The Genetic Analyses of French Canadians of Quebec Facilitate the Characterization of New Cancer Predisposing Genes Implicated in Hereditary Breast and/or Ovarian Cancer Syndrome Families

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Simple Summary: The French Canadian population of the province of Quebec has been investigated because of its genetic attributes and is known for making significant contributions to the medical genetics field. Their unique genetic background has been attributed to a small number of early settlers from France that contributed to the majority of the gene pool. The French Canadian population has been investigated for the role of known breast and ovarian cancer predisposing genes, such as BRCA1 and BRCA2. In this review we describe the merits of studying this population with respect to the discovery of new such cancer predisposing gene.

Abstract: The French Canadian population of the province of Quebec has been recognized for its contribution to research in medical genetics, especially in defining the role of heritable pathogenic variants in cancer predisposing genes. Multiple carriers of a limited number of pathogenic variants in BRCA1 and BRCA2, the major risk genes for hereditary breast and/or ovarian cancer syndrome families, have been identified in French Canadians, which is in stark contrast to the array of over 2000 different pathogenic variants reported in each of these genes in other populations. As not all such cancer syndrome families are explained by BRCA1 and BRCA2, newly proposed gene candidates identified in other populations have been investigated for their role in conferring risk in French Canadian cancer families. For example, multiple carriers of distinct variants were identified in PALB2 and RAD51D. The unique genetic architecture of French Canadians has been attributed to shared ancestry due to common ancestors of early settlers of this population with origins mainly from France. In this review, we discuss the merits of genetically characterizing cancer predisposing genes in French Canadians of Quebec. We focused on genes that have been implicated in hereditary breast and/or ovarian cancer syndrome families as they have been the most thoroughly characterized cancer syndromes in this population. We describe how genetic analyses of French Canadians have facilitated: (i) the classification of variants in BRCA1 and BRCA2; (ii) the identification and classification of variants in newly proposed breast and/or ovarian cancer predisposing genes; and (iii) the identification of a new breast cancer predisposing gene candidate, RECQL. The genetic architecture of French Canadians provides a unique opportunity to evaluate new candidate cancer predisposing genes regardless of the population in which they were identified.

Keywords: French Canadian; hereditary cancer syndrome; breast cancer; ovarian cancer; cancer predisposing gene
1. Introduction

Over the past 40 years, genetic epidemiology studies of breast cancer (BC) and ovarian cancer (OC) have provided unequivocal evidence for the role of genetic factors conferring risk for these diseases. Indeed, the estimated heritability of BC at 31% (95% confidence interval (CI) = 11–51%) and OC at 39% (95%CI = 23–55%) are among the highest for all cancer types [1]. That heritable risk factors are involved is reflected in the familial aggregation of these diseases, where females with at least one first-degree relative with BC have a two-fold increased lifetime risk of BC (relative risk = 1.7; 95%CI = 1.4–2) and those with one first-degree relative with OC have a four-fold increased risk for OC (relative risk = 4.6; 95%CI = 2.1–8.7) [2]. Evidence for the role of specific genetic factors conferring risk for these cancers culminated with the discoveries of \(\text{BRCA1}^{[3]}\) and \(\text{BRCA2}^{[4]}\), the BC and OC cancer predisposing genes. They were identified using a genetic linkage analysis and positional cloning approach that took advantage of multigenerational cancer families featuring premenopausal BC cases (hereditary BC (HBC) syndrome) with or without at least one OC case (hereditary BC and OC (HBOC) syndrome) and having a family structure consistent with the transmission of an autosomal dominant trait (reviewed in [5,6] (Appendix A). \(\text{BRCA1}\) and \(\text{BRCA2}\) have been established as high risk cancer predisposing genes, as heterozygous carriers of pathogenic variants (PVs; all variants in this review are germline unless otherwise stated) have absolute risks greater than 60% for BC and 13–58% for OC, depending on the gene involved [7]. Thus, carriers of a PV have a significantly higher risk for cancer as compared to the overall lifetime risk of BC at 12.9% and OC at 1.3% for North Americans [8]. The spectrum of PVs is multifaceted, where the genetic alteration could affect any region of \(\text{BRCA1}\) or \(\text{BRCA2}\), and over 2000 different PVs have been identified in each gene in different populations worldwide [9]. \(\text{BRCA1}\) and \(\text{BRCA2}\) are considered major cancer predisposing genes as they account for a significant proportion of HBC and HBOC syndrome families in all studied populations [10,11]. However, not long after their discovery in the mid 1990s [3,4], it became apparent that not all such cancer syndrome families could be explained by \(\text{BRCA1}\) and \(\text{BRCA2}\), suggesting that other high-risk genes have yet to be discovered [12–14].

The availability of a limited number of large multi-generational cancer families and thus the small chance of meiotic recombination events to help refine chromosomal regions for identifying gene candidates posed considerable challenges for discovering \(\text{BRCA1}\) and \(\text{BRCA2}\). With the completion of The Human Genome Project [15] and advances in understanding the biology of these genes, other strategies, largely favouring a candidate gene approach, have been applied in the identification of new candidate hereditary factors. With each new gene candidate reportedly accounting for only a small proportion of the remaining unexplained cancer syndrome families, it is apparent that another major BC and/or OC predisposing gene like \(\text{BRCA1}\) and \(\text{BRCA2}\) is unlikely. The rarity of carriers of PVs in new risk genes and the genetic heterogeneity of HBC and HBOC syndromes likely explain the difficulty of both identifying and establishing the role of new cancer predisposing gene candidates.

Gene discovery could be facilitated by investigating genetically unique populations that exhibit founder effects due to shared ancestry. A founder effect occurs when a small group of individuals have become isolated from the general population but continue to expand, resulting in a loss of genetic diversity due to genetic drift [16,17]. By chance, genetic drift can result in a significant increase in the frequency of carriers of specific rare disease-associated variants in populations [18]. In the context of HBC and/or HBOC predisposing genes, founder effects have been documented in the Ashkenazi Jewish of Eastern European ancestry [19], Icelandic [20], Finnish [21], and French Canadian (FC) of the province of Quebec, Canada [22,23] populations. In contrast to the general population, all of these populations have been shown to exhibit a limited spectrum of PVs in \(\text{BRCA1}\) and \(\text{BRCA2}\) [24]. Populations exhibiting founder effects have also provided an efficient and cost-effective means to investigate gene candidates in large pools of cancer cases and controls as carriers are readily identifiable due to targeted analyses of PVs [25,26]. However,
FCs exhibit a broader array of PVs in BRCA1 and BRCA2, each associated with different carrier frequencies, in contrast to three PVs in BRCA1 or BRCA2 in the Ashkenazi Jewish population [27,28] and one PV in BRCA2 in the Icelandic population [29] (reviewed in [24]).

We posit that the unique genetic architecture of FCs of Quebec provides an opportunity to evaluate new candidate BC and OC predisposing genes (Appendix B). To elaborate upon this working hypothesis, we reviewed studies of FCs of Quebec that described rare variants (minor allele frequency ≤1% in the general population) in known and new candidate cancer predisposing genes that had been identified in the context of HBC and/or HBOC syndrome families consistent with an autosomal dominant mode of inheritance. We also include new interpretations of missense and splice site variants predicted by selected high performing computational tools [30] (Supplementary Materials). We examined the merits of investigating this genetically unique population, especially for characterizing new cancer predisposing gene candidates. We begin this review by summarizing the methods that have been successfully used to identify new BC and OC predisposing gene candidates.

