GENETIC STUDIES OF OVARIAN CANCER IN JEWISH WOMEN

by

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ABSTRACT

Ovarian cancer may occur due to a mutation in either the *BRCA1* or *BRCA2* genes. Two mutations in *BRCA1* (185delAG and 5382insC) and one mutation in *BRCA2* (6174delT) are common in the Ashkenazi Jewish population.

I report the results of a hospital-based case-control study I conducted in association with Dr. Steven Narod on 249 Ashkenazi Jewish women with ovarian cancer recruited from fourteen medical centres in North America and Israel. One of the three founder mutations of *BRCA1* or *BRCA2* was present in 38.6% of the cases. Only one non-founder mutation was identified in a patient of mixed ancestry, and the three founding mutations accounted for most of the observed excess risk of ovarian and breast cancer in relatives of the cases. *BRCA2* mutation carriers had a significantly higher age of onset of ovarian cancer compared to the *BRCA1* mutation carriers and the non-carrier cases. One of the founder mutations was present in 72.5% of cases with a family history consistent with hereditary ovarian cancer. 29.5% of the cases in our study who did not have a significant family history of ovarian cancer had one of the three mutations tested.

The penetrance of breast cancer associated with the *BRCA1* and *BRCA2* mutations was estimated at 42.1% and 34.2% to age 75, respectively. The penetrance of the *BRCA1* and *BRCA2* mutations for ovarian cancer was estimated at 13.8% and 20.2% by age 75, respectively. These estimates are lower than those previously reported in studies of hereditary breast-ovarian cancer families. The risk for breast cancer in first-degree relatives of our cases appears to be lower and the risk of ovarian cancer higher than that found in studies of these same founder mutations in unselected Ashkenazi Jewish women with breast cancer.

Age and vaginal talc use were found to be risk factors for ovarian cancer among *BRCA1* and *BRCA2* mutation carrier cases as well as among the non-carrier cases. Height was found to be a risk factor for ovarian cancer among the non-carrier cases only. There was an over-representation of epithelial serous tumours of the ovary among the *BRCA1* and *BRCA2* mutation carriers, but the increase was not statistically significant. *BRCA1* and *BRCA2* mutation carriers had a significantly higher frequency (90.7%) of grade III tumours compared to the non-carriers (68.7%, *P*=0.002), but the stage of disease did not differ between these groups.

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DEDICATION

To my mother,
Shams Tayeri, for
her extraordinary
patience and her
absolute support

To my father, Manouchehr
Moslehi, for his keen
interest in my studies and
his belief in my abilities

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GENETIC STUDIES OF OVARIAN CANCER IN JEWISH WOMEN

CHAPTER 1

INTRODUCTION

Cancer arises when a single somatic cell escapes from the constraints of normal growth control. This involves the acquisition of multiple genetic abnormalities according to what is known as the multi-step theory (Solomon, 1991; Kinzler *et al.*, 1997). In this model each mutation confers a selective growth advantage which leads to an expanded cellular population. Subsequent mutations give rise to a further growth advantage and therefore expansion of that cell clone, and so on. Besides mutations, other changes such as alterations in methylation or mRNA processing may also lead to advantageous cell growth and survival (Solomon *et al.*, 1991; Kinzler *et al.*, 1997). The multi-step model is now well established for some tumours, for example, colon cancer (Lengauer *et al.*, 1997).

In order to understand the molecular basis of cancer, it is first necessary to localise and identify the genes that are altered in various human malignancies. Employing cytogenetic and molecular genetic methods, specific chromosome abnormalities and gene mutations have been found to be associated with particular forms of cancer. These genes are usually involved in the control of mutation repair, cell growth, differentiation, or death (Steel, 1994; Kinzler *et al.*, 1997). There are many such genes and they affect, and are affected by, each other and numerous other genes and their products. It is not surprising, therefore, that cancer is a common disease, affecting one in three people (Parkin *et al.*, 1997). The changes observed cytogenetically include loss of whole chromosomes (monosomy), deletions, insertions, inversions, reciprocal translocations, and amplification of parts of chromosomes (Solomon *et al.*, 1991). A wide variety of

factors have been implicated as causative and contributory agents in cancer. Exposure to these factors may lead to mutations or chromosome aberrations, which may in turn lead to cancer. These contributory agents include environmental factors such as exposure to carcinogens in pollution and cigarette smoke, exposure to sunlight (UV rays), dietary factors, viruses and other organisms and endogenous factors such as inherited predisposition and effects of the immune system (Steel, 1994; Solomon *et al.*, 1991; Vogel et al, 1997; Weinberg, 1991).

Cancer Genes

Currently, we classify most genes responsible for uncontrolled proliferation into three major groups, the proto-oncogenes, the tumour suppressor genes, and genes involved in repair of DNA damage (Kinzler *et al.*, 1997). Although this is likely to be an over simplification, most of the genes associated with the pathogenesis of cancer identified so far fall broadly into these three categories.

Proto-oncogenes are normal cellular genes that appear to exert an essential role in controlling cell proliferation and differentiation. The proto-oncogenes identified to date are classified into five main groups: 1) secreted growth factors (e.g. SIS), 2) Cell surface receptors (e.g. RET), 3) components of intracellular signal transduction (e.g. HRAS1), 4) DNA-binding nuclear proteins (e.g. MYC) and 5) components of the network of cyclins, cyclin-dependent kinases and kinase inhibitors which control the cell cycle (e.g. PRAD1) (Hunter, 1991; Steel, 1994).

A proto-oncogene can be converted to an oncogene (cancer causing gene) by variety of events including point mutations, small insertions and deletions, and

juxtaposition to other chromosome sequences. Oncogenes can be defined as mutated or over-expressed proto-oncogenes with the new and aberrant ability to promote cancer development. In cancerous cells, the activity of these genes is increased through overexpression of their normal protein, acquisition of a new function or by constitutive or otherwise inappropriate expression of their products. Viral integration and insertion can also result in over-expression of cellular proto-oncogenes (Steel, 1994).

The tumour suppressor genes, like proto-oncogenes, are normal cellular genes; they produce proteins that are believed normally to be involved in the negative regulation of proliferation or induction of apoptosis. Tumour suppressor genes contribute to tumour formation through their loss or a decrease in their function rather than through their activation. Loss of function may occur through chromosomal loss, deletion or mutation of the tumour suppressor gene. Their behaviour is recessive at the cellular level in that both copies of the gene must be inactivated for tumour formation to occur (Weinberg, 1991; Kinzler *et al.*, 1997).

The concept of tumour suppressor genes was first introduced by Knudson in 1971. Knudson put forward a hypothesis based on epidemiological data for a two-hit mechanism in the development of retinoblastoma, a tumour of the eye that afflicts young children. Knudson hypothesized that patients with multiple or bilateral tumours and those with a family history inherited a single mutation in every cell of their body from one parent. The development of another somatic mutation would lead to tumour formation. Sporadic retinoblastoma occurred when both genes were mutated in a progenitor cell (Knudson, 1971).

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Cell-hybrid studies and epidemiological data support the tumour suppressor gene concept. It was discovered later that both mutations occur in alleles at a single locus. Thus the two-hit model of tumourigenesis was proposed: the functional loss of both alleles of a tumour suppressor gene are necessary for tumourigenesis, with the first hit being either inherited or somatically acquired and the second hit being additionally acquired. The second hit is usually a gross chromosomal alteration, which results in loss of the wild type allele. The loss of one allele of a particular gene is known as loss of heterozygosity (LOH), and it is presumed to indicate that the gene in question is a tumour suppressor gene (Weinberg, 1991). There is now compelling molecular evidence that the Knudson hypothesis is correct for retinoblastoma and several other malignancies that occur in hereditary and sporadic forms (Kinzler *et al.*, 1997; Kinzler *et al.*, 1998).

DNA repair genes comprise the third major category of cancer genes. The products of these genes are responsible for repair of DNA damage caused by variety of factors such as ionizing radiation or errors of replication. Loss of function of these genes leads to unrepaired mutations of tumour suppressor and proto-oncogenes that can lead to development of cancer. Therefore, it would appear that DNA repair genes function in the manner of a tumour suppressor gene in that a two-hit mechanism of gene inactivation is required for tumourigenesis. However, tumour suppressor gene inactivation is by definition accompanied by a growth advantage. In contrast, mutations in the DNA repair genes do not directly alter the growth properties of the cell but increase the likelihood of occurrence of mutations in other cancer genes. Therefore, these genes are also referred to as "mutator" genes (Kinzler *et al.*, 1997; Lengauer *et al.*, 1997; Warburton *et al.*, 1997).

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Ovarian Cancer

Aetiology

Globally ovarian cancer is the 6th most common cancer among women. In North America, ovarian cancer is the fifth most common cancer in women and accounts for 4% of all cancers in this population (Parkin *et al.*, 1997). Ovarian cancer is the leading cause of death due to gynaecological malignancies.

The causes of ovarian cancer are poorly understood. Reproductive hormones are thought to be involved in the aetiology of this malignancy. Two main hypotheses, for which evidence has been obtained through epidemiological studies, have been suggested for ovarian carcinogenesis. The first hypothesis is the "incessant ovulation" theory that suggests that the risk of epithelial ovarian cancer increases with the number of ovulations (Risch, 1998; Schildkraut *et al.*, 1997). The traumatized epithelium of ruptured follicles is normally repaired post-ovulation. This hypothesis suggests that the rupture and repair process, which occurs during ovulation, allows the possibility of aberrant repair. This is based on the fact that it is during cellular proliferation and DNA replication that mutations may occur. The accumulation of these unrepaired mutations may lead to cancer as previously described in the multi-step process of cancer development. Therefore, with increasing number of ovulation cycles, the probability of developing ovarian cancer may also increase.

The second theory for ovarian cancer development is referred to as the "gonadotrophin" hypothesis. This theory predicts that high levels of pituitary gonadotrophins increase cancer risk by stimulating ovarian surface epithelium. The stimulation of these surface epithelial cells may cause increased proliferation of these

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cells and increased possibility of unrepaired mistakes occurring during DNA replication. One piece of evidence for this theory comes from the observation that both gonadotrophin levels and the age-specific incidence of epithelial ovarian cancer are highest during early post-menopausal years (Risch, 1998).

Pathology

Several different malignancies may arise from the ovary. The classification of ovarian neoplasms is based on the World Health Organization (WHO) system (Table 1). This system classifies ovarian tumours based on morphology and histogenesis. The three most common types of ovarian tumours are epithelial ovarian tumours, germ cell tumours, and sex cord/stromal tumours (Table 1).

Epithelial ovarian tumours comprise the largest group of primary malignant ovarian neoplasms and represent about 90% of all ovarian tumours (Altcheck *et al.*, 1996). Epithelial ovarian cancer arises in the surface or germinal epithelium that covers the ovary, in continuity with the peritoneal mesothelium. Histologically, this superficial lining of the ovary is quite similar to the peritoneal mesothelial lining. There are several subgroups of epithelial ovarian cancer. About 43% of epithelial ovarian tumours are serous adenocarcinomas, 15% are mucinous adenocarcinomas, 22% are endometrioid adenocarcinomas, 5% are clear cell tumours, 14% are mixed or unclassified epithelial tumours of the ovary and 1% are transitional cell or squamous cell tumours (Table 1) (Altcheck *et al.*, 1996; National Cancer Institute, 1999).

Ovarian cancer usually spreads via local shedding into the peritoneal cavity, followed by implantation on the peritoneum, and also by local invasion of bowel and

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bladder. The nodes usually become affected and the resulting impairment of lymphatic drainage of the peritoneum is thought to play a role in development of ascites in ovarian cancer. Also, transdiaphragmatic spread to the pleura is common. The prognosis of ovarian cancer is influenced by several factors, but multivariate analyses suggest that the most important favourable factors include younger age, cell type other than mucinous or clear cell, low degree of spread (stage), and high degree of differentiation (low grade) of the tumour (Altcheck *et al.*, 1996; National Cancer Institute, 1999).

The degree of differentiation of the tumour is determined histopathologically and is classified as grade. Grade $G_{0/3}$ indicates that the tumour is in situ or borderline. Grade $G_{1/3}$, $G_{2/3}$, $G_{3/3}$ indicate well-differentiated, moderately-differentiated, and poorly- or undifferentiated tumours, respectively. In general, the more differentiated the tumour, the better the prognosis (Altcheck *et al.*, 1996).

Ovarian cancer staging is based on surgical pathologic findings. An exploratory laparotomy, peritoneal washings, total abdominal hysterectomy, bilateral salpingo-oophorectomy (TAH-BSO), omentectomy, multiple peritoneal biopsies, and pelvic and para-aortic lymph node sampling are necessary for adequate staging. In 1971, the International Federation of Gynaecology & Obstetrics (FIGO) provided the first classification system for staging ovarian cancer. This system allowed more appropriate treatment, more accurate evaluation of treatments, and comparison of statistics on a world-wide basis. This classification system has since been revised in 1974 and 1987, reflecting the new information available (Table 2). The lower the stage of the tumour, the better the prognosis (Altcheck *et al.*, 1996).

Epidemiology

The life-time risk for a woman to develop ovarian cancer is about 1.4% in North America (Parkin *et al.,* 1997). Ovarian cancer occurs primarily in women in the 40 to 70 year age range. Peak incidence is in the 55 to 59 year age group, and the median age at time of diagnosis is 61 years (Altcheck *et al.,* 1996; National Cancer Institute, 1999). The prognosis for ovarian cancer can vary in different women based on individual profiles. Due to the asymptomatic nature of early ovarian cancer, only 24 percent of all cases are found at an early stage. Because many ovarian cancers are not detected early, the overall 5-year survival rate for women with ovarian cancer is only between 35 percent and 47 percent, depending upon the type of tumour (Altcheck *et al.,* 1996; National Cancer Institute, 1999). Because of the high mortality rate associated with ovarian cancer and the absence of effective screening tests, prophylactic oophorectomy has been advocated as a preventative approach for women at high risk.

Both reproductive and genetic factors have been implicated in ovarian cancer etiology (Risch, 1998). Epidemiological studies have indicated early age at menarche, nulliparity, and late age at menopause as risk factors for ovarian cancer (Whittemore *et al.,* 1992) (Riman *et al.,* 1998). The risk of ovarian cancer seems to be correlated with the length of time a woman has ovulated. There have been suggestions that suppression of ovulation by pregnancy, lactation, and oral contraceptives decreases the risk of ovarian cancer.

There are contradictory reports in the literature on the relationship between age at menarche and menopause and ovarian cancer. Some studies have estimated the relative

risks for early age of menarche and late age at menopause for ovarian cancer to be 1.2 and 2.0, respectively. However, other studies have found no such association. Therefore age at menarche and menopause are probably weak predictors of ovarian cancer risk (Riman *et al.*, 1998).

The protective effect of increasing parity on the risk of epithelial ovarian cancer is well established. Nulliparity is estimated to have a relative risk of 2.0 for ovarian cancer (Whittemore *et al.*, 1992). In a large case-control study, a 40% lowered risk of ovarian cancer was found for the first full-term pregnancy and another 14% risk reduction was found for each subsequent birth (Whittemore *et al.*, 1992; Riman *et al.*, 1998). The effect of age at first birth is not fully settled yet; however, there is evidence that later age at first birth may reduce epithelial ovarian cancer risk (Riman *et al.*, 1998).

Lactation suppresses the secretion of pituitary gonadotropins and leads to anovulation. Most studies have found a decreased risk of ovarian cancer with lactation. The magnitude of the risk reduction is usually weak with odds ratios 0.6-0.9 (Whittemore, 1992; Risch *et al.*, 1994).

Oral contraceptives (OC) exert their effects by suppressing mid-cycle gonadotropin surge and inhibiting ovulation. Epidemiological studies have provided strong evidence that OC use reduces ovarian cancer risk. A meta-analysis including 20 studies from the 1970s, 1980s and 1990s calculated a summary relative risk of 0.64 (95% confidence interval 0.57-0.73) for ever use of OC (Harkinson *et al.*, 1992). Longer duration of OC use seems to increase the protection against epithelial ovarian cancer. Several studies have documented 10-12% reduction in ovarian cancer risk with each year of OC use. The protective effect of OC use seems to last for a long time after the cessation of use. A 40-

70% ovarian cancer risk reduction persisted when at least 10 years had elapsed since last use (Whittemore *et al.*, 1992; Harkinson *et al.*, 1992).

The effect of other factors such as hormone replacement therapy (HRT), tubal ligation, and hysterectomy on ovarian cancer risk is not well established. The question of whether HRT alters the risk of epithelial ovarian cancer remains unanswered. Tubal ligation and hysterectomy, based on altering the hormonal surges, are believed to protect against ovarian cancer. The studies examining the association between these two surgical procedures and ovarian cancer risk suggest that there may exist a protective effect; however, the findings are not consistent (Riman *et al.*, 1998).

Perineal talc use has been indicated as a risk factor for ovarian cancer in several studies (Harlow *et al.*, 1995). This finding is controversial because of contradicting reports and lack of biological evidence. A recent prospective study of 78630 women between the ages of 30 and 55 found no association between perineal talc use and ovarian cancer risk overall (Gertig *et al.*, 2000). Gertig et al. (2000) reported a modest increase in risk of invasive serous ovarian cancer associated with perineal talc use (RR=1.40; 95% CI=1.02-1.91).

Epidemiological studies have found the strongest risk factor for ovarian cancer to be a family history of breast and ovarian cancer, particularly if it occurs in women below the age 50 (Amos *et al.*, 1994; Berchuck *et al.*, 1998; Lynch *et al.*, 1998; Narod *et al.*, 1994; Schildkraut *et al.*, 1998; Whittemore *et al.*, 1992). The younger age at which cancer is diagnosed in familial cases is interpreted as further evidence to support a genetic basis. This is based on the theory that familial cases carry one mutation in all cells in their bodies. Therefore, it will take less time for one mutation to occur in the

remaining allele than for two independent mutations to occur in the 2 alleles of a predisposing gene in an ovarian cell. Approximately 5-10% of all ovarian cancers are attributable to mutations in single cancer susceptibility genes (Berchuck *et al.*, 1999, Lynch *et al.*, 1998, Narod *et al.*, 1994). Three patterns of hereditary predisposition to ovarian cancer have been described: site-specific ovarian cancer, hereditary breast and ovarian cancer, and ovarian cancer as a component of hereditary nonpolyposis colorectal cancer (HNPCC) (Lynch *et al.*, 1998; Lynch *et al.*, 1991). More recently, the genetic basis for hereditary ovarian cancer has been identified in many families.

Site-specific ovarian cancer families and breast-ovarian cancer families account for the disease in 90% of hereditary ovarian cancer cases. The familial breast-ovarian cancer syndrome accounts for approximately 5% of all ovarian cancer cases in Canada (Narod *et al.* 1994). By studying these high-risk families, two genes, *BRCA1* and *BRCA2*, were identified (Miki *et al.*, 1994; Wooster *et al.*, 1995). Subsequently, the majority of families with this syndrome were found to carry a mutation in one of these two genes (Easton *et al.*, 1993; Narod *et al.* 1995a, Narod *et al.* 1995b). Other families are believed to segregate other single breast and/or ovarian cancer genes not yet identified.

Approximately 2% of hereditary ovarian cancer cases occur in the context of hereditary non-polyposis colorectal cancer (HNPCC) (Lengauer *et al.*, 1997; Lynch *et al.*, 1998, Lynch *et al.*, 1991). Mutations in five DNA mismatch repair genes are responsible for the disease in the majority of HNPCC families. These genes are MSH2, MLH1, MSH6, PMS1 and PMS2 (Lengauer *et al.*, 1997; Petersen *et al.*, 1999). Loss of function mutations in these DNA mismatch repair genes may lead to accumulation of mutations

and other changes in the DNA in the cells which may lead to colorectal and other cancers.

BRCA1

The *BRCA1* gene was localised to chromosome 17q12-21 in 1990, using genetic linkage analysis in 23 multiple-case Caucasian breast cancer families (Hall *et al.*, 1990). A further study indicated the significance of ovarian cancer associated with *BRCA1* by showing linkage to the region in 3 of 5 breast-ovarian cancer families (Narod *et al.*, 1991). It was estimated that more than 80% of breast-ovarian cancer families and approximately 50% of site-specific breast cancer families were linked to the *BRCA1* locus (Easton *et al.*, 1993; Ford *et al.*, 1998). In contrast, the majority of families with site-specific ovarian cancer and breast cancer arising in men (so called "male breast cancer") were not linked to *BRCA1* (Stratton *et al.*, 1994; Ford *et al.*, 1998). In addition to breast and ovarian cancer, the *BRCA1* gene was reported to confer an increased risk of prostate and colon cancer (Ford *et al.*, 1994).

The *BRCA1* gene was cloned in 1994 by narrowing down the region with tightly linked polymorphic DNA markers and then employing the candidate gene approach (Miki *et al.*, 1994). It covers approximately 100 Kb of genomic DNA. There are 24 exons, 22 of which are coding. Exons 1 and 24 are non-coding and exon 11 is unusually large, accounting for one half of the entire coding region of the gene. The transcript size is about 8Kb (Miki *et al.*, 1994), and the protein is 220-kDa (Chen *et al.*, 1995). *BRCA1* encodes a protein of 1863 amino acids. This protein's function is not fully known. It is believed that the *BRCA1* protein can function as a tumour suppressor because the normal

copy of *BRCA1* is invariably deleted in breast and ovarian cancers that arise in women who inherit a mutant copy of this gene.

The amino terminus of *BRCA1* contains a zinc RING finger motif. The biological function of this zinc finger domain remains unclear, although such structures may be involved in protein-protein interactions (Bienstock *et al.,* 1996). The observation was made that the *BRCA1* protein was localised in normal cells in the nucleus and in tumour cells in the cytoplasm (Chen *et al.,* 1995). Coene et al. (1997) reported localization of *BRCA1* in the perinuclear compartment of the endoplasmic reticulum-Golgi complex and in tubes invaginating the nucleus. This group found the nuclear detection of *BRCA1* to be dependent on the fixation method used. Chen et al. (1996) reported that expression of the *BRCA1* gene and the phosphorylation of the *BRCA1* protein are cell cycle dependent. The greatest levels of expression of the *BRCA1* gene and phosphorylation of the *BRCA1* protein seem to occur in S and M phases.

Initial speculation as to *BRCA1*'s involvement in the transcriptional process appears to be supported by the interaction of this protein with various transcription factors, including the repressor pair CtIP and CtBP (Li *et al.,* 1999). This particular interaction appears to repress the ability of *BRCA1* to transactivate the p21 promoter. This interaction is disrupted upon DNA damage, thereby allowing p21-mediated cell cycle inhibition and possible damage repair. In addition, the induction of *BRCA1* is followed immediately by the increased mRNA expression of GADD45, a DNA damage-response gene (Li *et al.,* 1999).

Although there is strong evidence to support a role for *BRCA1* in transcriptional repression, why alterations in this gene result in predominantly breast and ovarian cancer

has not been well understood. Recently, investigators have presented evidence linking the function of *BRCA1* as a transcriptional regulator to tissue specificity. In one study, wild-type *BRCA1*, in a dose-dependent manner, repressed E2-mediated transcriptional activation by the transcriptional activation function AF-2 of the estrogen receptor ER-α (Chen *et al.*, 1999). Since breast and ovarian cells are highly responsive to estrogen, *BRCA1*'s repression of E2-responsive, ER-α-mediated transcription is perhaps the strongest evidence to explain *BRCA1*'s role predominantly in breast and ovarian cancer. When *BRCA1* is mutated, E2-responsive transcription proceeds unimpeded and may stimulate breast cells already initiated by other factors. The biological significance of *BRCA1*'s interactions with other transcription factors remains to be proven.

At the distal carboxy-terminus (amino acids 1640-1863), *BRCA1* contains two BRCT (*BRCA1* C-terminal) repeats. These repeats have been found in many proteins involved in DNA repair including Rad9, XRCC1, and three eukaryotic DNA ligases (Bork *et al.*, 1997). The minimal binding region of the p53 binding protein, P53BP1, also contains BRCT repeats (Iwabuchi *et al.*, 1994), suggesting that such domains may be involved in protein-protein or protein-DNA interactions, possibly in response to DNA damage. Recently, the structure of the XRCC1 BRCT domain was resolved by x-ray crystallography at 3.2 A resolution (Zhang *et al.*, 1998). Structural analysis revealed that the BRCT domain comprises a four-stranded parallel beta-sheet surrounded by three alpha-helices that forms an autonomously folded domain. Recently, Yarden and Brody (1999) isolated other proteins that interacted with the *BRCA1* BRCT domain. These proteins were found to be components of the histone deacetylase complex. This may

explain a role for *BRCA1* in multiple processes such as transcription, DNA repair and recombination.

The involvement of *BRCA1* in DNA repair pathways is further underscored by its association with the Rad50/MRE11/p95 complex and with its colocalization with Rad51 (Zhong *et al.*, 1999). Rad51 is implicated in yeast homologous recombination repair and a similar role is suspected in mammalian cells. In addition, *BRCA1* also associates and coimmunoprecipitates with the Rad50 complex involved in non-homologous DNA double-strand break repair pathway (Zhong et al. 99). Precisely how *BRCA1* participates in various pathways of DNA repair through its interactions with proteins in response to DNA-damaging agents, remains to be determined. Nevertheless, *BRCA1* appears to have dual roles in response to DNA damage, leading to cell cycle arrest by upregulation of p21 expression and DNA repair by forming repair foci.

The 5'- and 3'-sequences of the *BRCA1* gene are highly conserved through evolution (Szabo *et al.*, 1996); suggesting they may have an important role. The *BRCA1* protein also appears to bind to another protein known as BARD1, which can attach to the ring finger motif of *BRCA1* (Wu *et al.*, 1996). BARD1 may have a role in *BRCA1*-mediated tumour suppression. Furthermore, there is a putative 'granin' consensus sequence (position 1214-1223) which one group has suggested is important (Jensen *et al.*, 1996). The granins are a family of acidic proteins which are involved in the processing of proteins, such as prolactin and growth hormones, which are secreted in response to signals from the extracellular environment. The granin consensus sequence is not totally conserved in murine or canine *BRCA1* (Szabo *et al.*, 1996), (Koonin *et al.*, 1996).

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The mouse homologue of human *BRCA1* has been cloned (*Brca1*) and maps to mouse chromosome 11. *Brca1* shares only 58% homology with *BRCA1* at the amino acid level (Chen *et al.*, 1999). Therefore, this protein may function differently in the two species. Nonetheless, animal studies have supported the role of *BRCA1* protein in transcriptional regulation, DNA repair, and growth and differentiation. The role of *Brca1* in mouse tumorigenesis has been difficult to study, limited by the embryonic lethality conferred by the *Brca1-/-* genotype. In addition, heterozygous *Brca1+/-* mice did not demonstrate any phenotypic abnormality up to 1 year of age (Gowen et al. 96, Hakem *et al.* 96). Recently the results of conditional deletion of *Brca1* in *Brca1+/-* mice were reported. Wap-Cre or MMTV-Cre-mediated excision of *Brca1* exon 11 in the mammary epithelial cells of these mice resulted in increased apoptosis and abnormal ductal development (Xu *et al.*, 1999). Mammary tumour formation was observed in both strains (Wap-Cre and MMTV-Cre) but at low frequency and after long latency. The tumours were associated with genetic instability characterized by aneuploidy and chromosomal rearrangements (Xu *et al.*, 1999).

Furthermore, *Brca1-/-* murine embryos were found to be hypersensitive to gamma irradiation. *Brca1* deficient mouse embryonic stem (ES) cells are also hypersensitive to ionizing radiation and hydrogen peroxide (Gowen *et al.*, 1998). These ES cells are unable to carry out transcription-coupled repair, a process in which DNA damage is repaired more rapidly in transcriptionally active loci compared to the whole genome. These cells also have impaired repair of chromosomal double-strand breaks by homologous recombination (Moynahan et al. 99). These results strongly suggest a role for *BRCA1* in preserving genomic integrity.

In humans, a large number of breast and breast-ovarian cancer kindreds has been screened for mutations in *BRCA1*. Several methods have been used to detect mutations of the *BRCA1* gene. Direct DNA sequencing can detect sequence variation. Single strand conformation polymorphism (SSCP) assay does not detect all sequence changes but can detect most DNA sequence variations. The protein truncation test (PTT) can also be used to detect the aberrant gene product. PTT does not detect mutations in the introns, exon-intron boundaries, splice junctions or promotor and enhancer regions. Sequencing of the genomic DNA needs to be done to determine the exact nature of the DNA sequence variation (Castilla *et al.*, 1995).

In July 2000, the Breast Cancer Information Core (BIC), a database for *BRCA1* and *BRCA2* mutations, contained 865 different *BRCA1* sequence variations (http://www.nhgri.nih.gov/intramural_research/Lab_transfer/Bic/Member/index.html). The majority of the alterations are frameshift (71%) or nonsense mutations (10%) which presumably lead to a truncated protein product. A number of missense mutations (14%) have also been identified, and most are located within the highly conserved amino- or carboxy- termini of the gene, resulting in disruption of the RING finger domain or BRCT repeats, respectively. The significance of some other mutations is unknown, and they may represent polymorphisms.

There appears to be genotype-phenotype correlation with respect to ovarian cancer in *BRCA1* kindreds. Mutations in the 5' end of the gene have an increased risk of ovarian cancer compared to the mutations in the 3' end of the gene (Gayther *et al.*, 1995).

BRCA2

Linkage in some breast cancer families to a second locus on 13q21 was suggested in 1994 (Wooster *et al.*, 1994). Simultaneously, a 300 kb homozygous deletion in the same region was identified in a pancreatic tumour (Schutte *et al.*, 1995). The combination of this information further defined the region and the *BRCA2* gene was cloned in 1995 (Wooster *et al.*, 1995, Tavatigan *et al.*, 1996). *BRCA2* was estimated to be responsible for approximately 45% of site-specific breast and breast/ovarian cancer families, and 80% of breast cancer families with male breast cancer (Couch *et al.*, 1996; Ford *et al.*, 1998).

The *BRCA2* gene spans approximately 200 kb of genomic DNA. It contains 26 coding exons, with an estimated transcript size of 10-12 kb. The protein consists of 3418 amino acids and is 348kDa in size (Jensen et al. 1996). There is a weak homology in one region in exon 11 to *BRCA1* (Connor *et al.*, 1997), and the 'granin' consensus sequence is not fully conserved (Jensen et al, 1996). There are a series of highly conserved repeat regions of unknown function in exon 11 (Bork *et al.*, 1996). The *BRCA2* mRNA has been shown to be regulated by the cell cycle and associated with proliferation in normal and tumour-derived breast epithelial cells (Chen *et al.*, 1999).

Similar to *BRCA1*, the *BRCA2* protein is reported to interact with RAD51 in vivo. Moreover, the cells of *BRCA2*-deficient tumours are aneuploid (Chen *et al.*, 1999) consistent with this locus participating in the maintenance of genome stability. Chen et al. (1998) reported the interaction of endogenous *BRCA2* with endogenous *BRCA1* in cultured human cell lines, nuclear colocalization of these two proteins, and similar responses of these proteins to DNA damage. This group suggested that endogenous

BRCA1 and *BRCA2* proteins coexist in a biochemical complex and jointly participate in at least one DNA damage pathway (Chen *et al.,* 1998).

The mouse homologue, *Brca2* has been cloned and maps to mouse chromosome 5. There is 59% homology at the amino acid level with human *BRCA2* and the protein is expressed in a variety of tissues (Connor *et al.,* 1997). *Brca2* heterozygous deficient mice, in one study, did not develop cancer up to 1 year of age (Lee *et al.,* 1999). *Brca2* nullizygous mice all reveal embryonic lethality, associated with a proliferation deficit (Gowen *et al.,* 1996). Furthermore, *Brca2* nullizygous embryos exhibit X-ray hypersensitivity (Sharan *et al.,* 1997). Cells of *Brca2* mutant mice reveal inefficient repair of DNA breaks and aberrant chromosomal structures (Patel *et al.,* 1998). They are also hypersensitive to DNA-adducting agents (Patel *et al.,* 1998). These findings may suggest a role for *BRCA2* in recombinational responses to DNA damage, as was suggested for *BRCA1*.

Direct DNA sequencing, SSCP, PTT and genomic DNA sequencing are some of the techniques that have been used to identify mutations in the *BRCA2* gene. 882 *BRCA2* mutations, polymorphisms and variants had been reported by July 2000 (BIC database) (http://www.nhgri.nih.gov/intramural_research/Lab_transfer/Bic/Member/index.html). The majority of mutations identified in *BRCA2* families include small deletions and insertions, which lead to frameshifts (approximately 70%) or nonsense mutations (7.5%). Splice site mutations account for approximately 4% of mutations identified thus far (BIC data base).

As in with *BRCA1*, a genotype-phenotype correlation has been proposed for *BRCA2*. Families with mutations in a 3300 bp region in exon 11, which partly overlaps

with the repeat region, have a higher incidence of ovarian cancer compared to those families with mutations in the 5'- and 3'- regions (Gayther *et al.*, 1997).

Clinical Significance of BRCA1 and BRCA2 Mutations

In July 2000, BIC contained 1747 different variants of the BRCA genes. The majority of these alterations are believed to be associated with a deleterious effect (http://www.nhgri.nih.gov/intramural_research/Lab_transfer/Bic/Member/index.html). More than 90% of these mutations lead to a non-functional truncated protein. In most reported cases, these truncating mutations are point or small sized mutations, and they are spread over the entire coding sequence that numbers 5592 nucleotides for *BRCA1* and 10,443 for *BRCA2*. Although the majority of these mutations are unique to a particular patient or family, some of the identified mutations in the *BRCA1* gene are recurrent, the most common being the 185delAG, 5382insC, 4184del4, 1294del40, and 1136insA. In a number of families with the same mutation, a common chromosome 17q haplotype is shared. As for *BRCA1*, a number of mutations in the *BRCA2* are recurrent, and there appears to be sharing of a common haplotype. The most common recurrent mutation in the *BRCA2* gene is the 6174delT mutation.

Recurrent mutations have been identified in almost all populations studied. Relatively small numbers of mutations are responsible for the disease in the majority of breast/ovarian cancer families from Finnish (Vehmanen *et al.*, 1997), Norwegian (Szabo *et al.*, 1997), Dutch, Belgian (Peelen t al., 1997), Icelandic (Thorlacius *et al.*, 1997), Swedish (Hakansson *et al.*, 1997), Spanish (Diez *et al.*, 1998; Diez *et al.*, 1999), French Canadian (Tonin *et al.*, 1998), Chinese (Khoo *et al.*, 1999), Pakistani (Moslehi *et al.*,

1998), Turkish (Balci *et al.*, 1999) and Jewish ancestries (Struewing *et al.*, 1995; Goldgar *et al.*, 1995; Levy-Lahad *et al.*, 1997; Moslehi *et al.*, 2000).

