Meeting report

13th General Meeting of The Breast Cancer Linkage Consortium, November 29–December 1, 1999, Amsterdam, The Netherlands

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Introduction

It was not particularly noted as an occasion for celebration, but the 13th meeting of the Breast Cancer Linkage Consortium (BCLC) did mark the 10th anniversary of the Consortium. Exactly 10 years ago, in November 1989, a small group of cancer geneticists and epidemiologists convened in Lyon, France, to initiate a joint effort with the aim being to find breast cancer predisposition genes through linkage analysis in multiple case families. Within a year, independent work by Dr King (then at Berkeley University, California, USA) led to the discovery of linkage to the *BRCA1* locus [1]. This provided a decisive incentive toward the creation of what is now known as the BCLC database, stimulating scientists to contribute linkage information to replicate this finding [2]. Currently, this database holds pedigree information on 1122 breast cancer families, and includes data on health and carrier status, as well as several thousand person-years of follow up. These pedigrees are from all over the world, and have been submitted to the database by more than 75 research centres. Genetic defects in *BRCA1* or *BRCA2* have been identified in 522 and 192 families, respectively. Linkage information at polymorphic markers flanking *BRCA1* and *BRCA2* is available in almost 300 families. This database has allowed cumulative estimates to be made of the cancer risks conferred by mutations in *BRCA1* [3] and *BRCA2* [4,5]. Furthermore, it has provided estimates of the proportions of families affected by mutations in either gene, given a certain phenotype of the family as defined by the number of patients with breast or ovarian cancer [4].

The Breast Cancer Linkage Consortium database

Doug Easton (University of Cambridge, Cambridge, UK) presented a number of recent analyses performed with the database. The combination of linkage and mutation information made it possible to estimate the sensitivity of mutation testing as currently applied by many laboratories. For *BRCA1*, a point estimate of 63% was derived, and 72% for *BRCA2*. Thus, we are apparently still missing a substantial proportion of disease-related mutations. Partly, this is due to technical limitations of the tests. Most testing currently performed worldwide is based on polymerase chain reaction, which misses large genomic rearrangements and intronic variants that interfere with messenger RNA processing. Sylvie Mazoyer (International Agency for Research on Cancer, Lyon, France) showed us that about 15 genomic rearrangements are known today in *BRCA1*. Depending on whether they display a founder effect in the population under investigation, they may represent anywhere between 5–25% of all mutations found. She also presented the results of a worldwide survey of the recently detected 6-kilobase duplication of exon 13 in *BRCA1*, and found it in 15 out of 3561 families tested, six of which were identified in the UK. Undoubtedly, though, we are also misclassifying unique missense variants as being of 'unknown significance'. This problem can only be addressed by assays that assess the functional relevance of the DNA change.

Genotype–phenotype correlations

Analysis of the BCLC database also confirmed the presence of a genotype–phenotype correlation for both *BRCA1* and *BRCA2*. Independent work by Bruce Ponder (University of Cambridge, Cambridge, UK) showed earlier that mutations in the carboxyl-terminal part of *BRCA1*, or those in the middle third part of *BRCA2*, display altered family-specific ratios of breast and ovarian cancer cases, relative to mutations in the remainder of the genes [6,7]. Among non-Cambridge families in the database, this effect was also seen, albeit with lower level of significance. For the ovarian cancer cluster region in *BRCA2*,

*See Appendix. BCLC = Breast Cancer Linkage Consortium; RR = relative risk.

the evidence from 163 *BRCA2*-linked families suggested that the ratio distortion is due to a reduced relative risk for breast cancer in conjunction with an increased relative risk for ovarian cancer, as compared with the nonovarian cancer cluster region regions of *BRCA2*.