2. Methods Applied in the Identification of HBC and/or HBOC Syndrome Predisposing Gene Candidates

Depending on the population studied, between 5% and 40% of HBC and HBOC cancer syndrome families have not been accounted for by PVs in BRCA1 and BRCA2 [5,31–34]. Although the wide range in proportion of BRCA-negative families has been attributed to different criteria used to define cancer families, a consistent feature among these reports is that HBC syndrome families are more likely BRCA-negative than HBOC syndrome families (Figure 1a,b) [12,35]. Indeed, the research community initially debated the significance of pursuing new high-risk genes in BRCA-negative HBOC families, as these families could be due to chance clustering of cancer cases [12,36]. The search for “BRCA3” began in earnest in the mid-1990s but the paucity of promising leads suggested that another major cancer predisposing gene explaining the remaining BRCA-negative cancer families was unlikely. Although linkage analyses identified promising chromosomal regions, they were unique to the population in which they were identified [37,38]. These observations suggested that HBC and HBOC syndrome families were more genetically heterogenous than previously expected, suggesting that the carrier frequencies of each high-risk gene candidate would be considerably lower relative to BRCA-carriers. This working hypothesis led to a variety of gene discovery studies which differed based on case selection, methodology, and analyses.
Figure 1. Representative carrier frequencies of frequently occurring pathogenic variants in HBC and HBOC predisposing genes in French Canadians of Quebec. Distribution of BRCA1 and BRCA2 variants in hereditary breast cancer syndrome (a), hereditary breast and ovarian cancer syndrome (b), and sporadic ovarian cancer cases (c). Carrier frequency of a PALB2 variant (d) and TP53 variants (f) in hereditary breast cancer. Carrier frequency of a PALB2 variant (e) and a RAD51D variant (g) in sporadic ovarian cancer cases. Data from [35] where 169 cancer families were analyzed. Selected variants in BRCA1 (n = 11) and BRCA2 (n = 9) were assessed in this study. Data from [39] where 439 sporadic ovarian cancer cases were analyzed. Selected variants in BRCA1 (n = 2) and BRCA2 (n = 4) were assessed in this study. Data from [40] where 48 hereditary breast cancer families and 238 sporadic serous ovarian cancer cases were analyzed. One PALB2 variant (c.2323C > T; p.Gln775Ter) was assessed in this study. Data from [41] where 52 hereditary breast cancer families were analyzed. Targeted sequencing of TP53 exons and splice sites was assessed. Data from [42] where 341 sporadic high-grade serous ovarian cancer cases were analyzed. One RAD51D variant (c.620C > T; p.Ser207Leu) was assessed in this study. Sporadic ovarian cancer cases are all derived from the same study group [39].

Observing a higher frequency of carriers of variants in familial cancer cases versus either unselected cancer cases or cancer-free controls is often the first step in proposing new gene candidates. In addition to the availability of participants, identifying BRCA-negative families suitable for gene discovery remains an obstacle due to their rarity. It has been estimated that the proportion of families with at least two first-degree relatives with BC or OC is approximately 8% and 2%, respectively, in the general population, regardless of BRCA1 or BRCA2 carrier status [43]. Over the past 20 years, national and international consortia have been developed to increase the pool of both familial and sporadic cancer cases and cancer-free controls suitable for research, a concept that was in part successful in identifying cases suitable for BRCA1 discovery and subsequent validation studies [44,45]. Some examples include The German Consortium for Hereditary Breast and...
Ovarian Cancer (GC-HBOC), which was established in 1996 (health-atlas.de/projects/2), The Breast Cancer Association Consortium (BCAC) (bcac.ccge.medschl.cam.ac.uk) and The Ovarian Cancer Association Consortium (OCAC), which were established in 2005 (ocac.ccge.medschl.cam.ac.uk), The Japanese HBOC Consortium (JHC) and The Asian BRCA Consortium [46], which were established in 2012 [47], and The Latin American Consortium for HBOC (LACAM), which was established in 2019 [48].

Since the discovery of BRCA1 and BRCA2, 12 new cancer predisposing genes have been proposed to play a role in BRCA-negative HBC/HBOC cancer syndrome families (Table S1). These genes were identified using a candidate gene approach based on the knowledge that BRCA1 and BRCA2 proteins function in the repair of double stranded DNA breaks by homologous recombination (HR) (reviewed in [5,31,34,49]). As examples, ATM [50], BARD1 [31,51,52], BRIP1 [53], CHEK2 (whereby a genetic linkage analysis was used in combination with a candidate gene approach) [45], PALB2 [54], RAD51C [55], and RAD51D [56] were selected as plausible candidates because they either directly interact with BRCA1 or BRCA2 or are involved at some level in the HR DNA repair pathway [34]. Most of these candidates were identified by investigating BRCA-negative families with at least three BC cases in HBC syndrome families, as with ATM [50], PALB2 [54], CHEK2 [45], BRIP1 [53], and RECQL [57], or families with at least two BC cases and one OC case in HBOC syndrome families, as with RAD51C [55] and RAD51D [56], attesting to the continuing importance of family-based studies for cancer predisposing gene discovery. A number of these studies, especially in those that characterized candidates in large case-control cohorts, have been facilitated with access to targeted next generation sequencing technologies using gene panels [58]. An important criterion for maintaining candidacy is demonstrating a role for proposed new candidates in independently ascertained cancer cases from the populations in which they were identified and in other populations, as this would strengthen their association with risk of HBC or HBOC [7].

3. Genetic Analyses of FC Cancer Cases Facilitate the Interpretation of Variants in BRCA1 and BRCA2

With the identification of BRCA1 and BRCA2, their roles in conferring risk for BC and OC in various populations were investigated by targeted gene sequencing analyses of cancer cases. A complex array of rare variants affecting any coding exon were reported, often unique to the family in which they were identified, initially hindering their clinical interpretation for genetic counselling purposes. The interpretation of variants was facilitated by data sharing where independently identified variants were deposited into databases. The Breast Information Core (BIC) database was the first (no longer actively curated) publicly accessible database for BRCA1 and BRCA2 variants identified in cases [59]. The BIC database also included information concerning the ethnic or geographic origins of the variant carriers found useful for medical geneticists. As more variants were deposited in the BIC database and new computational tools became available to predict biological effects, it was possible to infer their clinical relevance for carriers. The BIC database has since been supplanted by ClinVar (ncbi.nlm.nih.gov/clinvar/) [60] and BRCAExchange (brcaexchange.org) [9]. ClinVar also provides inferences of the clinical relevance for a variety of cancer predisposing genes and other risk genes, though information about ancestry is not usually included [60].

As described below, it is clear that BRCA variants found to occur in FCs were also reported in other populations, particularly from those with Western European ancestry. In the next section, we describe the unique spectrum of PVs identified in FC BC and OC families and cases and evidence to support that those carriers of the same variant could be due to common ancestors in the FC population, and relate these observations to other studied populations.