In the Ashkenazi Jewish population three recurrent mutations have been identified. Two of these mutations are in the *BRCA1* gene (185delAG and 5382insC) and are present at a frequency of 1.1% and 0.1%, respectively (Struewing *et al.*, 1995; Roa *et al.*, 1996). The third (6174delT) is in the *BRCA2* gene and is present at 1.4% frequency (Oddoux *et al.* 1996). The combined frequency of these three mutations in the Ashkenazi Jewish population is approximately 2.5%.

Current estimates of life-time risks in women carrying the *BRCA1* mutations are 50%-85% for breast cancer and 15%-45% for ovarian cancer (Gayther *et al.*, 1997; Lynch *et al.*, 1999). These risks are significantly elevated over the population life-time risks for breast and ovarian cancers at 10% and 1.4%, respectively. Women who carry *BRCA1* mutations also have an increased incidence of bilateral breast cancer, with a second primary breast cancer occurring in 40% to 60% of patients (Lynch *et al.*, 1999). *BRCA2* has a cancer risk profile similar, but not identical, to *BRCA1*. Women with a mutation in *BRCA2* have a 50% to 85% lifetime risk of developing breast cancer. Unlike *BRCA1*, *BRCA2* mutations confer a 6% risk of male breast cancer. This represents 100-fold increase over the general population risk. The life-time risk of ovarian cancer conferred by *BRCA2* mutations appears to be in the range of 10%-20% (Lynch *et al.*, 1999; Hopper *et al.*, 1999).

The higher risk estimates for breast and ovarian cancer in these ranges reflect penetrance figures derived from studies of families with multiple cases of breast and ovarian cancer. The lower estimates, on the other hand, are from the studies of *BRCA1*

and *BRCA2* mutations in the general population, for example, Ashkenazi Jewish individuals unselected for a family history.

Struewing et al. (1997) examined the risk for cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jewish women in the Washington, DC, area. Blood samples were collected from 5318 Jewish volunteers who had filled out epidemiological questionnaires. Carriers of the 185delAG and 5382insC mutations in *BRCA1* and the 6174delT mutation in the *BRCA2* were identified. Risks for breast and ovarian cancer by age 70 were estimated by comparing the cancer histories of relatives of carriers and non-carriers of the mutations. The estimated risk for breast cancer among carriers was 56% (95%CI: 40% to 73%), a lower estimate than that previously obtained from high-risk families. The estimated risk for ovarian cancer among carriers was 16% (95% CI: 6% to 28%) (Struewing *et al.*, 1997). The numbers were too small to allow statistically meaningful calculation of risk and comparison of the *BRCA1* and *BRCA2* carriers.

Effects of Risk Factors for Ovarian Cancer in BRCA1 and BRCA2 Mutation Carriers

Most non-genetic risk factors for breast and ovarian cancer have low predictive value, and the use of genetic tests may improve the predictive value of environmental factors. A new paradigm of the primary prevention of breast and ovarian cancers could be the identification and modification of environmental cofactors that lead to clinical disease among persons with susceptibility genotypes.

The variable age at onset of hereditary breast and ovarian cancers and the fact that some women who carry *BRCA1* and *BRCA2* mutations do not develop cancer suggest

that other host or environmental factors may modify the expression of these traits. Only a few studies have been reported on the role of environmental risk factors for breast and ovarian cancer among women with *BRCA1* and *BRCA2* mutations. Narod *et al.* (1995) examined whether known risk factors modified susceptibility to breast and ovarian cancer in 333 women with *BRCA1* gene mutations. An increased risk for breast cancer was associated with recent birth cohort and low parity (compared with that of the general population). However, the risk for ovarian cancer increased with increasing parity and decreased with increasing age at last childbirth.

More recently, our group studied the effect of oral contraceptive use on ovarian cancer risk in women with *BRCA1* and *BRCA2* mutations (Narod *et al.*, 1998). We conducted a case-control study of 207 women with hereditary ovarian cancer who had a mutation in either *BRCA1* or *BRCA2* and 161 of their healthy sisters whose carrier status was not known. The adjusted odds ratio for ovarian cancer associated with any past use of oral contraceptives was 0.5 (95% confidence interval, 0.3-0.8). The risk decreased with increasing duration of oral contraceptive use (P for trend <0.001); use for six or more years was associated with a 60% reduction in risk. In our study, oral contraceptive use protected against ovarian cancer for carriers of both *BRCA1* mutations (odds ratio=0.5, 95% confidence interval, 0.3-0.9) and *BRCA2* mutations (odds ratio=0.4, 95% confidence interval, 0.2-0.11) (Narod *et al.*, 1998).

Our group also studied the effect of smoking on breast cancer risk in carriers of *BRCA1* and *BRCA2* mutations. We found a decreased risk of breast cancer (odds ratio=0.46, 95% confidence interval, 0.27-0.80, two sided P=0.006) among the carriers of *BRCA1* and *BRCA2* mutations who smoked 4 pack-years (i.e., number of packs per day

multiplied by the number of years of smoking) in comparison to subjects with mutations who never smoked (Brunet *et al.,* 1998). This observation raises the possibility that smoking reduces the risk of breast cancer in carriers of *BRCA1* and *BRCA2* mutations. There are no reports on the effect of smoking on ovarian cancer risk among the *BRCA1* and *BRCA2* carriers.

Studies are now in progress to identify other genetic modifiers of breast and ovarian cancer risk among the *BRCA1* and *BRCA2* mutation carriers. Phelan *et al.,* (1996) demonstrated that *BRCA1* mutation carriers who have a rare allele of a variable number of tandem repeats (VNTR) locus located 1 kb downstream of the HRAS1 oncogene have an increased risk of ovarian cancer. Our group recently analysed two biallelic polymorphisms in introns 3 and 6 of the p53 gene for a possible risk-modifying effect for ovarian cancer. We studied 124 affected and 276 unaffected female carriers with known *BRCA1* and *BRCA2* mutations from high-risk breast-ovarian cancer families. We also studied 310 German Caucasian ovarian cancer patients and 364 healthy controls as part of this case-control study. Our data suggested that intronic polymorphisms of the p53 gene increase the risk for ovarian cancer but not in carriers of *BRCA1* and *BRCA2* mutations (Wang-Gohrke *et al.,* 1999).

Histopathology and Survival Associated with BRCA1 and BRCA2 Mutations

The clinical features of breast cancer associated with germline mutations in *BRCA1* have been well described. Histopathological studies of tumours in women who carry *BRCA1* mutations have shown these neoplasms to be characterised by a lower mean aneuploid DNA index, higher proliferation rates, and a high S-phase fraction - features

that are generally associated with a poor prognosis (Breast Cancer Linkage Consortium, 1997). Despite these features, the relationship between mutation status and survival remains unclear.

The characteristics of familial epithelial ovarian cancer are less well described. In one small study of site-specific familial ovarian cancer, no difference in grade was found between familial and sporadic ovarian tumours (Buller et al., 1993). Another study of familial ovarian cancer found a significantly higher proportion of serous cystadenocarcinoma in familial cases (83%) compared to non-familial cases (49%) (Chang et al., 1995). Piver et al. (1993) presented data suggesting that mucinous carcinomas of the ovary may be underrepresented in familial ovarian cancer. To address this issue, Narod et al. (1994) reviewed the histology of 49 ovarian cancers seen in 16 hereditary breast-ovarian cancer families shown to be linked to BRCA1. Five of the 49 (10.2%) tumours were mucinous. Three of the 5 mucinous tumours in this study occurred in the 4 cases that did not show linkage to the BRCA1 locus and 2 of the 5 mucinous tumours occurred in the 36 cases that showed linkage to the BRCA1 locus (Narod et al., 1994).

It has been suggested that papillary serous carcinoma of the peritoneum may be part of the BRCA1 and BRCA2 spectrum. Peritoneal cancer is a malignancy that diffusely involves peritoneal surfaces, sparing or only superficially involving the ovaries. Peritoneal cancer is histologically indistinguishable from serous epithelial ovarian cancer, and it may develop years after oophorectomy. Therefore, patients with BRCA1 or BRCA2 mutations may be at an increased risk for peritoneal cancer, although this has not been fully studied. Bandera et al. (1998) screened 17 consecutive cases of primary peritoneal

carcinoma for *BRCA1* mutations. They identified 2 Ashkenazi Jewish patients with the *BRCA1* 185delAG mutation and a non-Jewish patient with a novel mutation in exon 11 of the *BRCA1* gene. Among the three patients who tested positive for a *BRCA1* mutation, one of the Ashkenazi Jewish individuals had a significant family history of breast and ovarian cancer. The other Ashkenazi Jewish mutation carrier had a personal history of breast cancer. Bandera et al. (1998) concluded that germline *BRCA1* mutations occur in papillary serous carcinoma of the peritoneum with a frequency comparable to the *BRCA1* mutation rate in ovarian cancer.

Several studies have investigated the outcome for patients with familial ovarian cancer, but the results of these studies have been conflicting. Buller et al. (1993) found a 67% 5-year survival in 11 women from ovarian cancer families, compared to 17% in 34 age-matched controls with no family history of ovarian cancer. The disease stage in the two groups was similar. However, a slightly larger study found the survival of 28 cases of familial ovarian cancer to be similar to that of 84 control cases matched for age and stage (Chang *et al.*, 1995).

There have also been several published studies that investigated the influence of *BRCA1* mutations on survival in patients with ovarian cancer. Rubin et al. (1996) found a median survival of 77 months in 43 *BRCA1* mutation carriers with advanced ovarian cancer, compared to 29 months for age- and stage-matched controls, a difference that was highly statistically significant. This study was subsequently criticised because of several possible biases. In particular, the possibility that a family history in mutation carriers may have led to surveillance bias has been suggested, and likely differences in treatment between the two groups have been highlighted (Whitmore, 1997).

Johannsson et al. (1998) described survival rates among persons with breast and ovarian cancer in 21 families with germ-line *BRCA1* mutations from southern Sweden and compared their survival characteristics with all breast and ovarian cancer patients who were diagnosed in Sweden during the years 1958 to 1995. In addition, these researchers identified a subgroup that was age- and stage-matched. Their results led them to conclude that survival in *BRCA1* mutation carriers is similar to, or worse than, that for breast and ovarian cancer patients in general. There were several criticisms of this study, which arose from the fact that a small number of *BRCA1* cases were examined and that the difference in the age of onset of disease between *BRCA1* and sporadic cases was not considered in the analysis. An uncontrolled Canadian study found the median survival in 44 *BRCA1*-associated ovarian cancers to be 31 months, which is similar to that reported by Johannsson et al. (1998) for both mutation carriers and controls and to the controls in the study by Rubin et al. (1996) (Brunet *et al.*, 1997).

The only data on survival in ovarian cancer patients with mutations in *BRCA2* comes from two recent studies. Pharoah et al. (1999) estimated the overall survival in 151 patients from 57 *BRCA1* and *BRCA2* mutation positive families and compared it with that in 119 patients from 62 families in which a *BRCA1/2* mutation was not identified, as well as with that of an age-matched set of population control cases. Pharoah et al. (1999) compared the clinical outcome as well as the tumour histopathology, grade and stage. Their results indicated that survival in familial ovarian cancer cases as a whole was significantly worse than for population controls ($P = 0.005$). These researchers reported a greater frequency of mucinous tumours in the population cases (2 versus 12%, $P < 0.001$). They also found a greater frequency of advanced disease (stage III/IV) among the

familial cases (83% versus 56%; $P = 0.001$). Their results indicated that prognosis and survival among the familial cases was worse than the sporadic cases regardless of whether a BRCA1/2 mutation was identified (Pharoah et al., 1999).

Boyd et al. (2000) conducted a retrospective cohort study of a consecutive series of 189 Jewish ovarian cancer patients diagnosed and treated at a single institution over a 12-year period. The strength of this study compared to the previous ones is that it used a method of ascertainment that avoided two common biases. First, by retrospective selection of a series of consecutive cases of ovarian cancer and using tumour blocks for BRCA1 and BRCA2 mutation testing, this group avoided the bias of selecting cases with advantageous survival. Second, by selecting cases who were all diagnosed and treated at the same institution, they avoided the bias of differences in outcome related to treatment. Boyd et al. (2000) reported a longer survival for advanced-stage cases with BRCA1 or BRCA2 mutations compared to the nonhereditary group ($P=0.004$). Boyd et al. (2000) did not find a difference in histology, stage, or grade between mutation carriers and sporadic cases. They reported a longer disease-free interval following primary chemotherapy among the BRCA1 and BRCA2 mutation carriers (14 months) in comparison with the sporadic cases (7 months) ($P<0.001$) (Boyd et al. 2000).

Current Study

In North America, the incidence of ovarian cancer is higher among Ashkenazi Jewish women than among Sephardic Jews or than non-Jewish women. The rate of ovarian cancer among Israeli Jews born in Europe or North America is among the highest reported, and greatly exceeds the rate for Israeli non-Jews (Parkin *et al.* 1997). In 1993, the rate of ovarian cancer per 100,000 was 13.1 for Israeli born Jews and 17 for European or American born Jews. This rate for Asian and African born Jewish women was 6.9 and 9.2, respectively (Bar-Sade *et al.*, 1998).

Three common mutations are reported in the Ashkenazi Jewish population; it is important to measure directly the proportion of ovarian cancers in Jewish women that are attributable to these mutations for purposes of genetic counselling, screening and prevention. We ascertained a total of 249 Ashkenazi Jewish women with ovarian cancer, unselected for age or family history, from 14 hospitals in North America and Israel, and have evaluated 241 of these women for the presence of a founding mutation in *BRCA1* or *BRCA2*. I also collected family histories, risk factor data and pathology reports on these patients.

The purpose of this study was several fold:

1) To estimate the frequency of the three founder *BRCA1* (185delAG, 5382insC) and *BRCA2* (6174delT) mutations in this group of women.

2) To calculate the relative risk of breast, ovarian and other cancers in the first-degree relatives of the probands.

3) To estimate the penetrance of the three *BRCA1* and *BRCA2* mutations for breast and ovarian cancers

4) To assess the importance of reproductive and other risk factors on the development of ovarian cancer in this group of women.

5) To compare the histopathologic features of the ovarian tumour in patients with and without the three *BRCA1* and *BRCA2* mutations.

CHAPTER 2

MATERIAL AND METHODS

Data Collection

<u>Cases</u>

In association with Dr. Narod and his collaborators, I conducted a hospital-based study of Jewish women with epithelial ovarian cancer identified at 14 centres in North America and Israel. A total of 516 potential study subjects were identified through the Departments of Gynaecology and Oncology of the collaborating hospitals. In some hospitals, religious affiliation was recorded on the medical record. In other hospitals, I reviewed the patient lists for patients who were known to be of Jewish ancestry by the treating physician or who were likely to be Jewish based on surname. I asked the treating physicians to approach the patient by letter, requesting her permission for me or a local member of the study team to contact her. The letter described the general study goals and offered the patient an opportunity to participate (Appendix 1).

Of the 516 patients identified, 80 subjects were deceased, and it was not possible to locate 98 women. Therefore, 338 women were approached to be recruited into the study. These patients were contacted by a member of the study team. Forty-nine of these women refused to participate: 16 were too ill, eight were concerned about insurance implications of genetic testing, three did not speak English, two were part of other genetic studies, and two were concerned that participating would be stressful. For eighteen patients, the reason for refusal was not specified. Therefore, 289 women agreed to complete the family history questionnaire. I interviewed the majority (146) of these patients in person or by telephone for their family history and risk factor profile. Other patients were

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interviewed by the collaborating doctors or genetic counsellors at the participating centres. Patients confirmed that they were Jewish by birth (i.e. they were not adopted and had not converted). Five of the 289 patients identified themselves as Sephardic and 284 as Ashkenazi Jewish.

The study protocol also included the collection of a blood sample for genetic testing. Patients who wished to participate in the genetic testing protocol were given pre-test counselling by me (in the majority of cases) or by a genetic counsellor affiliated with the host institution. Patients were offered the option of receiving the results of the genetic testing in each center. They were asked, by a question as part of the consent form they signed for the study, whether they would like to receive the results of their DNA analysis (Appendix 2).

I was able to obtain a copy of the report on the ovarian tumour pathology for 85% of these patients. I reviewed all pathology reports for tumour stage (FIGO classification), grade and histologic subtype. Review of the pathology reports led to the exclusion of some cases originally ascertained as ovarian cancer from the analyses. Thirty-five cases were excluded based on the fact that reports indicated a diagnosis other than invasive epithelial ovarian cancer. Table 3 lists these diagnoses and the number of cases in each category. Overall 289 cases were ascertained from 14 centres. Of the 284 Ashkenazi Jewish cases, 14 were found to have had borderline tumours of the ovary, 6 had adenofibromas and 2 had mesoblastomas. In addition, 3 cases were found to have been primary fallopian tube tumours, 7 were primary peritoneal tumours and 3 were sex cord-stromal ovarian tumours. These 35 cases were excluded from all analyses (Table 3).

Interestingly, all but one of these 35 cases was found to be negative for the three founder mutations in the *BRCA1* and *BRCA2* genes tested. One primary peritoneal cancer patient was found to carry a *BRCA1* 185delAG mutation. This patient had a family history of breast cancer (Appendix 3).

Excluding these cases reduced the total number of patients in the study to 249 Ashkenazi Jewish patients with epithelial ovarian cancer. Two hundred and forty one of these Ashkenazi Jewish patients and all 5 Sephardic Jewish patients gave a blood sample for genetic testing.

Epidemiological and demographic information was collected by questionnaire, and a detailed family history was taken by interview. The questionnaire also inquired about the patient's ethnic origin and the birthplace of her parents and grandparents (Appendix 4). Three-generation pedigrees were drawn to include all known cases of breast and ovarian cancer. Appendix 5 illustrates an example of a pedigree drawn on participants in this study. The current age, or age of death, of all the first-degree relatives was recorded on the pedigree. The ages at diagnosis and the sites of cancer were recorded for all affected relatives. The diagnosis of cancer in the proband was confirmed by review of pathology reports, but, in general, it was not possible to confirm cancer in the relatives.

Controls

Controls were used in order to evaluate the importance of family history and risk factors for ovarian cancer in Jewish women. Controls were Jewish women with no personal history of breast or ovarian cancer. They were selected from two sources. First, staff members of several of the collaborating hospitals were approached to participate. These controls included 124 employees or volunteers of the Montreal Jewish General

Hospital, the Cedars-Sinai Hospital, the Columbia Presbyterian Medical Center, the Albert Einstein Medical Center, and the Yale University Medical Center. I interviewed all of these 124 controls. Six Israeli controls were selected and interviewed by our collaborator at the Emek Central Hospital. A second group of 200 controls was obtained by sending a mailed invitation to women on the membership lists of a Toronto synagogue and a Jewish women's group. One individual from these lists refused to participate in the study when contacted. These controls were interviewed by a research assistant affiliated with Dr. Narod's group. Controls were aware that they were participating in a study of breast and ovarian cancer, but were unaware that family history and ovarian cancer risk factors were the principal factors under study. The controls provided a detailed family history as well as the answers to the epidemiologic questionnaire but did not supply a blood sample for genetic testing. All controls signed a consent form detailing the procedures involved (Appendix 6).

After the interview was complete, the controls were de-briefed on the study and offered genetic counselling if the family history was positive for breast or ovarian cancer. I gave genetic counselling to all controls, with a family history of breast or ovarian cancer, among the 124 individuals I interviewed. I received training in pre-test counselling of individuals from high-risk breast and ovarian cancer families from Dr. Friedman and Dr. McGillivray as part of my Masters project. Subsequently, I was hired by Dr. Narod and Dr. Rosenblatt as a genetic counsellor in Montreal before initiating my Ph.D. project, where I was trained in pre- and post-test counselling of individuals from high risk cancer families.

Mutation Analysis

Technicians in Dr. Narod's lab in Toronto performed the mutation analysis. High molecular weight DNA was extracted from whole blood. Red blood cells were lysed by absorbing to ammonium ions in RBC lysis buffer. The leukocytes were isolated and stored at -70°C. Leukocytes were then digested by adding SE buffer (NaCl, EDTA), 100µl of 20mg/ml Proteinase K, 4µl of 1U/µl Rnase A and 250µl of 20% SDS to this solution. DNA was then extracted using standard phenylchloroform procedures. Exons 2 and 20 in the *BRCA1* gene and exon 11 in the *BRCA2* gene were amplified using standard PCR amplification protocols. Exon 20 of *BRCA1* was evaluated for 5382insC mutations by SSCP analysis and exon 2 of *BRCA1* was evaluated for 185delAG mutations by heteroduplex analysis.

The protein truncation test (PTT) was used to screen for truncating mutations in exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2*. Truncating mutations in these exons represent approximately 70% of the mutations found to date in families with deleterious mutations in these genes. PTT of exon 11 is also used to identify the abnormal band corresponding to the *BRCA2* 6174delT mutation.

The aberrant bands generated by each of these techniques were sequenced by annealing 5µl exon 2 (185delAG) PCR product, 1µl exon 20 (5382insC) and 7µl exon 11 (6174delT) PCR products to template and primers and sequenced using a standard protocol as outlined in Amersham sequencing kits, US70170 and US79750. All samples were tested for all three founder mutations and each mutant was confirmed by direct sequencing.

Genetic Counselling

All cases were asked, at the time of testing and as part of the consent form, whether they wished to receive the results of *BRCA1* and *BRCA2* mutation analysis (Appendix 4). Of the 241 cases, all but three wished to receive their results. The three who declined to be informed of their results were too ill at the time and were worried that finding out their results might cause them further anxiety. All three, however, said that they may want to find out their results in the future or they may wish for their relatives to receive their results.

I prepared mutation reports indicating the type of mutation and the gene involved for all cases in the study (Appendix 7). I used these reports for post-test counselling of the participants. I also prepared and sent mutation reports to the genetics personnel at the collaborating hospitals. All cases collected from Cedars Sinai hospital received post-test genetic counselling from myself and Dr. Steven Narod. Patients from other centres received genetic counselling from the geneticist at the local centre. All cases positive for a mutation were asked to give another blood sample for confirmation of their positive *BRCA1/BRCA2* mutation results. They were also told of the possibility of getting confirmation by sending a blood sample to a commercial laboratory. Patients with positive mutation results were told of the possibility of testing their at-risk relatives as part of a separate research protocol. Many at-risk relatives contacted me and were tested for all three common mutations in the *BRCA1* and *BRCA2* genes. Appendix 8 contains the consent form that was used for the relatives of ovarian cancer cases.

Patients with a significant family history of breast and ovarian cancer who tested negative for the three mutations were offered further testing on their DNA samples. The additional tests included *BRCA1* and *BRCA2* mutation screening as well as a search for another breast/ovarian cancer locus (*BRCA3*) performed at collaborating laboratories. Those who consented were told they would be informed if they are found to carry a predisposing mutation in *BRCA1* or *BRCA2* or another gene.

Statistical Analysis

I performed all statistical analyses for this thesis. Each case of ovarian cancer was classified as familial or non-familial, based on the presence of at least one case of ovarian cancer (other than the proband) or two cases of early-onset breast cancer (less than age 50 at diagnosis) in the first and second-degree relatives of the proband. The positive and negative predictive values of a family history for carrying a *BRCA1* or *BRCA2* mutation were calculated using standard formulas (Appendix 10). The proportion of individuals with and without mutations was calculated by age-of-onset and by histologic type, grade, and stage.

I compared the cases with *BRCA1* mutations to those with *BRCA2* mutations and those with mutations to those without any of the three mutations for age of onset of ovarian cancer, using the T-test statistic. I also used T-test to compare the carrier and the non-carrier cases with the controls with regards to height.

I compared the cancer risks among the relatives of the ovarian cancer cases and the relatives of the healthy Jewish controls. To do this, the cumulative incidence of breast and ovarian cancer was calculated for all first-degree relatives of the cases and controls. Each relative was considered to be at risk for cancer from birth until he or she developed

cancer, the time of interview, the time of prophylactic mastectomy or oophorectomy, or until death. The cumulative cancer risks were calculated by the Kaplan-Meier survival method and the significance was assessed with the log-rank test. The relative risk (RR) for cancer was then estimated by comparing the incidence rates for the relatives of the ovarian cancer patients with the relatives of controls, using a Cox proportional hazards model. Risks were calculated for the entire patient population, and then separately for the subgroups of cases with *BRCA1* or *BRCA2* mutations. Because I recorded cancer histories on all first-degree relatives, I was able to estimate the penetrance of breast and ovarian cancer for each of the three mutations, using the kin-cohort method described by Struewing et al. (1997). This method is based on the assumption that one-half of the first-degree relatives of the mutation carriers are also expected to be carriers, and that relatives are also at risk of carrying a different mutation, consistent with the population estimates of frequency.

The importance of reproductive and hormonal risk factors on ovarian cancer risk was assessed by two methods. First a matched case-control approach was used for assessing the importance of all dichotomous cofactors. Each of the mutation-positive cases of ovarian cancer was country- and age-matched with a case of mutation-negative ovarian cancer and with a healthy control. The cases and controls were age-matched within two years of the birth date of the case. The cases and controls were divided and matched, according to country of residence, to Canada, the United States, and Israel. The variables tested in this analysis were pregnancy (history of having ever been pregnant versus no pregnancy), parity (less than three pregnancies versus three or more pregnancies), breast feeding (yes versus no), oral contraceptive use of greater than 1 year

(yes versus no), tubal ligation (yes versus no), and use of talcum powder in the vaginal area (yes versus no). Odds ratios (OR) and 95% confidence intervals (CI) were estimated from the ratio of concordant to discordant pairs and significance assessed with the McNemar's test. In these analyses, exposures in the cases and matched controls were considered only prior to the date of diagnosis in the case.

The second method used for risk factor analysis was logistic regression. Some of the variables used in the matched analysis as well as several continuous variables and those showing an association with breast or ovarian cancer risk in the general population were evaluated using this method. The dependent variable in this analysis was the diagnosis of ovarian cancer. The variables tested were age at menarche, parity, height, oral contraceptive use of greater than one year, pack-years of smoking and vaginal talc use. Age at the time of interview was also included as a covariate in these analyses. Risk factors in all carriers and non-carriers were compared to the controls, and odds ratios and P-values were estimated using both univariate and multivariate logistic models. For multivariate analysis, the logistic model was tested by both the forward conditional and backward conditional methods, in order to ensure consistency of the results. I calculated the power for the risk factor analyses using UCLA Statistics, a web-based power calculator (http://home.stat.ucla.edu/calculators/powercalc).

I reviewed the pathology and surgical reports on all the Ashkenazi Jewish women. Based on ovarian tumour histology, I classified the cases into several categories of epithelial ovarian tumours. Cases with and without any of the three *BRCA1/BRCA2* mutations were compared with regards to ovarian tumour histology using a chi-square statistic.

I recorded the degree of differentiation of the ovarian tumour as reported on the pathology reports. I classified the cases into three groups based on grade of the tumour (Grade I-III). Cases with and without the three *BRCA1/BRCA2* mutations were compared with regards to the ovarian tumour grade using the Kruskal-Wallis test.

Next, I recorded the stage of ovarian tumour as reported on the surgical notes of the patients. I classified the cases into four major groups based on stage (Stages I-IV). Cases with and without the three mutations were compared with regards to the tumour stage using the Kruskal-Wallis test.

CHAPTER 3

RESULTS

Mutation Analysis

Table 4 summarizes the number of cases and controls collected from each centre. These cases represent the majority of living cases of ovarian cancer in Jewish women under active follow-up at the Departments of Gynecology and Oncology of the 14 participating centres. Overall, 254 cases and 330 controls were collected. Five of the cases were Sephardic and 249 were Ashkenazi Jewish. None of the controls were Sephardic. I interviewed 146 cases and 124 controls from 6 centres. The rest were interviewed by the research personnel of the collaborating hospitals.

Our study attempted to recruit all prevalent cases, and the median time since diagnosis was 2.2 years (range 0 to 25.6 years). The average age of diagnosis of ovarian cancer was 56.8 years (range 19 to 88 years), and the average age of interview of the cases was 60.1 years (range 22 to 89 years). The average age of the controls at the time of interview was 52 years (range 25-87).

Patients with at least one relative diagnosed with ovarian cancer or with two relatives with early-onset (age<50) breast cancer were classified as familial. In total, 52 of the 249 Ashkenazi Jewish cases (20.9%) for whom complete family histories on second-degree relatives were available satisfied this definition of familial cancer. Out of the five Sephardic cases, two could be classified as familial. Eleven of the 330 controls (3.3%) had a family history consistent with hereditary ovarian cancer as defined above (Table 5).

Two hundred and forty one of the 249 Ashkenazi Jewish cases were tested for the three common mutations in the *BRCA1* and *BRCA2* genes. A total of 93 founder mutations were found among the 241 patients of the Ashkenazi Jewish ancestry (38.6%) (Table 6). These included 63 *BRCA1* mutation carriers (48 with the 185delAG and 15 with the 5382insC) and 30 *BRCA2* 6174delT carriers. One of the five Sephardic cases was a carrier of the 185delAG mutation.

Complete pedigrees were available on all the 241 Ashkenazi Jewish cases analyzed for mutations. 37 of the 93 mutation carriers (39.8%) were classified as familial. 18 of the 48 *BRCA1* 185delAG carriers, 9 of the 15 *BRCA1* 5382insC carriers and 10 of the 30 *BRCA2* 6174delT carriers could be classified as familial (Table 7). 14 of the 148 non-carriers (9.5%) were familial. The one 185delAG carrier among the five Sephardic Jews had no significant family history of breast or ovarian cancer.

As expected, mutations were more common among the familial cases than among the non-familial cases (Table 7). 37 of the 51 cases (72%) with hereditary ovarian cancer had one of the three *BRCA1/BRCA2* mutations. 27 of these 37 carriers had a *BRCA1* mutation, and 10 had the *BRCA2* 6174delT mutation. 14 of the 51 cases with hereditary ovarian cancer (27%) had none of the three mutations tested. 56 of the 190 cases (29.5%) without a significant family history of ovarian cancer had one of the three mutations. 36 of these 56 carriers had a *BRCA1* and 20 had the *BRCA2* mutation (Table 7). The positive and negative predictive values of the family history of ovarian cancer were 72.5% and 29.5% respectively (Appendix 10).

Some of these non-familial cases had a family history of breast or ovarian cancer but did not fit our criteria for hereditary ovarian cancer. Table 8 lists the frequency of

mutations in the cases by family history. 143 of the 241 cases analyzed had no family history of breast or ovarian cancer (Table 8). 33 of these 143 cases (23.1%) had one of the three *BRCA1/BRCA2* mutations. 57 of the cases had first-degree relatives with breast cancer diagnosed below 50 years but no relatives with ovarian cancer. 31 of these 57 cases (54%) had one of the three mutations. 26 of the 241 cases tested had one relative with ovarian cancer at any age but no relatives with breast cancer. 18 of these 26 cases (69%) had one of the three mutations. 15 of the 241 cases tested had a family history of early-onset breast cancer in one or more relatives as well as a family history of ovarian cancer in other relatives. 11 of these 15 cases (73%) had one of the three mutations (Table 8). In all categories, *BRCA1* mutations were more frequent than the *BRCA2* mutation. About 70% of individuals with site-specific ovarian cancer were positive for one of the three *BRCA1* and *BRCA2* mutations tested.

Table 9 summarizes the average age of onset of ovarian cancer among the different mutation carrier cases. Women with *BRCA1* mutations were diagnosed with ovarian cancer at a younger age, on average, than cases for whom no mutation was detected (51.5 years and 58.3 years respectively, $P=0.0001$). In contrast, women with *BRCA2* mutations were older at diagnosis (62.9) than cases with *BRCA1* mutations ($P=0.0001$) and cases for whom no mutation was detected ($P=0.27$). There was no significant difference in the age of diagnosis of ovarian cancer between cases with the 185delAG mutation (51.6) and those with the 5382insC mutation (51.1).

Table 10 summarizes the frequency of the three *BRCA1* and *BRCA2* mutations by age of diagnosis of the cases. 51 of the 129 women diagnosed between the ages 30 and 60 (39.5%) had a *BRCA1* mutation versus 11 out of the 108 women diagnosed above the age

60 (10.2%) (*P*<0.0001 for difference). *BRCA2* mutations were more numerous than *BRCA1* mutations in women diagnosed with ovarian cancer after age 60 (19 cases versus 11 cases, respectively). A *BRCA2* mutation was found in 9.3% of women diagnosed between 30 and 60 and in 17.6% of women diagnosed after age 60 (*P* =0.07) (Table 10).

Relative Risk Estimates among First Degree Relatives

Table 11 summarizes the data on the probability of cancer among the first-degree relatives of Ashkenazi Jewish cases and controls. These analyses were based on the observations in 1158 first-degree relatives (580 females and 578 males) of ovarian cancer cases and 1596 first-degree relatives (791 females and 805 males) of the controls. Overall, the first-degree relatives of the cases had a significantly higher cumulative risk of any cancer to age 75 compared to the relatives of the controls (Table 11, Figure 1). This analysis was based on the observation of 211 cases of cancer among the first-degree relatives of ovarian cancer cases versus 165 cases of cancer among the first-degree relatives of the controls.

Female first-degree relatives of the cases had a significantly higher risk of any cancer to age 75 compared to the relatives of the controls (Table 11). There were 138 cases of any cancer to age 75 among the first-degree female relatives of the cases versus 99 cases among the first-degree relatives of the controls. The probability of any cancer except for breast and ovarian cancer among the first-degree relatives of the cases was not significantly different from the estimates of this risk for the relatives of the controls (Table 11, Figure 2). This analysis was based on the occurrence of 54 cases of any cancer

but breast and ovarian in the female first-degree relatives of the cases versus 49 cases in the first-degree relatives of the controls.

The cumulative incidence of any cancer among the male first-degree relatives of the cases was slightly higher but not significantly different from the estimates of the risk among the relatives of the controls (Table 11, Figure 3). There were 72 cases of cancer to age 75 among the male first-degree relatives of cases and 62 cases among the first-degree relatives of the controls.

Among the first-degree female relatives of the Ashkenazi Jewish cases, the cumulative incidence of breast cancer was significantly higher than the risk in the first-degree relatives of the controls (Table 11, Figure 4). This was based on the occurrence of 68 and 41 cases of breast cancer among the female first-degree relatives of the cases and controls, respectively. The cumulative incidence of ovarian cancer was also significantly higher in the first-degree relatives of the cases compared to the relatives of the controls (Table 11, Figure 5). This comparison involved 20 cases of ovarian cancer among the female first-degree relatives of the cases and 12 cases of ovarian cancer among the relatives of the controls.

Among all the other cancer types, which occurred in the relatives of the cases and controls, prostate and pancreatic cancers as well as cancers of an unknown primary were significantly more frequent among the relatives of the cases compared to the controls. Prostate cancer occurred in 17 male first-degree relatives of cases and 3 first-degree relatives of the controls. There were 11 cases of pancreatic cancer among the first-degree relatives of the cases and 10 cases of pancreatic cancer among the first-degree relatives of the controls. There were 6 cases of cancer of an unknown primary among the first-degree

relatives of both cases and controls. The cumulative incidences of uterine, colon, lung, and head and neck cancers as well as melanomas among the first-degree relatives of the cases were not significantly different from those of the relatives of the controls (Table 11).

