LETTER TO JMG

Sex ratio skewing of offspring in families with hereditary susceptibility to breast cancer

S M Domchek, S L Merillat, J Tigges, A J Tweed, M Weinat, J Stopfer, B L Weber

J Med Genet 2005;42:511–513. doi: 10.1136/jmg.2004.027722

The function of *BRCA1* is complex and includes roles in DNA damage repair, cell cycle control, regulation of transcription, and X chromosome inactivation.^{1,2} TSIX is thought to control X chromosome inactivation by blocking the accumulation of XIST on the active X chromosome.³ *BRCA1* co-localises with XIST inactive X chromosomes (Xi) and stabilises Xi. Because of this interaction, the loss of *BRCA1* is associated with altered Xi chromatin structure and increased expression of silenced Xi genes.^{1,2} Mouse models have suggested a link between aberrant X chromosome inactivation and sex ratio skewing, with a bias towards male births when TSIX activity is abolished.³ In addition, non-random X chromosome inactivation has been seen in *BRCA1* mutation carriers with ovarian cancer.⁴ With these data as background, a skewed sex ratio in offspring of *BRCA1* mutation carriers was reported,⁵ with a bias towards females, compared with offspring of women with *BRCA2* mutations and those without mutations in either gene. Others have not found a difference in the sex ratios of offspring of *BRCA1* and *BRCA2* mutation carriers.^{6–8} Here, we analysed the sex ratio of offspring in a large cohort of *BRCA1* and *BRCA2* mutation carriers, as well as in HBOC families who tested negative for *BRCA1* and *BRCA2* mutations or were not tested.

METHODS

Database

We included data from 1276 families evaluated between 1992 and 1994 (University of Michigan) and between 1994 and 2003 (University of Pennsylvania) in breast cancer risk evaluation clinics and considered to be at increased risk for having a breast cancer susceptibility gene mutation. Individuals were either self referred or physician referred for assessment of inherited susceptibility to breast or ovarian cancer. All individuals providing data signed their informed consent before genetic testing and entry into our database, which includes detailed pedigree information. Data collection between 1992 and 1994 was done with the approval from the University of Michigan institutional review board; subsequent data collection and analyses were done with approval of the University of Pennsylvania institutional review board.

All tested individuals had at least a 10% likelihood of having a *BRCA1* or *BRCA2* mutation, based on prevalence tables or various prediction models (reviewed by Domchek *et al*⁹). Untested individuals also had at least a 10% prior probability of having a detectable *BRCA1* or *BRCA2* mutation, based on one or more of the following criteria:

- both breast and ovarian cancer in the same lineage of a three generation pedigree;
- at least one breast cancer diagnosis before age 35;
- at least one case of male breast cancer;
- at least three breast cancers in the same lineage diagnosed before age 60;
- prior probability of a *BRCA1* or *BRCA2* mutation calculated as above;

Key points

- A previous report suggested that there is a skewed sex ratio in offspring of *BRCA1* mutation carriers compared with *BRCA2* carriers or families with evidence of hereditary breast and ovarian cancer (HBOC) but without known mutations, possibly because of *BRCA1* involvement in X chromosome inactivation. This association was investigated in a large cohort of HBOC families.
- Of the 795 offspring of 180 *BRCA1* mutation carriers, 57.6% were female; in 268 offspring of 99 *BRCA2* mutation carrier offspring, 54.1% were female ($p=0.32$).
- In 997 mutation negative or untested families, 57.4% and 59.1% of offspring, respectively, were female ($p=0.93$, $p=0.50$).
- In *BRCA2* mutation carriers, mutations in the 3' end of the gene (exons 12–27) were associated with a significant reduction in female offspring (40.3% female) compared with 5' mutations (exons 1–11) (58.7% female, $p=0.009$), and compared with all other HBOC families, suggesting that *BRCA2* may play an as yet undefined role in X chromosome inactivation or other sex determination mechanisms.

- breast cancer with any other primary cancer in the same individual.¹⁰

All patients were offered clinical testing for *BRCA1* and *BRCA2* mutations; 668 of the 1276 families were tested for *BRCA1* and *BRCA2* mutations. Of these, 180 families had *BRCA1* mutations (26.9%), 99 had *BRCA2* mutations (14.8%), and 389 had no detectable mutation in either gene (58.2%). Families identified with variants of unknown significance (VUS) were not included with these 1276 families. In all, 608 families opted not to undergo testing.

Genetic testing

Mutation testing methods included direct sequencing in 56% of individuals (either comprehensive or screening for the three Ashkenazi Jewish founder mutations; Myriad Genetics, Utah, USA); conformation sensitive gel electrophoresis (CSGE) in 37%¹¹; and other methods in 7% (Southern blot analysis, single strand conformational polymorphism analysis, or protein truncation assay).¹² The same proportion of individuals was tested by direct sequencing in the mutation positive and mutation negative groups (both 56%).