3.1. Haplotype Analyses Suggest Common Ancestors of Frequently Occurring BRCA1 and BRCA2 Variants in the FC Population

Genetic studies of populations with unique genetic architecture, such as the FCs, have provided important insights into evolutionary origins of PVs in BRCA1 and BRCA2, which
have also impacted medical genetic testing practices of these genes. In 1994, the first report of PVs in BRCA1 in FCs described multiple carriers of BRCA1 c.4327C > T; p.Arg1443Ter (historically known as C4446T), which was attributed to the possibility of shared common ancestors in this population [61]. In 1995, a report of an unusually large FC family with 21 cases of BC showed linkage to the BRCA2 locus on chromosome region 13q12 [62]. Following these initial reports, and with the discovery of BRCA2 [4], genetic analyses of BRCA1 and BRCA2 in larger defined cohorts of FC HBC and HBOC families identified a limited number of PVs in the FC population [63]. Haplotype analysis of FCs harbouring the most commonly occurring PVs [63] suggested that carriers of each specific variant likely shared a common ancestor.

Haplotyping is a form of genotyping analysis that makes use of polymorphic genetic markers (usually single nucleotide polymorphisms) to investigate genetic regions harbouring rare potentially PVs. The similarity of a variant-bearing haplotype in carriers might indicate identity by descent, having inherited sequences from a common ancestor, in contrast to carriers who share similar nucleotide sequences and are identical by state. Moreover, the size of a haplotype can aid in determining the age of a rare variant in a population. In FCs, the average size of chromosomal regions suggesting identity by descent is 21.3 centimorgans as compared to 8 centimorgans in individuals of North-Western European origin [64], which is not surprising given that many of the present day FCs can be genealogically traced back to common ancestors [65] (Appendix B). There is no evidence to suggest that variants have arisen independently in FCs as different haplotypes of PV-bearing alleles have not been identified.

A more likely hypothesis is that frequently occurring PVs in BRCA1 and BRCA2 are the consequence of common ancestry. BRCA1 (NM_007294.4): c.4327C > T; p.Arg1443Ter remains the most common variant reported in the FC population (Table 1, Figure 2), and haplotype analysis suggests a common ancestor in this population for carriers of this variant [66]. Genealogical reconstruction suggested that carriers of this BRCA1 variant could be traced to a couple from France and Portugal that were married in 1761 in Quebec. Interestingly, this variant is also one of the most common PVs in BRCA1 reported in North American populations of Western European ancestry [39]. Haplotype analysis of FC and other populations suggests that BRCA1 c.4327C > T; p.Arg1443Ter may have arisen independently in different populations [66].

### Table 1. Frequently occurring pathogenic variants in BRCA1 and BRCA2 in French Canadians of Quebec 1.

| Gene | Coding Change 2 | Protein Change 2 | Historical Nomenclature | Shared Haplotype in Carriers | Sources(s) |
|------|-----------------|-----------------|-------------------------|-----------------------------|------------|
| **BRCA1** | | | | | |
| c.962G > A | p.Trp321Ter | 1018G > A | - | | 35, 63, 85 |
| c.1054G > T | p.Glu352Ter | E352X | - | | 63, 83 |
| c.1961dup | p.Tyr655ValfsTer15 | 2086insA | - | | 83 |
| c.2125_2126insA | p.Phe709TyrfsTer3 | 2244insA | - | | 83, 83, 84, 86 |
| c.3649_3650insA | p.Ser1217TyrfsTer2 | 3768insA | Yes | | 35, 59, 63, 85, 87, 88 |
| c.3756_3759del | p.Ser1253ArgfsTer10 | 3875delGTCT | - | | 35, 85, 88 |
| c.4041_4042del | p.Gly1348AsnsfsTer7 | 4160delAG | | | 35, 83 |
| c.4327C > T | p.Arg1443Ter | C4446T | Yes | | 35, 59, 67, 77, 79, 83–86, 88 |
| c.5102_5103del | p.Leu1701GlnfsTer14 | 5221delITG | - | | 35, 77, 83 |
| **BRCA2** | | | | | |
| c.2588dup | p.Asn863LysfsTer18 | 2816insA | Yes | | 35, 59, 83, 86 |
| c.2808_2811del | p.Ala938ProfsTer21 | 3034del4 | Yes | | 35, 83, 86 |
| c.3170_3174del | p.Lys1057ThrsfsTer8 | 3398del5 | Yes | | 35, 84–86, 88 |
| c.3545_3546del | p.Phe1182Ter | 3773delTT | - | | 35, 63, 84, 86 |
| c.5857G > T | p.Glu1953Ter | G6085T | Yes | | 35, 59, 63, 78–79, 84–86, 88 |
| c.6275_6276del | p.Leu2092ProfsTer7 | 6803delTT | | | 35, 59, 78, 83, 86 |
| c.8537_8538del | p.Glu2846GlyfsTer22 | 8765delAG | Yes | | 35, 59, 63, 77–79, 81, 83–86, 88 |
| c.9004G > A | p.Glu3002Lys | E3002K | - | | 63, 68, 86 |

- Data not available. 1 See Table S3 for more information on variants. 2 All annotated variants are based on the Human Genome Reference GRCh37/hg19 and the Human Genome Variation Society (HGVS) nomenclature guidelines.
Figure 2. The most frequently occurring pathogenic variants \textit{BRCA1}, \textit{BRCA2}, \textit{PALB2}, and \textit{RAD51D} in French Canadians of Quebec and their allele frequency in other worldwide non-cancer populations. Source of the data: gnomAD v2.1.1 (gnomad.broadinstitute.org).

Similar observations have been made for the most commonly reported variants in \textit{BRCA2} (NM_000059.4), c.5857G > T; p.Glu1953Ter (historically known as G6085T) and c.8537_8538del; p.Glu2846GlyfsTer22 (historically known as 8765delAG) (Table 1, Figure 2). Haplotype analysis also suggests that carriers of these variants likely shared a common ancestor in the FC population [63]. Furthermore, haplotype analysis showed that ancestral origins of FC carriers of 8765delAG likely differed from carriers of the same variant reported in the Yemenite Jewish and Sardinian populations, which likely have arisen independently of each other [67,68]. These observations are not surprising due to the purported increased mutability of the AG dinucleotide repeat sequence of exon 20 of \textit{BRCA2} where this variant resides [68].

Other specific PVs in \textit{BRCA1} and \textit{BRCA2} have also been reported in unrelated FCs but occur less frequently in FC cancer families than those described above (Appendix B, Table 1, Table S2). Among these PVs, haplotype analysis has suggested that carriers of \textit{BRCA2} c.3170_3174del; p.Lys1057ThrfsTer8 (historical name 3398del5), as an example, also shared a common FC ancestor [69].

While loss-of-function variants are readily interpretable for their potential to affect risk, missense variants are more difficult to understand. The genetic architecture of the FC population has been useful in classifying such variants. For example, the rare missense \textit{BRCA2} c.9004G > A; p.Glu3002Lys reported in a number of unrelated cancer families from
the North American population was initially classified as a variant of uncertain clinical significance in the BIC database [70]. In addition to identifying this variant in unrelated FC cancer cases, it was shown to segregate with cancer cases in FC HBC families, suggesting that it might indeed be pathogenic [71]. This interpretation was supported by subsequent in cellulo assays revealing that it encoded a protein with aberrant HR function, and this finding led to its reclassification as pathogenic [72].