Table 12 summarizes the data on the probability of cancer among the first-degree relatives of mutation carrier cases compared to the relatives of the non-carrier cases. These analyses were based on the observations in 416 first-degree relatives (219 female and 197 male) of mutation carrier cases and 742 first-degree relatives (361 female and 381 male) of the non-carrier cases, by age 75. Overall, the first-degree relatives of the carrier cases had a significantly higher cumulative risk of any cancer to age 75 compared to the relatives of the non-carrier cases (Table 12, Figure 6). This analysis was based on the occurrence of cancer in 93 first-degree relatives of the carrier cases and 118 first-degree relatives of the non-carrier cases.

The probability of any cancer in female first-degree relatives of the carriers was significantly higher than the probability in the first-degree relatives of the controls (Table 12). There were 78 cases of cancer in the female first-degree relatives of the carrier cases and 68 cases of cancer in the female first-degree relatives of the non-carrier cases. The probability of any cancer except for breast and ovarian cancer among the first-degree relatives of the carriers was not significantly different from the estimates of this risk for the relatives of the non-carriers (Table 12, Figure 7). This analysis was based on the occurrence of 18 cases of any cancer but breast and ovarian in the female first-degree relatives of the carrier cases and 36 cases among the female first-degree relatives of the non-carrier cases.

The cumulative incidence of any cancer among the male first-degree relatives of the carrier cases was not significantly different from the estimates of the incidence among the relatives of the non-carrier cases (Table 12, Figure 8). There were 25 cases of cancer among the male first-degree relatives of the carrier cases and 47 cases among the relatives of the non-carrier cases.

Among the first-degree female relatives of the mutation carriers, the cumulative incidence of breast cancer to age 75 was significantly higher than the cumulative incidence of breast cancer among the first-degree female relatives of the non-carriers (Table 12, Figure 9). There were 36 cases of breast cancer among the female first-degree relatives of the carrier cases and 32 cases among the relatives of the non-carriers. The cumulative incidence of ovarian cancer was also significantly higher for the first-degree relatives of the carriers compared to the non-carriers (Table 12, Figure 10). There were 13 cases of ovarian cancer among the female first-degree relatives of the carrier cases and 7 cases among the relatives of the non-carrier cases.

Among all the other cancer types in the relatives of the cases, uterine cancer occurred more frequently among the relatives of the mutation carriers (3.9% to age 65) compared to the relatives of the non-carriers (0.0% to age 65) (P=0.011) (Table 12). This analysis was based on the occurrence of one case of uterine cancer to age 65 among the first-degree relatives of the carrier cases versus none in the relatives of the non-carrier cases. There was no significant difference between the mutation carriers and the non-carriers with respect to the risk of any other cancers listed in table 12.

Table 13 summarizes the data on the probability of cancer among the first-degree relatives of *BRCA1* carriers with ovarian cancer compared to the risk in the relatives of

the *BRCA2* carriers with ovarian cancer. These analyses are based on the observations among 274 first-degree relatives (144 female and 130 male) of *BRCA1* mutation carrier cases and 142 first-degree relatives (75 female and 67 male) of *BRCA2* mutation carrier cases, by age 75.

The risk of any cancer in the relatives of *BRCA1* carriers was not significantly different compared to the risk of cancer among the first-degree relatives of *BRCA2* carriers (Table 13, Figure 11). This analysis was based on the occurrence of 55 cancer cases among the relatives of *BRCA1* mutation carriers and 38 cancer cases among the relatives of the *BRCA2* mutation carriers. The probability of any cancer to age 55 in female first-degree relatives of the *BRCA1* carriers was significantly higher than the risk among the female first-degree relatives of the *BRCA2* carriers (Table 13). There were 26 cases of cancer among the 104 female first-degree relatives of the *BRCA1* mutation carriers by age 55 versus 7 cases of cancer among the 45 female first-degree relatives of the *BRCA2* mutation carriers by age 55. The probability of any cancer except for breast and ovarian cancer among the first-degree relatives of the *BRCA1* carriers was not significantly different from the estimates of this risk for the relatives of the *BRCA2* carriers (Table 13, Figure 12). There were 10 cases of any cancer but breast and ovarian to age 75 in the female first-degree relatives of the *BRCA1* mutation carriers and 8 such cases among the relatives of the *BRCA2* mutation carriers.

The cumulative incidence of any cancer to age 65 among the male first-degree relatives of the *BRCA2* carriers was significantly higher than the risk in the relatives of the *BRCA1* carriers (Table 13, Figure 13). There were four cases of cancer by age 65

among the 102 male first-degree relatives of *BRCA1* carriers versus 8 cases among the 54 relatives of *BRCA2* carriers.

Among the first-degree female relatives of the *BRCA1* carriers, the cumulative incidence of breast cancer was estimated at 26.3% by age 65. This estimate was significantly higher than the cumulative incidence of breast cancer among the first-degree female relatives of the *BRCA2* carriers, which was 18.8% to age 65 (P=0.0256) (Table 13, Figure 14). Breast cancer by age 65 occurred in 24 first-degree relatives (n=126) of *BRCA1* carriers and in 9 first-degree relatives (n=66) of *BRCA2* carriers. The cumulative incidence of ovarian cancer was estimated at 11.9%, and 8.7% to age 65 for the first-degree relatives of the *BRCA1* and *BRCA2* carriers, respectively. This difference was not statistically significant. (Table 13, Figure 15). This analysis was based on the occurrence of 7 cases of ovarian cancer by age 75 among the first-degree relatives of the *BRCA1* carriers and 6 cases among the relatives of *BRCA2* carriers. There was no significant difference in the risk of any other cancer among the first-degree relatives of the *BRCA1* and *BRCA2* carriers (Table 13).

The cumulative incidence of breast and ovarian cancer was compared for the mothers and sisters of the ovarian cancer patients (Table 14). Among the subgroup of mutation-positive cases, the risk of breast cancer to age 65 in the sisters (31%) moderately exceeded that in the mothers (20%), but the difference was not significant (P = 0.29) (Table 14, Figure 16). There were 15 breast cancer cases among 61 sisters of the mutation carriers versus 16 breast cancer cases among 36 mothers of the mutation carriers. The risk of ovarian cancer in the carriers' sisters (14%) was also higher than that of the carriers' mothers (9%), but the difference was not statistically significant (P=0.40)

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(Table 14, Figure 17). This comparison was based on the occurrence of 6 cases of ovarian cancer in the sisters and 7 cases of ovarian cancer in the mothers of the mutation carriers.

The probability of breast and ovarian cancers by age 65 in the mothers of *BRCA1* carriers were 22% and 6% respectively. These estimates were not significantly different from the estimates of breast and ovarian risks in the sisters of *BRCA1* carriers (Table 14, Figures 18 and 19). There were 11 cases of breast cancer and four cases of ovarian cancer among the sisters (n=42) of the *BRCA1* carriers versus 12 cases of breast cancer and three cases of ovarian cancer among the mothers (n=24) of the *BRCA1* carriers.

The probability of breast cancer by age 65 in the mothers of *BRCA2* carriers was estimated at 17%. This estimate was not significantly different from the estimate of breast cancer risk in the sisters of *BRCA2* carriers (Table 14, Figure 20). Breast cancer was observed in four of the sisters (n=19) and four of the mothers (n=12) of the *BRCA2* mutation carriers. The probability of ovarian cancer in the mothers of *BRCA2* carriers was estimated at 15%. This estimate was elevated compared to the risk of ovarian cancer in the sisters of *BRCA2* carriers (10%) but the difference was not statistically significant (P=0.0784) (Table 14, Figure 21). Ovarian cancer occurred in two of the sisters and four of the mothers of the *BRCA2* mutation carriers.

The cumulative incidence data on breast and ovarian cancer in the first-degree relatives of the *BRCA1* and *BRCA2* carriers were used to estimate the penetrance of breast and ovarian cancer for the mutations in these genes (see methods). The cumulative risk of breast cancer among the first-degree relatives of *BRCA1* and *BRCA2* carriers was 18.4% and 6.0% to age 55, respectively (Table 13). The risk of breast cancer among the first-degree relatives of the non-carriers in this study was 6.9% (Table 12). Appendix 9

outlines how these values were used to calculate the penetrance of breast cancer for the *BRCA1* and *BRCA2* mutations based on a method by Struewing et al. (1997).

Table 15 summarizes the penetrance values obtained using this method. The penetrance of breast cancer to ages 55 and 75 for the *BRCA1* mutations was estimated at 29.9% and 42.1% respectively. The penetrance of breast cancer to age 55 and 75 for the *BRCA2* mutation was estimated at 5.1% and 34.2% respectively. The difference in the breast cancer penetrance to age 55 of *BRCA1* and *BRCA2* mutations is striking (29.9% and 5.1%, respectively) (Table 15). The relatives of the non-carriers had 6.9% and 16.0% risk of developing breast cancer by ages 55 and 75 respectively (Table 15).

Table 16 summarizes the penetrance values of ovarian cancer for the *BRCA1* and *BRCA2* mutations. The penetrance of ovarian cancer for the *BRCA1* mutations was estimated at 12.6% and 13.8% to ages 55 and 75 respectively. This penetrance for the *BRCA2* mutation was estimated at 13.4% and 20.2% to ages 55 and 75 respectively. The number of ovarian cancers observed among the relatives of the mutation carriers was small, and the difference was not significant. Risk of ovarian cancer to age 55 and 75 for the relatives of the non-carriers was 1.6% and 3.6% respectively (Table 16).

Risk Factor Analysis

I used two approaches to analyse the risk factor data in ovarian cancer cases with and without mutations compared to the appropriate controls. The first approach was by a matched pair analysis using the McNemar's test (see methods). I compared the mutation positive cases and the mutation negative cases with the controls for a range of factors relating to reproductive history and contraceptive history (Table 17). Odds ratios (OR)

were estimated from the ratio of concordant to discordant pairs, and confidence intervals (CI) and significance were assessed with McNemar's test.

The first variable examined was parity versus nulliparity. Carriers and non-carriers were compared to the controls with regards to having ever been pregnant. I found a suitable control match for 86 carrier and non-carrier cases for this analysis. A history of pregnancy was associated with an Odds Ratio (OR) of 0.71 (95%CI: 0.20-2.50) in carriers and 0.82 in non-carriers (95%CI: 0.31-2.12). There were no significant differences between the carrier cases and controls or between the non-carrier cases and controls (Table 17).

Parity was dichotomized in to fewer than three pregnancies and more than three pregnancies, and carriers and non-carriers were compared to controls. I found a suitable control match for 72 carrier and non-carrier cases for this analysis. A history of fewer than three pregnancies was associated with OR of 1.90 in carriers (95%CI: 0.84-4.38) and 1.07 in non-carriers (95%CI: 0.49-2.35). The differences between carrier cases and controls and between non-carrier cases and controls were not significant with regards to parity (Table 17).

A history of having ever breast fed versus no breast-feeding was compared in cases versus the controls. I found a suitable control match for 77 carrier and non-carrier cases. History of breast-feeding was associated with an OR of 1.36 in carriers (95%CI: 0.65-2.85) and 1.50 in non-carriers (95%CI: 0.73-3.11). This comparison was not significant between carrier cases and controls or between non-carrier cases and controls (Table 17).

A history of oral contraceptive use was compared in cases versus the controls. For this analysis, I found a suitable control match for 69 carrier and non-carrier cases. Among

carrier cases, use of oral contraceptives (at least one year) was associated with OR of 0.44 (95%CI: 0.18-1.08). Oral contraceptive use was associated with OR of 1.0 for non-carrier cases in our sample (95%CI: 0.46-2.16). The comparison was not significant for either the carrier cases versus the controls or the non-carrier cases versus the controls (Table 17).

Next I compared cases and controls with regards to history of tubal ligation. I found a suitable control match for 82 carrier and non-carriers cases. Among carrier cases, tubal ligation was associated with a non-significant increased risk for ovarian cancer (OR=1.38, 95%CI: 0.51-3.73) compared to the controls. Among non-carrier cases the odds ratio for tubal ligation was 0.78 (95%CI: 0.26-2.27), but the decrease in risk was not significant (Table 17).

I compared the cases and controls with regards to history of talcum powder use in the vaginal area. Vaginal talc use was reported by 30% of the cases and 21% of the controls. I found a suitable control match for 80 carrier and non-carrier cases. Vaginal talc use was associated with an increase in risk of ovarian cancer for both carriers (OR=1.90, 95%CI: 0.84-4.38) and non-carriers (OR=2.40, 95%CI: 1.10-5.37). The comparison was significant for the non-carriers versus the controls (P=0.026) (Table 17).

The second approach to assessing risk factors among cases and controls was through logistic regression. The carrier and non-carrier cases were compared to the appropriate controls for current age, age at menarche, parity, height, oral contraceptive use of greater than 1 year, vaginal talc use and pack-years of smoking. Initially country of residence (Canada versus United States versus Israel) was also used as a covariate in all analyses. However, no association was found between country of residence and ovarian

cancer risk in any of the analyses. Removing this factor did not affect the results of the analyses. In these analyses 89 carrier cases and 136 non-carrier cases for whom information on these variables was available were compared to 303 controls.

All variables were compared in the cases with one of the three *BRCA1* or *BRCA2* mutations and the controls using univariate and multivariate logistic models. Table 18 lists the odds ratios (OR), 95% confidence intervals (CI), and *P*-values for these analyses. Age was found to be a risk factor for ovarian cancer (OR=1.04 per year, 95%CI: 1.02-1.06, *P*<0.0001) using both univariate and multivariate logistic models (Table 18). Vaginal talc use was also found to be a risk factor (OR=2.34, 95%CI: 1.30-4.20, *P*=0.0054 with multivariate analysis). Age at menarche, parity, height and pack-years of smoking did not show an association with ovarian cancer risk in these cases. No history of oral contraceptive use or use of less than one year was associated with OR of 1.14 among the carrier cases (OR=1.14, 95%CI: 0.92-1.41 with univariate analysis); however, the difference was not significant (Table 18).

Table 19 lists the odds ratios (OR), 95% confidence intervals (CI), and *P*-values for risk factors in ovarian cancer cases who did not carry one of the three *BRCA1* or *BRCA2* mutations and the controls. Age was a risk factor for ovarian cancer using both univariate and multivariate analysis (OR=1.06 per year, 95%CI: 1.04-1.08, *P*<0.0001 with multivariate model) in these cases (Table 19). Height was also a risk factor for ovarian cancer in these analyses (OR=1.06 per cm, 95%CI: 1.02-1.09, *P*=0.0006 with multivariate model) (Table 19). Non-carrier cases were 1.7 cm taller on average (163.3 cm) than controls (161.6 cm; *P*=0.02). There was no significant difference in height between carrier cases (162.0 cm) and the controls (*P*=0.70). There was no significant

difference in height between the *BRCA1* carriers (163.1 cm) and the *BRCA2* carriers (159.6 cm; *P*=0.09).

Vaginal talc use also showed a positive association with ovarian cancer among the non-carrier cases (OR=2.26, 95%CI: 1.30-3.94, *P*=0.0042). Age at menarche, parity, and pack-years of smoking did not show an association with ovarian cancer risk in these analyses. No history of oral contraceptive use or use of less than one year was associated with an OR of 1.40 in the non-carrier cases (95%CI: 0.94-2.08 with univariate analysis). This comparison was not significant (*P*=0.0999) (Table 19).

The range of power for the risk factor analyses for the mutation carrier cases versus the controls was between 53.5% to 71.0% and for the non-carrier cases versus the controls was between 52.8% and 77.4%.

Ovarian Tumour Histology

I was able to obtain pathology and surgical reports on 203 of the 249 Ashkenazi Jewish women with ovarian cancer. Table 20 summarizes the histopathological subtypes of the ovarian tumours reported on these cases. The majority of the tumours among both carriers and non-carriers were serous adenocarcinomas (112 out of 203). The majority of mutations were found in women with serous tumours (Table 20). Overall, there was a non-significant increased frequency of serous tumours among the carrier cases (62.8%) compared to the non-carrier cases (49.6%, P=0.061) (Table 21).

Table 22 summarizes the data on the degree of differentiation (grade) of the ovarian tumour in carriers and non-carriers. I was able to obtain information on ovarian tumour grading on 171 patients. The mutation carrier cases had a significantly higher frequency of grade III tumours (90.7%) compared to the non-carrier cases (68.7%) (P=0.002) (Table

22). 68 of the 141 (48.2%) of the women with grade III tumours were mutation-positive (Table 22).

I was able to obtain information on ovarian tumour staging on 189 cases. Table 23 summarizes these data obtained from reviewing pathology and surgical reports. There was no significant difference in the stage distribution between the *BRCA1* or *BRCA2* mutation-positive cases and *BRCA1* or *BRCA2* mutation-negative cases (Table 23).

Case Reports

Three unique families were identified among the cases in this study. Family R014 was found to contain an individual who is heterozygous for mutations in both the *BRCA1* and *BRCA2* genes. The family was ascertained through individual 45 who was a participant in our study (Figure 22). I interviewed the proband at a participating centre. The proband had unilateral breast cancer diagnosed at age 30. She had recurrence of cancer in the same breast at age 38, four months post partum. She then developed ovarian cancer at age 39. She passed away at age 41 from ovarian cancer.

The family history (Figure 22) was remarkable for ovarian cancer at age 36 in the mother of the proband (individual 38). There is a family history of early-onset breast cancer in the proband's maternal great grandfather's family. The proband had two maternal great great aunts with early-onset breast cancer and two distant cousins with early-onset breast cancer. There is no family history of breast or ovarian cancer in the proband's maternal grandmother's family.

The proband was tested for the 185delAG, 5382insC, and 6174delT mutations. She tested positive for the 185delAG mutation in the *BRCA1* gene. Genetic testing was

offered to other family members. Three other first-degree relatives, individuals 39, 47, and 46, were tested for the three mutations. The proband's brother and father tested negative for the three mutations. The proband's sister, individual 46, tested positive for both the *BRCA1* 185delAG and the *BRCA2* 6174delT mutations. Individual 46, who was 36 years old, had never been diagnosed with cancer and had not undergone any prophylactic surgery. Paternity was confirmed in individuals 39 and 46 by using five polymorphic markers: D1S249, D2S293, D2S172, D6S434 and D8S537. All participating individuals in this family received genetic counselling, based on genetic testing results, from a genetic counsellor at the participating centre.

The second unique family (R023) was found to include members who are carriers of all three *BRCA1* or *BRCA2* mutations tested. This family was ascertained through two related individuals in separate cancer genetics centres (Figure 23). The first individual (40) was recruited into the study based on the diagnosis of ovarian cancer and her Jewish ancestry. I interviewed this individual at a participating centre. The second individual (39) was an affected family member of a kindred referred to a cancer genetics centre because of a history of breast and ovarian cancer. Her blood sample was sent to Dr. Narod's laboratory in Toronto for genetic testing. The two women were subsequently found to be members of the same extended family (Figure 23).

Individual 40 was diagnosed with ovarian cancer at the age of 48 and breast cancer at the age of 49. Mutation analysis identified her to be a carrier of the *BRCA1* 5382insC mutation. The second proband (individual 39) was diagnosed with primary cancers of the breast, ovary and colon at ages 37, 58 and 64, respectively. Mutation analysis revealed a *BRCA1* 185delAG mutation in this individual. Paternal cousins of this proband

(individuals 31 & 31) also underwent genetic testing. These sisters were found to carry the *BRCA2* 6174delT mutation. One of these carriers (individual 31) was diagnosed with carcinoma of the breast at age 61 and carcinoma of the ovary at age 65. The other individual (32) was diagnosed with breast cancer at age 49. More recently, the niece of individual 39 (individual 45) was tested and also found to carry the *BRCA1* 185delAG mutation. This woman was diagnosed with breast cancer at the age of 34. Dr. Narod and I gave genetic counselling to individual 40 in a subsequent visit to Los Angeles, based on her genetic testing results. The rest of the individuals in this family who were tested for *BRCA1* and *BRCA2* mutations received genetic counselling from other geneticists and genetic counsellors.

The third unique family (R013) included a wife and a husband who were carriers of two different *BRCA1* and *BRCA2* mutations. Family R013 was ascertained through individual 11, who was diagnosed with breast cancer at age 57 and ovarian cancer at age 58 (Figure 24). I interviewed this patient and her husband at a collaborating hospital. Individual 10 had recently been diagnosed with prostate cancer at age 64. I gave him genetic counselling because of a history of early-onset breast and prostate cancer in his siblings. Both the proband and her husband were tested for the three *BRCA1* and *BRCA2* mutations. She was found to carry the *BRCA2* 6174delT mutation. Her husband was found to carry the *BRCA1* 185delAG mutation. This couple has two daughters who are at risk of having inherited one or both mutations from their parents. The parents and daughters received counselling from a genetic counsellor at the participating centre. The daughters were given an opportunity to be tested for the three mutations; they declined testing at this time.

Search for other BRCA1/2 mutations among ovarian cancer cases

A founder mutation was not identified for 148 Ashkenazi Jewish cases out of 241 tested (Table 7). 14 of these 148 cases had a significant family history of breast and ovarian cancer (Table 7). In addition, more than 50 patients had at least one first-degree relative with either breast or ovarian cancer; no mutations were identified in about half of these cases (Table 8). We postulated that there may be other mutations in the *BRCA1* and *BRCA2* genes that occur at a lower frequency in this population. In order to estimate the frequency of these non-founder mutations in Jewish ovarian cancer cases, we performed PTT analysis on the large exons of *BRCA1* and *BRCA2* (which comprise the majority of the coding regions of the two genes) for 122 of these cases. A technician in Dr. Narod's lab in Toronto performed this analysis.

No *BRCA1* mutation was identified in these cases. A single truncating mutation in the *BRCA2* gene (6696delTC) was found in the proband from family R99 (Figure 25). Family R99 was ascertained through individual 18, who was diagnosed with ovarian cancer at age 40. She had previously been diagnosed with breast cancer at age 32. I interviewed this individual at a participating centre. At the time of interview, the patient identified herself as Ashkenazi Jewish. Further investigation of the family revealed that the proband's mother was not Jewish. Although the Jewish paternal aunt of the patient was also affected with early-onset ovarian cancer, the proband's father tested negative for *BRCA1* and *BRCA2* mutations. Therefore, the mutation was inherited from proband's non-Jewish mother. Surprisingly, the sister of the proband was diagnosed with early-onset bilateral cancer but was found not to be a carrier of the *BRCA2* 6696delTC mutation upon testing. The proband's brother, who was diagnosed with basal cell

carcinoma at age 46, also tested positive for the *BRCA2* 6696delTC mutation. This family had been tested independently at a commercial laboratory where the 6696delTC mutation had been found prior to testing at our centre. The commercial laboratory had published a report identifying this mutation as a novel non-founder Jewish mutation (Frank *et al.*, 1998).

CHAPTER 4

DISCUSSION

Mutation Analysis

Our study is the first large survey of mutations in both *BRCA1* and *BRCA2* genes in Jewish women with ovarian cancer, unselected for age or family history, published to date. I recruited and interviewed the majority of the cases (146) and about half of the controls (124). The rest of the controls were approached and interviewed by one other person. This along with our method of recruitment contributed to the consistency and accuracy of the information gathered and avoided the bias of collecting primarily individuals with a family history of cancer.

The participation rate among the cases in our study was 85.5%; 289 out of 338 cases approached agreed to participate in the study (see results). The most common reasons for refusal to participate given by the cases involved their health condition and concerns around confidentiality of the genetic testing results as it applied to implications for insurance purposes for both the patients and their immediate relatives. The only reason for refusal given by a control subject who declined to participate was concerns about confidentiality.

35 cases were excluded from the analyses because review of their medical reports revealed a diagnosis other than primary epithelial ovarian cancer (Table 3). Among these 35 cases, only one individual with primary peritoneal cancer was found to carry a *BRCA1* 185delAG mutation (Appendix 3). This individual had a family history of breast cancer but did not fit our criteria of familial breast-ovarian cancer syndrome (Appendix 3). Although the number of cases in the excluded categories was small, these findings

indicate that borderline and non-epithelial tumours of the ovary as well as fallopian tube tumours may not be part of the spectrum of *BRCA1* and *BRCA2* mutations. These findings are also consistent with suggestions that primary peritoneal cancer may be part of the *BRCA1* spectrum (Bandera *et al.*, 1998).

Bandera et al. (1998) concluded that germline *BRCA1* mutations occur in serous carcinoma of the peritoneum at a frequency comparable to the *BRCA1* mutation rate in the ovarian cancer cases. In our study, the mutation rate was much higher among the ovarian cancer cases (38.6%) than the peritoneal cancer cases (1 in 7). Furthermore, we did not find an increased risk of primary peritoneal cancer among the first-degree relatives of the cases with mutations compared to the non-carrier cases or controls. This may suggest a lower frequency of *BRCA1* mutations among peritoneal cancer cases or a lower penetrance of mutations in this gene for peritoneal cancer. The number of peritoneal cancers in our study was too small to draw conclusions (Table 6).

We identified a very high proportion (20.9%) of hereditary ovarian cancers among unselected cases of epithelial ovarian cancer in Ashkenazi Jewish women from North America and Israel. We found that two out of five Sephardic cases in our study had a family history of breast and ovarian cancer. These fractions are much higher than the reported hereditary proportions of ovarian cancer (5-10%) as well as the hereditary proportions of other common adult cancers for any ethnic group studied to date (2-5%) (Narod *et al.*, 1994, Lynch *et al.*, 1998, Berchuck *et al.*, 1999). A recent study of twins found a higher than previously reported heritability for certain neoplasms including breast cancer but not for the majority of other malignancies including ovarian cancer (Lichtenstein *et al.*, 2000). Lichtenstein et al. (2000) concluded that inherited genetic

factors make a minor contribution to susceptibility to most types of neoplasms including ovarian cancer.

The elevated estimates of hereditary breast and ovarian cancer in our sample may be due to the high frequency and penetrance of the three founder mutations in the Ashkenazi Jewish population studied here. Our estimates may also be too high as a result of ascertainment bias. There have been reports of better survival in individuals with ovarian cancer who have a family history of breast and ovarian cancer compared to individuals with non-hereditary ovarian cancer (Buller et al., 1993). There are also reports of better survival in ovarian cancer cases with BRCA1 and BRCA2 mutations compared to non-carrier ovarian cancer cases (Rubin et al., 1996; Boyd et al, 2000). Since we studied the prevalent ovarian cancer cases, we may have a bias in selecting those individuals with better survival who belonged to hereditary ovarian cancer families.

3.3% of the controls in our study had a family history consistent with hereditary ovarian cancer, according to the definition used in this investigation (Table 5). There has not been a study to date estimating the proportion of unaffected individuals from the general population who belong to hereditary breast and ovarian cancer families. Based on the reports of the frequency of BRCA1 and BRCA2 mutations in the Ashkenazi Jewish population, we would expect 2.5% of the controls in our study to be carriers of one of the three founder mutations studied (Struewing et al., 1995; Roa et al., 1996; Oddoux et al., 1996).

We found that 38.6% (93 out of 241) of the Ashkenazi Jewish ovarian cancer patients in our study carried one of the three common BRCA1 or BRCA2 mutations (Table 6). Two Israeli studies reported on the prevalence of BRCA1 and BRCA2

mutations in Israeli Jewish women with ovarian cancer (Abeliovich *et al.* 1997; Levy-Lehad *et al.* 1997). These studies focused on women selected either for early age-of-onset or a positive family history. Abeliovich *et al.* (1997) found 13 of 21 (62%) of women with ovarian cancer had one of the three founder mutations. Because these patients included women referred from a cancer genetic counselling clinic, it is not surprising that their estimate is higher than ours. No *BRCA1* 5382insC mutation was found in this sample. Levy-Lehad *et al.* (1997) studied 22 incident cases of ovarian cancer among Ashkenazi Jewish women presenting to the Division of Gynecologic Oncology at the Shaare Zedek Medical Center in Jerusalem between September 1995 and May 1996. One of the three founder mutations was present in 10 of the 22 cases (45%). This estimate is higher than ours; but the number of cases in the study by Levy-Lahad *et al.* (1997) was small.

The frequency of the two *BRCA1* mutations among the mutation carriers was 68% (63 out of 93 mutation carriers) (Table 6). The frequencies of the 185delAG and 5382insC mutation carriers among the total number of carriers were 52% (48 out of 93) and 16% (15 out of 93) (Table 6). The frequency of the *BRCA2* 6174delT carriers among the total number of carriers was 32% (30 out of 93 carriers) (Table 6). These findings are similar to previous findings of higher frequency of *BRCA1* than *BRCA2* mutations among Jewish women with breast or ovarian cancer (Abeliovich *et al.*, 1997; Levy-Lahad *et al.*, 1997; Tonin *et al.*, 1996). Muto *et al.* (1996) found that 6 of 31 (19.3%) unselected Jewish patients with ovarian cancer in Massachusetts carried a 185delAG mutation, and Modan *et al.* (1996) found that 15 of 79 (19.0%) Ashkenazi women with ovarian cancer

in Israel carried a 185delAG mutation. For this single mutation, our estimate is 19.9% (48 out of 241 cases), which is similar to the previous findings (Table 6).

The frequencies of the *BRCA1* 185delAG, *BRCA1* 5382insC, and *BRCA2* 6174delT mutations among the general Ashkenazi Jewish population are reported as 1.1%, 0.1%, and 1.4%, respectively (Struewing *et al.*, 1995; Roa *et al.*, 1996; Oddoux *et al.*, 1996). Based on these frequencies, we would have expected to find a much lower proportion of *BRCA1* 5382insC to *BRCA2* 6174delT mutation carriers in our sample. There are two possibilities that can explain the higher than expected frequency of *BRCA1* 5382insC mutations in our sample. One possibility is that the penetrance of ovarian cancer is higher for the *BRCA1* 5382insC mutation compared to the *BRCA2* 6174delT mutation. Alternatively, it is possible that the frequency of the 5382insC mutation in the Ashkenazi Jewish population is higher than previously reported. I will discuss this further in the section on penetrance of *BRCA1* and *BRCA2* mutations.

As expected, mutations in the *BRCA1* and *BRCA2* genes were more common in cases with hereditary ovarian cancer compared to cases with no significant family history of the disease. We found that 72% (37 of 51) of the hereditary cases had one of the three mutations while 29.5% (56 of 190) of the non-familial cases had a mutation (Table 7). 143 out of the 190 cases classified as non-familial had no family history of breast or ovarian cancer (Table 8). 23.1% of these 143 cases had one of the three *BRCA1* or *BRCA2* mutations (Table 8). More interestingly, 60% (56 out of 93) of the mutation carriers did not have a significant family history of breast or ovarian cancer (Table 7). Thus, in our study, a family history of breast and ovarian cancer was an indicator of the risk of carrying one of the three *BRCA1* or *BRCA2* mutations (positive predictive value of

72.5%) (Appendix 10). However, the absence of a family history of breast or ovarian cancer was not an indicator for a low risk of being a *BRCA1* or *BRCA2* mutation carrier (negative predictive value of 29.5%) (Appendix 10). Based on these findings, we recommended that all Ashkenazi Jewish women with ovarian cancer be tested for the three common *BRCA1* and *BRCA2* mutations regardless of family history (Moslehi *et al.*, 2000).

9.5% (14 out of 148) of the cases who did not carry the *BRCA1* and *BRCA2* mutations tested had a significant family history of breast and ovarian cancer (Table 7). Furthermore, the risk of ovarian cancer by age 75 among the relatives of these non-carrier cases was estimated at 3.6% (Table 12), which is more than twice the life-time risk of ovarian cancer for women in the general population (1.4%). This high proportion of cases may carry another mutation in the *BRCA1* or *BRCA2* genes. Alternatively, these cases may have a mutation in another ovarian cancer susceptibility gene not yet identified. To find out if other *BRCA1* or *BRCA2* mutations are responsible for the disease in these families, we tested 122 of these 148 cases who do not carry any of the founder mutations using a technique that detects about 70% of mutations in each of these genes (see results). Overall, 93 of the 94 identified mutations in our sample were founder mutations, and the single non-founder mutation was inherited from a non-Jewish ancestor (Figure 25). These results are consistent with previous suggestions that the three founder mutations account for most of the heritability of ovarian cancer in the Jewish population (Abeliovich *et al.*, 1997; Berchuck *et al.*, 1999). Our results also suggest that although a high percentage of the familial clustering of ovarian cancer in the Jewish population is attributable to these

three founder mutations, there may be other *BRCA1* or *BRCA2* or other ovarian cancer susceptibility gene mutations involved in this population.

The prevalence of *BRCA1* and *BRCA2* mutations in Ashkenazi Jews with ovarian cancer is many times higher than in non-Jewish patients. In a study of 374 women with ovarian cancer in the United Kingdom, Stratton *et al.* (1997) estimated that only 3.5% of all cases carry a *BRCA1* mutation. In a smaller study, Rubin *et al.* (1998) found one *BRCA2* mutation and 10 *BRCA1* mutations in 113 hospital-based cases of ovarian cancer, for an overall prevalence of 9.7%. Janezic et al. (1999) found a pathogenic *BRCA1* mutation in only two of 107 unselected ovarian cancer cases in California. The proportions of Jewish patients in these studies are not known.

The average age of diagnosis of ovarian cancer among the Ashkenazi Jewish cases in our study was 56.8 years. This is within the range of the average age of diagnosis of ovarian cancer in the general population, which is between 55 and 59 (Altcheck *et al.*, 1996). The average age of diagnosis of ovarian cancer in the *BRCA1* mutation carriers (51.5 years) was significantly lower than the average age of diagnosis of ovarian cancer in the *BRCA2* mutations carriers (62.9 years) and the non-carriers (58.3 years) (Table 9). While only 19.1% of mutation-positive cases who were diagnosed with ovarian cancer below the age 50 carried the *BRCA2* 6174delT mutation, 63.3% of mutation positive cases diagnosed with ovarian cancer above the age 60 were carriers of this mutation (Table 10). Our findings are consistent with previous reports. Levy-Lahad et al. (1997) found the mean age of diagnosis in the *BRCA2* carriers (68.3 years) to be much older than that of the *BRCA1* carriers (50.5 years). These patients are also described by Beller *et al.* (1997).

Based on the results of studies on the structure and function of the *BRCA1* and *BRCA2* proteins, it can be assumed that these proteins are responsible for protecting the integrity of the genome. Therefore, *BRCA1* and *BRCA2* genes may belong to the category of either "tumour suppressor" or "mutator" genes. Knudson's hypothesis as it applies to the tumour suppressor genes can be extended to the "mutator" genes. This means that individuals with an inherited mutation of a tumour suppressor or a "mutator" gene are at an increased risk of early-onset disease. Therefore, we would expect the *BRCA1* and *BRCA2* mutation carriers, in our study, to have an earlier age of diagnosis of ovarian cancer than the non-carrier cases or than women in the general population. The earlier age of onset of ovarian cancer in the *BRCA1* carriers is consistent with this theory. However, the *BRCA2* carriers in our study had a later age of onset of ovarian cancer than the *BRCA1* carriers and the non-carriers.