Abbreviations: CSGE, conformation sensitive gel electrophoresis; HBOC, hereditary breast and ovarian cancer

Table 1 Sex ratio in hereditary breast and ovarian cancer families

	Families	Individuals	Tested mutation carriers	Obligate mutation carriers	Total offspring	Female offspring	*p Value
BRCA1	180	475	453	22	795	458 (57.6%)	
BRCA2	99	173	172	1	268	145 (54.1%)	0.32
All tested negative	389	431	–	–	556	319 (57.4%)	0.93
Affected, tested negative	329	355	–	–	496	288 (58.1%)	0.87
Untested (affected, untested)	608	1315	–	–	1459	862 (59.1%)	0.50

*Compared with BRCA1 mutation carriers.

Pedigree analysis

Offspring were counted through query of our Progeny database. All offspring of obligate and test confirmed *BRCA1* and *BRCA2* mutation carriers were counted. In the tested negative group, offspring of all tested individuals were counted. In the untested group, offspring of all individuals affected with breast or ovarian cancer were included.

Statistical methods

The χ^2 test was used to evaluate differences in offspring ratios. STATA (release 8.0, Stata Corporation, College Station, Texas, USA) was used for all statistical analyses.

RESULTS

Of 795 offspring of *BRCA1* mutation carriers, 458 (57.6%) were female, while of 268 offspring of *BRCA2* mutation carriers, 145 (54.1%) were female (percentage of female offspring not significantly different, $p = 0.32$). In families testing negative, 319 of 556 offspring (57.4%) were female. This ratio was unchanged when we restricted counting to offspring of individuals testing negative who were affected by breast or ovarian cancer, who accounted for 85% of those testing negative (288 of 496; 58.1%). Likewise, 862 of 1459 offspring (59.1%) of individuals affected by breast or ovarian cancer in families not tested for *BRCA1* or *BRCA2* were female. This ratio was not significantly different from that in offspring of *BRCA1* mutation carriers (table 1).

Of 180 families with *BRCA1* mutations, 94 (52.2%) had one of the Ashkenazi Jewish founder mutations (68 with 185delAG, 26 with 5832insC). The sex ratio was not significantly different in the offspring of carriers of these specific *BRCA1* mutations compared with other *BRCA1* mutations (data not shown). Forty seven of 99 families (47.5%) with *BRCA2* mutations had the Ashkenazi Jewish founder mutation 6174delT. Interestingly, 6174delT mutation carriers had a greater proportion of female offspring than carriers of other *BRCA2* mutations (72/117 (61.5%) *v* 73/151 (48.3%), $p = 0.032$) (table 2). Thus we asked whether positional effects might account for this difference. This analysis revealed that the offspring of individuals with mutations proximal to and including exon 11 (encompassing 6174delT) were significantly more likely to be female. Indeed, we found that 118 of 201 offspring (58.7%) in families with 5' mutations (exons 1–11, which includes 6174delT) were

female, while 27/67 (40.3%) offspring of those with mutations at the 3' end (exons 12–27) were female ($p = 0.009$). No positional effect was seen in analysis of *BRCA1* mutations (data not shown).

DISCUSSION

de la Hoya and colleagues examined the sex distribution of offspring in 17 families with *BRCA1* mutations and in 15 families with *BRCA2* mutations seen in their risk evaluation clinic, and reported a skewed ratio favouring female offspring in *BRCA1* mutation carriers (table 3).⁵ In contrast, using a larger, statistically more powerful dataset, we found no difference in sex ratios in any of the three groups of HBOC families we analysed when the groups were analysed as a whole, although all groups had an excess of female offspring probably reflecting ascertainment bias. However, in evaluating potential biases stemming from overrepresentation of Ashkenazi founder mutations in our dataset, we did find that offspring sex ratios of *BRCA2* mutation carriers varied depending on the position of the mutation, with a decrease in female offspring associated with mutations in the 3' end of *BRCA2*. As the percentage of female offspring of individuals with 5' *BRCA2* mutations is similar to that of *BRCA1* mutation carriers and others with HBOC, it seems more likely that those with 3' *BRCA2* mutations would have a decrease in female offspring, although it cannot be excluded that this is related to an excess of male offspring.

In considering other sources of bias in our dataset, it should be noted that offspring under the age of 18 are not kept in our database, for consent and confidentiality reasons. However, both female and male offspring under 18 are deleted from the pedigrees and therefore this practice should not induce a sex bias.