Identifying frequently occurring variants in populations that have undergone genetic drift, such as the FCs, is important as it supports the notion that PVs in \( \text{BRCA1} \) and \( \text{BRCA2} \) are least likely to arise from de novo mutagenesis in the germline. Indeed, there are no credible reports of de novo germline variants in these genes, though there is evidence that PVs can arise due to this mechanism for other high-risk cancer predisposing genes, such as those reported in \( \text{RB1} \) in the rare non-hereditary forms of pediatric retinoblastoma [73]. The stable origin of heritable PVs also provides a means of cost-effective genetic testing for PVs found most commonly in founder populations for research and medical genetic purposes, the exemplars being the three PVs in \( \text{BRCA1} \) and \( \text{BRCA2} \) found to account for almost all \( \text{BRCA} \)-carriers in the Ashkenazi Jewish population [74]. As shown in an early targeted analysis of 20 variants in FCs, 84% of \( \text{BRCA1} \) and \( \text{BRCA2} \) positive HBC and HBOC syndrome families harbour one of five specific PVs in these genes accounting for the high frequency of these PVs observed in BC- and OC-affected individuals in this population [35]. Unlike the high (1.1–2.5%) carrier frequency of the three founder \( \text{BRCA1} \) and \( \text{BRCA2} \) PVs observed in the Ashkenazi Jewish population [75–77], there is no evidence to suggest that the overall \( \text{BRCA} \)-carrier frequency in FCs of Quebec is higher than 0.25% carrier frequency estimated for Northern Americans [78,79]. Indeed, a recent study has shown that \( \text{BRCA1} \) and \( \text{BRCA2} \) variants are rare (<0.2%) in the non-cancer FC population with no personal or family history of cancer relative to cancer cases [80].

### 3.2. The Spectrum of \( \text{BRCA1} \) and \( \text{BRCA2} \) Variants in FCs

Genetic studies of the FC population have helped validate the role of rare potentially PVs in \( \text{BRCA1} \) and \( \text{BRCA2} \) in cancer syndrome families, sporadic cancer cases regardless of family history of cancer, and the general population. The overall frequency of \( \text{BRCA} \)-carriers in FCs with sporadic BC [81,82] or OC [83] (Figure 1c) is within the range reported for BC (5–10%) and OC (12–15%) from North American, European, and other populations [51,84]. Since the initial reports of \( \text{BRCA1} \) and \( \text{BRCA2} \) PVs in FCs, the spectrum of frequently occurring variants identified in FC BC and/or OC cases has expanded to a total of 25 variants, including 18 PVs [35,39,63,80–83,85–91] (Table 1, Figure 3, Table S2), of which the majority are nonsense and frameshift variants that are expected to result in the loss of the protein function.
Figure 3. Pathogenic variants and variants of uncertain significance reported in French Canadians of Quebec mapped to full length BRCA1 (a) or BRCA2 (b) transcripts. Variants are predicted to be pathogenic or have uncertain significance based on ClinVar and/or ACMG guidelines. RING = Really Interesting New Gene domain; NES = Nuclear export signal; NLS = Nuclear localization signal (BRCA1: [92]; BRCA2: [93]); SCD = Serine cluster domain [94]; BRCT = BRCA1 C Terminus domain; BRC repeats = BRCA2 repeats; HD = Helical domain; OB = Oligonucleotide binding; Tower = Domain essential for DNA binding [95]. BRCA1 GenBank: AAC37594.1 [96], BRCA2 GenBank: AAB07223.1 [97], DNA binding domain [98]. See Table S2 for more information about variants.

In reviewing the literature, 36 rare variants have been reported only once in BRCA1 and BRCA2 in FCs with BC or OC [35,63,87,89,99] (Table S2). The ease of gene sequencing enabled the identification of new variants in the FC population using targeted gene sequencing of all exons and splice site regions. Of these 36 variants, the most promising PVs are the 11 that are classified as pathogenic and 13 of uncertain significance, the remainder being benign based on ClinVar [60] and American College of Genetics and Genomics (ACMG) guidelines (Figure 3, Table 1, Supplementary Materials). These variants include 11 missense, 6 frameshift, 4 nonsense, 2 splicing, and 1 in-frame deletion. The classification of the majority of these variants is consistent between in silico tools and functional characterizations, though some are not, which is in line with the approximately 90% accuracy of these tools [30] (Supplementary Materials). Although none of the in silico splicing tools predicted that BRCA1 c.81-6T > C affects splicing, a biological assay has shown that there is an effect on RNA splicing [100] (Table S2). This is not surprising as the in silico tools used in that study, which predicted that this variant would affect splicing, differed from those applied in this review. Currently, the splicing tools that have the best predictive performance have not been systematically investigated, unlike the established list of best performing in silico tools suggested for classifying missense variants [30]. BRCA2 c.7007G > A; p.Arg2336His is predicted to affect splicing by all four splicing tools used (Table S2). Of the 11 missense variants, seven were potentially pathogenic using our in silico tools...
Of note, two of these missense variants, \(BRCA1\) c.736T > G; p.Leu246Val and \(BRCA2\) c.8850G > T; p.Lys2950Asn, did not affect the function of the HR pathway [101,102] (Table S2). Although \(BRCA2\) c.9976A > T; p.Lys3326Ter introduces a stop codon predicted to truncate the BRCA2 protein, its clinical significance remains controversial (reviewed in [103,104]), and this is due to: (1) the fact that its carrier frequency at 0.6% in the general population, though rare, is higher than that of other PVs in \(BRCA2\) (0–0.001%) (Table S2); and (2) its location in the C-terminus where it has been proposed to exert the least effect on the function of the protein [105]. Independent studies of sporadic and familial cancers have shown an increased risk for BC and OC in carriers of this \(BRCA2\) variant [105,106]. Although this variant has not been investigated to the same extent as other PVs in the FC population, targeted gene sequencing analysis identified \(BRCA2\) c.9976A > T in two out of 256 (0.8%) unrelated HBC syndrome families [87], placing it among the least frequently occurring \(BRCA2\) PVs in FC cancer families.

Complex and difficult to detect large deletions or genomic rearrangements in \(BRCA1\) and \(BRCA2\), which are rarely found in the general population, are also likely rare in FCs, as suggested by a study of BC and OC cases from cancer families that applied the established multiplex ligation probe amplification (MLPA) analysis technique and found no examples of carriers of such variants [107]. As observed with variants in other populations, there is no obvious clustering of PVs in any protein encoding or splice site region of \(BRCA1\) or \(BRCA2\) (Figure 3). PVs that occur in the defined BC Cluster and OC Cluster Regions in \(BRCA1\) and \(BRCA2\) have been statistically associated with increased risk of BC, or OC, respectively [108]. However, this has not been studied in FCs due to the overall low frequency of carriers in this population enabling statistical associations of each variant with risk of BC or OC (Figure 3).

