# Original Articles

# Insulin-Like Growth Factor I Polymorphism and Breast Cancer Risk in Jewish Women

Arie Figer MD[1]*, Yael Patael Karasik MD[2]*, Ruth Gershoni Baruch MD[3], Angela Chetrit MSc[4], Moshe Z. Papa MD[5], Revital Bruchim Bar Sade MSc[2], Shulamith Riezel MD[6] and Eitan Friedman MD PhD[2]

[1] Oncology Institute, Sourasky Tel Aviv Medical Center, Tel Aviv, Israel
[2] Oncogenetics Unit, Gertner Institute of Genetics, Tel Hashomer, Israel
[3] Genetics Institute, Rambam Medical Center, and Technion Faculty of Medicine, Haifa, Israel
[4] Gertner Institute for Epidemiology and Health Policy Research, Tel Hashomer, Israel
[5] Department of Oncologic Surgery, Sheba Medical Center, Tel Hashomer, Israel
[6] Institute of Oncology, Sheba Medical Center, and Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

Key words: breast cancer risk, insulin-like growth factor-I polymorphism, BRCA mutations, modifier genes

## Abstract

**Background:** Genes that confer mild or moderate susceptibility to breast cancer may be involved in the pathogenesis of sporadic breast cancer, modifying the phenotypic expression of mutant *BRCA1/BRCA2* alleles. An attractive candidate is the insulin-like growth factor I, a known mitogen to mammary ductal cells *in vivo* and *in vitro*, whose serum levels were reportedly elevated in breast cancer patients.

**Objective:** To evaluate the contribution of the IGF-1 gene polymorphism to breast cancer risk by genotyping for a polymorphic allele size in breast cancer patients and controls.

**Methods:** We analyzed allele size distribution of the polymorphic CA repeat upstream of the IGF-I gene in 412 Israeli Jewish women: 268 women with breast cancer (212 sporadic and 56 carriers of either a *BRCA1* or *BRCA2* mutation), and 144 controls. Genotyping was accomplished by radioactive polymerase chain reaction of the relevant genomic region and size fractionation on polyacrylamide gels with subsequent autoradiography.

**Results:** Among women with breast cancer, with or without BRCA germline mutations, 196 and 198 basepair alleles were present in 4.7% (25/536 alleles), compared with 9% (26/288) controls ($P = 0.02$). This difference was more pronounced and significant in the non-Ashkenazi population. Conversely, the smaller size allele (176 bp) was present in the breast cancer group only (3/536, 0.6%).

**Conclusions:** The IGF-I polymorphism may serve as a marker for breast cancer risk in the general Jewish population, in particular non-Ashkenazi Jews, but extension and confirmation of these preliminary data are needed.

*IMAJ 2002;4:759–762*

An estimated 5–10% of breast cancers occur in the context of an inherited predisposition to cancer, heralded by familial clustering of breast and/or ovarian cancer [1,2]. In these cases, inactivating germline mutations within *BRCA1* or *BRCA2* have been shown to occur in the majority of families [1,3]. These mutant genes are highly penetrant and confer a high lifetime risk for developing breast/ovarian cancer [3,4]. However, the incomplete penetrance of mutant BRCA genes has been attributed in part to the actions of so-called modifier genes: genes that modulate the effects of mutant alleles and, in concert with various environmental exposures, determine the phenotypic expression of mutant *BRCA1/BRCA2*. Indeed, modifier genes of *BRCA1/BRCA2* mutation carriers have been reported for both ovarian cancer (H-ras polymorphic variable number of tandem repeats) [5] and breast cancer (the size of the CAG repeat of androgen receptor) [6]. In addition to functioning as an expression modifier of major cancer susceptibility genes, modifier genes may serve as breast cancer risk modulators in the general population. Most breast cancers occur sporadically and represent a multigenetic multifactorial disease, where mutations in multiple genes – each individually conferring only a slight cellular growth advantage – combine to result in frank neoplastic proliferation. Thus, a compound genetic profile encompassing relevant gene mutations ultimately determines the individual's risk for developing breast cancer. The precise genes involved in breast cancer risk in the general population are currently unknown. One attractive candidate is the insulin-like growth factor-I. There is substantial experimental evidence that IGF-I and the signaling cascade that it activates are operative in breast cellular proliferation. Animal studies have shown that IGF-I or growth hormone treatment leads to mammary cellular hyperplasia [7]; and that transgenic animals that over-express growth hormone, an IGF-I precursor, have an increased incidence of breast cancer [8,9]. Furthermore, at the cellular level, IGF-I has been shown to act as a mitogen and anti-apoptotic in normal mammary tissue [10]. Epidemiologic evidence suggests that IGF-I or bioavailable IGF-I is an indicator for breast cancer risk in premenopausal women [11–14]. These latter results have not been fully substantiated or confirmed by all investigators [15,16].