In the study by de la Hoya and colleagues, the number of *BRCA2* mutation carriers was small ($n = 15$) and there were no 6174delT mutation carriers in the sample set. When we excluded 6174delT mutations from our analysis, which represent the majority of 5' *BRCA2* mutations in our dataset, the trend persisted but we lost the statistical power to see a positional effect and our results appear similar to those of de la Hoya. Thus both small overall sample size and absence of 6174delT mutation carriers in the Spanish dataset may explain the discrepancies between these studies. However, we cannot exclude a mutation specific effect of 6174delT.

Table 2 *BRCA2* mutation carrier offspring data by mutation position

	Families	Individuals	Total offspring	Female offspring	p Value
All <i>BRCA2</i>	99	174	268	145 (54.1%)	
6174delT	47	71	117	72 (61.5%)	0.032
Other <i>BRCA2</i>	52	103	151	73 (48.3%)	
Exons 1–11*	82	138	201	118 (58.7%)	0.009
Exons 12–27	17	36	67	27 (40.3%)	
Exons 1–11 excluding 6174delT	35	67	84	46 (54.7%)	0.077
Exons 12–27	17	36	67	27 (40.3%)	

*Includes 6174delT.

Table 3 Comparison of sex ratio data

	Total families	Mutation	Male	Female	p Value*
de la Hoya <i>et al</i> ²	17	BRCA1	65 (33%)	133 (67%)	
	15	BRCA2	85 (47%)	97 (53%)	0.006
	36	Tested negative	315 (48%)	344 (52%)	<0.001
UPenn	180	BRCA1	337 (42%)	458 (58%)	
	99	BRCA2	123 (46%)	145 (54%)	0.32
	389	Tested negative	237 (42.6%)	319 (57.4%)	0.93
	608	Untested	597 (40.9%)	862 (59.1%)	0.50

*Compared with BRCA1 mutation carriers.
UPenn, University of Pennsylvania (current study).

When the offspring ratio in our study participants was compared with US census data from 2000 (www.census.gov) an excess of female offspring is evident in all families seen in our breast cancer risk evaluation clinic ($p<0.0001$). The most likely explanation for the overall increased ratio of female offspring of women attending breast cancer risk evaluation clinics is a selection bias for participation, as a significant predictor for use of genetic testing is the perception of benefit to family members.¹³ With breast and ovarian cancer susceptibility being the most widely known risks associated with *BRCA1* and *BRCA2* mutations, many patients give concern for their daughters as their primary reason for presenting for risk evaluation and genetic testing. As overall cancer risks for male mutation carriers are lower,¹⁴ woman with only sons may be less likely to seek evaluation than women with one or more daughters. Alternatively, mutations in genes associated with hereditary breast and ovarian cancer syndromes could indeed be associated with a skewed offspring ratio, perhaps because of abnormalities in X chromosome inactivation in mutation carriers.

These data suggest the possibility that expressing truncated *BRCA2* has different biological consequences from expressing no *BRCA2* at all. This explanation is consistent with the recent observation that individuals homozygous for *BRCA2* mutations have Fanconi anaemia, but it appears that at least one mutation in a *BRCA2* homozygote must be in the 3' region of the gene or the embryo may not survive.^{15 16} In addition, a polymorphism in exon 10 of *BRCA2* (N372H) has been reported to result in an excess of male births. Although this altered sex ratio is in the opposite direction to what we have observed, this may reflect a difference between polymorphisms and deleterious mutations resulting in protein truncation.¹⁷

Conclusions

We found no difference overall in sex ratios of offspring of *BRCA1* mutation carriers, *BRCA2* mutation carriers, and HBOC families without a known *BRCA1* or *BRCA2* mutation. However, differences in offspring sex ratios became apparent when *BRCA2* mutation carriers where analysed by mutation position. These data, although very preliminary, are intriguing and suggest that additional functional analysis of *BRCA2* may reveal a role for *BRCA2*, as well as *BRCA1*, in X inactivation or in other sex determination mechanisms.

ACKNOWLEDGEMENTS

This research was supported by NIH award K12-HD43459 to SMD, and by the Breast Cancer Research Foundation and the Abramson Family Cancer Research Institute to BLW.

Authors' affiliations

S M Domchek, S L Merillat, J Tigges, A J Tweed, M Weinat, J Stopfer, B L Weber, Abramson Cancer Center, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Competing interests: none declared

Correspondence to: Dr Susan Domchek, 14 Penn Tower, Hospital of the University of Pennsylvania, 3400 Spruce St, Philadelphia, PA 19104, USA; susan.domchek@uphs.upenn.edu

Received 30 September 2004
Revised version received 3 January 2005
Accepted for publication 5 January 2005

REFERENCES

1 Venkitaraman AR. Cancer susceptibility and the functions of *BRCA1* and *BRCA2*. *Cell* 2002;**108**:171–82.

2 Ganesan S, Silver DP, Greenberg RA, Avni D, Drapkin R, Miron A, Mok SC, Randrianarison V, Brodie S, Salstrom J, Rasmussen TP, Klimke A, Marrese C, Marahrens Y, Deng CX, Feunteun J, Livingston DM. *BRCA1* supports XIST RNA concentration on the inactive X chromosome. *Cell* 2002;**111**:393–405.