The spectrum of \(BRCA1\) and \(BRCA2\) PVs described in the FC population is not surprising given the European origins of FC ancestors. Indeed, all PVs identified in FCs in \(BRCA1\) and \(BRCA2\) have been reported in other populations (Table S2). Early studies from our group showed that carriers of the most commonly reported \(BRCA1\) c.4327C > T; p.Arg1443Ter had grandparents with ancestral ties to different geographic regions across Quebec [63], whereas carriers of the less frequently reported variants were each from a smaller defined region within Quebec even though they were identified in unrelated families [63,69,71].

The unique genetic architecture of FCs thus affords an opportunity to investigate this population for new candidate variants in known cancer predisposing genes as well as help validate new cancer predisposing genes for heritable BC and OC, as elaborated upon further below.

4. Genetic Analyses of FC Cancer Cases Helps Define the Role of New Candidate HBC/HBOC Predisposing Genes

The genetic analyses of new cancer predisposing genes in the FC population has provided support for their role in hereditary BC and OC (Table 2, Table S1). In this section, we describe studies of FCs involving PVs in new risk genes, especially those associated with HBC and HBOC syndrome families exhibiting an autosomal dominant mode of inheritance [34]. However, before doing so, it is important to mention the few studies of FC HBC and HBOC families involving established cancer predisposing genes, \(TP53\) and \(STK11\), which are known to play a role in Li–Fraumeni (MIM:151623) [109] and Peutz–Jeghers (MIM:175200) [110,111] syndrome families, respectively. These genes are plausible candidates to account for the fact that \(BRCA\)-negative HBC families as heterozygous carriers of PVs in these genes also have significant absolute risks for premenopausal BC: exceeding 60% for \(TP53\) carriers [7] and 40–60% for \(STK11\) carriers [7]. Our group has reported seven rare variants in \(TP53\) in FC familial or sporadic BC cases [41,112], where five are classified as PV by \textit{in silico} analyses. Interestingly, there were two carriers of the same PV in \(TP53\) (NM_000546.6), namely c.638G > A; p.Arg213Gln or c.685T > C; p.Cys229Arg, among the BC cases, where the latter PV was identified in cases not known to be related to each other [41,112]. The overall estimated carrier frequency of PVs in \(TP53\) in HBC families
at 3.8% [41] (Figure 1f) and 1.2% in sporadic BC cases [112] was higher than expected given the estimated 1 in 5000 to 20,000 TP53-carriers in the general population worldwide (reviewed in [113]). The carrier frequency of PVs in TP53 in the FC population and whether the carriers of the same PV share a common ancestor have yet to be determined. Only one rare variant in STK11 was identified in a study of 96 BRCA-negative HBC families, where the carrier family did not exhibit clinical features consistent with Peutz–Jeghers syndrome [114] (Table S4). This STK11 missense variant (c.1062C > G; p.Phe354Leu) is benign/likely benign in ClinVar and was not predicted to be a PV based on our in silico analysis. There are no reliable estimates of the carrier frequency of STK11 PVs in HBC or HBOC families, though it is likely rarer than for carriers of TP53 variants. While there are other studies of established cancer predisposing genes in FC cancer cases, such as those involved in Cowden syndrome (MIM:158350) and Lynch syndrome (MIM:120435; 609310), which also feature BC and OC cancer, they have not been systematically explored in BRCA-negative HBC and HBOC families or in BC or OC cases, and thus are not included in this review. Regardless, it is apparent from studies of FCs and of other populations that established BC and OC cancer predisposing genes are implicated in a small proportion (0–1%) of BRCA-negative cancer families, further supporting the hypothesis that other cancer predisposing genes have yet to be discovered.

Table 2. Frequently occurring potentially pathogenic variants in new candidate cancer predisposing genes in French Canadians of Quebec 1.

| Gene     | Canonical Transcript | Coding Change | Protein Change | Shared Haplotype in Carriers | Source(s) |
|----------|----------------------|---------------|----------------|-----------------------------|-----------|
| BARD1    | NM_000465.4          | c.1075_1095dup| p.Leu359_Pro365dup | -                           | 134       |
|          |                      | c.1930G > A    | p.Val644Ile      | -                           | 134       |
|          |                      | c.2212A > G    | p.Ile738Val      | -                           | 134       |
| BRIP1    | NM_032043.3          | c.577G > A     | p.Val193Ile      | 126                         |           |
|          |                      | c.2097 + 7G > A|                |                            | 126       |
| CHEK2    | NM_007194.4          | c.1100del      | p.Thr367MetfsTer15| -                           | 85, 105   |
|          |                      | c.1217G > A    | p.Arg406His      | -                           | 129       |
| MRE11    | NM_005590.4          | c.1516G > T    | p.Glu506Ter      | -                           | 136       |
| PALB2    | NM_001005735.2       | c.226A > G     | p.Ile76Val       | -                           | 108       |
|          |                      | c.1273G > A    | p.Val425Met      | -                           | 105       |
|          |                      | c.1676A > G    | p.Gln559Arg      | -                           | 105       |
|          |                      | c.2232C > T    | p.Gln775Ter      | Yes                         | 84, 85, 107, 108, 107 |
|          |                      | c.2590C > T    | p.Pro864Ser      | -                           | 105       |
| RAD51D   | NM_002878.4          | c.620C > T     | p.Ser207Leu      | Yes                         | 119       |
| RECQL    | NM_032941.2          | c.643C > T     | p.Arg215Ter      | -                           | 53        |
| TP53     | NM_000546.5          | c.638G > A     | p.Arg213Gln      | -                           | 102, 103  |
|          |                      | c.703A > G     | p.Asn235Asp      | -                           | 103       |
|          |                      | c.730G > A     | p.Gly244Ser      | -                           | 103       |
|          |                      | c.742C > T     | p.Arg248Trp      | -                           | 103       |
|          |                      | c.844C > T     | p.Arg282Trp      | -                           | 103       |

: Data not available. 1 See Table S3 for more information on variants. 2 All annotated variants are based on the Human Genome Reference GRCh37/hg19 and the HGVS nomenclature guidelines.

4.1. A Predominant PV in PALB2 Frequently Occurs in FC Hereditary BC Cases

PALB2 is the most promising of the newly proposed BC predisposing genes [54]. In 2007, after an independent report described PALB2 as a BRCA2 binding partner [115], targeted sequencing of PALB2 as a new candidate gene for hereditary BC in Finnish HBC families determined a statistical association with one of the identified loss-of-function variants in this gene [54]. Further targeted genotyping analysis showed that the carrier frequency of this variant was higher in BC cases versus controls in the Finnish population (Table S1). Soon thereafter, targeted sequencing analysis of PALB2 in 50 FC early-onset or fa-
milial BC cases identified one carrier of PALB2 (NM_001005735.2): c.2323C > T; p.Gln775Ter, and this variant was also identified in 2/356 BC cases but not in controls [116]. Carriers were subsequently identified in 2% of FC HBC families BRCA-negative for the five most commonly occurring PVs observed in FCs (Table 2, Figures 1d and 2). This variant is predicted to introduce a stop codon at amino acid position 775 of PALB2, and if expressed would render the truncated protein non-functional, suggesting its role in pathogenicity [116]. Haplotype analysis of unrelated FC carriers suggested that they may have inherited this PALB2 variant from a common ancestor [40,88,114]. PALB2 c.2323C > T accounts for 0.7% of FCs with early-onset BC not selected for family history of BC [88]. In contrast, carriers of this PALB2 variant are rare in cancer-free FC controls, as none were found in approximately 2000 cancer-free individuals [80,88]. PALB2 c.2323C > T is the first example of a newly proposed BC risk gene shown to play a role in BRCA-negative HBC syndrome families in the FC population.