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* Both co-authors contributed equally to this manuscript.
IGF-1 = insulin-like growth factor-1
bp = basepair

Taken together, we postulate that IGF-I polymorphism might be used as a possible genetic marker for breast cancer risk. To test this notion we analyzed allele sizes of a CA repeat polymorphism located upstream of the IGF-I gene in Jewish Israeli women with sporadic and inherited breast cancer.

## Patients and Methods

### Patient populations

Several subsets of individuals were analyzed, as follows
- *Unselected breast cancer patients*. Jewish women with histopathologically proven breast cancer, who were treated at one of the participating medical centers, were eligible for participation. Pertinent data on those agreeing to participate were collected from medical records and a detailed questionnaire conducted by a personal interview.
- *BRCA1/BRCA2 mutation carriers*. Individuals who were counseled at either the oncogenetic services of the Sheba or Rambam Medical Centers were eligible for participation. All mutation carriers included in this study had breast cancer and two additional first- or second-degree relatives with these neoplasms, bilateral disease, or age at onset under 40 years.
- *Population screen (controls)*. Jewish individuals of diverse ethnic origin who came for prenatal counseling at the Genetics Institutes of the Sheba and Rambam Medical Centers served as "ethnic controls." No information regarding family history of cancer or any other relevant details is available on these seemingly healthy individuals. All tested individuals were unrelated to each other, and were tested anonymously for being mutation carriers of one of the three predominant Jewish mutations in *BRCA1/BRCA2*. The ethnic origin of all the above-mentioned individuals was determined by an interview and by dating parental origin as far back as possible, but at least three generations.

The institutional review board of the participating medical centers approved the study, and each participant signed a written informed consent.

## Methods

- *DNA extraction*. Genomic DNA was prepared from anticoagulated venous blood samples with standard techniques using the Gentra Systems kit (Gentra Inc., Minneapolis, MN, USA), as recommended by the manufacturer.
- *Polymerase chain reaction*. Non-radioactive PCRs were performed in a final volume of 50 µl containing 3–5 µl template DNA (about 50–100 ng), 20 pM of each primer, 200 mM of each dNTP, 10X standard PCR buffer (1.5 mM MgCl2), and 1 U of thermostable DNA polymerase (Rhenium, Bioprobe System, Motrueil-Sous-Bois, France). Thermal cycling was accomplished by PTC-100-60 thermocycler (M.J. Research Inc., Watertown, MA, USA). The cycling profile included: an initial denaturation at 94°C for 5 minutes, followed by 34 cycles at 60°C annealing temperature for

3 min, extension at 72°C for 1 min and denaturation at 94°C for 45 seconds, with a final extension cycle at 72°C for 5 min. PCR reactions for IGF-I genotyping were performed in a final volume of 15 µl and contained a radioactively labeled (33P) dCTP in the PCR reaction mixture, 10 pM of each primer, in addition to the "regular" PCR ingredients mentioned above.

- *Mutation analysis of the predominant Jewish mutations in BRCA1 and BRCA2*. Mutational analyses for the three predominant mutations (185delAG 5382InsC in *BRCA1* and 6174delT in *BRCA2*) were carried out by restriction enzyme digest of amplified PCR products using modified amplification primers to generate novel restriction sites, followed by restriction enzyme analysis to distinguish the mutant from the wild-type allele, as previously described [17,18].
- *Analysis of the IGF-I polymorphism*. Primer sequences flanking the CA polymorphism located upstream of the IGF-I gene were as previously described [19]. PCR amplification, gel electrophoresis and autoradiography were performed using standard protocols, as previously described by Rosen et al. [20]. To assign the appropriate size allele on each gel, we ran the M13 marker in adjacent lanes.