This observation may be explained if the *BRCA2* protein is not as essential in keeping the genomic integrity of the cell as the *BRCA1* gene product. It may be that the *BRCA1* protein is involved in many pathways within the cell while the *BRCA2* protein is only involved in a single pathway. This would mean that *BRCA2* mutations would not lead to the initiation of the cell as effectively as *BRCA1* mutations would. Another explanation could be formed based on the report that *BRCA1* and *BRCA2* proteins interact with each other and with the same proteins of the repair pathway, such as Rad51 (Zhong *et al.*, 1999; Chen *et al.*, 1998). It is possible that the endogenous *BRCA1* protein can compensate, to an extent, in the absence of the *BRCA2* protein, so that the cell's repair mechanism is not compromised. However, the *BRCA2* protein cannot compensate

in the absence of the *BRCA1* protein, which is very likely if the *BRCA1* protein is involved in several repair and tumour suppression pathways within the cell.

Other explanations as to the why *BRCA1* mutation carriers have an earlier age of onset of ovarian cancer compared to the *BRCA2* mutation carriers may involve the difference in the length of time it takes for the LOH to occur. It may be that LOH of chromosome 17 harbouring the *BRCA1* gene occurs with a higher likelihood compared to the LOH of chromosome 13 containing the *BRCA2* gene. This may be due to the presence of many tumour suppressor genes, such as the p53 gene, on chromosome 17, which may be the targets for the LOH. More data on the functions of the *BRCA1* and *BRCA2* gene products are required before the mechanisms of action and compensation can be identified. Further studies on the mechanism of inactivation of these genes may provide us with an explanation as to the differences between the age of onset of ovarian cancer associated with these two genes.

The frequency of mutations in the *BRCA1* and *BRCA2* genes in the Jewish population at large is much greater than the frequency in non-Jews (1 in 800 to 1 in 10,000) (Roa *et al.,* 1996; Oddoux *et al.,* 1996). This high prevalence accounts, to a large extent, for the greater population-attributable risk observed in the Jewish population; however, it is also possible that the risk of ovarian cancer associated with the Jewish founder mutations exceeds that of other *BRCA1* or *BRCA2* mutations. I estimated the penetrance of these three mutations from our data. In order to estimate the penetrance, I first calculated the risk of cancer associated with these mutations in the first-degree relatives of the cases and controls.

Relative Risk and Penetrance Estimates for Breast and Ovarian Cancer among the First Degree Relatives

Although the first-degree relatives of cases had a significantly higher risk of any cancer to age 75 compared to the relatives of the controls, this excess risk was due to higher probability for cancers of the breast, ovary, prostate and pancreas only (Table 11). The high risk of cancer in female relatives of the cases was primarily due to cancers of breast and ovary since when these cancers were excluded from the analysis, there was no difference between the risk of cancer in female relatives of cases and controls (Table 11). The high risk of cancer in men was solely due to the increased risk of prostate and pancreatic cancers by age 75 in male relatives of cases.

The same pattern was observed when comparing the relatives of carrier and non-carrier cases. The high risk of cancer by age 75 among the first-degree relatives of the carrier cases was primarily due to cancers of breast and ovary (Table 12). There was also an increased risk of uterine cancer among the first-degree relatives of the carriers to age 65, but this estimate was based on only one case of uterine cancer.

The relatives of the *BRCA1* carriers had a significantly higher risk of breast cancer by age 65 compared to the relatives of *BRCA2* carriers. This observation is consistent with reports of an earlier age of onset of breast cancer for *BRCA1* mutations than for *BRCA2* mutations (Tonin *et al.*, 1996, Warner *et al.*, 1999). There is no significant difference in the risk of any other cancer among the first-degree relatives of *BRCA1* and *BRCA2* carriers, but the numbers are small. The only case of uterine cancer occurred among the relatives of the *BRCA1* carriers (Table 13). The relationship between uterine cancer and mutations in the *BRCA1* and *BRCA2* genes warrants further investigation.

Based on previous reports, I expected to find a significantly higher number of pancreatic cancer cases among the relatives of the *BRCA2* carriers compared to the relatives of the *BRCA1* carriers. In a previous study, *BRCA2* mutations were present in three of 26 unselected Jewish cases with pancreatic cancer (Ozcelik *et al.* 1997). I found a significantly higher frequency of pancreatic cancer in the relatives of ovarian cancer cases compared to the controls (Table 11). However, no significant difference was observed in the frequency of pancreatic cancer in the relatives of mutation carrier cases versus non-carrier cases and *BRCA1* carriers versus *BRCA2* carriers, but the numbers were small (Tables 12, 13). Despite the non-significant *P*- values, four of the five pancreatic cancer cases among the relatives of mutation carriers were seen in the relatives of the *BRCA2* carriers. Therefore, our data neither supports nor refutes the data by Ozcelik et al. (1997).

Previous studies have suggested that male carriers of *BRCA1* or *BRCA2* mutations may be at an increased risk for prostate cancer (Warner *et al.* 1999). My analysis is consistent with an increased risk of early-onset prostate cancer among the relatives of the mutation carriers. However, due to the small number of prostate cancers (17) observed among the relatives of the cases, the comparison did not reach statistical significance when comparing relatives of mutation positive and negative cases (Table 12). There have been reports recommending PSA screening of men with *BRCA1* or *BRCA2* mutations from age 50 (Lynch *et al.*, 1999; Warner *et al.*, 1999). Based on this analysis, our group agrees with these colleagues that PSA screening should be offered to male carriers of *BRCA1* and *BRCA2* mutations.

I did not find an increase in the risk of colon cancer in the relatives of the *BRCA1* or *BRCA2* carriers, compared to the non-carriers or to healthy controls. No excess of colon cancer was seen in the family members of *BRCA2* carriers in the study of the Breast Cancer Linkage Consortium (1999). Although the data of Ford and her colleagues (1994) suggested that colon cancer may be a feature of the *BRCA1* spectrum, our study and other investigations (Lynch *et al.*, 1999) do not support such an association.

It has been reported that the risk of breast and ovarian cancer appears to be increasing from generation to generation in some families with mutations (Narod *et al.* 1995). The present study provided the opportunity to evaluate directly the possibility of a cohort effect among the carriers. I compared the incidence of breast and ovarian cancer in the mothers and sisters of the carriers. Under the assumption that cancer risk is increasing with birth year, I would expect the age-adjusted incidence in sisters to exceed that of mothers. This was not found, and our data set did not support the phenomenon of genetic anticipation or a cohort effect (Table 14).

Based on the kin-cohort method, I estimated the penetrance of breast cancer by age 55 to be 29.9% for carriers of the *BRCA1* mutations and 5.1% for the *BRCA2* 6174delT mutation by age 55. The penetrance of ovarian cancer by age 55 was estimated at 12.6% for the *BRCA1* mutations and 13.4% for the *BRCA2* 6174delT mutation.

There is a range of estimates of breast and ovarian cancer penetrance attributed to *BRCA1* and *BRCA2* mutations from studying different families. Easton et al. (1993) estimated the penetrance for the *BRCA1* and *BRCA2* mutations to be close to 85% for breast cancer by age 75. Their estimates of risk for ovarian cancer were 45% for *BRCA1* mutations and 25% for *BRCA2* mutations (Easton *et al.*, 1993). These estimates were

based on studying families ascertained through multiple cases of breast and ovarian cancer. Therefore these may be over-estimations of the penetrance of the *BRCA1* and *BRCA2* mutations for women with no family history of breast or ovarian cancer. Since then several groups have estimated the penetrance for *BRCA1* and *BRCA2* genes by studying unselected Ashkenazi Jewish breast and ovarian cancer patients. Struewing *et al.* (1997) estimated the penetrance of breast and ovarian cancers to be 56% and 16%, respectively, for *BRCA1* and *BRCA2* mutation carriers in the Jewish population. Their estimates, however, were based on only 11 observed ovarian cancers in 306 female relatives of *BRCA1* and *BRCA2* mutation carriers. Our estimates of breast and ovarian cancer penetrance are generally much smaller than previous estimates. This is probably due to the fact that our estimates are based on cases unselected for a family history of breast and ovarian cancer.

The risk estimates currently given to individuals seeking counselling at genetics centres are probably not appropriate for all *BRCA1* or *BRCA2* mutation carriers and may have to be corrected as more data on the penetrance of *BRCA1* and *BRCA2* mutations emerges. These higher estimates may be accurate when counselling individuals from families with breast and ovarian cancer syndrome but may not apply to women positive for the *BRCA1* or *BRCA2* mutations who do not belong to hereditary breast-ovarian cancer families.

Another factor that must be taken into consideration during genetic counselling of individuals at risk for *BRCA1* or *BRCA2* mutations is the type of cancer in the family. I compared the penetrance values obtained from our study to those reported by Warner et al. (1999). This group recently reported the results of a study of *BRCA1* and *BRCA2*

mutations in 412 Ashkenazi Jewish women with breast cancer. The method of calculation of penetrance in the study by Warner et al. (1999) was identical to ours. For *BRCA1* mutations, I estimated the penetrance for breast cancer by age 75 to be 42.1%. Warner et al. (1999) estimated the penetrance of breast cancer for the same mutations in the *BRCA1* gene to age 75 to be ~60%. The first-degree relatives of breast cancer patients with the same mutations in the *BRCA1* genes seem to be at an increased risk for breast cancer compared to the first-degree relatives of ovarian cancer cases. My estimate of penetrance of breast cancer for the *BRCA2* 6174delT mutation was 34.2% which was higher than the estimate by Warner et al. (1999) (28%); however, the numbers of the *BRCA2* 6174delT carriers in both studies may be too small to allow a meaningful comparison.

I estimated the penetrance of ovarian cancer by age 75 for the three *BRCA1* and *BRCA2* mutations in our study by age 75 to be 13.8% and 20.2%, respectively. Warner et al. (1999) did not report estimates of ovarian cancer penetrance for the patients in their study, due to the small number of ovarian cancer cases (2) among the relatives of their probands. It seems as if the first-degree relatives of the ovarian cancer patients with these three mutations in the *BRCA1* and *BRCA2* genes are at an increased risk for ovarian cancer compared to the first-degree relatives of breast cancer cases with the same mutations. Therefore, individuals from families ascertained by breast cancer may have a higher risk for breast cancer compared to individuals from families ascertained by ovarian cancer. Accordingly, the penetrance of ovarian cancer for the same mutations in the *BRCA1* and *BRCA2* genes may be higher in families ascertained by ovarian cancer than in families ascertained by breast cancer. This observation may be explained by the

presence of modifying factors in these families that increase the risk of one cancer over the other in the presence of *BRCA1* and *BRCA2* mutations.

It is important to identify and quantify the modifying effects of risk factors for ovarian cancer in women who carry mutations in the *BRCA1* and *BRCA2* genes. The contributions of these modifiers of ovarian cancer risk may be significant. Identifying such modifiers of risk may lead to more accurate counselling and medical management of women with *BRCA1* and *BRCA2* mutations.

There may be genetic as well as environmental modifiers of the ovarian cancer risk present in a family. The genetic modifiers may be mutations or polymorphisms that exert their effects in a fashion compatible with multifactorial or polygenic models. Phelan et al. (1996) demonstrated that *BRCA1* carriers with a rare allele of a VNTR locus in the proximity of the HRAS1 oncogene have an increased risk of ovarian cancer. Similar studies on genetic modifiers of ovarian cancer risk among the *BRCA1* and *BRCA2* mutation carriers are currently in progress and may lead to identification of many important loci. Environmental factors may also be shared among relatives and contribute to the risk of breast and ovarian cancer. Brunet et al. (1999) suggested that smoking may reduce the risk of breast cancer in the carriers of *BRCA1* or *BRCA2* mutations. Narod et al. (1998) reported that oral contraceptive use may reduce the risk of ovarian cancer in women who carry *BRCA1* or *BRCA2* mutations. There may be other such factors that have a familial component and modify the risk of cancer associated with *BRCA1* and *BRCA2* mutations in different families.

The Jewish founder mutations represent only three of the hundreds of mutations in *BRCA1* or *BRCA2* that have been identified to date. The risk of ovarian cancer is believed

not to be the same for all *BRCA1* and *BRCA2* mutations and may vary according to the position of the mutation along the gene. In particular, the 6174delT mutation is in the ovarian cancer cluster region (OCCR) of *BRCA2* as defined by Gayther *et al.* (1997). *BRCA2* mutations outside this region of exon 11 are believed to carry a much lower risk of ovarian cancer. This is consistent with the high risk of ovarian cancer associated with the 6174delT mutation in our study. Similarly, truncating mutations in the first two-thirds of the coding region of *BRCA1* are thought to be associated with a higher risk of ovarian cancer than mutations in the last third of the gene (Gayther *et al.* 1995). The 185delAG mutation is in the extreme 5' end of the gene and the 5382insC mutation is in the extreme 3' end of the gene. This in combination with a lower population frequency of the 5382insC mutation is consistent with our findings of a higher frequency of the *BRCA1* 185delAG mutations compared to the *BRCA1* 5382insC mutations in our sample. From the reported population frequency of the 5382insC mutation (one tenth that of the 185delAG mutation), we would have expected a much lower number of carriers of this mutation among our sample. This may indicate a higher penetrance for ovarian cancer associated with the 5382insC mutation or a higher frequency of this mutation in the Jewish population than previously reported.

Risk Factor Analysis

The most significant risk factor for ovarian cancer for both carrier and non-carrier cases in our sample was age (Tables 18, 19). The controls in our study were on average younger than the cases (52 versus 60.1 years). It is known that age is a risk factor for ovarian cancer in the general population; the highest risk period for ovarian cancer is the

first few years post menopause (Altchek *et al.*, 1996). Age difference between the cases and the controls was one of the limitations for comparing the two groups in regards to risk factors. Ideally, the controls for this type of analysis should be matched to the cases within two years of age at the time of collection.

Height was also found to be a significant risk factor for ovarian cancer among the non-carrier cases in our study (Table 19). Non-carrier cases were 1.7 cm taller on average (163.3 cm) than controls (161.6 cm; $P=0.02$). Carrier cases were also taller (162.0 cm) than controls, but the difference was not significant ($P=0.70$). This difference in height is not explained by the year of birth difference between cases and controls, or between carrier cases and non-carrier cases. Cases were born an average of 8.4 years earlier than the controls, and height has generally increased with calendar year. There are no reports of an increase in ovarian cancer risk with increasing height in the general population. Gunnell et al. (1998) found a positive correlation between childhood leg length and adult cancer mortality. This association was strongest for cancers related to sex hormones, including ovarian. It is not yet clear if the biological basis of this relationship is nutritional or is due to genetic variation in hormones that influence growth.

Another factor that was associated with a significantly increased risk of ovarian cancer among the carrier and non-carrier cases in our analysis was vaginal talc use. Among the non-carrier cases, individuals who used talc in the vaginal area were 2.3 times more likely to have had ovarian cancer (Table 17). Vaginal talc use was associated with a significant increased risk of ovarian cancer among the carrier cases (Table 17). Vaginal talc use has been associated with an increased risk of ovarian cancer in women in the general population (Riman *et al.*, 1998).

None of the other risk factors studied showed a significant association with ovarian cancer risk. History of any pregnancy has been associated with a lower risk of ovarian cancer in women in the general population (Risch *et al.*, 1994; Whittemore *et al.*, 1992). This protection is believed to increase with increasing parity (Risch *et al.*, 1994; Whittemore *et al.*, 1992). Our data suggested a decrease in risk of ovarian cancer with any history of pregnancy for both carriers and non-carriers, but the difference was not significant (Table 17). Furthermore, increasing parity above 3 births in our data was associated with a decrease in risk of ovarian cancer for both carriers and non-carriers, but again the trend was not statistically significant (Tables 17, 18, 19).

Breast-feeding is also believed to protect against ovarian cancer in women in the general population (Risch *et al.*, 1994; Whittemore *et al.*, 1992). Our analyses showed no significant association between risk of ovarian cancer and breast-feeding in either carriers or non-carriers (Table 17). The modifying effect of this risk factor seems to be small in the presence of *BRCA1* and *BRCA2* mutations.

Tubal ligation was not associated with a significant alteration in risk of ovarian cancer in carriers or non-carriers (Table 17). The effect of tubal ligation on ovarian cancer risk in the general population is not yet clarified (Riman *et al.*, 1998). Earlier age at menarche and later age at menopause are associated with an increased risk for ovarian cancer in the general population (Riman *et al.*, 1998) but in our study, age of menarche did not affect ovarian cancer risk for either carriers or non-carriers (Table 18). When age of menarche was treated as a dichotomous variable (<14 and >14) or when age at menopause was also included in the logistic regression model, the results remained the same. Smoking may reduce the risk of breast cancer in women with *BRCA1* or *BRCA2*

mutations (Brunet *et al.*, 1998), but our results showed no difference in risk of ovarian cancer for either carrier or non-carrier cases with pack-years of smoking (Table 18).

Oral contraceptive use of greater than one year was not associated with a decreased risk of ovarian cancer in either carriers or non-carriers in our study, in contrast to the findings in the general population (Table 17, 18, 19). There have been suggestions that unaffected carriers of *BRCA1* and *BRCA2* mutations be put on oral contraceptives for protection against ovarian cancer (Narod *et al.*, 1998). The data presented here do not support this recommendation as the magnitude of this protection in the presence of the *BRCA1* and *BRCA2* mutations appears to be small, although the power for this analysis (~53%) was inadequate for both mutation carrier and non-carrier cases. It is essential to gather more data on this and other risk factors for ovarian cancer in this group of women in order to be able to offer sound medical advice to these patients and their families.

Ovarian Tumour Histology

The majority of the cases (112 out of 203) in our study had serous adenocarcinoma of the ovary (Table 20). There were higher numbers of mutation carriers with serous adenocarcinoma (62.8%) than non-carrier cases with serous adenocarcinoma (49.6%) (Table 21). This difference was not statistically significant (P=0.061), although this may have been due to inadequate power because of small sample size. Our results are consistent with the previous findings of a trend towards greater frequency of serous adenocarcinomas of the ovary among the familial cases of ovarian cancer versus the sporadic cases (Chang *et al.*, 1995). Although epithelial serous histology was indicative of a higher risk of carrying *BRCA1* or *BRCA2* mutations in our sample, histology other

than serous was not predictive of a low risk of carrying a *BRCA1* or *BRCA2* mutation. *BRCA1* and *BRCA2* mutations occurred in all other histologic subtypes of epithelial ovarian tumours in our sample except for transitional cell and Brenner tumours of the ovary (Table 20). The numbers in these categories were too small to exclude these subtypes as part of the spectrum of *BRCA1* and *BRCA2* mutations.

The carrier cases in our study had a significantly higher number (90.7%) of grade III tumours than the non-carrier cases (68.7%, *P*=0.002) (Table 22). Severity of the disease is believed to increase with the decrease in the degree of differentiation. Therefore, based on our data, we would expect the tumours in the *BRCA1* or *BRCA2* mutation carriers to be histologically more·aggressive than sporadic tumours. However, our data on the stage of the ovarian tumour suggested that there was no significant difference between mutation carriers and non-carriers as far as the spread of the disease was concerned. Thus, there does not seem to be a difference in terms of prognosis between carrier and non-carrier cases despite the increased histological severity of the tumours among the carrier cases.

Rubin *et al.* (1996) reported that ovarian cancer patients with *BRCA1* mutations have a better ten-year survival than women without mutations, after adjusting for stage. This study has been criticized because the selection criteria favored long-term survivors among the carriers (Modan *et al.* 1997). However, a recent study based on pathology specimens confirmed the previous results (Boyd *et al.* 2000). If these reports are correct, it is surprising to find that the mutation carriers in our study presented with high-grade tumours. It may be that the high-grade, *BRCA1-* or *BRCA2*-positive tumours lack the capacity to repair DNA damage and are therefore particularly sensitive to the cytotoxic

effects of chemotherapy. This suggestion is based on the reports that poorly-differentiated tumours are associated with mutations in many important genes including those involved in the DNA repair mechanisms (Chang *et al.*, 1995). This theory is also consistent with the report of a longer-disease free interval following primary chemotherapy among the *BRCA1* and *BRCA2* carrier cases in comparison to the non-carrier cases (Boyd *et al.*, 2000).

Case Reports

Family R014 raised new counselling issues with regards to genetic testing for *BRCA1* and *BRCA2* mutations (Figure 17). Individual 46 in this family was the first reported case of an unaffected woman inheriting mutations in both the *BRCA1* and *BRCA2* genes from one parent and the second reported case of an unaffected *BRCA1/BRCA2* double heterozygote. The proband's father (individual 39) was negative for all three mutations. Therefore, the proband's mother (individual 38) is assumed to have been a 185delAG/6174delt carrier. Individual 38 is deceased, and tumour blocks are not available for testing.

Individual 16, the mother of individual 38, is 88 years old and has never had cancer or prophylactic surgery. According to the current estimates of penetrance of the *BRCA1* and *BRCA2* mutations, individual 16 has about 8% probability of carrying either the *BRCA1* 185delAG or the *BRCA2* 6174delT mutations without developing breast or ovarian cancer by age 88 (Easton *et al.*, 1993; Struewing *et al.*, 1997). The observation that many female relatives of individual 16 lived to an old age and never developed breast or ovarian cancer supports the assumption that there is no mutation segregating in

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that side of the family. Therefore, there is a much higher likelihood that individual 38 inherited both mutations from her double heterozygote father, individual 17. This could then be a case of a two-generation inheritance of two mutations in the *BRCA1* and *BRCA2* genes from one parent to one child.

The high frequency of the *BRCA1* 185delAG and the *BRCA2* 6174delT mutations in the Jewish population make it possible for a Jewish individual to be a double heterozygote; this probability is 0.014%. So finding one case in a study the size of ours is not unlikely.

Friedman et al. (1998) reported a breast cancer patient of Ashkenazi Jewish origin with both the *BRCA1* 185delAG and the *BRCA2* 6174delT mutations. This individual may have inherited both mutations from her mother, who had ovarian cancer. This group also reported three other Ashkenazi Jewish women, two of whom were affected with either breast or ovarian cancer, who were double heterozygotes for the 185delAG/6174delT mutations. The third woman in their study was a 50 year old asymptomatic Ashkenazi Jewish woman with a maternal family history of breast and ovarian cancer and a paternal history of prostate cancer. None of the parents in this case were available for testing (Friedman *et al.,* 1998).

Another group reported an Ashkenazi Jewish individual with breast cancer and both the *BRCA1* 185delAG and the *BRCA2* 6174delT mutations (Ramus *et al.,* 1997). There is also a report of a non-Jewish breast cancer patient with germline mutations in the *BRCA1* and *BRCA2* genes (Liede *et al.,* 1998).

Friedman et al. (1998) reported that two of their double heterozygote patients had reproductive problems. Individual 46 in our family did not report any problems with

fertility or premature menopause, but two of her seven pregnancies ended in miscarriages. We do not have a detailed reproductive history on individual 38.

Due to the small number of *BRCA1* 185delAG and *BRCA2* 6174delT double heterozygotes identified to date, current data are insufficient to estimate the risk of breast or ovarian cancer in these individuals. There has been a suggestion that the phenotypic effects of double heterozygosity for *BRCA1* and *BRCA2* germline mutations may not be cumulative (Friedman *et al.,* 1998). In our family, individual 46 with both the *BRCA1* 185delAG and *BRCA2* 6174delT mutations remained unaffected to age 36 while her sister with the *BRCA1* 185delAG mutation was diagnosed with breast cancer at age 30. Individual 38 who is inferred to have carried both of these mutations also did not develop breast or ovarian cancer until age 36.

There is no information available on the risk of other cancers in individuals who carry mutations in both *BRCA1* and *BRCA2* genes. The implication for a male of carrying both the *BRCA1* 185delAG and the *BRCA2* 6174delT mutation is also unclear. In our family, individual 17, who is inferred to have carried both of these mutations, lived to age 70 without developing cancer. He died in his 70's of an unknown cause.

Individual 46 in our family was counselled as having at least the same risk of breast and ovarian cancer as a carrier of just the *BRCA1* 185delAG mutation, such as her sister. Individual 46 subsequently decided to have prophylactic oophorectomy and bilateral mastectomy.

Identifying individuals with mutations in both the *BRCA1* and *BRCA2* genes has both clinical and scientific relevance. Accurate genetic counselling and medical management of *BRCA1/BRCA2* double heterozygotes will depend on studying the

reproductive, medical and family history of such individuals. These studies may also

enhance understanding of the mechanism of action of these genes and their interactions.

Moreover, our finding further emphasizes that all Ashkenazi Jewish individuals at high

risk for breast and ovarian cancer should be tested for all three common Ashkenazi

Jewish mutations, regardless of the mutation previously identified in the family.

We also identified a family segregating all three common *BRCA1* and *BRCA2*

mutations. Family R023 is the first breast-ovarian cancer family described with three

distinct mutations in the *BRCA1* and *BRCA2* genes (Figure 23). Parents of individual 40

and other relatives who were tested were not available to establish paternal or maternal

transmission of the mutation identified. The *BRCA1* 185delAG mutation was associated

with the youngest ages of onset for breast cancer (34 and 37 years) in this family. All

three mutations were associated with a diagnosis of ovarian cancer (individuals 31, 39,

and 40). The *BRCA2* 6174delT mutation was found in one woman with post-menopausal

breast cancer and ovarian cancer and in another with peri-menopausal breast cancer at

age 49. This supports the hypothesis of a lower age of onset of breast cancer with *BRCA1*

185delAG and 5382insC mutations compared to the *BRCA2* 6174delT mutation.

Family R013 is similar in that the members of a couple each carry a different

mutation in the *BRCA1* and *BRCA2* genes (Figure 24). In this family the side of the

kindred segregating the *BRCA2* 6174delT mutation is associated with post-menopausal

breast cancer (age 57, in the proband) and post-menopausal ovarian cancer (age 58 in the

proband and age 80 in her mother). There is also a history of stomach cancer in that side

of the family, which may actually have been pancreatic cancer; cancer of the pancreas

has been reported as being part of the *BRCA2* spectrum (Ozcelik *et al.*, 1997). The side of

the kindred segregating the *BRCA1* 185delAG mutation is associated with prostate cancer and early-onset breast cancer (age 20 in individual 9). These observations are consistent with the results reported in this thesis as well as other published studies on *BRCA1* and *BRCA2* genes (Lynch *et al.*, 1999; Warner *et al.*, 1999; Struewing *et al.*, 1997; Tonin *et al.*, 1996).

The observations in these families are not unexpected, considering the high frequency of *BRCA1* and *BRCA2* mutations in the Ashkenazi Jewish population (Roa *et al.*, 1996; Oddoux *et al.*, 1996). The informal method of ascertainment of the two branches of family R023 relied on reports of breast and ovarian cancer in relatives and resulted in the construction of an extended breast-ovarian cancer kindred. However, if all descendants of individuals 1 and 2 were systematically ascertained, the number of at-risk individuals would be undoubtedly greater than represented in Figure 23.

Although the results for these Ashkenazi Jewish kindreds are not unexpected, they do have significant implications for how clinicians should approach genetic testing for Ashkenazi Jewish families. These observations further emphasize that it is not sufficient to test for only a known mutation in order to classify individuals as carriers or non-carriers in families where one *BRCA1* or *BRCA2* mutation is known to be segregating. It is also important to address the limitations of testing for common mutations within a specific ethnic population during pre-test genetic counselling for individuals and families (Lynch *et al.*, 1999; Liede *et al.*, 1998). Sometimes a negative result may be ambiguous in the absence of a known family mutation. After testing negative for all three mutations of *BRCA1* and *BRCA2*, members of family R023 were given population risks for breast and ovarian cancer. In the absence of a known *BRCA1* or *BRCA2* mutation in an

Ashkenazi Jewish family with multiple cases of breast and ovarian cancer, a negative result for the three common mutations may warrant more comprehensive testing of *BRCA1* and *BRCA2* genes. The issues discussed here are also applicable to other ethnic groups with a high frequency of mutations in *BRCA1*, *BRCA2* or other cancer predisposing genes.

In conclusion, we found a high frequency (38.6%) of three founder *BRCA1* and *BRCA2* mutations among unselected Ashkenazi Jewish women with ovarian cancer. The *BRCA2* 6174delT carriers in our study had a significantly higher age of onset of ovarian cancer compared to the *BRCA1* 185delAG and *BRCA1* 5382insC carriers. Lack of a family history of the breast or ovarian cancer was not a predictor of the low risk for carrying a *BRCA1* or *BRCA2* mutation in our sample. We feel that our data support the recommendation that Jewish women with ovarian cancer should be offered testing for *BRCA1* and *BRCA2* mutations regardless of their family history. Factors that predicted the presence of a mutation among the cases in our study included tumour grade, age-of-onset, histology and family history. Tumour grade appears to be the most discriminating of the four predictive factors. Based on these data, we expect that the majority of Jewish women who develop ovarian cancer in the context of a screening program for women at high familial risk will also carry a *BRCA1* or a *BRCA2* mutation. Because of the high risk of ovarian cancer associated with each of these mutations, and because of the limitations of ovarian cancer screening, we have recommended that the option of prophylactic oophorectomy be raised with mutation carriers who have not yet developed cancer (Moslehi *et al.*, 2000).

Struewing *et al.* (1995) found no difference in the rate of ovarian or peritoneal carcinoma in oophorectomized versus non-oophorectomized women. Statistical correction of raw data in this study suggested a 45% reduction in the risk of ovarian or peritoneal carcinoma following prophylactic oophorectomy in high-risk women; however, the confidence intervals were widely overlapping for the two groups. Therefore, this study did not have adequate statistical power to resolve the question of the degree of protection afforded by oophorectomy. Prospective data on the residual risk of cancer and on the complications of pre-menopausal surgical oophorectomy are needed. Data are emerging that prophylactic oophorectomy may also be effective in reducing the risk of breast cancer in *BRCA1* carriers (Rebbeck *et al.*, 1999). If efficacy of prophylactic oophorectomy in high-risk women is proven, based on the age distributions of the mutation-positive ovarian cancers (Table 10), it may be reasonable to wait until the time of the natural menopause to perform oophorectomy for *BRCA2* carriers. However, for maximum protection, the operation should be performed at a younger age in women who carry *BRCA1* mutations.

We estimated the penetrance of breast and ovarian cancer for the three common mutations of the *BRCA1* and *BRCA2* genes for Ashkenazi Jewish ovarian cancer cases unselected for a family history. We found that the penetrance of breast and ovarian cancer for these three mutations may be different in families ascertained through different selection criteria. These differences in life-time risks of breast and ovarian cancer in different families should be considered in counselling and medical management of individuals at risk.

We found the reproductive and other risk factors (except for vaginal talc use) associated with ovarian cancer in the general population to have a small impact on the ovarian cancer risk among the carriers of the three *BRCA1* and *BRCA2* mutations tested. The impact of some of these factors, such as oral contraceptive use, on ovarian cancer risk among the mutation carriers may have to be re-evaluated for accurate counselling and management of women at high risk. Studies with higher statistical power are required to re-evaluate the impact of vaginal talc use and height among the *BRCA1* and *BRCA2* mutation carriers and non-carriers.

Identification of an individual heterozygous for mutations in both the *BRCA1* and *BRCA2* genes and two families with members carrying different founder mutations in these two genes further emphasizes the possibility of such occurrences in the Ashkenazi Jewish population because of the high prevalence of these mutations. These observations suggest that it may be appropriate to discuss the possibility of the presence of different *BRCA1* and *BRCA2* founder mutations in different branches of the family during the counselling session of Ashkenazi Jewish individuals at high risk of being carriers. In such cases, it may also be appropriate to discuss the possibility of double heterozygosity. Furthermore, observations in these three unique families emphasize that Ashkenazi Jewish individuals eligible for *BRCA1* and *BRCA2* testing be tested for all three founder mutations in these two genes regardless of the mutation previously identified in the family.