While there is mounting evidence from the research community supporting PALB2 conferring an increased risk for BC, its role in hereditary OC is unclear [7,117,118]. Our analysis of PALB2 c.2323C > T in sporadic FC OC cases only identified one carrier who had OC at the age of 58 years among 238 (0.2%) cases (Figure 1e). Interestingly, this carrier also had BC at the age of 52 years [118]. A report of 524 PALB2 PV carrier families of European ancestry estimated the associated relative risks with BC as 7.2 (95%CI = 5.8–8.8; p = 6.5 × 10−76) and OC as 2.9 (95%CI = 1.4–6; p = 4.1 × 10−3) [118]. A targeted sequencing analysis of 54 candidate genes selected based on their function in HR repair in OC and controls by OCAC only identified a statistical association of potentially PVs in PALB2 with OC [119]. The National Comprehensive Cancer Network (NCCN) guidelines reported estimates that the absolute risk for OC in carriers of PALB2 PVs is between 3 and 5% compared to the absolute risk for BC: 41–60% [7].

Other PALB2 variants in FC BC cases have been reported [114,116] that were predicted as potentially pathogenic by our selected in silico tools, with the exception of two variants (Table 2, Table S3). Large germline deletions or insertions have been investigated in BC cases from FC HBC or HBOC families using MLPA, and though none were identified [114,116], they have been reported in studies of other populations [120,121]. Potentially PVs in PALB2 have since been reported in diverse populations, providing support for its role in BC risk. A large multi-center study involving familial and sporadic cases from diverse populations estimated that the risk for female BC in PALB2 carriers to age 80 is 53% (95%CI = 44–63%), adding PALB2 to the list of validated high-risk BC predisposing genes [118].

4.2. A Frequently Occurring Missense Variant in FCs Supports a Role for RAD51D in Hereditary OC

Genes encoding members of the RAD51 family are directly involved in the HR repair pathway and as such have been investigated as plausible new BC and OC predisposing gene candidates in BRCA-negative families [34]. The earliest reports appeared in 2010 for RAD51C [55] and 2011 for RAD51D [56]. The sequencing of protein encoding and splice regions of these genes in BRCA-negative HBC and HBOC families identified rare potentially PVs. A higher frequency of carriers from HBOC families with either OC or BC, but not BC cases from HBC families, were found to harbour one of these variants as compared to the controls. Subsequent studies further supported the strong association of PVs in RAD51C and RAD51D with OC [117,122,123]. Estimates of the absolute risk for OC in carriers of RAD51C or RAD51D PVs are greater than 10% [7], which is in line with established cancer predisposing genes conferring a significant risk of cancer, as for carriers of PVs in BRCA1 and BRCA2. The relative risk for OC is estimated to be as high as 40% if carriers of variants in these genes have a first-degree relative with OC [117]. However, the role of RAD51C and RAD51D in BC risk is less clear [7], though population-based studies suggest that carriers of PVs in these genes are more likely found among BCs classified as estrogen receptor-negative or triple-negative [51,52] (tumours defined by the absence of estrogen and progesterone receptor expression accompanied with no overexpression of human epidermal growth factor receptor 2).
Studies of a rare RAD51D variant found in FCs provided further evidence in support of this gene playing a role in OC risk. RAD51D (NM_002878.4): c.620C > T; p.Ser207Leu was classified at the time of the study as a variant of uncertain significance in ClinVar [60] as it was rare in the general population [122,124,125] (Table S3). Following initial reports of carriers of RAD51D c.620C > T, a number of OC and BC cases from FC HBC and HBC families were found to carry the same variant in medical genetic units by multi-gene panel testing [42], although an early study of HBC and HBOC families, which included a small number of FC families, did not identify any potentially PVs in RAD51D [126] (Table S4). This missense variant was investigated in sporadic BC and OC cases not selected for age of diagnosis to further determine its role in conferring risk for cancer in FCs [42]. The results revealed a significantly higher carrier frequency in OC cases relative to controls (3.8% vs. 0.2%) (Figures 1g and 2, Table 2). In sporadic OC cases, the carrier frequency of this variant was comparable to carriers of BRCA1 c.4327C > T; p.Arg1443Ter (3.4%), the most prevalent PV in FCs [42]. Interestingly, in this study, there were no co-occurring carriers of these specific RAD51D and BRCA1 variants, nor with the other five most common BRCA1 and BRCA2 variants found in FCs [39,42]. In cellulo assays showed that this variant encodes an aberrant protein, RAD51D p.Ser207Leu, that affects the HR pathway function [42], and thus may be pathogenic and play a role in conferring risk for OC in carriers. This is reflected in the conflicting interpretations of RAD51D c.620C > T in more recent updates of ClinVar [60] (Accession ID: VCV000142102.11) as a variant of uncertain significance (three submissions), likely pathogenic (five submissions), and pathogenic (two submissions), and a variant of uncertain significance by ACMG guidelines (Table S3). Haplotype analysis has suggested that carriers of RAD51D c.620C > T likely shared a common ancestor [42]. The high frequency of carriers of RAD51D c.620C > T in the OC cases was not expected given the rarity of carriers of this missense variant or any PV in this gene in FC and other non-FC populations [124]. These findings place RAD51D c.620C > T; p.Ser207Leu among one of the most commonly observed PVs conferring risk of OC in FCs.

Less is known about RAD51C in FCs, though one report described a targeted sequencing analysis of this gene in 152 BRCA-negative FC HBC and HBOC families [127]. In this report, no loss-of-function or potentially pathogenic missense variants were identified, suggesting that these variants in RAD51C are rare in this population and may not significantly contribute to FC HBC and HBOC families.

4.3. BRIP1 and CHEK2 in FC BC and OC Cases

Relative to BRCA1 and BRCA2, less is known of the role of BRIP1 and CHEK2 cancer predisposing genes in conferring risk of BC and OC in FCs. Studies have shown that variants in these genes likely confer risk of BC that is lower than for PVs in BRCA1, BRCA2, PALB2, RAD51C, and RAD51D [45,53,128]. These genes were plausible BC and/or OC predisposing candidates because of their role in directly interacting with BRCA1 protein through its binding to BRIP1 protein [129] or playing a central role in the cellular response to double stranded DNA breaks, as shown with CHEK2 protein (reviewed in [34,130,131]). Targeted sequencing analysis of BRIP1 in FC BC cases with a family history of BC identified missense variants [132], where three of them are predicted as potentially PVs by our in silico analysis (Table S3, Supplementary Materials). A population-based study by OCAC investigating sporadic cancer cases estimated that carriers of BRIP1 variants had a relative risk of 11.2 for OC (95% CI = 3.2–34.1; \( p = 1 \times 10^{-4} \)) [133], but not for BC [51,52]. The NCCN guidelines reported an estimated absolute risk for OC in carriers to be greater than 10%, but had limited evidence-based data on the risk for BC [7]. In one study of FCs, the frequency of CHEK2 (NM_007194.4): c.1100del; p.Thr367MetfsTer15, was 2% in BC families with at least two or more BC cases diagnosed before the age of 65 years, which is lower but comparable to the 3.7% carrier frequency reported in BC families from the general population [45] (\( p = 0.7 \) using Fisher’s exact test). In another study, the carrier frequency of this variant was 1.1% in FCs with young age of onset sporadic BC [88], which is comparable to the 1% carrier frequency reported in the general population [134]. Targeted gene sequencing analyses
identified other CHEK2 variants in FC BC cases, with c.1217G > A; p.Arg406His being the most promising candidate based on our in silico analysis (Table 2, Table S3), though the carrier frequencies were not significantly different between BC cases and controls [135]. The absolute risk for BC in CHEK2 PV carriers has been estimated to be in the range of 15–40%, but with no evidence for increased risk for OC [7]. The identification of BRIP1 and CHEK2 variants in FC HBC or HBOC syndrome families, which have also been reported in BC and OC cases from other populations (Table S3), supports their role in the risk of these cancers.

