatic mutation in a breast cancer tumor, whereas it was not present in adjacent normal tissue or in blood leukocytes [50]. A high proportion of mitochondrial somatic tumor-specific variants are also known mtDNA SNPs, which is consistent with the hypothesis that tumor cells are prone to acquire the same mutations that segregate into mtDNA by selective adaptation when humans migrated out of Africa and confronted new environments [51]. Interestingly, the germline variant G13708A has already been shown to be inversely associated with familial breast cancer risk (with the same direction of the association), with a breast cancer odds ratio of 0.47 (95% CI, 0.24 to 0.92) [52]. None of these SNPs have been described in the context of ovarian cancer.

The corrected *P*-value obtained using ALTree in studying clade T is 0.02, which is not highly significant. A replication step should be performed to validate these results. However, it will be difficult to include enough women in this replication step, given the specific profile studied here. In fact, the estimations of *BRCA2* pathogenic mutations in the general population range from 0.068% [5] to 0.69% [53]. T1a1 represents only a small percentage of European haplogroups (from 1% to 2%). The number of women who have this association is therefore low. However, women carrying such mutations are confronted with drastic choices regarding the prevention of breast cancer, notably prophylactic mastectomy or complete hysterectomy. If breast cancer risk is really reduced by a factor of 2 for women with T1a1, this could be an important fact to take into account for breast cancer prevention.

Conclusions

This study and our results suggest that mitochondrial haplogroup T1a1 may modify the individual breast cancer risk in *BRCA2* mutation carriers. For now, this observation cannot be extended to the general population. Further investigation of the biological mechanism behind the associations we observed may further reinforce the hypothesis that the mitochondrial genome is influential in breast cancer risk, particularly among carriers of *BRCA2* mutations, and, if validated, is of a level to influence cancer risk management choices.

Additional files

Additional file 1: List of ethical committees that approved the access to the data analyzed in this study.

Additional file 2: SNPs selected for downstream analyses.

Additional file 3: Description and results of the procedure used to estimate the accuracy of our haplogroup inference methodology.

Additional file 4: Absolute and relative frequencies of imputed haplogroups by population. Table containing absolute and relative frequencies of imputed haplogroups for *BRCA1* and *BRCA2* mutation carriers.

Additional file 5: Correlated evolution index for all non-monomorphic sites observed in short haplotype sequences of subclade T. Table containing correlated evolution index for all non-monomorphic sites observed in short haplotypes sequences of subclade T.

Additional file 6: Details of haplogroups inference results for subclade T.

Additional file 7: Methods used to compute coevolution index.

Abbreviations

BCAC: Breast Cancer Association Consortium; CI: Confidence interval; CIMBA: Consortium of Investigators of Modifiers of *BRCA1/2*; COGS: Collaborative Oncological Gene-environment Study; DSB: Double-strand break; GWAS: Genome-wide association study; HR: Hazard ratio; LHON: Leber's hereditary optic neuropathy; MAF: Mean allele frequency; mtDNA: Mitochondrial DNA; mtTree: Phylogenetic tree of the mitochondrial genome; OCAC: Ovarian Cancer Association Consortium; *pop1*: *BRCA1* mutation carrier; *pop2*: *BRCA2* mutation carrier; PRACTICAL: Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome; ROS: Reactive oxygen species; RSRS: Reconstructed Sapiens Reference Sequence; SNP: Single-nucleotide polymorphism.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Conception and design: DGC and GT. Development of methodology: SB, CBa and VD. Acquisition of data: LM, SHe, DB, ALe, JD, KBK, PS, MBT, WKC, DEG, SSB, RJ, LT, NT, CMD, EJvR, SLN, YCD, AMG, BE, FCN, TvOH, AO, JBe, RA, ES, JNW, MThe, PP, PR, VP, RDo, BB, BP, DZ, GSc, SMan, LV, GLC, LP, LO, DY, IK, JGa, UH, AD, ABr, CBr, CF, DGE, DF, DE, FDo, JCo, JA, JBa, LW, LI, LES, MJK, MTi, MTR, MEP, PJM, RP, RE, RDa, SHo, TCo, AKG, CI, KC, KDL, AM, AG, BW, CS, CE, DN, DS, HP, KK, KR, ND, NA, RV, RKS, SP, NB, SW, AdP, CLe, CLas, DL, ER, FDa, GSC, HD, LB, LG, NU, VB, VS, YB, JCa, LVL, MP, PAD, MdlH, TCa, HN, KA, AJag, AMvdO, CMK, CMA, FEvL, FBH, HEM, JCO, KvR, MAR, PD, RBvdL, EO, OD, AT, CLaz, IB, JDV, AJak, GSu, JGr, JLu, KD, KJ, BAA, CM, AA, MM, MRT, ABS, WF, CO, NLi, VSP, CIS, ALinc, LJ, MC, MR, JV, ABe, AF, CFS, CR, DGK, GP, MTe, MHG, PLM, GR, EI, AMM, GG, ILA, ST, AET, ISP, MTho, TAK, UBJ, MAC, EF, JZ, YL, ALind, BM, BA, NLo, RR, OIO, RLN, SR, KLN, SMD, TRR, BKA, GM, BYK, JLe, SO, DSL, GT, JS, FJC, KO, DFE, GC, ACA, SMaz, CMP and OMS. Analysis and interpretation of data: SB, DGC and ACA. Writing of the manuscript: SB, DGC, ACA, SH, ABS, GC, SLN, AET, ILA, JCO, KO, MTho and GM. All authors read and approved the final manuscript.

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