Table 1

World Health Organization
Histological Classification of Ovarian Tumours

Surface Epithelial-Stromal Tumours

Serous tumours
Mucinous Tumours
Endometrioid Tumours
Clear Cell Tumours
Transitional Cell Tumours
Squamous Cell Tumours
Mixed Epithelial Tumours
Unclassified Tumours

Sex Cord-Stromal Tumours

Granulosa-Stromal Cell Tumours
Sertoli-Stromal Cell Tumours
Sex Cord Tumours with Annular Tubules
Gynandroblastoma
Steroid (Lipid) Cell Tumours
Unclassified Tumours

Germ Cell Tumours

Dysgerminoma
Yolk Sac Tumour
Embryonal Carcinoma
Polyembryoma
Choriocarcinoma
Teratomas
Mixed Germ Cell Tumours

Gonadoblastoma

Germ Cell-Sex Cord-Stromal Tumour of
Non-gonadoblastoma Type

Tumours of Rete Ovarii

Mesothelial Tumours

Tumours of Uncertain Origin

Secondary (metastatic) Tumours

Table 2

International Federation of Gynecology and Obstetrics (FIGO) Staging System for Ovarian Cancer

FIGO	Definition
-	Primary Tumour cannot be assessed
-	No evidence of primary tumour
I	Tumour limited to one or both ovaries
IA	Tumour limited to one ovary; capsule intact, no tumour on ovarian surface, no malignant cells in ascites or peritoneal washings
IB	Tumour limited to both ovaries; capsule intact, no tumour on ovarian surface, no malignant cells in ascites or peritoneal washings
IC	Tumour limited to one or both ovaries with any of the following: capsule ruptured, tumour on ovarian surface, malignant cells in ascites or peritoneal washings
II	Tumour involves one or both ovaries with pelvic extension
IIA	Extension and/or implants on the uterus and/or tube(s); no malignant cells in ascites or peritoneal washings
IIB	Extension to other pelvic tissues; no malignant cells in ascites or peritoneal washings
IIC	Pelvic extension (IIa or IIb) with malignant cells in ascites or peritoneal washings
III	Tumour involves one or both ovaries with microscopically confirmed peritoneal metastasis outside the pelvis and/or regional lymph node metastasis
IIIA	Microscopic peritoneal metastasis beyond the pelvis
IIIB	Microscopic peritoneal metastasis beyond the pelvis 2 cm or less in greatest dimension
IIIC	Microscopic peritoneal metastasis beyond the pelvis 2 cm or less in greatest dimension and/or regional lymph node metastasis
IV	Distant metastasis (excludes peritoneal metastasis)

Table 3

Cases Excluded from the Analysis Based on Tumour Histology

	Frequency
Total No. of Cases Ascertained	289
Total No. of Ashkenazi Jewish Cases	284
Borderline Tumours	14
Adenofibroma	6
Mesoblastoma	2
Primary Fallopian Tube	3
Sex Cord Ovarian Tumour	3
Primary Peritoneal	7
Total Cases Excluded	35
Total Cases Included in the Analysis	249

Table 4

Ashkenazi Jewish Cases and Controls from Participating Centers

Hospital	Cases	Controls
Jewish General Hospital	18	27
Cedars Sinai Medical Center	53	33
Sunnybrook Regional Hospital	12	
Toronto General Hospital	6	
Long Island Jewish Hospital	19	
Emek Central Medical Center	20	6
Columbia Presbyterian Medical Center	13	33
Kaplan-Rehovot Medical Center	8	
University of Pennsylvania Medical Center	6	
Albert Einstein Medical Center	24	9
Beth Israel Hospital	10	
Prentice Women's Medical Center	36	
Yale University Medical Center	11	22
Ontario Ovarian Tumour Registry	13	
Jewish Women's Group		200
Total	249	330

Table 5

Frequency of Hereditary Ovarian Cancer Among the Ashkenazi Jewish Cases and Controls

	Cases	Controls
Hereditary Ovarian Cancer	52	11
Total	249	330
Frequency of BOCS	20.9%	3.3%

Table 6

Frequency of the Three Common *BRCA1* and *BRCA2* Mutations Among the Ashkenazi Jewish Cases

	Number	Frequency
Total number of cases analysed	241	
Total number of *BRCA1* or *BRCA2* carriers	93	38.6%
Number of *BRCA1* carriers	63	26.1%
185delAG carriers	48	
5382insC carriers	15	
Number of *BRCA2*, 6174delT carriers	30	12.4%

Table 7

Frequency of Hereditary Ovarian Cancer Among the *BRCA1* and *BRCA2* Mutation Carriers

	Familial	Non-Familial	Total
BRCA1	27	36	63
185delAG	18	30	48
5382insC	9	6	15
BRCA2, 6174delT	10	20	30
BRCA1 and *BRCA2*	37	56	93
Non-carriers	14	134	148
Total	51	190	

Table 8

Frequency of Mutations in Ashkenazi Jewish Women with Ovarian Cancer by Family History

Family History	Total No. of patients	No. (%) Positive for Mutations in		
		BRCA1	BRCA2	Either
None	143	22 (15.4)	11 (7.7)	33 (23.1)
Breast cancer (no relatives with ovarian cancer):				
1 instance of breast cancer under age 50 years	47	16 (34.0)	7 (14.9)	23 (48.9)
2 or more instances of breast cancer under age 50 years	10	7 (70.0)	1 (10.0)	8 (80.0)
Ovarian cancer (no relatives with breast cancer):				
1 or more instances of ovarian cancer	26	10(38.5)	8 (30.8)	18 (69.2)
Breast and Ovarian cancer:				
1 instance of breast cancer under age 50 years	9	5 (55.6)	2 (22.2)	7 (77.8)
2 or more instances of breast cancer under age 50 years	6	3 (50.0)	1 (16.7)	4 (66.7)
Total cases analysed and carriers of the BRCA1/2 mutations	241	63	30	93

Table 9

Mean Age of Onset of Ovarian Cancer Among Ashkenazi Jewish Cases (n=241)

	Age of Onset of Ovarian Cancer
BRCA1 Carriers	51.5
185delAG	51.6
5382insC	51.1
BRCA2, 6174delT Carriers	62.9
Non- Carriers	58.3

Table 10

Frequency of Mutations in Ashkenazi Jewish Cases of Ovarian Cancer by Age at Diagnosis

Age Group (Years)	Total No. of Patients	No. (%) Positive For Mutations In		
		BRCA1	*BRCA2*	Either
19-29	4	0 (.0)	0 (.0)	0 (.0)
30-39	20	7 (35.0)	1 (5.0)	8 (40.0)
40-49	61	27 (44.3)	3 (4.9)	30 (49.2)
50-59	48	17 (35.4)	8 (16.6)	25 (52.1)
60-69	57	9 (15.8)	11 (19.3)	20 (35.1)
70-90	51	2 (3.9)	8 (15.7)	10 (19.6)
Total	241	63 (26.1)	30 (12.4)	93 (38.6)

Table 11

Cumulative Incidence of Cancer in First-Degree Relatives of Ashkenazi Jewish Ovarian-Cancer Patients and Jewish Controls

CUMULATIVE INCIDENCE OF CANCER (%) TO AGE

Site	55 Years[a]				65 Years[a]				75 Years[a]			
	Control	Case	P^b	RR^c	Control	Case	P^b	RR^c	Control	Case	P^b	RR^c
Breast	4.1	9.4	0.0012	2.26	7.8	14.3	0.0004	1.83	10.2	20.2	0.0001	1.97
Ovary	1.1	3.7	0.0233	3.41	2.2	4.8	0.029	2.19	2.9	6.0	0.0349	2.08
Prostate	0.0	0.6	0.081		0.3	0.6	0.339	3.05	2.6	7.5	0.007	3.50
Uterus	0.3	0.6	0.644	1.58	0.3	1.5	0.153	3.22	0.6	2.2	0.163	2.67
Colon	0.4	0.8	0.29	1.88	1.6	2.2	0.310	1.48	3.5	5.3	0.152	1.51
Pancreas	0.0	0.4	0.031		0.1	1.5	0.002	12.66	1.0	2.0	0.027	3.15
Lung	0.1	0.1	0.756	1.55	0.3	1.0	0.167	2.64	2.4	3.4	0.319	1.46
Head & Neck	0.1	0.1	0.747	1.57	0.3	0.8	0.316	2.11	0.5	0.8	0.512	1.58
Melanoma	0.2	0.2	0.656	1.56	0.2	0.2	0.656	1.56	0.4	0.8	0.323	2.09
Primary Site Unknown	0.0	0.6	0.013		0.3	1.0	0.036	4.73	1.5	1.4	0.542	1.37
Any:												
Women[d]	3.9	4.4	0.7865	1.13	6.4	9.2	0.1049	1.43	12.7	16.9	0.0825	1.33
Women	7.4	16.4	0.0001	2.41	15.1	27.2	0.0001	2.06	25.6	40.4	0.0001	1.85
Men	2.8	4.0	0.5118	1.43	6.9	10.9	0.2638	1.59	20.7	27.3	0.0864	1.32
All	5.9	10.0	0.003	1.69	11.7	18.9	0.0003	1.62	22.8	33.5	0.0000	1.47

[a] Kaplan-Meier estimates.

[b] Log-rank test.

[c] Relative risks are obtained from a univariate Cox proportional hazard model. Baseline: index controls.

[d] Any cancer except for breast or ovarian.

Table 12

Cumulative Incidence of Cancer in First-Degree Relatives of Ashkenazi Jewish Women with Ovarian Cancer: Carriers Versus Non-Carriers

CUMULATIVE INCIDENCE OF CANCER (%) TO AGE

Site	55 Years[a] Noncarrier	carrier	P^b	RR^c	65 Years[a] Noncarrier	Carrier	P^b	RR^c	75 Years[a] Noncarrier	Carrier	P^b	RR^c
Breast	6.9	13.5	0.004	1.95	9.0	23.3	0.0073	2.58	16.0	27.4	0.0006	1.72
Ovary	1.6	7.3	0.007	4.47	1.6	10.2	0.0014	6.21	3.6	10.2	0.0051	2.82
Prostate	0.0	1.5	0.078		0.0	1.5	0.078		5.3	9.3	0.109	2.47
Uterus	0.0	1.6	0.078		0.0	3.9	0.011		1.1	3.9	0.053	6.53
Colon	0.7	1.1	0.593	1.54	2.3	2.2	0.978	0.99	5.3	5.7	0.895	1.06
Pancreas	0.5	0.3	0.830	0.77	1.5	1.4	0.945	0.95	1.5	2.9	0.448	1.61
Lung	0.0	0.3	0.211		1.1	0.3	0.590	0.54	3.1	3.4	0.759	1.20
Head & Neck	0.2	0.0	0.427	0.00	1.3	0.0	0.115	0.00	1.3	0.0	0.115	0.00
Melanoma	0.4	0.0	0.250	0.00	0.4	0.0	0.250	0.00	0.9	0.7	0.582	0.54
Primary Site Unknown	0.5	0.4	0.828	0.77	0.8	0.9	0.961	1.05	1.4	0.9	0.798	0.80
Any:												
Women[d]	4.4	4.4	0.8893	1.01	8.9	9.9	0.8322	1.11	17.3	16.1	0.9132	0.93
Women	11.4	24.0	0.002	2.13	18.5	40.2	0.0001	2.31	35.4	48.6	0.001	1.76
Men	3.6	4.6	0.8484	1.29	11.6	9.5	0.4343	0.81	28.0	26.2	0.6161	0.94
All	7.5	14.6	0.0053	1.94	15.1	25.4	0.0114	1.68	31.1	37.9	0.0001	1.22

[a] Kaplan-Meier estimates.

[b] Log-rank test.

[c] Relative risks are obtained from a univariate Cox proportional hazard model. Baseline: index non-carrier cases.

[d] Any cancer except for breast or ovarian.

Table 13

Cumulative Incidence of Cancer in First-Degree Relatives of Ashkenazi Jewish Women with Ovarian Cancer: Women Positive for BRCA1 Mutations Versus BRCA2 Mutations

CUMULATIVE INCIDENCE OF CANCER (%) TO AGE

Site	55 Years[a]				65 Years[a]				75 Years[a]			
	BRCA1	BRCA2	P[b]	RR[c]	BRCA1	BRCA2	P[b]	RR[c]	BRCA1	BRCA2	P[b]	RR[c]
Breast	18.4	6.0	0.175	0.33	26.3	18.8	0.0256	0.71	29.0	25.1	0.3327	0.87
Ovary	7.1	7.5	0.3878	1.06	8.7	11.9	0.934	1.37	8.7	11.9	0.559	1.37
Prostate	1.0	2.7	0.618	2.00	1.0	2.7	0.618	2.00	8.0	12.0	0.626	1.45
Uterus	2.8	0.0	0.233	0.00	6.6	0.0	0.092	0.00	6.6	0.0	0.092	0.00
Colon	1.0	1.2	0.914	0.88	1.0	4.4	0.275	2.61	5.3	6.4	0.699	1.30
Pancreas	0.4	0.0	0.450	0.00	0.4	2.9	0.289	3.39	1.5	5.0	0.308	2.46
Lung	0.5	0.0	0.450	0.00	0.5	0.0	0.450	0.00	4.6	1.8	0.398	0.40
Head & Neck	0.0	0.0			0.0	0.0			0.0	0.0		
Melanoma	0.0	0.0			0.0	0.0			0.0	1.8	0.182	
Primary Site Unknown	0.0	1.1	0.188		0.0	2.5	0.066		0.0	2.5	0.066	
Any:												
Women[d]	5.2	3.2	0.941	0.62	9.8	10.0	0.8936	1.02	14.1	18.5	0.703	1.31
Women	28.8	16.6	0.041	0.48	41.2	38.8	0.249	0.73	46.6	51.0	0.508	0.85
Men	2.9	7.8	0.2143	2.68	4.3	18.5	0.0334	4.31	21.9	33.7	0.0769	1.54
All	15.9	12.3	0.3387	0.78	23.1	28.9	0.593	1.25	34.8	42.5	0.3606	1.22

[a] Kaplan-Meier estimates.
[b] Log-rank test.
[c] Relative risks are obtained from a univariate Cox proportional hazard model. Baseline: positive for BRCA1 mutations.
[d] Any cancer except for breast or ovarian.

Table 14

Risk of Cancer in the Mothers Versus the Sisters of Ashkenazi Jewish Women with Ovarian Cancer

Type of Analysis	Probability to age 65, mothers	Probability to age 65, sisters	p value to age 65
Breast cancer in mothers & sisters of mutation carriers	20.1%	31.2%	0.2889
Ovarian cancer in mothers & sisters of mutation carriers	9.1%	13.9%	0.4018
Breast cancer in mothers & sisters of BRCA1 carriers	21.8%	41.0%	0.4645
Ovarian cancer in mothers & sisters of BRCA1 carriers	5.9%	17.1%	0.8178
Breast cancer in mothers & sisters of BRCA2 carriers	17.1%	20.6%	0.3434
Ovarian cancer in mothers & sisters of BRCA2 carriers	14.6%	10.3%	0.0784

Table 15

Penetrance of Breast Cancer for the *BRCA1* and *BRCA2* Mutations

	Penetrance to Age 55	Penetrance to Age 75
BRCA1	29.9%	42.1%
BRCA2	5.1%	34.2%
Noncarriers[a]	6.9%	16.0%

[a] Risk of breast cancer in the female relatives of non-carrier cases

Table 16

Penetrance of Ovarian Cancer for the *BRCA1* and *BRCA2* Mutations

	Penetrance to Age 55	Penetrance to Age 75
BRCA1	12.6%	13.8%
BRCA2	13.4%	20.2%
Noncarriers[a]	1.6%	3.6%

[a] Risk of ovarian cancer in the relatives of non-carrier cases

Table 17

Risks factors and Ovarian Cancer: Matched Analysis

	Carrier Cases			non-carrier cases		
	OR[α]	95% CI	P[α]	OR[β]	95% CI	P[B]
Pregnancy						
yes versus no	0.71	[0.20, 2.50]	0.773	0.82	[0.31, 2.12]	0.8231
Parity						
< 3 births	1.90	[0.84, 4.38]	0.137	1.07	[0.49, 2.35]	1.00
Breast Feeding	1.36	[0.65, 2.85]	0.486	1.50	[0.73, 3.11]	0.311
Oral contraceptives						
>1 year	0.44	[0.18, 1.08]	0.078	1.0	[0.46, 2.16]	0.8551
Tubal ligation	1.38	[0.51, 3.73]	0.646	0.78	[0.26, 2.27]	0.8026
Vaginal Talc	1.90	[0.84, 4.38]	0.1374	2.40	[1.10, 5.37]	0.0258

[α] Odds ratios (OR) and Confidence Intervals (CI) are based on the ratio of concordant and discordant pairs while P-values are based on McNemar's test. The carrier cases are compared to the controls.

[β] Odds ratios (OR) and Confidence Intervals (CI) are based on the ratio of concordant and discordant pairs while P - values are based on McNemar's test. The non-carrier cases are compared to the controls.

Table 18

Logistic Regression Analysis of Risk factors for Ovarian Cancer in Cases with *BRCA1* and *BRCA2* Mutations Versus the Controls

Variable	Definition	Univariate Analysis OR (95%CI)	P	Multivariate Analysis OR (95%CI)	P
Age	continuous	1.04 (1.02-1.06)	0.0000	1.04 (1.02-1.06)	0.0001
Age at menarche	continuous	1.01 (0.88-1.16)	0.8772	0.96 (0.83-1.10)	0.5408
Parity	continuous	0.98 (0.84-1.14)	0.7888	0.95 (0.81-1.12)	0.5519
Height	continuous	1.00 (0.99-1.02)	0.4091	1.00 (0.99-1.02)	0.6070
Oral Contraceptives	< 1 year	1.14 (0.92-1.41)	0.2380	1.08 (0.90-1.29)	0.4124
Pack-years	continuous	1.01 (0.99-1.02)	0.2865	1.00 (0.99-1.01)	0.9869
Vaginal Talc	binary	2.43 (1.38-4.27)	0.0020	2.34 (1.30-4.20)	0.0054

OR = odds ratio; CI = confidence interval. In multivaraite model, I also adjusted for residence (Canada, US, Israel).
The carrier cases were compared to the controls. All *P* - values are based on the conditional logistic regression model.

Table 19

Logistic Regression Analysis of Risk Factors for Ovarian Cancer in Non-Carrier Cases Versus the Controls

Variable	Definition	Univariate Analysis OR (95%CI)	P	Multivariate Analysis OR (95%CI)	P
Age	continuous	1.05 (1.03-1.07)	0.0000	1.06 (1.04-1.08)	0.0000
Age at menarche	continuous	1.08 (0.96-1.21)	0.2159	0.98 (0.87-1.12)	0.8189
Parity	continuous	1.07 (0.95-1.20)	0.2831	1.04 (0.92-1.18)	0.5336
Height	continuous	1.02 (1.00-1.05)	0.0431	1.06 (1.02-1.09)	0.0006
Oral Contraceptives	< 1 year	1.40 (0.94-2.08)	0.0999	1.08 (0.90-1.29)	0.389
Pack-years	continuous	1.01 (1.00-1.02)	0.0966	1.00 (0.99-1.01)	0.5118
Vaginal Talc	binary	2.20 (1.32-3.67)	0.0025	2.26 (1.30-3.94)	0.0042

OR = odds ratio; CI = confidence interval. In multivaraite model, I also adjusted for residence (Canada, US, Israel).
The non-carrier cases were compared to controls. All *P* - values are based on the conditional logistic regression model.

108

Table 20

Ovarian Tumour Histology in Carrier Cases Versus Non-Carrier Cases (n=203)

Histology	Mutation Carriers	*BRCA1*	*BRCA2*	Non-Carriers	Total
Serous	54	35	19	58	112
Mucinous	1	1	0	7	8
Endometrioid	6	3	3	6	12
Clear Cell	1	1	0	6	7
Mixed	4	3	1	8	12
Mullerian	5	3	2	5	10
Transitional Cell	0	0	0	1	1
Brenner Tumour	0	0	0	1	1
Unclassified	15	12	3	25	40

Table 21

Analysis of Ovarian Tumour Histology in Carrier Cases Versus Non-Carrier Cases

	Carrier Cases	Non-Carrier Cases
Total	86	117
Serous Tumours (%)	54 (62.8)	58 (49.6)
Other Tumours (%)	32 (37.2)	59 (50.4)

P- value =0.061 calculated using the Chi-Square tests.

Table 22

Degree of Differentiation (Grade) of the Ovarian Tumour in Carrier Cases Versus the Non-Carrier Cases (n=171)

Grade	Carrier Cases	*BRCA1*	*BRCA2*	Non-Carriers	Total
GI (%)	2 (2.7)	2	0	13 (13.5)	15
GII (%)	5 (6.7)	1	4	17 (17.7)	22
GIII(%)	68 (90.7)	48	20	66 (68.7)	134

P- value =0.002, estimated using the Kruskal-Wallis Test.

Table 23

Stage of Ovarian Tumour in Carrier Cases Versus Non-Carrier Cases (n=189)

Stage	Carrier Cases	*BRCA1*	*BRCA2*	Non-Carriers	Total
Stage I (%)	9 (10.8)	7	2	23 (21.7)	32
Stage II (%)	9 (10.8)	7	2	7 (6.6)	16
Stage III (%)	59 (71.1)	35	24	69 (65.1)	128
Stage IV (%)	6 (7.2)	6	0	7 (6.6)	13

Figure 1

Probability of cancer in first-degree relatives of cases & controls
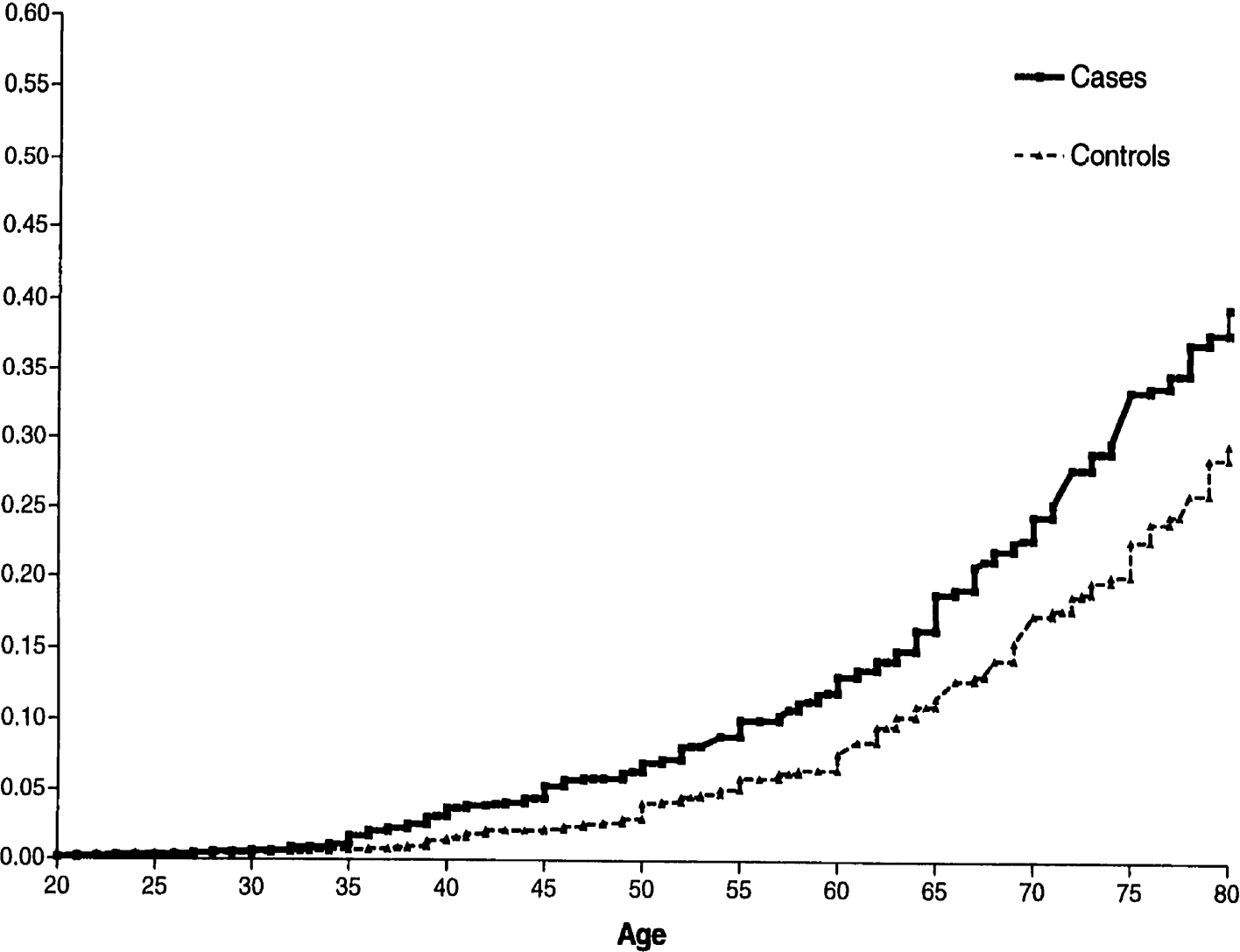
Figure 2

Probability of any cancer but breast & ovary in female first-degree relatives of cases & controls

114
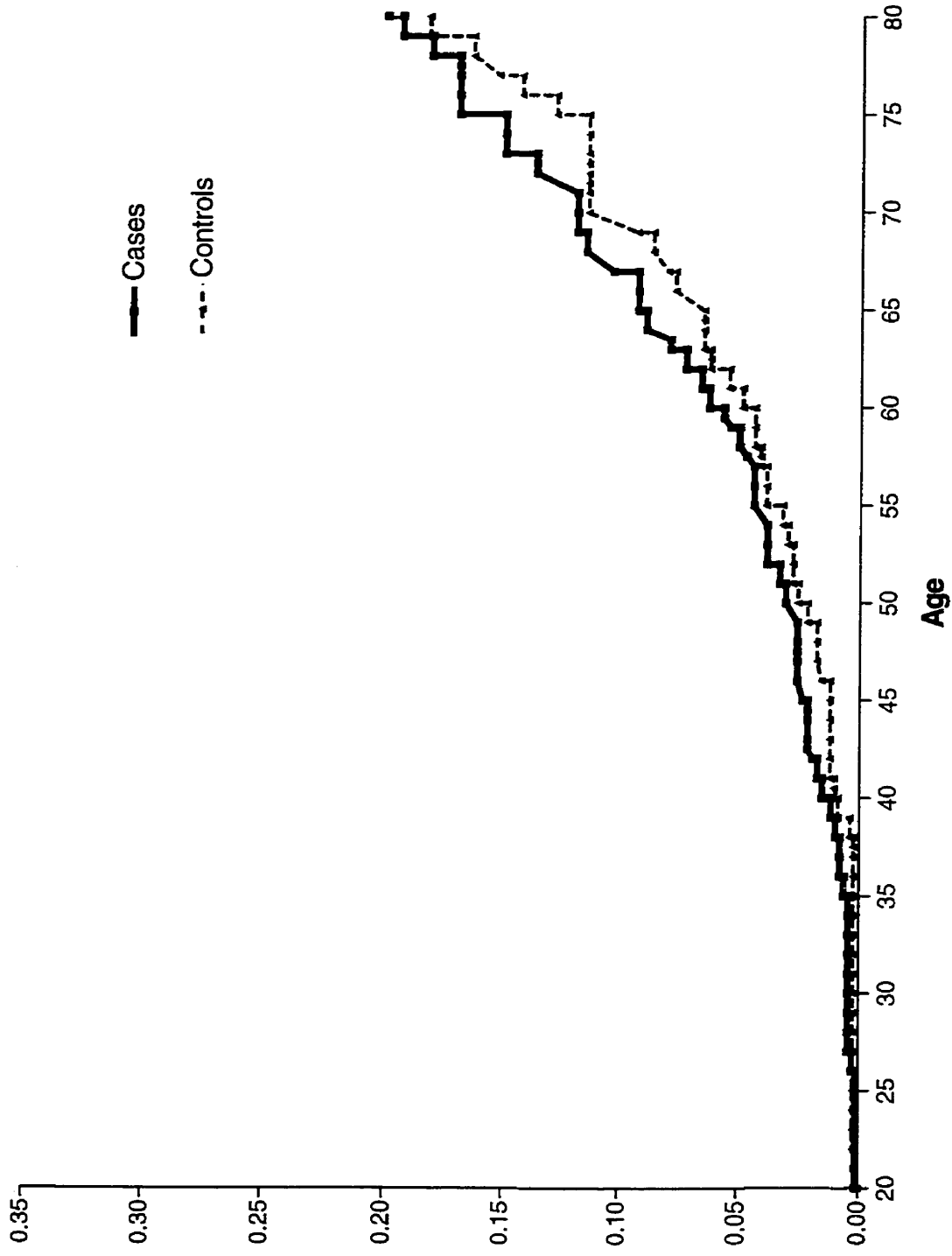
Figure 3

Probability of cancer in male first-degree relatives of cases & controls

Cases
Controls

Age

115
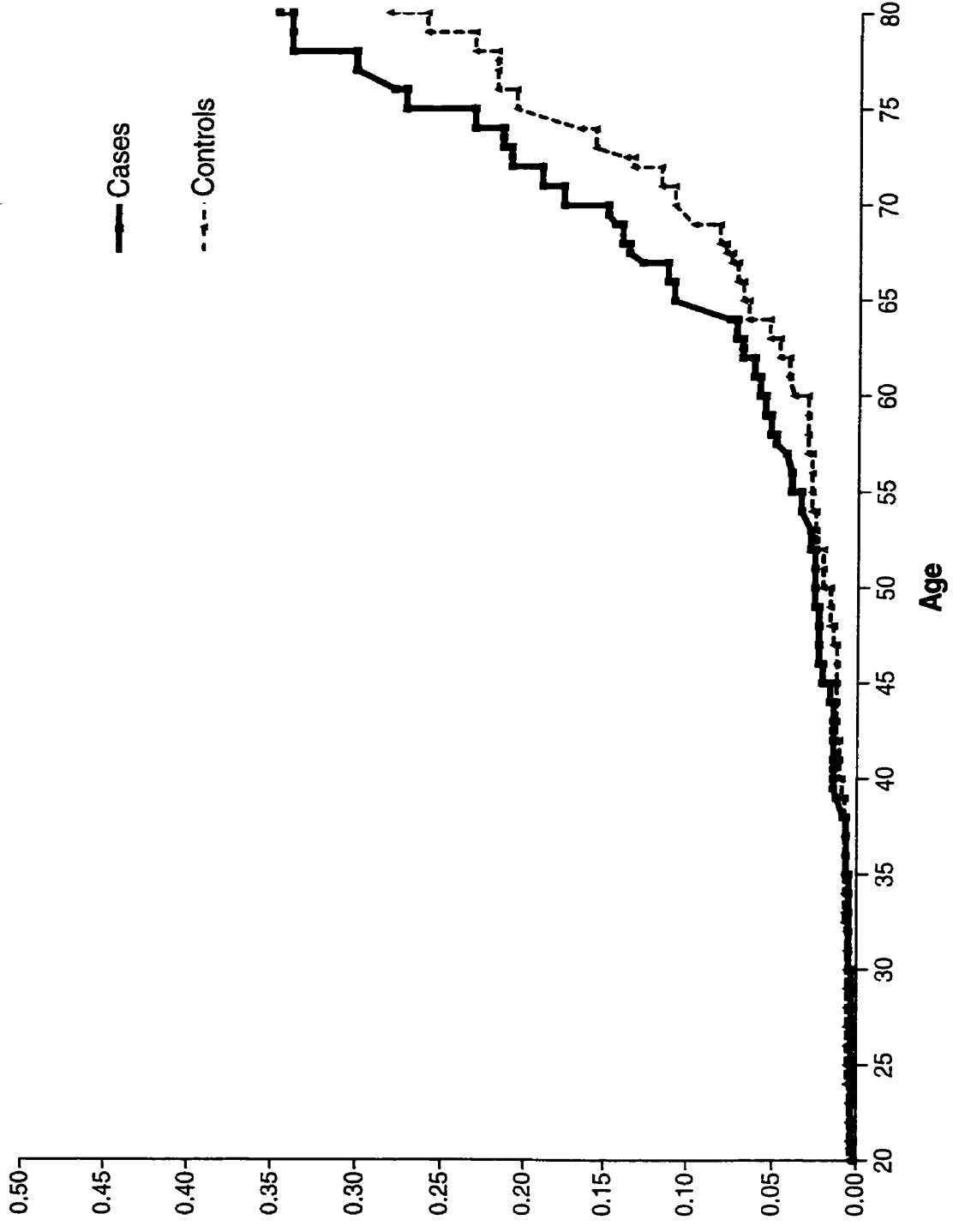
Figure 4

Probability of breast cancer in female first-degree relatives of cases & controls

116
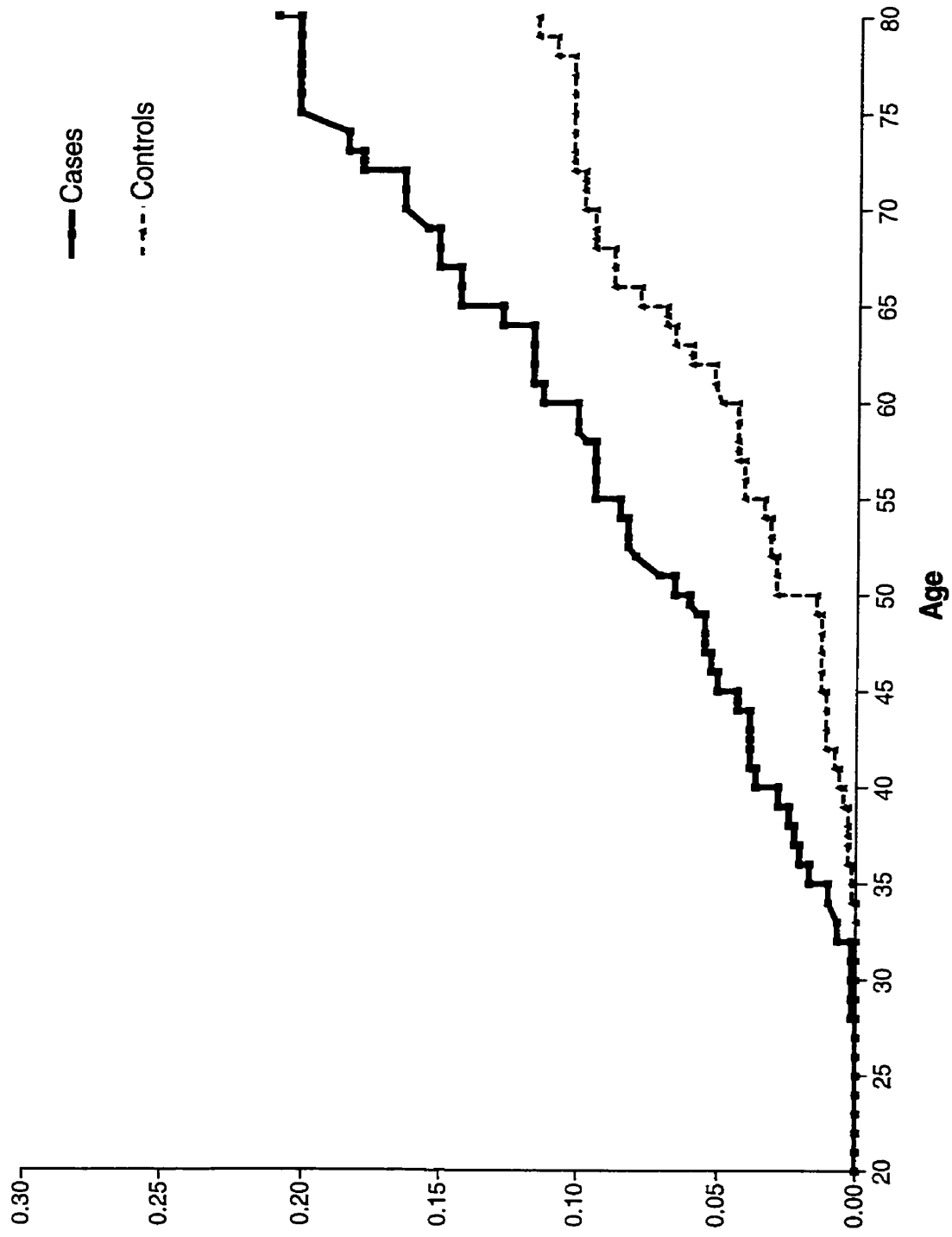
Figure 5

Probability of ovarian cancer in female first-degree relatives of cases & controls