4.4. The Role of Proposed Cancer Predisposing Genes in FCs

New candidate genes where the association with HBC and/or HBOC is still unknown include BARD1, MRE11, RAD50, and NBN (Table S1). BARD1, which encodes a BRCA1 interacting protein, was proposed as a candidate risk gene soon after the discovery of BRCA1 and BRCA2 [31,136,137]. MRE11, RAD50, and NBN, which were proposed as candidates a decade after the discovery of BRCA1 and BRCA2 [138,139], encode proteins of the MRN complex, a multi-protein structure that has been shown to play an important role in sensing double stranded DNA breaks for DNA repair (reviewed in [34]). Thus far, there have been reports investigating variants in BARD1, MRE11, and NBN in FCs, but not RAD50. Four rare variants in BARD1 in BC cases from FC HBC families have been described that were identified using targeted sequencing analyses [140]. Two BARD1 missense variants have been classified as likely benign or benign based on ACMG guidelines, which is consistent with the prediction with our in silico analysis (Table 2, Table S3). BARD1 (NM_000465.4): c.1075_1095dup; p.Leu359_Pro365dup was found in four unrelated BC cases (4/96 cases vs. 2/87 controls), though this observation was not surprising given the genetic architecture of FCs [140]. The sequencing of NBN in BC cases with a family history of BC identified carriers of several different variants where none were found in more than one FC BC case [141]. The one missense variant NBN (NM_002485.5): c.283G > A; p.Asp95Asn reported in this study has been classified as a variant of uncertain significance, though it was predicted as potentially pathogenic by our in silico analysis (Table S3). However, a recent study of MRE11 c.1516G > T; p.Glu506Ter, which has been reported in multiple FC cancer cases and also found in other populations, suggested that it may not to be associated with BC risk [142], which is in line with recent findings from a large BC case–control study [52] (Table 2, Table S3). In a study of FCs, this rare loss-of-function variant was not identified in 1920 BC and 341 OC cases but in 4/1891 (0.2%) adult cancer-free controls and 1/1932 (0.01%) newborns. Though the differences in MRE11-carrier frequency between cancer cases and controls were not significant, the findings questioned its classification as a PV (Table S3). Immunohistochemistry analysis of BC and OC tumours from carriers showed strong protein expression of MRE11, and genetic analyses of tumour tissues revealed the presence of both parent of origin MRE11 alleles [142]. With these findings, the authors of this study questioned the candidacy of MRE11 as a BC predisposing gene. Thus, the unique genetic architecture of FCs may also aid in the resolution of potentially benign candidate variants found to occur in other populations. With the exception of one BARD1 variant, all of the rare variants in BARD1, MRE11, and NBN that were identified in FC cancer cases have also been reported in studies of BC and OC from other populations (Table S3).

5. Discovery of New Candidate HBC/HBOC Predisposing Genes Identified in the FC Population

RECQL was identified as a new candidate BC predisposing gene by focusing on the study of FC and Polish cancer cases as both of these populations exhibit genetic drift, suggesting a genetic architecture amenable for the investigation of new candidate genes [57]. A complex but effective strategy was used that included the genetic analyses of three independent BC groups per population (Table S1). With respect to the studies of FC study groups, whole exome sequencing was performed on 51 index BC cases selected from HBC/HBOC syndrome families and/or if they were diagnosed at a young age. All BC
cases were not carriers of PVs in BRCA1, BRCA2, PALB2, CHEK2, and NBN that frequently occur in FC and Polish populations. Rare loss-of-function variants were prioritized as top candidates if they were identified in at least two unrelated BC cases. Using this approach, three carriers of loss-of-function variants in RECQL were identified: two in index cases from HBOC families (Table 2, Table S3). These findings were then validated by targeted sequencing in 475 familial BC cases who were not carriers of any of the most commonly occurring BRCA1 and BRCA2 PVs found in FCs. Though no additional carriers of the candidate variants were found, two carriers of a newly identified loss-of-function variant RECQL (NM_032941.2): c.643C > T; p.Arg215Ter were identified (Table 2). Targeted genotyping of RECQL c.643C > T in a third group of FC BC cases with a family history of this disease or who had BC before the age of 50 years identified additional carriers (5/538; 0.93% in cases vs. 1/7136; 0.014% in controls; p < 0.0001 using Fisher’s exact test). This strategy, replicated with the Polish study groups, identified three potentially pathogenic RECQL variants, which differed from those found in FCs, and one was found in unrelated cases in this population [57]. The journal that published the identification of RECQL as a candidate BC predisposing gene in FC and Polish populations also reported the identification of the same candidate gene by studying the Chinese Han BC population [143]. All potentially pathogenic RECQL variants identified in Chinese Han cancer cases differed from those found in the study of the FC and Polish populations. The clinical significance of RECQL has yet to be determined, though subsequent genetic studies of RECQL have been conflicting, even questioning its role in BC predisposition [144]. This may be a result of not using fully matched control cohorts, which may lead to spurious associations [52]. Noteworthy is that while the focus of research in these RECQL discovery studies was focused on identifying new BC predisposing genes, pedigree inspection of RECQL-carrier families clearly showed the presence of OC cases [57]. Therefore, further research is necessary to resolve the role of RECQL in BC and OC predisposition.

6. Perspectives

The contribution of FC participants to the study of a variety of genetic disorders has been recognized by researchers and the health care community [22,23]. Genetic analyses of FCs of Quebec, Canada have contributed to our understanding of the role of rare PVs in cancer predisposing genes that confer high risks for the hereditary form of BC and OC inherited in an autosomal dominant manner. An important consideration in studying populations with unique genetic architecture, such as FCs, is the possibility of identifying carriers of candidate variants that are rare in the general population but can be shown to be benign [145].