### Statistical analyses

All statistical analyses were performed using the SPSS software (version 6.1). Comparisons between the different subgroups with regard to IFG-I genotype and *BRCA* gene mutation carrier status were analyzed using the chi-square test and Fisher's exact test. Significance was assumed at $P < 0.05$.

## Results

- *Unselected breast cancer patients*. A total of 212 sporadic breast cancer patients was analyzed: 57.7% were of Ashkenazi origin (East European) and 42.3% were non-Ashkenazi. Age at diagnosis ranged from 26 to 79 years (mean SD 50.0 ± 12.6). None had a family history of breast or ovarian cancer in a first-degree relative, and none was a carrier of either one of the three predominant Jewish *BRCA1* or *BRCA2* mutations.
- *BRCA1 or BRCA2 mutation carriers*. There were 56 carriers of one of the three predominant mutations in *BRCA1* and *BRCA2*: 37 (66.1%) harbored the 185delAG *BRCA1* mutation, 8 (14.3%) were 5382InsC *BRCA1* mutation carriers, and 13 (23.2%) displayed the 6174delT *BRCA2* mutation. Average age at diagnosis for carriers was 42.3 ± 8.5 (range 26–62 years) with 30% of women diagnosed with breast cancer under 40 years of age. The majority of mutation carriers was Ashkenazi (55/56, 98.1%), and only one was non-Ashkenazi (1.8%). Twenty-eight women (50%) had a family history of breast or ovarian cancer in a first-degree relative.
- *Controls*. Of the 144 controls analyzed, 56 were Ashkenazi (38.9%) and 88 were non-Ashkenazi (61.1%). Age range was 22–44 years, and none was a carrier of either of the predominant *BRCA1* or *BRCA2* Jewish mutations.
- *Allele size distribution in the analyzed populations*. The results of the allele size distribution within the tested populations are given in Table 1. As shown, in both the patients and the controls the

PCR = polymerase chain reaction

**Table 1.** Allele size distribution of the polymorphic CA repeats at the 5'UTR of the IGF-I gene

| Allele size (bp) | Breast cancer patients (n=268) alleles (n= 536) | Control patients (n=144) alleles (n=288) | P |
|---|---|---|---|
| 198 | 4 (0.75%) | 7 (2.4%) | |
| 196 | 21 (3.9%) | 19 (6.6%) | 0.02* |
| 194 | 106 (19.8%) | 61 (21.2%) | NS** |
| 192 | 373 (69.6%) | 186 (64.6%) | NS |
| 190 | 21 (3.9%) | 12 (4.2%) | NS |
| 188 | 7 (1.7%) | 1 (0.3) | |
| 186 | 1 (0.19) | 2 (0.7) | |
| 176 | 3 (0.56%) | 0 (0%) | **NS |

*P value for the combined 196+198 bp alleles
** Significant when combining the shorter alleles (176, 186 and 188 bp) only
NS = not significant

most prevalent allele was the 192 bp allele: 373/536 (69.6%) in the breast cancer patients – sporadic or familial, and 186/288 (64.6%) in the controls. The two longer alleles, 196 and 198 bp, combined were detected in 25/511 alleles in the breast cancer group (4.7%) and in 26/262 alleles in the controls (9%), a statistically significant difference (P = 0.02). Furthermore, subsequent analysis by ethnic origin showed that this trend was maintained for both Ashkenazi and non-Ashkenazi women: for non-Ashkenazi women the longer alleles were detected in 2.7% of breast cancer patients (5/182 alleles), compared with 9.1% of controls (16/176) (P = 0.02). In Ashkenazi individuals, 5.9% (21/356 alleles) were in the affected group as compared with 8.9% (10/112 alleles) in the controls, though this trend did not reach statistical significance. Conversely, the shorter alleles (188, 186 and 176 bp combined) were detected in 11/526 alleles in the breast cancer group (2.1%) and in 3/288 control alleles (1%), a statistically insignificant difference. The smallest detectable allele (176 bp) was only noted in the breast cancer patients (3 alleles), whereas it was not detected in any of the controls.