— Cases
- · - Controls

Age

117
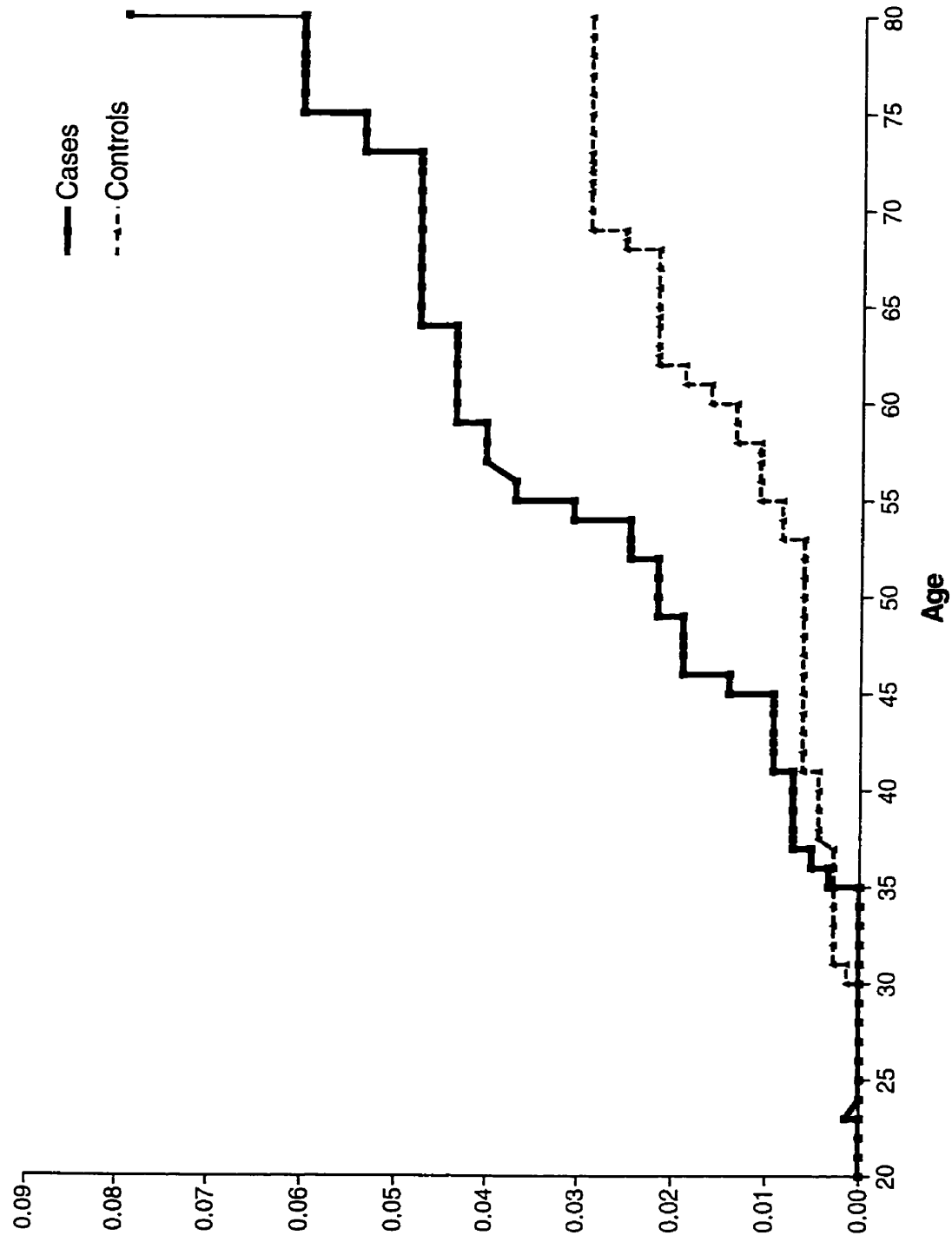
Figure 6

Probability of cancer in first-degree relatives of *BRCA1* & *BRCA2* mutation carriers & non-carriers

— non-carriers

---- carriers

Age

118
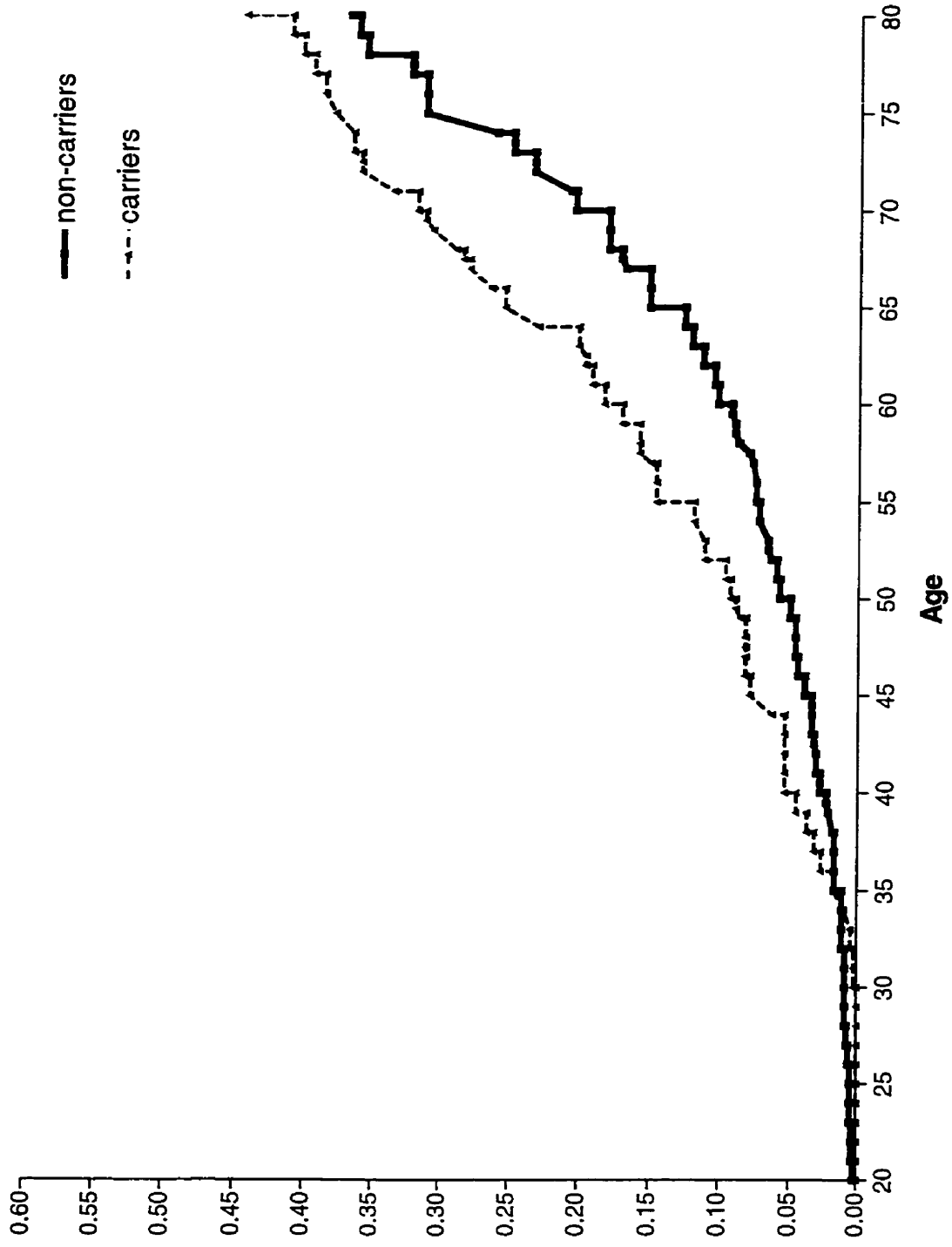
Figure 7

Probability of any cancer but breast & ovarian in female first-degree relatives of *BRCA1* & *BRCA2* mutation carriers & non-carriers
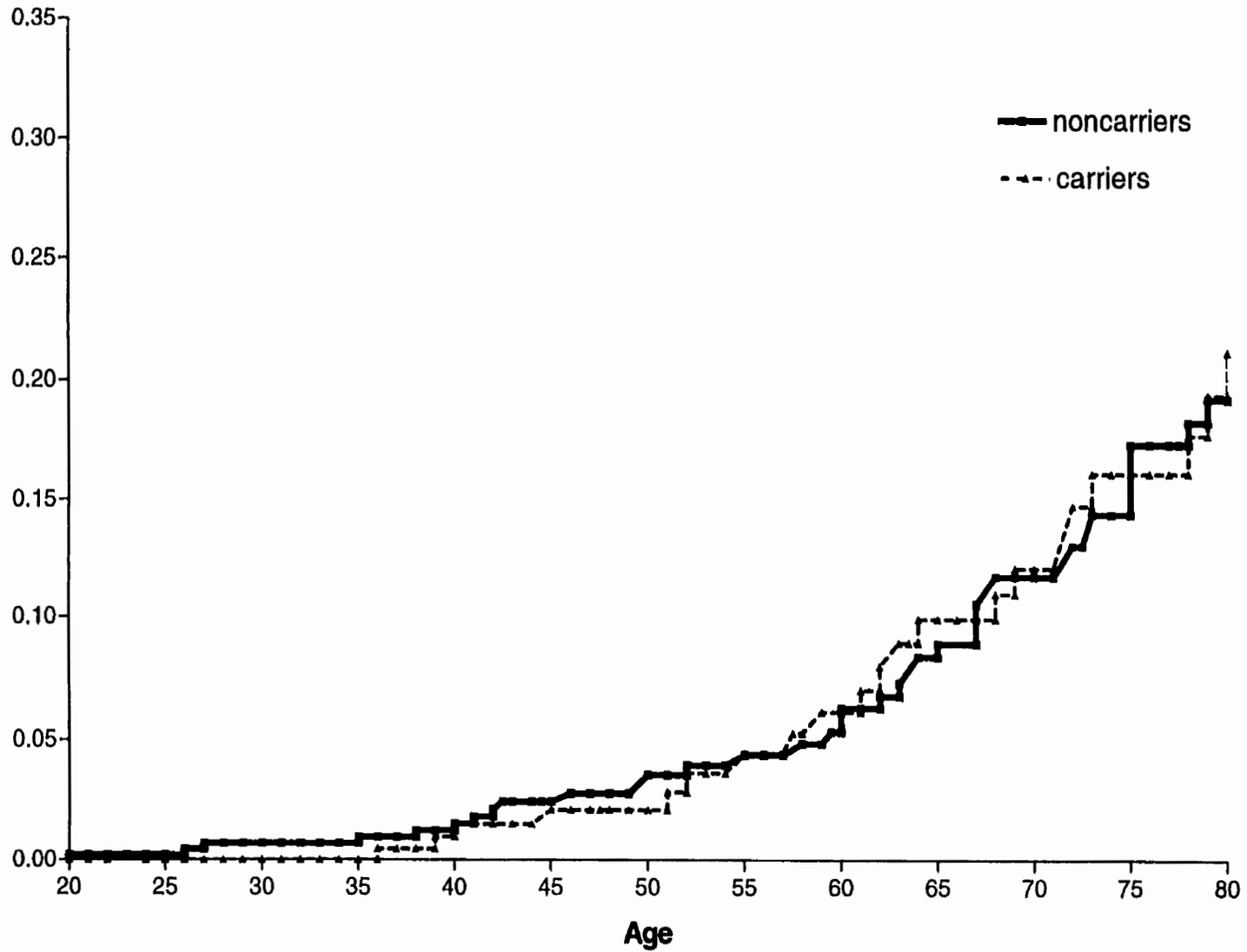
Figure 8

Probability of cancer in male first-degree relatives of *BRCA1* & *BRCA2*
mutation carriers & non-carriers
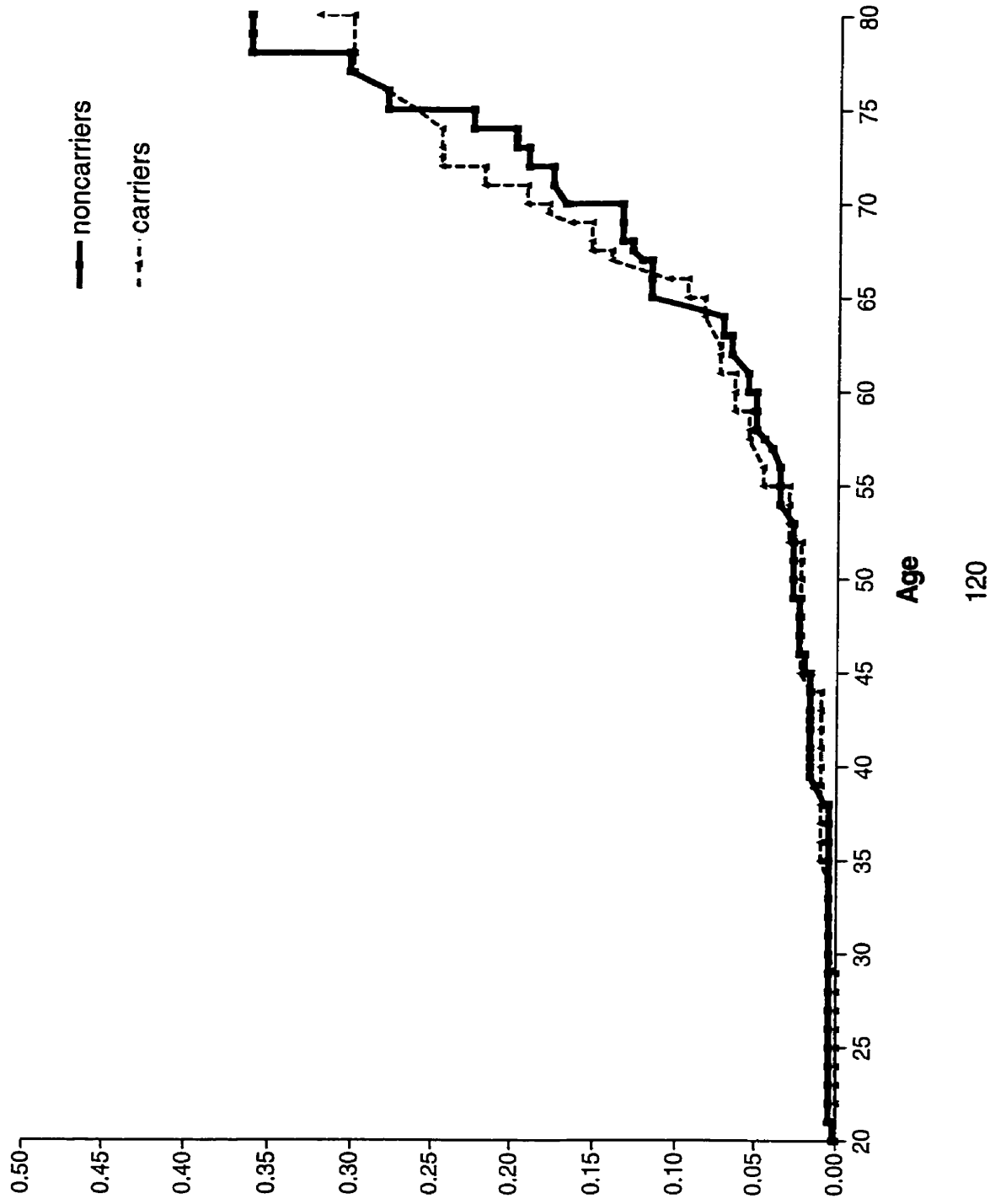
Figure 9

Probability of breast cancer in female first-degree relatives of *BRCA1* & *BRCA2* mutation carriers & non-carriers

121
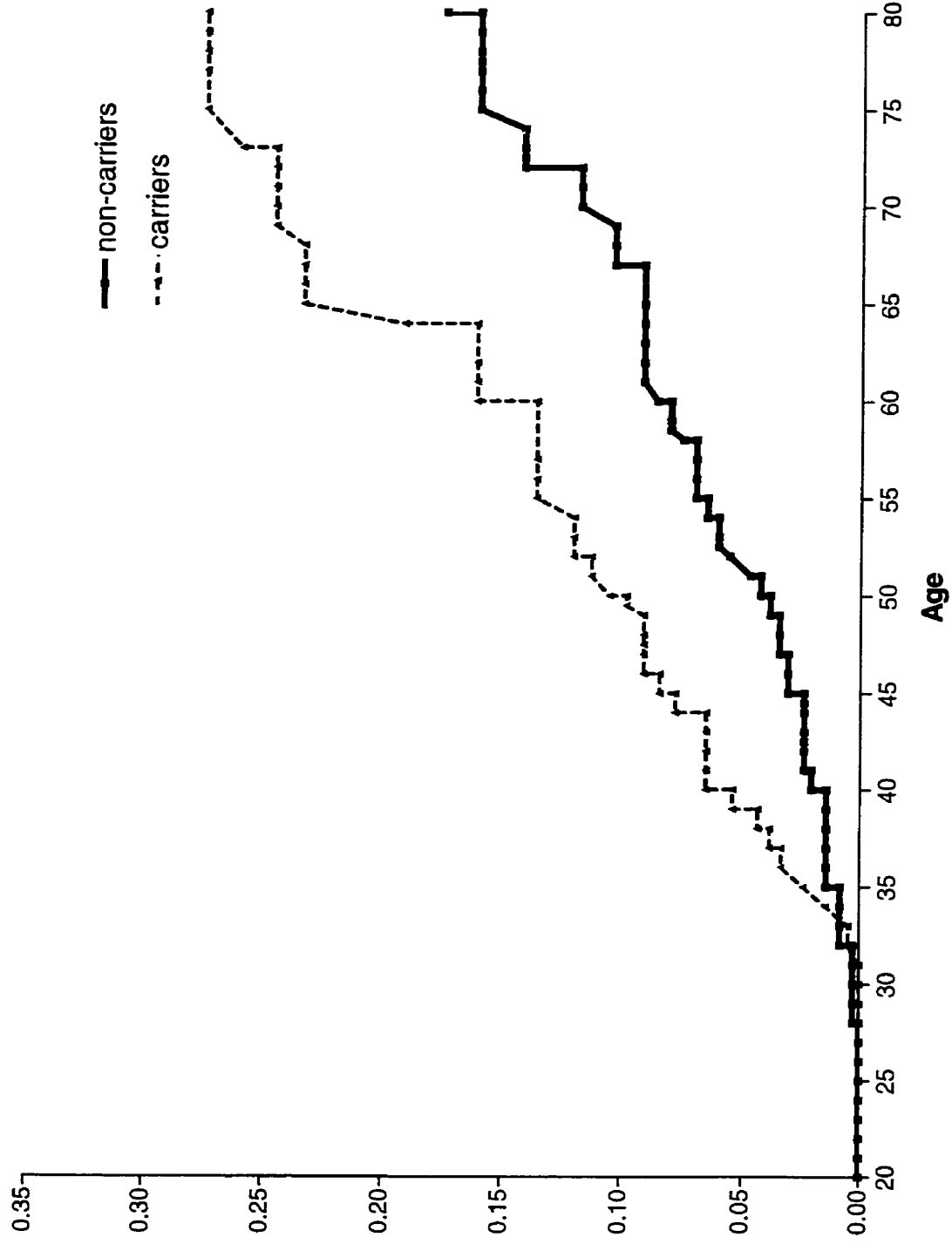
Figure 10

Probability of ovarian cancer in female first-degree relatives of *BRCA1* & *BRCA2* mutation carriers & non-carriers
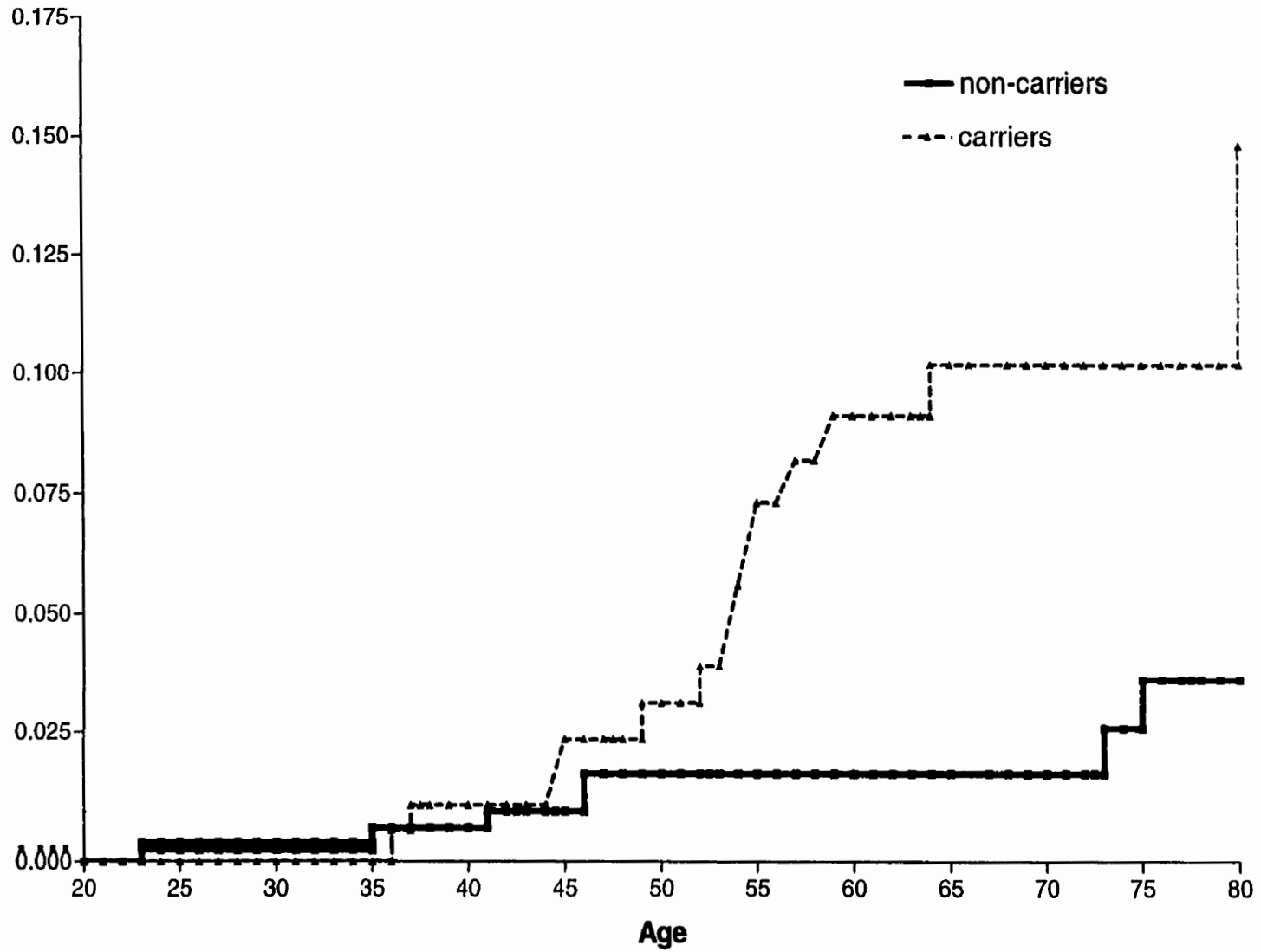
Figure 11

Probability of cancer in first-degree relatives of *BRCA1* & *BRCA2* mutation carriers

123
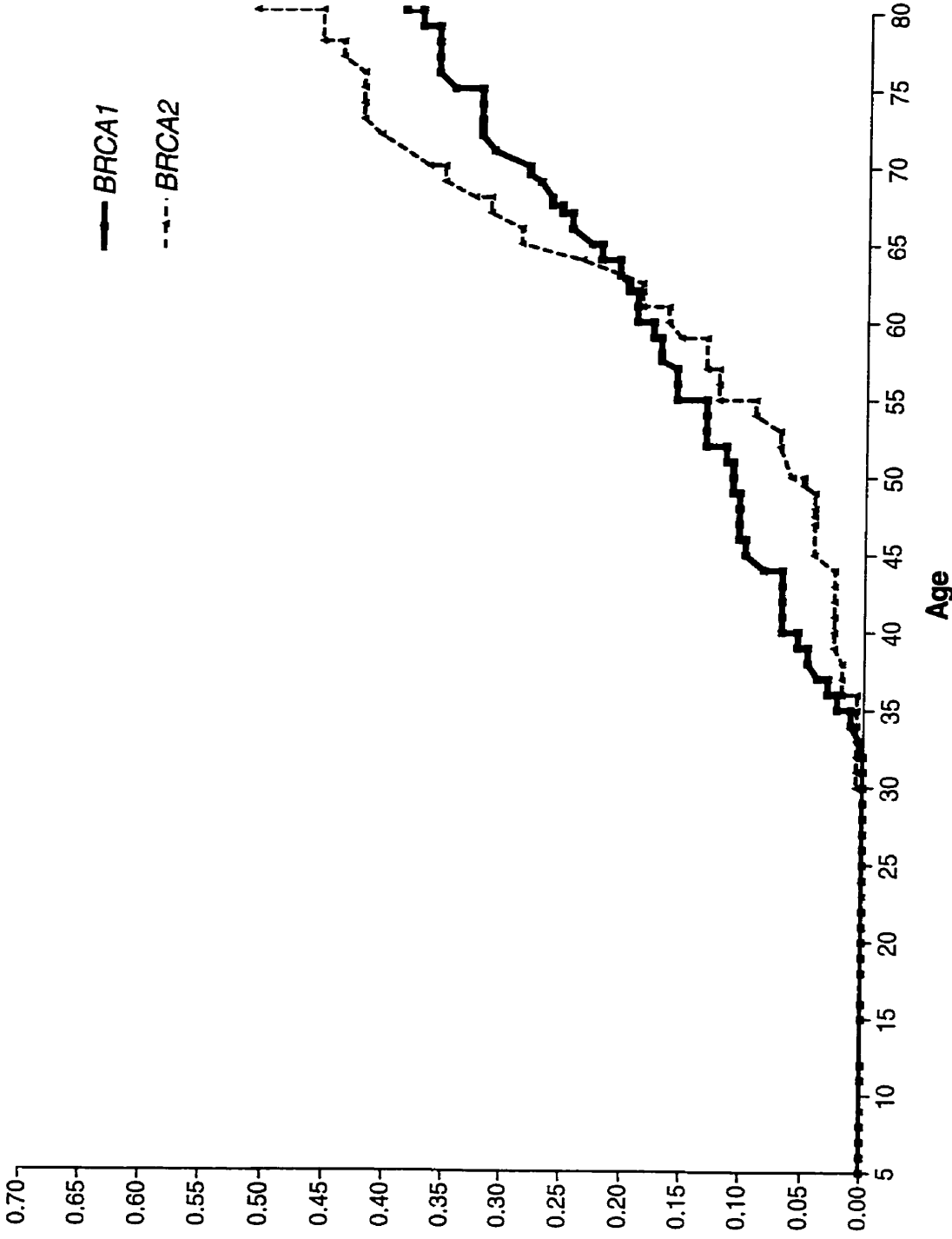
Figure 12

Probability of any cancer but breast and ovarian in female first-degree relatives of *BRCA1* & *BRCA2* mutation carriers
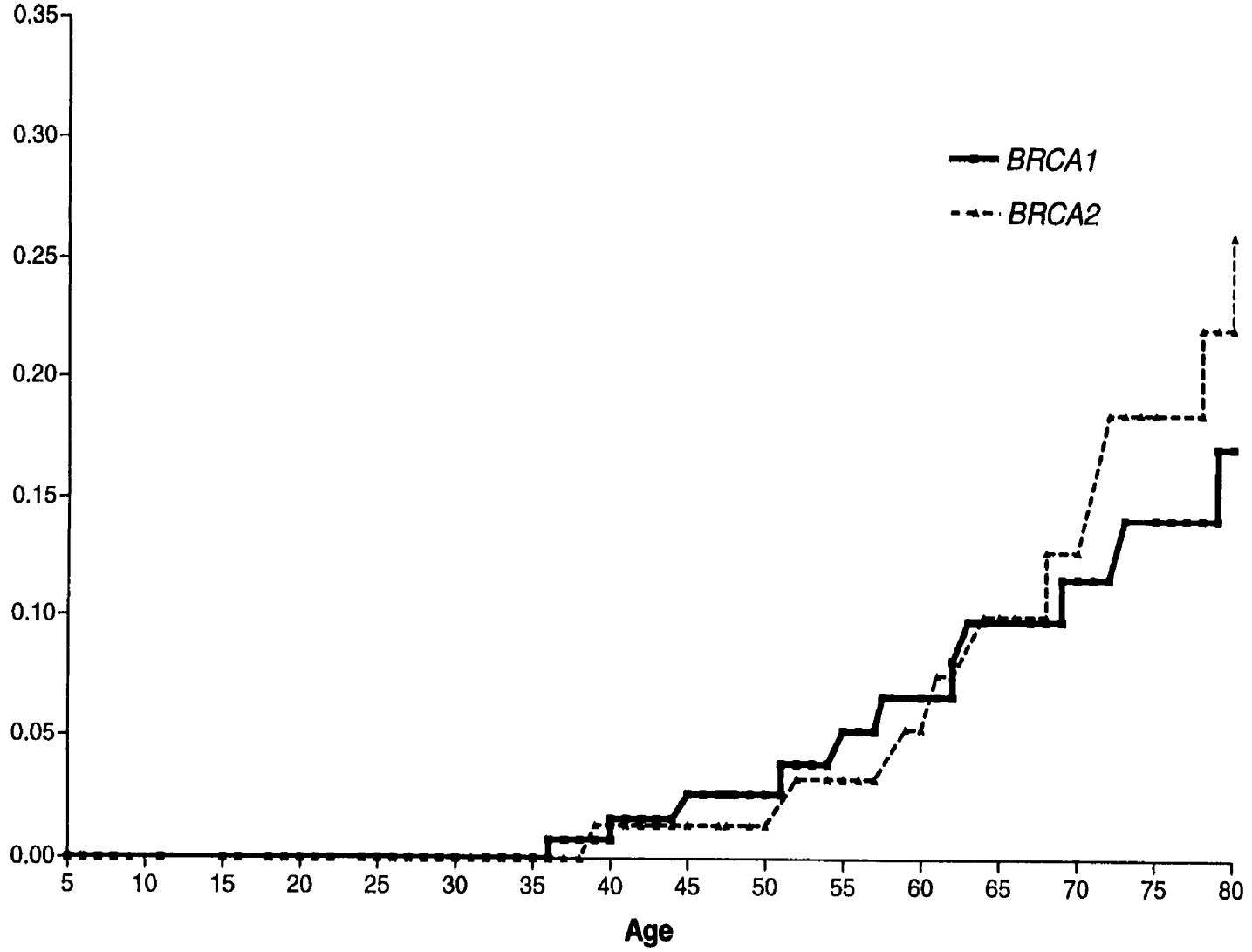
Figure 13

Probability of cancer in male first-degree relatives of *BRCA1* & *BRCA2* mutation carriers

125
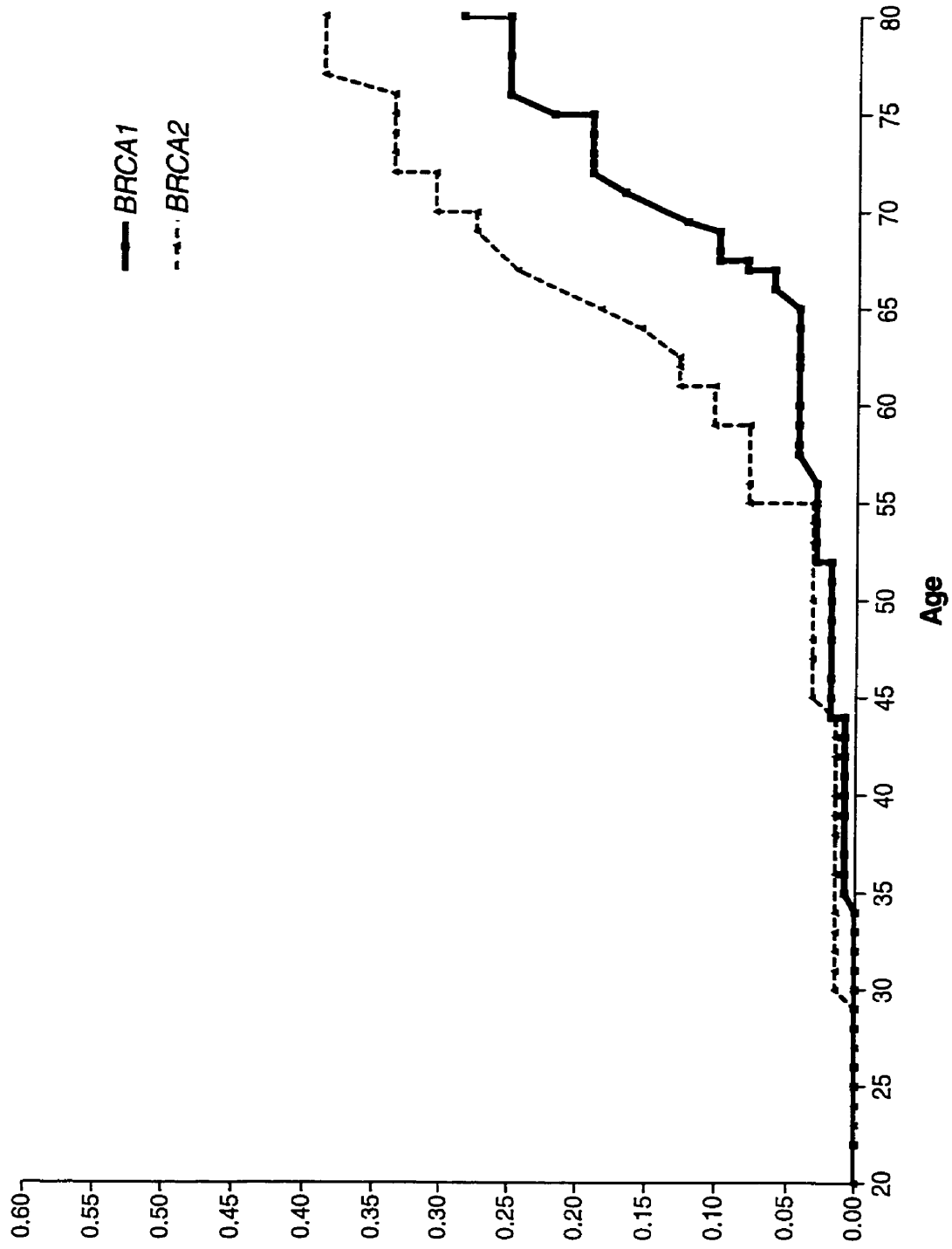
Figure 14

Probability of breast cancer in female first-degree relatives of *BRCA1* & *BRCA2* mutation carriers
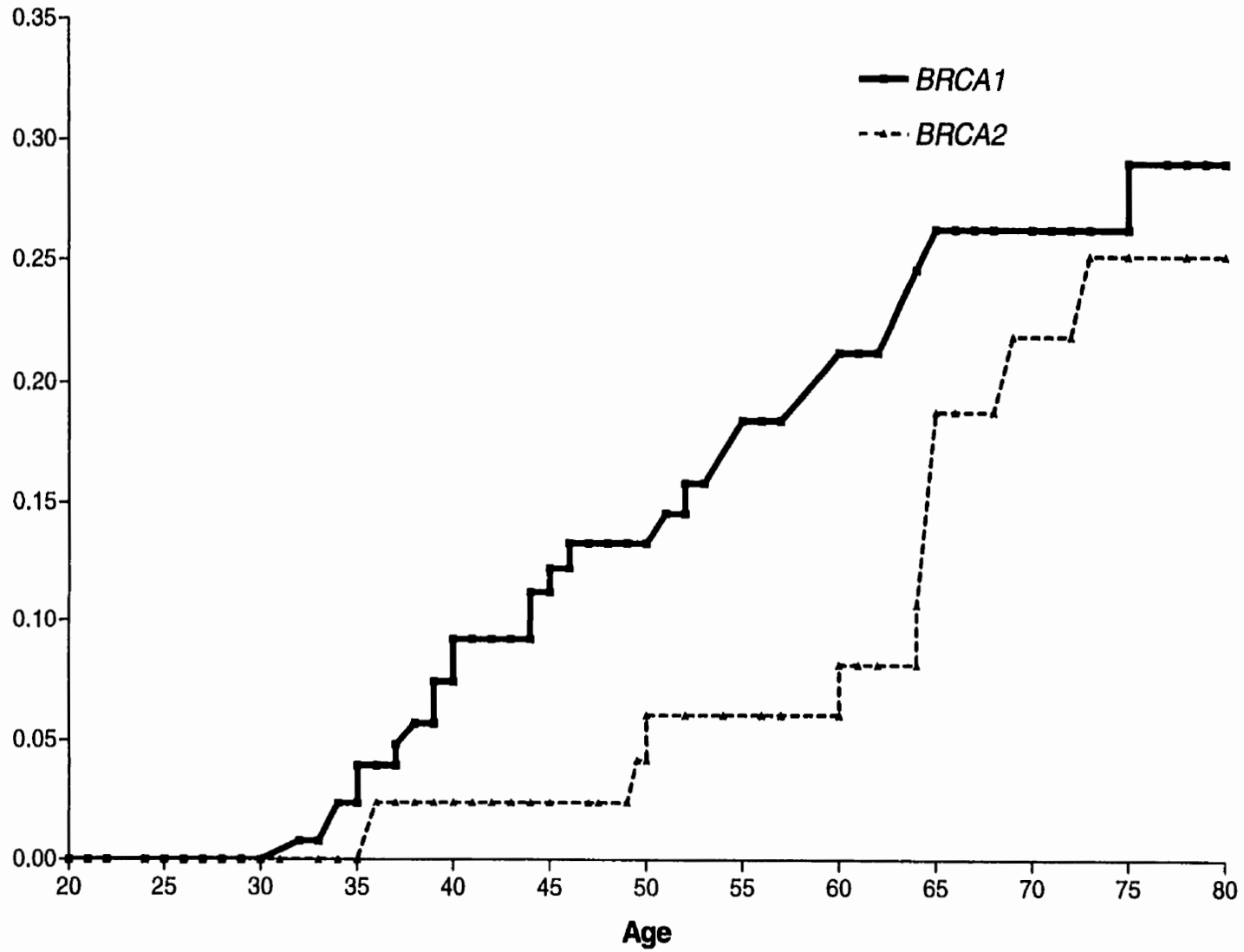
Figure 15