More work remains on the role of new BC and OC gene predisposing gene candidates in FCs, such as FANCC [146], FANCM [147,148], and RAD50, that have been described in other populations but have not yet been reported for FCs. Studies of FCs of Quebec have sparked new initiatives geared towards the resolution of rare disease-associated variants identified in this genetically unique population. One of the first population-based cohorts useful for interpreting the frequency of candidate variants included a collection of DNA samples from newborns from the Quebec City area, where the majority of inhabitants are FC [149–151], and as described above has been successfully used to study carrier frequencies of PVs in BRCA1 and BRCA2 [88], PALB2 [116], and RAD51D [42]. The launch of CARTaGENE (cartagene.qc.ca) [152], which is part of the Canadian Partnership for Tomorrow’s Health (CanPath), a Canada-wide population-based cohort including over 330,000 participants with the objective of improving knowledge about chronic diseases (can-path.ca) [153], has provided population-matched controls for genetic studies. CARTaGENE is a prospective population-based biobank including over 43,000 participants from Quebec between the ages of 40 and 69 years, with the aim of improving the prevention, diagnosis, and treatment of chronic diseases, including cancer. The availability of not only biological specimens but also over 600 detailed health, socio-demographic, and physiological metrics allows for the interpretation of genetic data in the context of these metrics. Personal and
first-degree family history of cancer is included. Other epidemiological factors, such as oral contraceptive pill use, reproductive factors, and/or hormone replacement therapy, have been associated with BC [154] and OC [32], and these can be investigated in candidate variant carriers. As data become available from these projects, candidate variants identified in cancer families and cases can be investigated in CARTaGENE cohorts to evaluate their frequency in population-matched controls and thereby their relevance as candidates.

7. Conclusions

Over the past 25 years since the discovery of BRCA1 and BRCA2, it is increasingly clear that FCs of Quebec, Canada have played a significant role in defining the genetic landscape of cancer predisposing genes. Variants in BRCA1 and BRCA2 have been well characterized in FCs and the investigation of other HBC/HBOC predisposing genes has allowed for their identification and characterization. Studies of the FC population have provided evidence that RECQL is a new HBC/HBOC predisposing gene. The unique genetic architecture of the FC population should provide the opportunity to identify future cancer predisposing genes.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/cancers13143406/s1, Supplementary Method, Table S1. Breast and/or ovarian cancer predisposing genes and the approach used in their identification, Table S2. Rare BRCA1 and BRCA2 variants reported in the literature in the French Canadians with breast and/or ovarian cancers, Table S3. Rare variants in breast and/or ovarian cancer predisposing genes other than BRCA1 and BRCA2 identified in French Canadians in the literature, Table S4. All studies in French Canadians of Quebec where new candidate HBC/HBOC genes were investigated in the literature.

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Appendix A. BRCA1 Discovery: A Paradigm for Genetic Linkage Analyses and Positional Cloning Approach for Identifying Cancer Predisposing Gene Candidates

BRCA1 and BRCA2 were discovered using a genetic linkage analysis and positional cloning approach [3,4]. The seminal 1990 study by Mary-Claire King’s group [155] reporting chromosomal region 17q12-21 as the most likely location of a putative major breast cancer predisposing gene was based on the hypothesis that familial BC could be explained by the transmission of an autosomal dominant trait with incomplete penetrance. This hypothesis was formulated by the following observations: (1) anecdotal clinical data from multi-generational families reporting clustering of BC cases that cannot be explained by chance; and (2) empirical data from population-based studies suggesting that BC risk was associated with having first- and second-degree relatives with BC, especially if they had a young age (<50 years) of BC diagnosis [156,157]. King’s group sought to identify candidate chromosomal regions that could harbour this putative BC susceptibility gene using a genetic linkage analysis approach that took advantage of germline DNA available.
from multi-generational BC families and an emerging new panel of polymorphic genetic markers representative of different chromosomal loci in the human genome that could be used to track the inheritance of origin of alleles [155]. Candidate regions were identified by using logarithm of odds (LOD) score analysis, which estimated whether the observed degree of concordance of a genetic marker with BC signified genetic linkage between the two (reviewed in [158]). A high cumulative LOD score (>3) for a genetic region, through the analyses of many families, identified the chromosomal region 17q12–21 associated with S74(CMM86) marker as having a high likelihood of harbouring a putative BC risk gene [155]. The study was initially met with skepticism as it was difficult to fathom that a common disease could be attributed to a major susceptibility gene inherited as an autosomal dominant trait. The observation in the initial report that only 45% of BC families were linked to the 17q21-12 region fueled this debate. The study had immediate clinical implications for linked families, as it was possible to identify carriers of the putative predisposing gene and thus those at significantly increased risk for BC. As more and more BC families were independently found genetically linked to 17q12-21, the race began to identify the underlying gene, especially when in 1991 Steven Narod and colleagues reported a strong association of the 17q12-21 locus with BC cancer families also having at least one OC case, suggesting that the putative BC susceptibility gene also conferred increased risk of OC [159]. These observations led to defining HBC and HBOC cancer syndromes most likely to harbour a putative cancer susceptibility gene: (1) multi-generational BC families with or without OC consistent with autosomal dominant mode of inheritance; and (2) average age of BC diagnosis less than 54 years. Meiotic recombination mapping was then applied to refine the localization of the region containing the putative gene in families demonstrating segregation (“linkage”) of 17q12-21 markers with BC. Using linked genetic markers as a starting point, additional polymorphic markers that physically map upstream and downstream from the linked allele were investigated until an allele was found that no longer co-segregated with BC and/or OC in a 17q12-21 linked family. Thus, a defined region on 17q12-21 was identified that was feasible for generating a physical map of that region suitable for cloning candidate genes. BRCA1 (named by Mary-Claire King in 1991 [160]) was identified by mapping candidate cDNAs that were cloned from a bacterial artificial chromosome containing the 17q21 region of interest as defined by meiotic recombination mapping [3]. From gene maps created with the aid of sequenced cDNAs, gene candidates were vetted by sequencing germline DNA from linked families and demonstrating the segregation of protein truncating PVs with BC in a family. A second locus identified at chromosomal region 13q12-13 was reported as a candidate region for BRCA2 identified by a genetic linkage analysis using families found negative for linkage to chromosome 17q12-21 markers [161]. BRCA2 was reported in 1995 using a similar cloning strategy but was facilitated by the observation of a large deletion in a cancer case in a BC linkage family [4].

Appendix B. The Unique Genetic Architecture of FCs of Quebec, Canada

It has been estimated that many of the present-day FC population of six million are descendants of 10,000 Western Europeans largely from France who settled in New France/Nouvelle France (along the St. Lawrence river) approximately 400 years ago in 1608 [22,65]. It has been said that only 8483 settlers contributed to population expansion [22]. However, the genetic architecture of modern-day FCs is complex, as both anthropological and genetic studies have shown [145,151]. This has been attributed to the waves of local population expansion until 1759 [150] and the influence of admixture from migration and integration of other populations [162]. Regions of identity by descent are larger in FCs than in European populations [64]. Heterogeneity in FCs of Quebec is reflected in the spectrum of BRCA1 and BRCA2 PVs observed in this population, where some variants account for a high frequency of BRCA carriers. This is also reflected in carriers of few PVs in PALB2, RAD51D, and RECQL, new cancer predisposing genes, which are rarely identified in other populations. The FC population can be viewed as an open population as it is not isolated
from integration from other gene pools. The FC population has often been referred to as a founder population but there is no evidence of reduced genetic diversity [151], as has been described for the Ashkenazi Jewish population of Eastern European ancestry [163]. The frequencies of carriers of specific variants in FCs are therefore likely a result of genetic drift that occurred following a population bottleneck.

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