3 Lee JT. Homozygous Tsix mutant mice reveal a sex-ratio distortion and revert to random X-inactivation. *Nat Genet* 2002;**32**:195–200.

4 Buller RE, Sood AK, Lallas T, Buekers T, Skilling JS. Association between nonrandom X-chromosome inactivation and *BRCA1* mutation in germline DNA of patients with ovarian cancer. *J Natl Cancer Inst* 1999;**91**:339–46.

5 de la Hoya M, Fernandez JM, Tosar A, Godino J, Sanchez de Abajo A, Vidart JA, Perez-Segura P, Diaz-Rubio E, Caldes T. Association between *BRCA1* mutations and ratio of female to male births in offspring of families with breast cancer, ovarian cancer, or both. *JAMA* 2003;**290**:929–31.

6 Struwing JP, Hartge P, Wacholder S, Tucker MA, Greene MH. *BRCA1* and sex ratio. *Eur J Hum Genet* 2004;**12**:663–7.

7 Feunteun J, Chompret A, Helbling-Leclerc A, Stoppa-Lyonnet D, Belotti M, Nogues C, Bonaiti-Pellie C. Sex ratio among the offspring of *BRCA* mutation carriers. *JAMA* 2004;**292**:687–8.

8 Gal I, Sadetzki S, Gershoni-Baruch R, Oberman B, Carp H, Papa MZ, Diestelman-Menachem T, Eisenberg-Barzilai S, Friedman E. Offspring gender ratio and the rate of recurrent spontaneous miscarriages in Jewish women at high risk for breast/ovarian cancer. *Am J Hum Genet*. 2004;**74**: 1270–5, Epub 2004 Apr 28.

9 Domchek SM, Eisen A, Calzone K, Stopfer J, Blackwood A, Weber BL. Application of breast cancer risk prediction models in clinical practice. *J Clin Oncol* 2003;**21**:593–601.

10 Shih HA, Nathanson KL, Seal S, Collins N, Stratton MR, Rebbeck TR, Weber BL. *BRCA1* and *BRCA2* mutations in breast cancer families with multiple primary cancers. *Clin Cancer Res* 2000;**6**:4259–64.

11 Couch FJ, DeShano ML, Blackwood MA, Calzone K, Stopfer J, Campeau L, Ganguly A, Rebbeck T, Weber BL. *BRCA1* mutations in women attending clinics that evaluate the risk of breast cancer. *N Engl J Med* 1997;**336**:1409–15.

12 Unger MA, Nathanson KL, Calzone K, Antin-Ozerkis D, Shih HA, Martin AM, Lenoir GM, Mazoyer S, Weber BL. Screening for genomic rearrangements in families with breast and ovarian cancer identifies *BRCA1* mutations previously missed by conformation-sensitive gel electrophoresis or sequencing. *Am J Hum Genet* 2000;**67**:841–50.

13 Armstrong K, Calzone K, Stopfer J, Fitzgerald G, Coyne J, Weber B. Factors associated with decisions about clinical *BRCA1/2* testing. *Cancer Epidemiol Biomarkers Prev* 2000;**9**:1251–4.

14 Thompson D, Easton DF. Cancer incidence in *BRCA1* mutation carriers. *J Natl Cancer Inst* 2002;**94**:1358–65.

15 Offit K, Levran O, Mullaney B, Mah K, Nafa K, Batish SD, Diotti R, Schneider H, Deffenbaugh A, Scholl T, Proud VK, Robson M, Norton L, Ellis N, Hanenberg H, Auerbach AD. Shared genetic susceptibility to breast cancer, brain tumors, and Fanconi anemia. *J Natl Cancer Inst* 2003;**95**:1548–51.

16 Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, Persky N, Grompe M, Joenje H, Pals G, Ikeda H, Fox EA, D'Andrea AD. Biallelic inactivation of *BRCA2* in Fanconi anemia. *Science* 2002;**297**:606–9, Epub 2002 Jun 13.

17 Healey CS, Dunning AM, Teare MD, Chase D, Parker L, Burn J, Chang-Claude J, Mannermaa A, Kataja V, Huntsman DG, Pharoah PD, Luben RN, Easton DF, Ponder BA. A common variant in *BRCA2* is associated with both breast cancer risk and prenatal viability. *Nat Genet* 2000;**26**:362–4.