Probability of ovarian cancer in female first-degree relatives of *BRCA1* & *BRCA2* mutation carriers

127
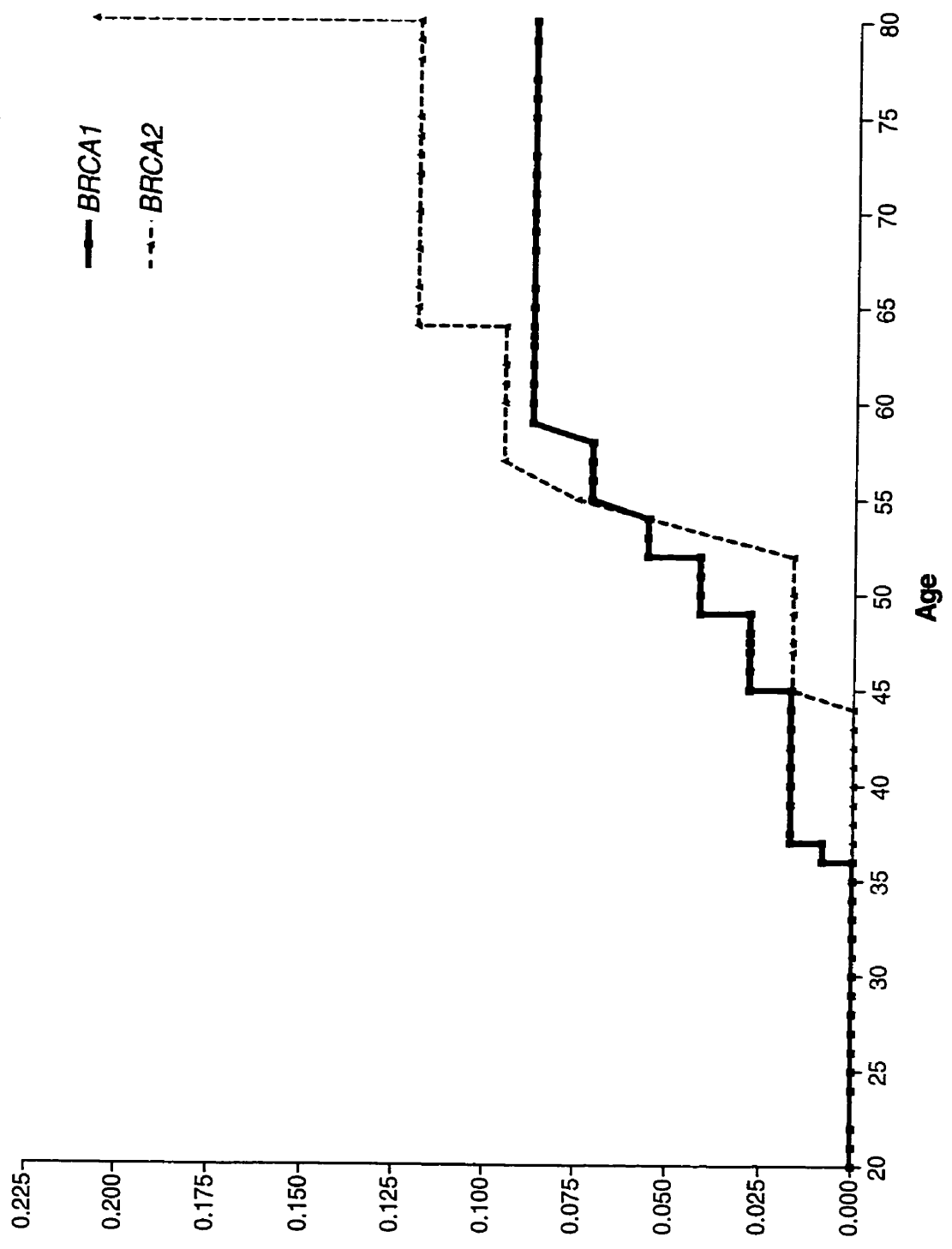
Figure 16

Probability of breast cancer in mothers & sisters of *BRCA1* & *BRCA2* mutation carriers with ovarian cancer

128
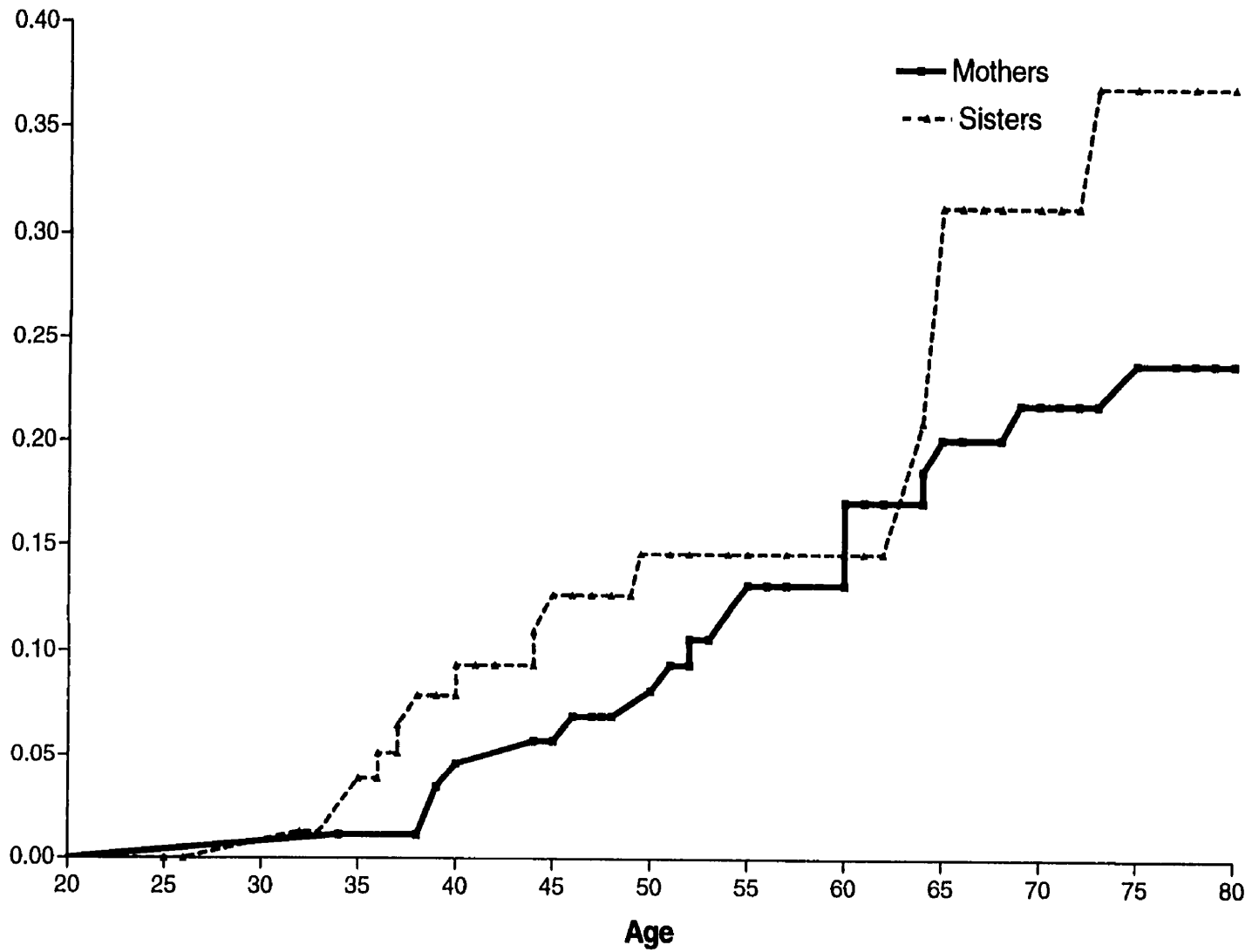
Figure 17

Probability of ovarian cancer in mothers & sisters of *BRCA1* & *BRCA2* mutation carriers with ovarian cancer
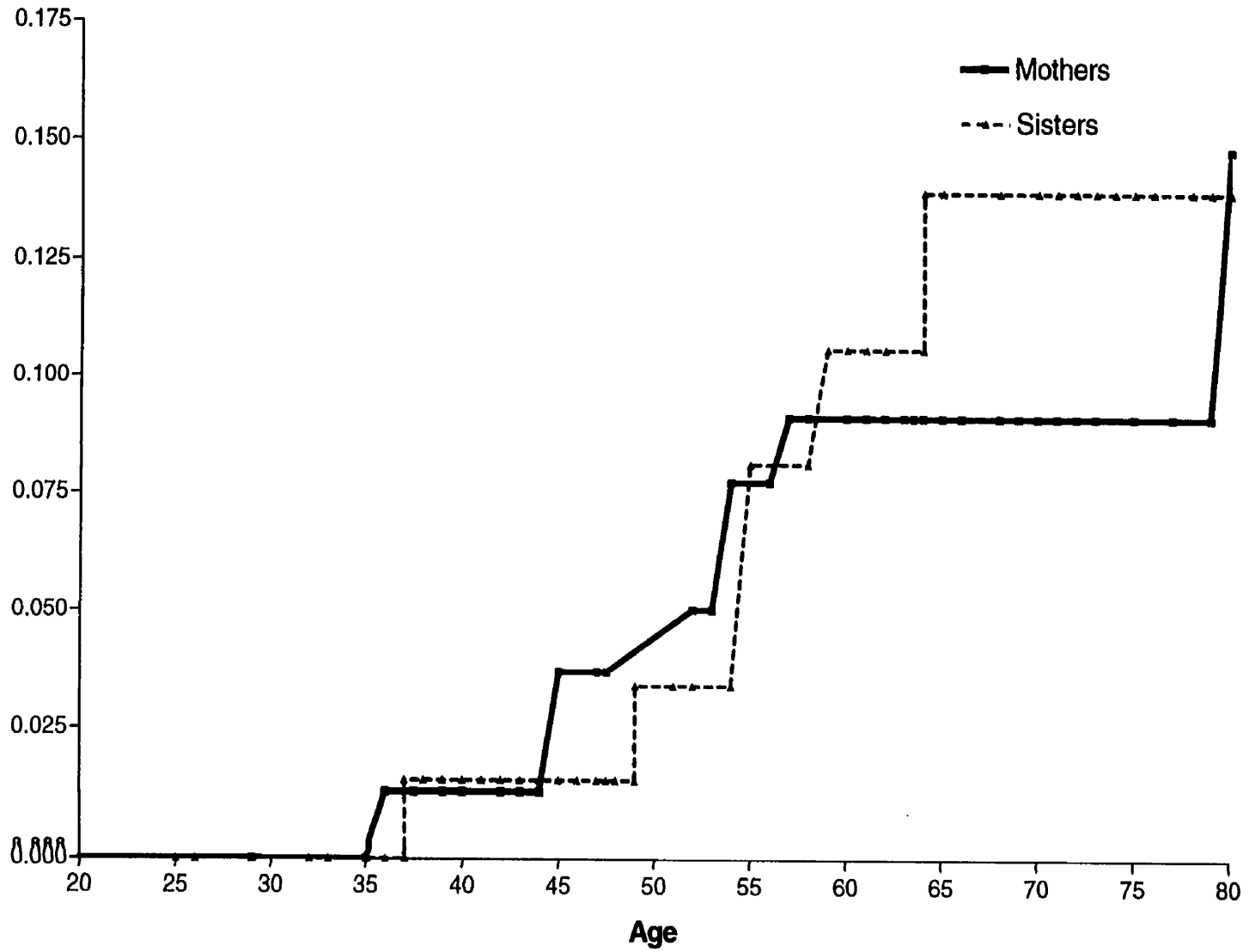
Figure 18

Probability of breast cancer in mothers & sisters of *BRCA1* mutation carriers with ovarian cancer
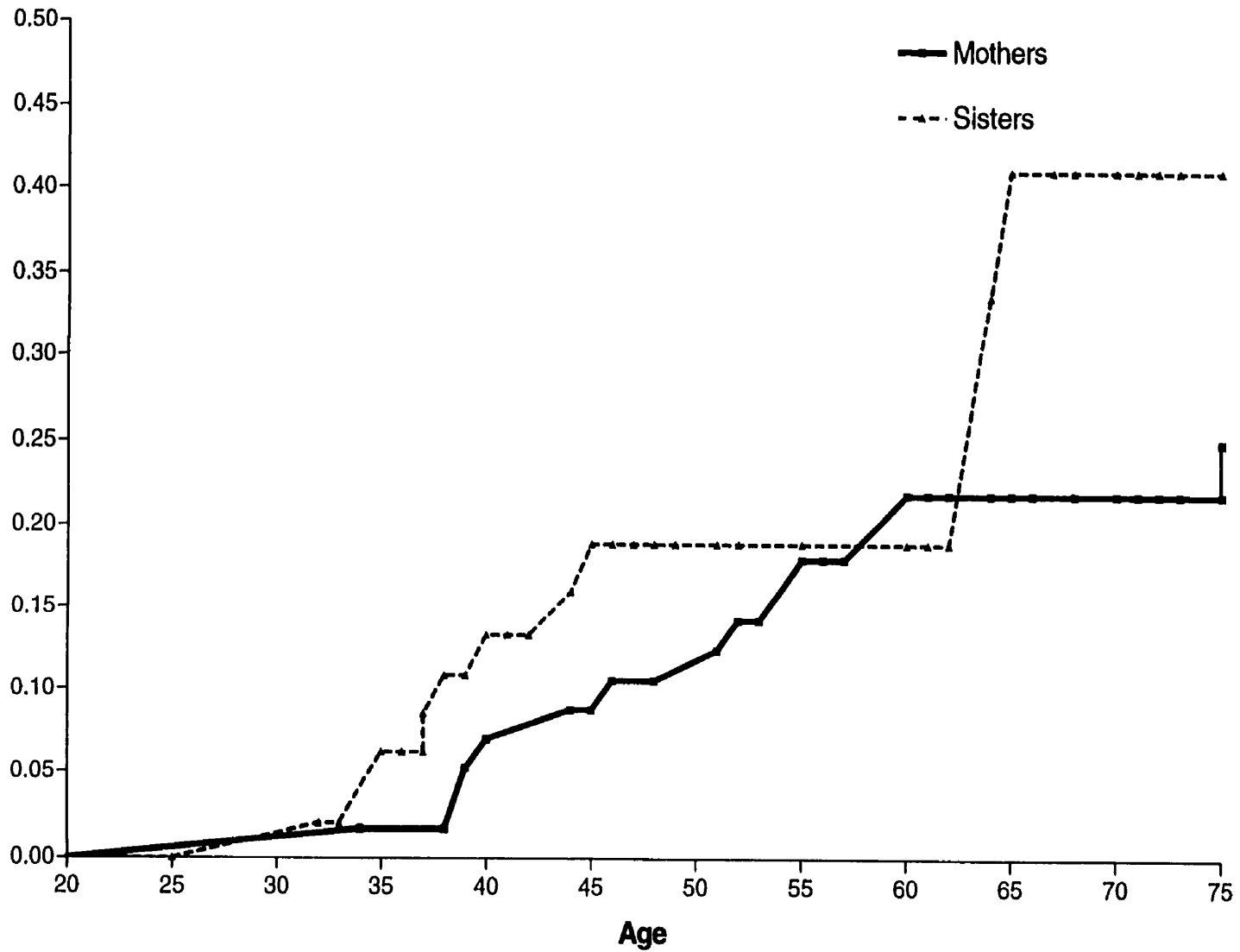
Figure 19

Probability of ovarian cancer in mothers & sisters of *BRCA1* mutation carriers with ovarian cancer
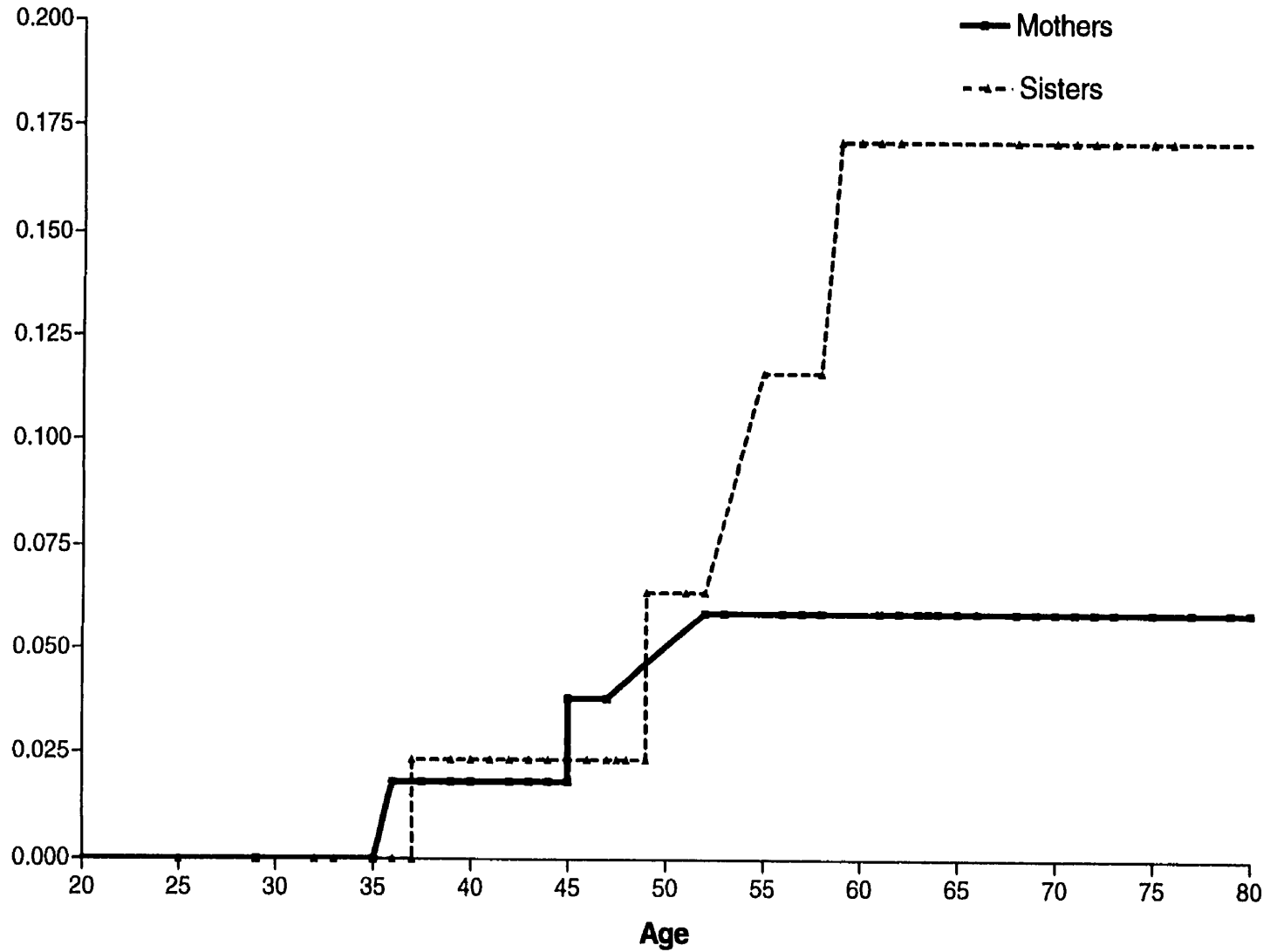
Figure 20

Probability of breast cancer in mothers & sisters of *BRCA2* mutation carriers with ovarian cancer
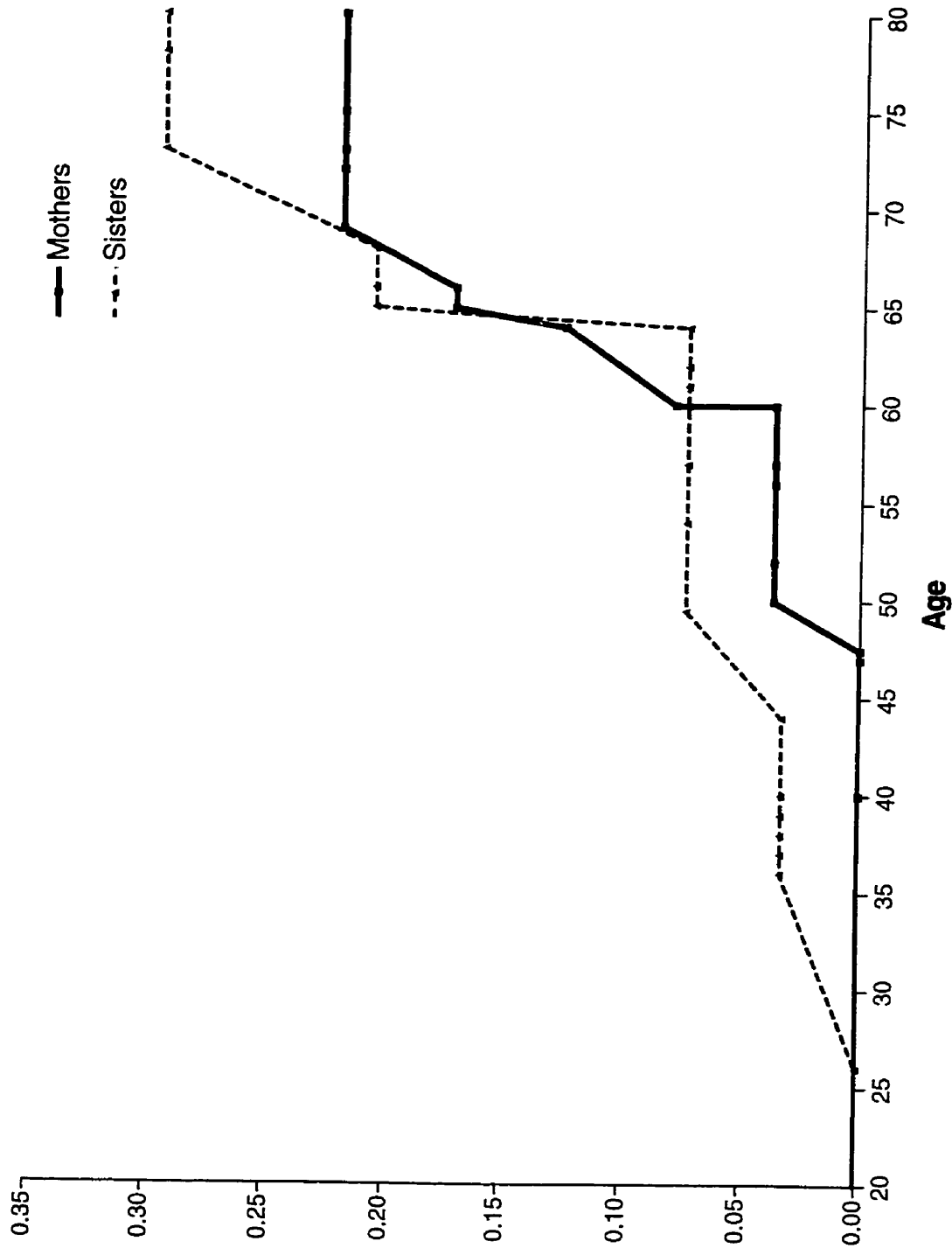
Figure 21

probability of ovarian cancer in mothers & sisters of *BRCA2* mutation carriers with ovarian cancer

133
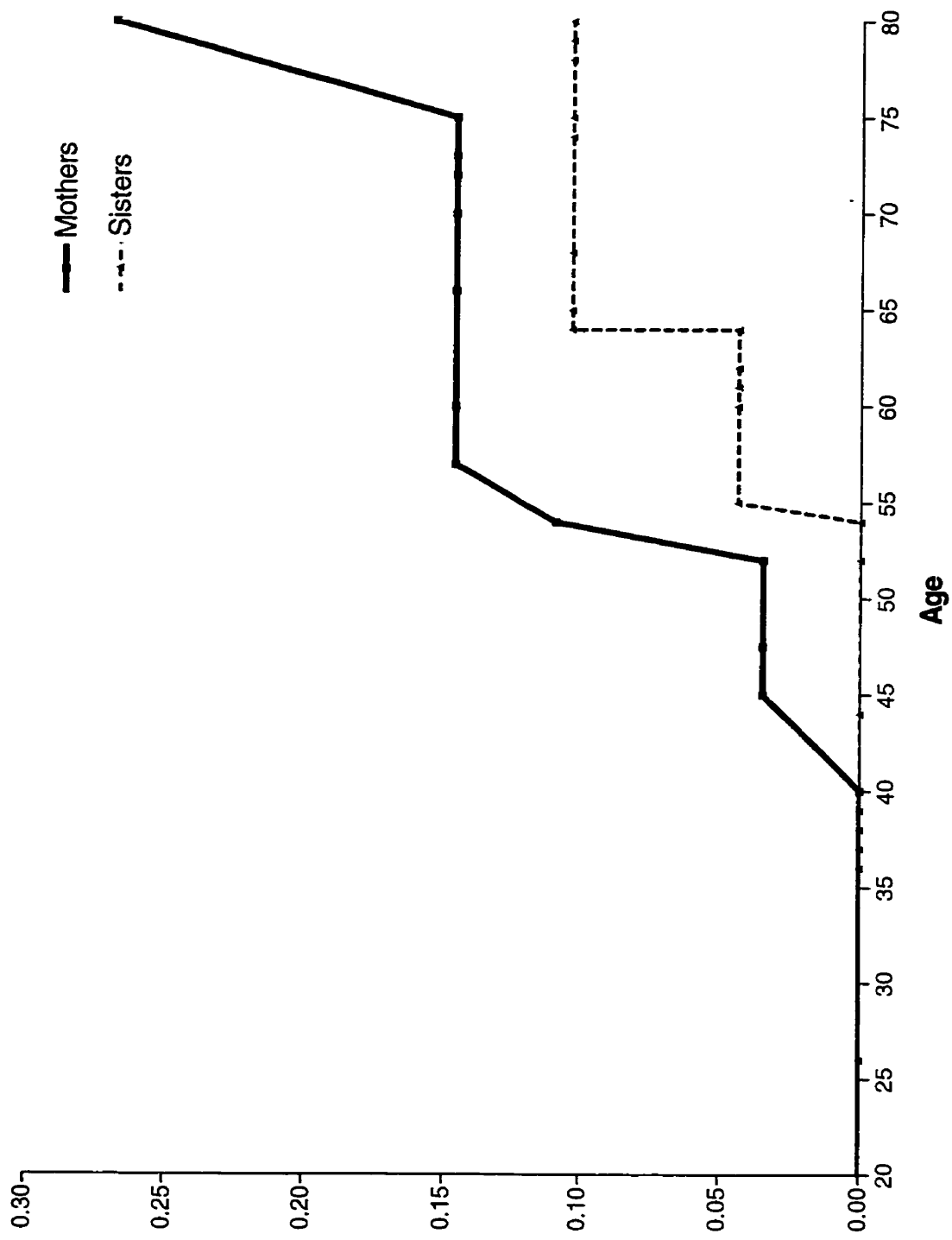
FIGURE 22

Pedigree of Family R014 Containing an Individual Heterozygous for Mutations in

Both the *BRCA1* and *BRCA2* Genes

Family R014

Breast <50 Breast <50

Breast Breast

Breast 60s

Ovarian 36

melanoma
50

(BRCA1 185delAG)
(BRCA2 6174delT)

Breast 30,38
Ovarian 39
(BRCA1 185delAG)

135
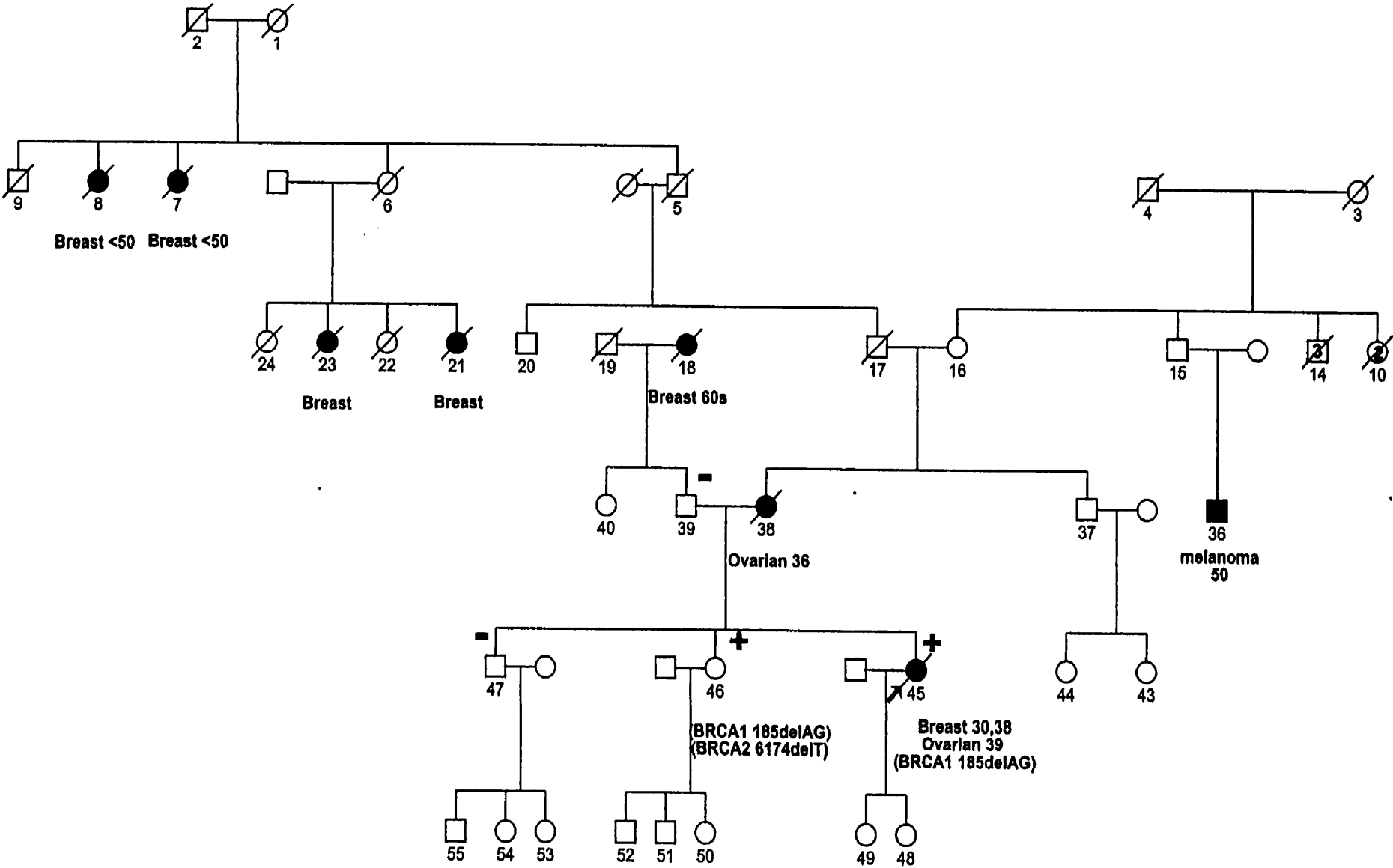
FIGURE 23

Pedigree of Family R023 Segregating All Three Common Mutations in the *BRCA1*

and *BRCA2* genes Tested

Family R023
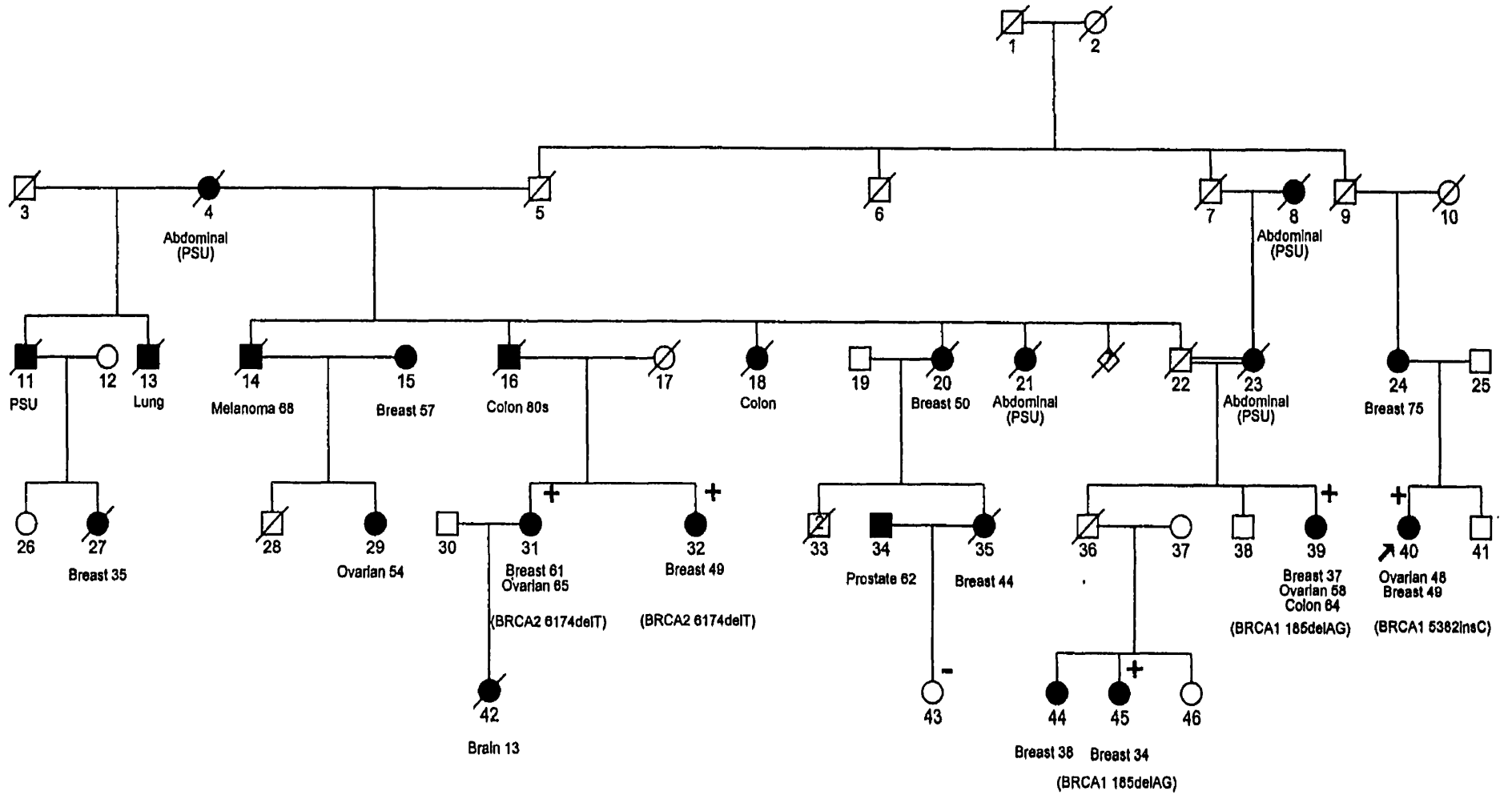
FIGURE 24

Pedigree of Family R013 with a Wife and a Husband Who are Carriers of Two Different *BRCA1* and *BRCA2* Mutations