There were no statistically significant differences in allele size distribution between sporadic breast cancer patients and those with breast cancer and a BRCA1/BRCA2 germline mutation: 20/424 (4.7%) of the alleles in the sporadic cases and 6/112 (5.4%) of the alleles in controls were the longer alleles (not significant). Similarly, no differences in allele size distribution were detected when analyzed by age at diagnosis at 40 years old or less compared with diagnosis over that age (data not shown). In addition, there were no statistically significant differences in the rate of homozygous carriers of the 192 bp allele between the various groups analyzed: sporadic non-carrier patients (99/212, 46.7%) compared with affected mutation carriers (26/56, 46.4%) (P = 0.9); and patients with breast cancer regardless of mutation status (125/268, 46.6%) and controls (62/144, 43.1%).

## Discussion
In this study, allele sizes of a polymorphic site at the 5' UTR of the IGF-I gene differed between women with breast cancer and the general population. The larger alleles were more prevalent in the

general population, whereas the shorter alleles were more common in breast cancer patients. This association is intriguing, as some previous studies observed a several-fold increase in IGF-I serum levels in premenopausal women with breast cancer [13,14]. These elevated levels, in turn, may be regulated by the CA repeat allele size in an inverse manner: the shorter the allele the higher the serum levels. In a previous study correlating IGF-I allele size with serum levels, Rosen and co-workers [20] noted that homozygosity for the most common allele (192 bp) was associated with the lower IGF-I levels than any other combination, but no data on IGF-I levels for the lower or upper allele sizes (188-198 bp) can be extracted from their article. The exact relationship between this polymorphism and the regulation of IGF-1 expression is presently unknown. However, the CA repeat is located about 1 Kb upstream of the transcription start site, a region typically known to contain specific regulatory elements [21]. Therefore it seems plausible that allelic size variation at this specific locus may affect transcription and eventual IGF-I levels. Another, equally plausible mechanistic explanation is the existence of another sequence in linkage disequilibrium with the CA repeat that, in turn, affects IGF-I mRNA stability or half-life.

Regardless of the mechanism of involvement in breast cancer pathogenesis, the results of this study, if confirmed in a larger prospective study, may provide a unique genetic marker for assessing breast cancer risk in the general population. This could facilitate identification of individuals at higher than average risk; these individuals could then be enrolled into early detection schemes or even offered prophylactic interventions.

There were no differences in allele sizes between the sporadic and inherited breast cancer patients, defined as being BRCA1 or BRCA2 mutation carriers and having breast cancer. There was also no association between allele size and age at onset. Thus, it seems that IGF-I is unlikely to play a role as a modifier of the phenotypic expression of mutant BRCA genes.

The drawbacks of this study should be pointed out. This is a retrospective study that encompassed only a limited number of BRCA1/BRCA2 mutation carriers. Second, we only analyzed Jewish women, so that the application of this analysis to other ethnically diverse populations remains to be determined. Third, since the family background of the so-called general population was unknown, we relied on "not being a mutation carrier of one of the predominant mutations" as indirect evidence that the analyzed controls truly represent the "average risk" population. Interestingly, for Jewish women worldwide, there are very few published reports on mutations in both genes [22], and the three predominant ones account for the majority of familial breast/ovarian cancer cases in Israel [17]. Lastly, we did not measure IGF-I or IGF binding protein-3 levels in the tested individuals at the time of diagnosis, so we can only speculate as to the levels based on previous studies.

In conclusion, we have shown that in Jewish women with breast cancer, the allele size of a polymorphic site upstream of the IGF-I gene is significantly shorter than in unaffected average-risk women. This might provide a tool for assessing the risk of the general population to develop breast cancer.

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**Correspondence:** Dr. E. Friedman, Susanne Levy Gertner Oncogenetics Unit, Danek Gertner Institute of Genetics, Tel Hashomer 52621, Israel.
Fax: (972-3) 644-1891 or 535-7308
email: eitan211@sheba.health.gov.il