Family R013

139
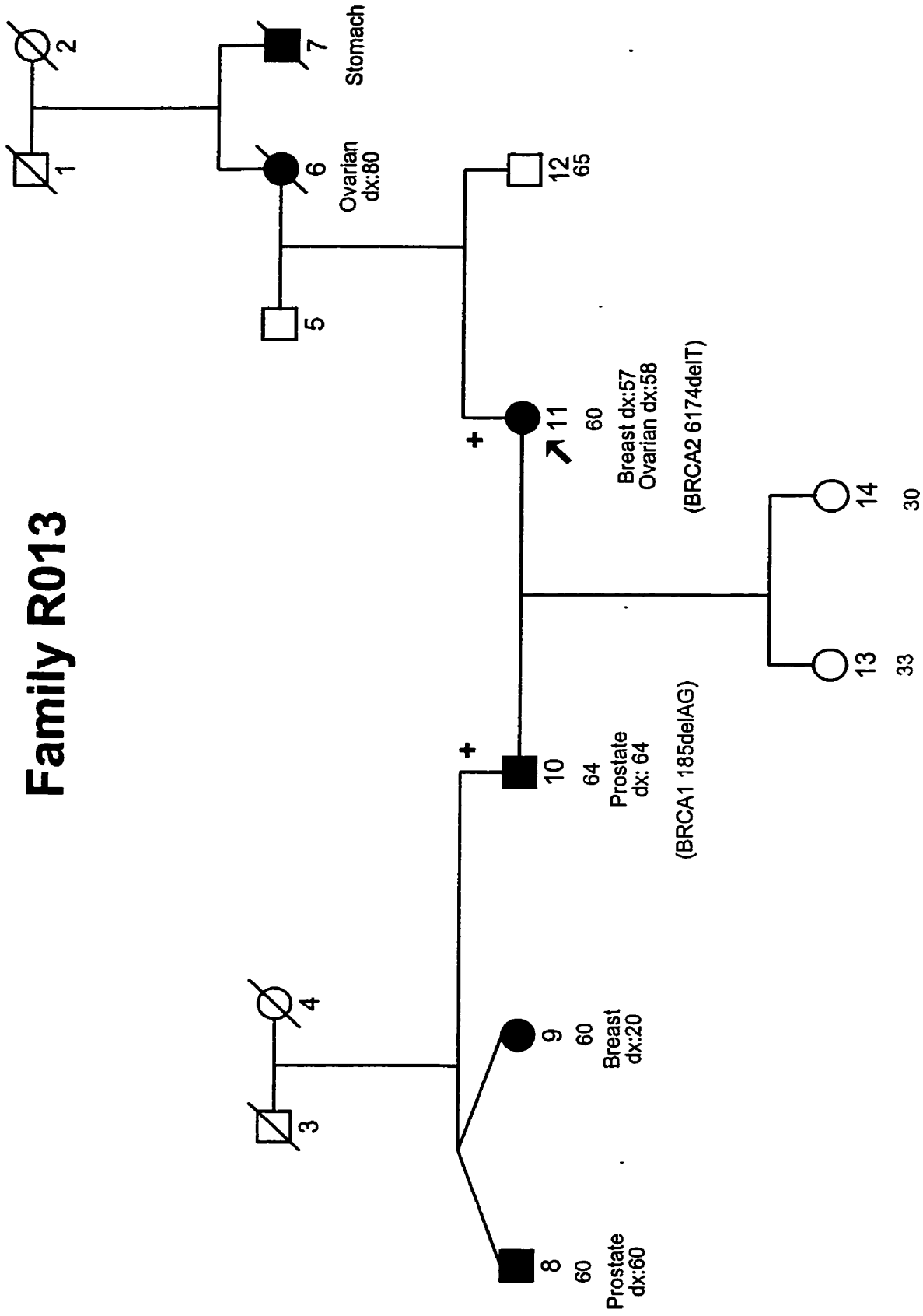
FIGURE 25

Pedigree of Family R99 Segregating a *BRCA2* 6696delTC Mutation

Family R99
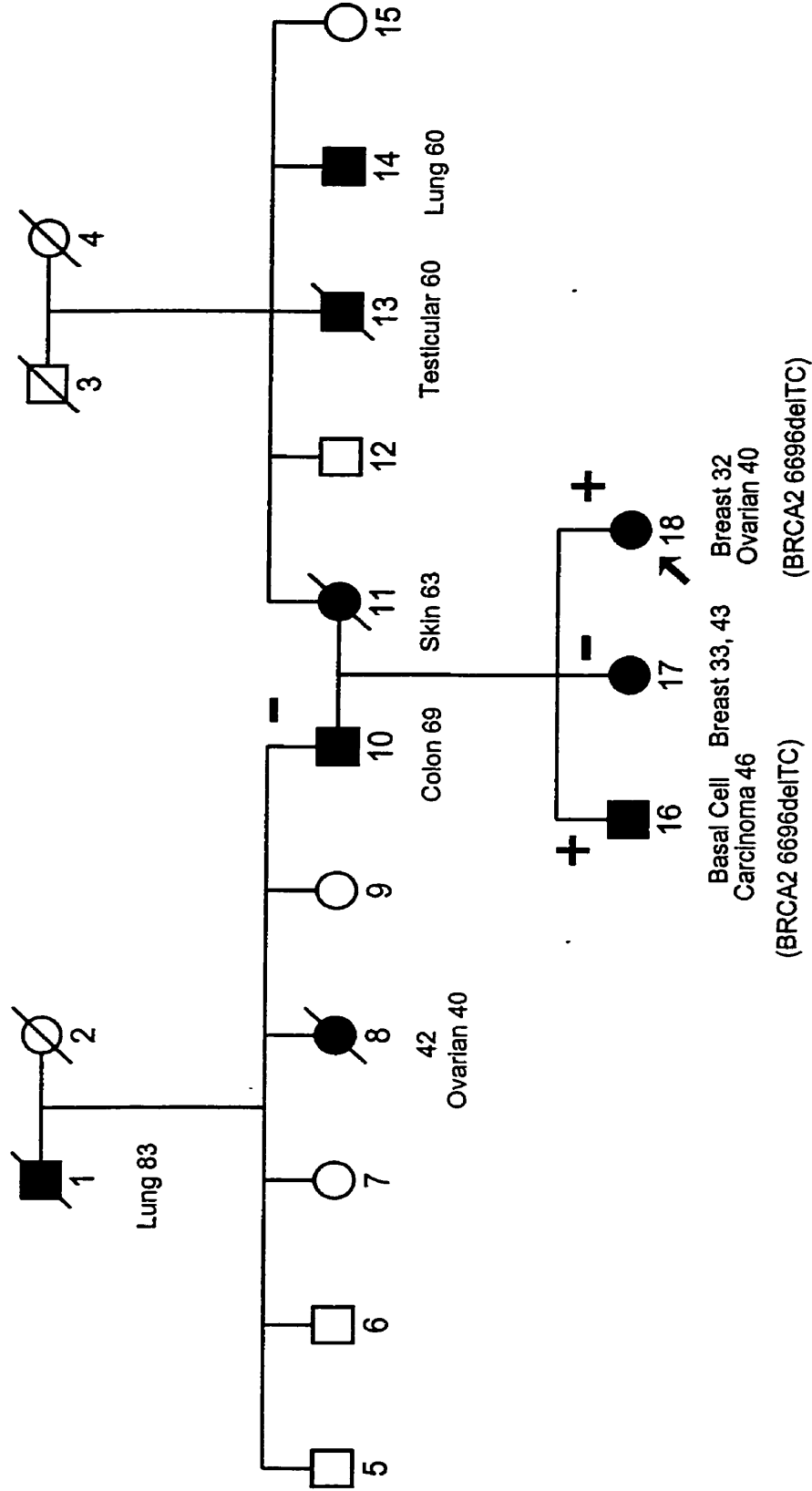
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APPENDIX 1

Letter of Initial Contact for the Jewish Women with Ovarian Cancer

Dear Ms. _____,

We at the _____ Medical Center are dedicated to developing a program of early detection and prevention of ovarian cancer. Recently, there have been several important advances in our understanding of the hereditary aspects of ovarian cancer, which we think may prove to be useful to our patients. We are therefore undertaking an evaluation of the use of this new genetic information in the health care of our patients.

We are particularly interested in the clustering of ovarian cancer in Jewish women. With your permission we would like to call you and ask you about your family history of cancer, as well as a few questions about your medical history. We may also request a blood sample. The purpose of this study is to estimate what proportion of ovarian cancers in Jewish women are likely to be hereditary. We feel that the answer to this question will be of great help to us in counseling patients and developing therapeutic and preventative strategies.

If you prefer not to be contacted, please call our office at _____ and leave a message. We will then remove your name from our list of study subjects. Otherwise, you can expect a call from us in the next few weeks. We thank you in advance for your help in this valuable project.

Sincerely,

APPENDIX 2

Consent Form for the Probands in the Study of "Genetics of Ovarian Cancer in Jewish Women"

Genetic Factors in Ovarian Cancer among Jewish Women

Subject Consent Form

Principal Investigators:
Roxana Moslehi, Women's College Hospital, Toronto & University of B.C., Vancouver
Dr. Steven Narod, Medical Genetics, Women's College Hospital, Toronto

PURPOSE OF RESEARCH

I understand that I have been asked to participate in a research project of ovarian cancer. The purposes of this study are 1) to estimate how many cases of ovarian cancer in Jewish Women can be attributed to genetic factors and 2) to estimate the frequency of cancer susceptibility gene mutations among Jewish Women with ovarian cancer.

I understand that my participation in this study is voluntary and if I agree to participate I may withdraw my consent and discontinue my participation from the study at any time without prejudice or loss of benefits to which I am otherwise entitled. I understand that my participation may be terminated with or without my consent.

PROCEDURE

I will be asked questions about my medical history and the medical history of my family members. I will be asked a number of questions about my use of hormonal medications and my reproductive history.

I have been asked to provide a blood sample to the investigator. I understand that 20cc of blood will be obtained from my vein and provided to the investigator. I understand that testing will be done on this blood with the purpose of identifying genetic markers of cancer risk. The investigators will use this blood to look for mutations in the genes, which predispose to hereditary breast and ovarian cancer. The DNA extracted from this blood will be stored in the laboratory of the principal investigator. DNA will be stored for twenty years and then destroyed. A portion of this DNA may be sent to the laboratory of collaborating investigators without identifying information. This DNA will not be used for commercial purposes.

RISKS

I understand that there may be some bruising at the site of the needle puncture.

BENEFITS

This study may provide information to me and my family about specific risks of cancer of the breast and ovary. This information could be of potential benefit for the prevention of cancer. Genetic counselling will be offered to all study participants and their family members if desired,

including discussion of individual disease risks and preventive strategies.

CONFIDENTIALITY

I understand that medical and all other information produced by this study will be strictly confidential and will be held securely in the Division of Medical Genetics of The University of Toronto and will be subject to the confidentiality and privacy regulations of The University of Toronto and The Women's College Hospital. This information will not become part of my personal medical record and no information will be released to any other party.

REQUEST FOR MORE INFORMATION

I understand that I may ask more questions about the study at any time. Dr. _____ is available to answer my questions and concerns at Tel # _____. I have received a copy of this consent form.

If the genetic analysis done in this study leads to information about my personal risk of cancer, I request that this information be communicated to me:

_____ Yes, I wish to be given the results of the genetic test

_____ No, I do not want to be told the results of the genetic test

I may be contacted in 2 to 3 years for a follow-up on my medical history:

_____ Yes, I agree to be contacted for follow-up questions

_____ No, I do not agree to be contacted for follow-up questions

CONFIRMATION OF PARTICIPATION

_____   _____
Participant   Date

I have explained to _____ the purpose of this research, the procedures required and the possible risks and benefits of the study.

_____   _____
Investigator   Date

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APPENDIX 3

Pedigree of Family R054 with the Proband diagnosed with Primary Peritoneal

Cancer

Family R054

few sisters died from
female cancers

Stomach 73

92

Breast 70

72   Breast 56

Breast 56

70's

70's

Breast 56

70

90's

Breast 40's?

26

35

38

33

39

primary peritoneal 38
(BRCA1 185delAG)

163
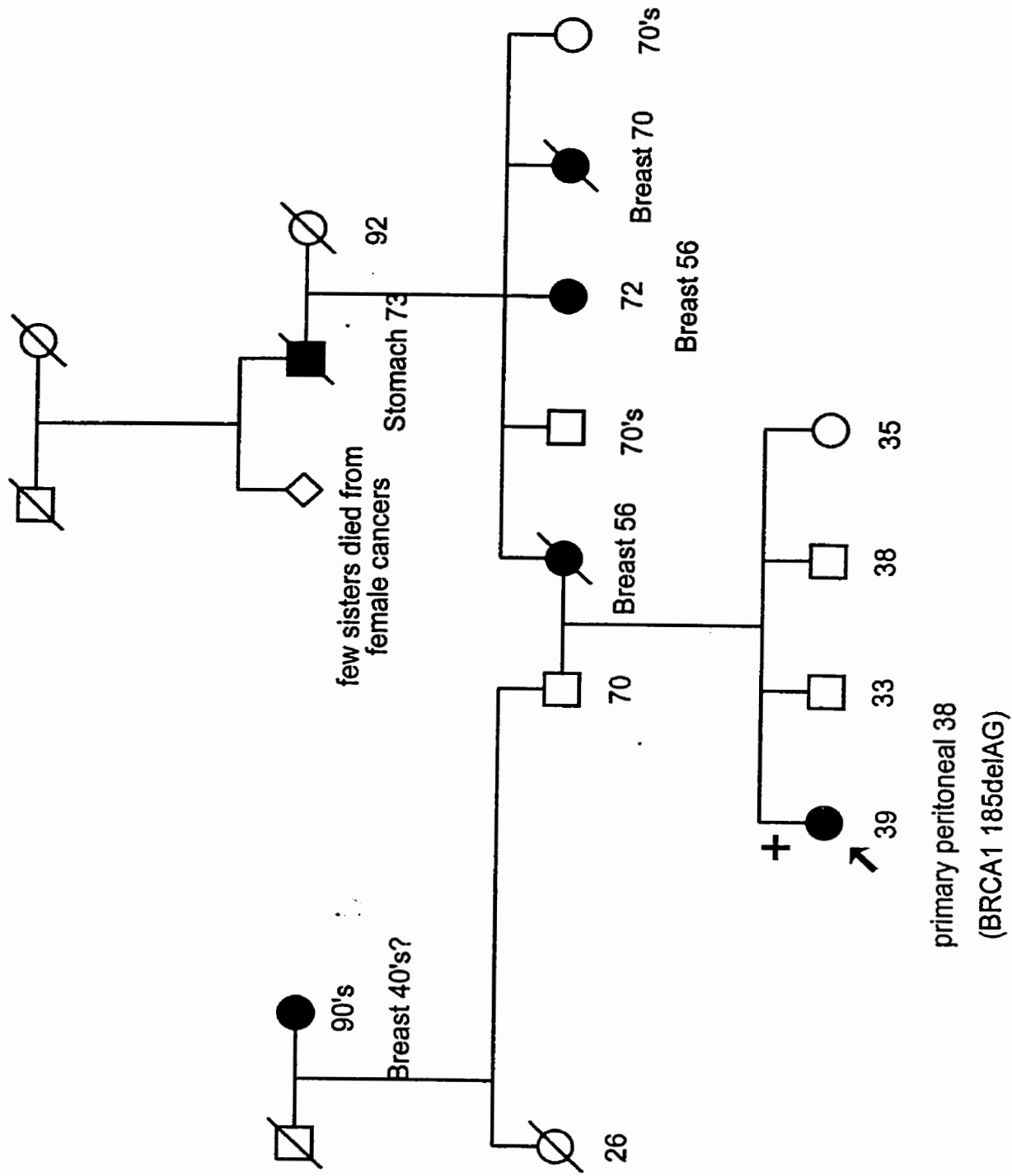
APPENDIX 4

Questionnaire for Cases and Controls in the Study of "Genetics of Ovarian Cancer

in Jewish Women"

Genetic Analysis of Ovarian Cancer among Jewish Women

Questionnaire

Patient Identification Number: |_|_|_|

Hospital

Document Number: |_|_|

Date of Interview: |_|_||_|_||1|9|9|5|
                                                                       day-month

1.   Have you ever been pregnant?
            1= Yes
            2= No - Go to Question 3 |_|

2.   If yes, what was the outcome of your pregnancies?

Pregnancy	Year	Code	Breast feeding (months)
1	19|_|		
2	19|_|		
3	19|_|		
4	19|_|		
5	19|_|		
6	19|_|		
7	19|_|		
8	19|_|		
9	19|_|		

Codes:
1 = boy
2 = girl
3 = twins (males)
4 = twins (female)
5 = twins (male, female)
6 = miscarriage
7 = stillborn
8 = abortion
9 = ectopic (tubal)

2.1   Have you ever taken medication to stop breast feeding?
            1 = Yes
            2 = No (Go to Question 3) |_|

2.2   Indicate the name of the medication and how many times?
            2.2.1   Medication_____ |_|
            2.2.2   Number of times_____ |_|

3.   Have you ever taken a medication to become pregnant?
            1 = Yes
            2 = No (Go to Question 5) |_|

/1

4.   If yes, for each treatment, indicate the name of the medication, how old you were, and how many months you took it.

Medication	Code	Age Started	Duration (months)

5.   How old were you when you had your first menstrual period? _____ |__|__|

6.   At your adult age, your periods were: 1 = always regular
                                                     2 = usually regular
                                                     3 = never regular |__|

7.   How many days from the first day of one period to the first day of the next period, on average? _____ |__|__|

8.   Have you ever taken oral contraceptives to regulate your cycles?
             1 = Yes
             2 = No (Go to question 10) |__|

9.   If yes, how old were you? _____ |__|__|

10.   Have you ever taken oral contraceptives for reasons of birth control?
             1 = Yes
             2 = No (Go to question 12) |__|

11.   If yes, From what age to what age (without forgetting to exclude when you stopped)?

        11.1   1st time   _____   from |__|__| to |__|__|
        11.2   2nd time   _____   from |__|__| to |__|__|
        11.3   3rd time   _____   from |__|__| to |__|__|
        11.4   4th time   _____   from |__|__| to |__|__|

12.   Do you currently have menstrual periods?
             1 = Yes - Go to Question 17
             2 = No |__|

13.   How old were you when your periods stopped completely? _____ |__|__|

14.   For what reason?   1 = natural menopause
                                2 = Total hysterectomy (ovaries removed)
                                3 = Partial hysterectomy (ovaries intact)
                                4 = chemotherapy that stopped periods
                                5 = other_____ |__|

/2

15. Have you taken hormone replacement therapy for menopause?
     1 = Yes
     2 = No (Go to question 17)

|__|

16. If yes, indicate the name of the hormone and from what age to what age you took it.

Hormone	Code	From Age	Duration (months)

17. Have you ever been told you are at risk for developing ovarian cancer?
     1 = Yes
     2 = No (Go to question 19)

|__|

18. If yes, by whom? _____

|__|

19. Have you had a tubal ligation?
     1 = Yes
     2 = No (Go to question 21)

|__|

20. If yes, how old were you? _____

|__|__|

21. Have you ever had other surgery on your reproductive organs?
     1 = Yes
     2 = No (Go to question 23)

|__|

22. If yes, what was the reason and the year or the age at surgery?

Type of Surgery and Reason	Code	Year or Age

23. Have you ever had another operation on your abdomen?
     1 = Yes
     2 = No (Go to question 25)

|__|

24. If yes, for each operation, indicate the reason and the year or the age at surgery.

Type of Surgery and Reason	Code	Year or Age

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/3

25. Have you ever been told that you were at risk for breast cancer?
  1 = Yes
  2 = No (Go to question 27)

    |_|

26. If yes, by whom?_____

    |_|

27. Have you ever been treated for cancer?
  1 = Yes
  2 = No (Go to question 29)

    |_|

28. If yes, please indicate:

Type of Cancer	Code	Year or Age of diagnosis
	|||	
	|||	
	|||	

29. Have you had other illnesses?
  1 = Yes
  2 = No (Go to question 31)

    |_|

30. If yes, describe_____

    |_|

31. Have you ever smoked?
  1 = Yes
  2 = No (Go to question 33)

    |_|

32. If yes, at what age did you start smoking? _____
  32.1 What age did you stop smoking? _____
  32.2 How many packs did you smoke per week? _____

    |_|_|
    |_|_|
    |_|_|

33. Have you ever been a regular drinker of alcohol?
  1 = Yes
  2 = No (Go to question 35)

    |_|

34. If yes, from what age to what age? from |_|_| to |_|_|

35. Have you ever been a regular user of talcum powder?
  1 = Yes
  2 = No (Go to question 37)

    |_|

36. If yes, did you apply it to : 1 = vaginal area
         2 = sanitary napkins
         3 = on other parts of the body

    |_|

37. What is your current height?

    |_|_|_| or |_|_|_|
    ft.in. cm.

/4

38. What is your current weight?

|_|_|_| or |_|_|_|
lbs.   kgs.

39. How much did you weigh at: 20 years?

                                30 years?

                                40 years?

                                50 years?

_	_	_	or	_	_	_
_	_	_	or	_	_	_
_	_	_	or	_	_	_
_	_	_	or	_	_	_
lbs.   kgs.

40. What is the most you have ever weighed (excluding pregnancy)?

|_|_|_| or |_|_|_|
lbs.   kgs.

    40.1   How old were you?

|_|_|_|

41. How many years of high school have you completed?

|_|_|

42. Did you study at University, college or professional school?
        1 = Yes
        2 = No

|_|

43. If yes, which studies or what degree did you obtain? _____

|_|

44. Please indicate the place of birth (country and city) of the following relatives:

Relative	Code	Country and City of birth	Code
Mother	|_|		|_|
Maternal grand-mother	|_|		|_|
Maternal grand-father	|_|		|_|
Father	|_|		|_|
Paternal grand-mother	|_|		|_|
Paternal grand-father	|_|		|_|

45. Have any of your relatives had cancer?
        1 = Yes
        2 = No (End of questionnaire)

|_|

46. If yes, please indicate their relationship to you (i.e. maternal or paternal relation), the type of cancer, and their ages of diagnosis.

Relative	Code	Type of Cancer	Code	Age
	|_|		|_|	
	|_|		|_|	
	|_|		|_|	
	|_|		|_|	
	|_|		|_|	
	|_|		|_|	

/5

Personal Information

Date: ___/___/_____
mm / dd / yy

Patient's Name: _____
            maiden   first name   (married name)

Date of Birth: ___/___/_____
           mm / dd / yy

Telephone number: _____

Ashkenazi Jewish: Yes_____   No _____

                 Case____   · Control ____

Hospital: _____

Hospital Unit #: _____

Thank you for your participation.

For Office Use:

Date of diagnosis: |_|_| |_|_| |1|9|_|_|
                                            D - M - YR

Date of surgery: |_|_| |_|_| |1|9|_|_|
                                            D - M - YR

Type of surgery:

Pathology specimen: _____

Diagnosis: _____

Revised by Roxana Moslehi
August 27 / 1996

/6

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APPENDIX 5

Example of the Detailed Pedigrees Drawn at the Time of Interview of the Cases and

Controls

not known

dying

Ovarian Cancer dx:56 d:56

Possible liver cancer? d:early 60s

dying Melanoma

89

d:80 natural causes

d:30s heart

Bilat. Breast Cancer dx:44,64 d:64

84 Unilat. Breast Cancer dx:68

Ovarian Cancer dx:80,81 d:81

Lymphoma dx:83 d:93

late 40s

53

40s 50s

40s 50s

51

51 on Tamoxifen

Breast Cancer dx:40 d:41,48

M.S.

75 perished Breast Cancer in the war d:75

All perished in the war

dying W.W.g

d:80s natural causes

d:90s natural causes

d:90s natural causes

All O.K.

70's-80's All O.K.

dying 60 in the war

dying in the war

45 Breast Cancer dx:40

5+ OOPH 52

Adopted in

51 Ovarian Cancer dx:50

27 23

Jan. 29/96
drawn by Roxana Mitchell

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IT

APPENDIX 6

Consent Form for the Controls in the study of "Genetics of Ovarian Cancer in Jewish Women"

Genetic Factors in Ovarian Cancer among Jewish Women

Control Consent Form

Principal Investigators:
Roxana Moslehi, Women's College Hospital, Toronto & University of B.C., Vancouver
Dr. Steven Narod, Medical Genetics, Women's College Hospital, Toronto

PURPOSE OF RESEARCH
I understand that I have been asked to participate in a research project of ovarian cancer. The purpose of this study is to estimate 1) how many cases of ovarian cancer in Ashkenazi Jewish Women can be attributed to genetic factors and 2) the frequency of cancer susceptibility gene mutations among Ashkenazi Jewish Women with ovarian cancer.

I understand that my participation in this study is voluntary and if I agree to participate I may withdraw my consent and discontinue my participation from the study at any time without prejudice or loss of benefits to which I am otherwise entitled. I understand that my participation may be terminated with or without my consent.

PROCEDURE
I will be asked questions about my medical history and the medical history of my family members. I will be asked a number of questions about my use of hormonal medications and my reproductive history.

BENEFITS
This study may provide information to me and my family about specific risks of cancer of the breast and ovary. This information could be of potential benefit for the prevention of cancer. Genetic counselling will be offered to all study participants and their family members if desired, including discussion of

individual disease risks and preventive strategies.

CONFIDENTIALITY
I understand that medical and all other information produced by this study will be strictly confidential and will be held securely in the Division of Medical Genetics of The University of Toronto and will be subject to the confidentiality and privacy regulations of The University of Toronto and The Women's College Hospital. This information will not become part of my personal medical record and no information will be released to any other party.

REQUEST FOR MORE INFORMATION
I understand that I may ask more questions about the study at any time. Dr. Narod is available to answer my questions and concerns at Tel # 416-351-3765. I will receive a copy of this consent form upon request.

CONFIRMATION OF PARTICIPATION

_____   _____

Participant   Date

I have explained to _____ the purpose of this research, the procedures required and the possible risks and benefits of the study.

_____   _____

Investigator   Date

APPENDIX 7

Report on the Results of *BRCA1* and *BRCA2* Mutation Analysis Made for All Cases and Relatives of Cases Who Were Tested

Familial Cancer Research Laboratory

DATE:	
KINDRED NAME & NUMBER:	
ETHNICITY:	
PATIENT'S NAME:	
DATE OF BIRTH:	
CANCER HISTORY:	
DNA LAB #:	

ANALYSIS:

Over 100 different mutations have been identified in BRCA1 and BRCA2. Certain mutations are found with high frequency in certain ethnic groups and may account for the majority of BRCA1 or BRCA2 mutations in those groups. Many other mutations are rare. Ethnic background partially determines which tests are performed.

ANALYSES PERFORMED:

This sample was screened for alterations in BRCA1 and BRCA2 as indicated by an asterisk in the table below.

BRCA1		BRCA2	
185delAG		6174delT	
5382insC		8764delAG	
188del11		SSCA (all exons)	
PTT (exon 11)		Other	
Other			

RESULTS:

COMMENTS:

This analysis is based on current knowledge of the molecular genetics of BRCA1 and BRCA2. Unless specifically stated it has been assumed that family relationships and ethnicity are as indicated and that the diagnosis of cancer in individuals presented as affected on the pedigree is correct. Conclusions and risk estimates do not include the possibility of sample mix-up or laboratory error. This analysis was performed in a research laboratory. For samples which have been identified as carrying a mutation we recommend that the finding be confirmed at an accredited diagnostic laboratory.

Steven A. Narod
Laboratory Director

APPENDIX 8

Consent Form for the Relatives of the Probands Who Were Tested for the *BRCA1* and *BRCA2* Mutations

CONSENT FORM
for GENETIC ANALYSIS OF FAMILIAL CANCER

PRINCIPAL INVESTIGATOR: Dr. Steven Narod

SITE: Women's College Hospital and Princess Margaret Hospital

PURPOSE OF RESEARCH:
I understand that I have been asked to participate in a research project of familial cancer. The purpose of this study is to identify gene(s) which are associated with increased risk of developing breast or ovarian cancer.

I understand that my participation is voluntary and if I agree to participate I may withdraw my consent and discontinue my participation at any time without prejudice to or loss of my medical care or the benefits to which I, or my family, are otherwise entitled. I understand that my participation will not affect my choice of, or access to, treatment or screening. I understand that my participation may be terminated with or without my consent.

PROCEDURE:
I understand that if I agree to participate I will be asked questions about my medical history and family history. I have been asked to provide a blood sample of 20 cc (four tablespoons) to the investigator. I understand that testing will be done on this blood with the purpose of identifying genetic markers of cancer risk. The DNA extracted from this blood will be stored in the laboratory of the principal investigator and will become the property of the principal investigator.

Samples of DNA may be sent to other academic institutions for additional studies of the hereditary basis of cancer, in which case no identifying information will be provided. I understand that the DNA will not be used for purposes other than the study of familial cancer.

The results of this testing may provide information regarding my individual risk of developing breast or ovarian cancer during my lifetime. I understand that DNA testing may not be 100% accurate. At my request this information will be provided to me or to my physician (or both).

CONFIDENTIALITY:
I understand that medical and all other information produced by this study will be strictly confidential and will be held securely at and will be subject to the confidentiality and privacy regulations of Princess Margaret Hospital, Women's College Hospital and University of Toronto. This information will not become part of my personal medical record. This information will be available to the study research team, and not released to any other party, except upon my express written consent. Information regarding my medical history or test results will not be disclosed to any other member of my family. Information pertaining to my relatives medical history or test results will not be disclosed to me.

BENEFITS:
Genetic counselling will be available to me and members of my family. The research team will be available to provide the most current information regarding genetic risk assessment and will provide referral to screening centres for breast and ovarian cancer if requested. I understand that I may have access to any information regarding my personal risk of developing cancer upon my request. I may also participate in the research without being informed of my personal risk of developing cancer.

Other than medical care which will be provided, I understand that there is no compensation available for my participation in this research study. I understand that this is not a waiver of my

legal rights. I understand that representatives of the United States Army, Department of Defence (the research granting agency) may inspect the research records.

RISKS:
Any potential risks of this testing are primarily of a psychological nature for those individuals who choose to be informed of their test results. A non-informative result can be frustrating and can intensify the ambiguity of the risk situation or can provide relief. An increased as well as a decreased risk can lead to serious psychological consequences including feelings of depression, futility, despair and stress. I understand that counselling will be provided to me to help me adjust to the information given to me. The only discomfort is minimal and is in drawing a blood sample usually from a vein in the arm. I understand that I will be given all necessary medical care for injury or illness which results from giving a blood sample.

REQUEST FOR MORE INFORMATION:
I understand that I may ask more questions about the study. Dr. Narod is available to answer my questions and concerns (Tel. 416-351-3765).

CONFIRMATION OF PARTICIPATION:
I confirm that the purpose of the research, the study procedures that I will undergo and the possible discomforts as well as benefits that I may experience have been explained to me in sufficient detail.

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care.

I give permission to Dr. Narod and the study team to contact me by telephone if additional information is needed.
YES_____   Telephone _____
NO _____

I would like to be told the results of any genetic testing which could provide information about my personal risk of breast or ovarian cancer.
YES _____   NO_____

_____   _____
Participant's Signature   Date

I have explained to _____ the purpose of this research, the procedures required and the possible risks and benefits of the study.

_____   _____
Investigator's Signature   Date

APPENDIX 9

Calculation of the Penetrance of *BRCA1* and *BRCA2* Mutations for Breast Cancer

to age 55

According to the formula by Struewing et al. (1997):

$$R_+ = (P/2 + \tfrac{1}{2})S_1 + (1/2 - P/2)\,S_0 \qquad\qquad R_- = P\,S_1 + (1-P)\,S_0$$

$$S_1 = 2R_+ - R_-$$

R_+ : Probability of disease among the relatives of the mutation carriers

R_-: Probability of disease among the relatives of the non-carriers

P: Frequency of the mutant allele

S_1: Risk of disease among the mutation carriers (i.e., penetrance)

S_0: Risk of disease among the non-carriers

Penetrance for breast cancer by age 55 for *BRCA1*:

$S_1 = 2(0.184) - (0.069)$ [see tables 12 and 13] =29.9% (see table 15)

Penetrance for breast cancer by age 55 for *BRCA2*:

$S_1 = 2(0.060) - (0.069)$ [see tables 12 and 13] =5.1% (see table 15)

Appendix 10

Positive and Negative Predictive Values of Family History of Ovarian Cancer

	BRCA1/BRCA2 Mutation positive	*BRCA1/BRCA2* Mutation negative	
Familial	37	14	51
Non-familial	56	134	190
	93	148	241

positive predictive value= 37 / 37+14   = 72.5%

negative predictive value = 56 / 134+56 = 29.5%