Modifiers of risk

There is currently much interest in whether the cancer risks conferred by *BRCA1* and *BRCA2* mutations can be modified by other factors, including genetic factors. The evidence for such modifiers stems mainly from observations that cancer risk estimates derived from high-risk families differ from those from population-based studies, and that cancer risks apparently differ substantially between families and populations [8]. Despite some positive findings [9,10], formal unbiased evidence that *BRCA1*/*BRCA2* risk-modifying genes exist is lacking, and David Goldgar (IARC, Lyon, France) discussed the statistical problems in finding them. Success strongly depends on the extent of the conferred modifying effect [expressed as relative risk (RR) or odds ratio]. If the RR is greater than 2.0, traditional linkage analysis in a well-defined set of nuclear families may suffice. For smaller effects ($1.3 < RR < 2.0$), association studies with candidate genes seem to be the only way forward. Kate Nathanson (University of Pennsylvania School of Medicine, Philadelphia, USA) presented some data using the former approach. An initial finding of linkage with two markers on 5q in 19 *BRCA1*-linked families was lost on adding four new families. Timothy Rebbeck (University of Pennsylvania School of Medicine, Philadelphia, USA) presented work by a North American Consortium of eight research groups, using the alternative approach with 506 *BRCA1* and 225 *BRCA2* mutation carriers. They found that polymorphisms in the *AR*, *AIB1*, *GSTM1* and *XRCC1* genes could significantly modify breast cancer risks. These observations now need replication in independent populations of carriers. Doug Easton showed some power calculations indicating that at least 500 cases are needed to detect a RR of 1.5, and more than 1000 cases are needed to detect a RR of 1.3 at a significance level of 10^{-4}.

Population studies

Much work on the prevalence of *BRCA1* and *BRCA2* mutations among breast and ovarian cancer cases unselected for family history from populations with strong founder effects at both genes has been published in recent years. These mostly deal with the Ashkenazi Jewish and Icelandic populations in which a few specific mutations occur at high frequency. The data suggest that *BRCA1* and *BRCA2* together largely explain the excess familial breast cancer risk in these populations, and that both genes confer considerably lower cancer risks than previously estimated by the BCLC on high-risk families [8]. Few data are as yet available on populations in which *BRCA1* and *BRCA2* mutations are less frequent, and Paul Pharoah (CRC Human Cancer Genetics Group, University of Cambridge, Cambridge, UK) presented data on the East Anglian population in the UK. Among 1500 population-based breast cancer cases diagnosed before age 55 years, he found eight *BRCA1* and 16 *BRCA2* mutations, which would only explain 17% of the excess familial risk in this population. This is very similar to the estimates obtained in other UK subpopulations [11]. The cumulative breast cancer risks conferred by *BRCA1* mutations were slightly lower than those derived by the BCLC (ie approximately 50% by age 60 years), but for *BRCA2* mutations comparable estimates were found (approximately 70% by age 60 years). Other investigators reported on similar work that is as yet uncompleted, and is ongoing in The Netherlands, Poland, Hungary, Sweden and Finland.

Other breast cancer predisposition genes

Clearly, there is considerable room for additional breast cancer predisposing genes, and previous work by the BCLC [4] has shown that, in particular, they may be found among families without individuals with ovarian cancer or male breast cancer, and only four or five breast cancer cases. However, a linkage search carried out jointly by David Goldgar and Mike Stratton (Institute of Cancer Research, Belmont, Sutton, UK) in a subset of approximately 50 such BCLC families, which were carefully selected for not being due to *BRCA1* or *BRCA2*, has remained negative so far. Assuming *BRCA3* is the only gene causing breast cancer in all these families, 89% of the genome has been excluded for harbouring it with lod scores lower than −2. Assuming that 50% of the examined families are due to *BRCA3*, and the remainder due to other genes, only 8% of the genome is excluded at the same significance level. This illustrates the difficulty in finding *BRCA3*, and the idea that '*BRCA3*' actually consists of an unknown number of different genes (*BRCA3*, *BRCA4*, *BRCA5*, etc) is therefore gaining weight. Olli Kallioniemi (National Human Genome Research Institute, Bethesda, USA) presented data from a Nordic/NHGRI collaborative group, stressing the importance of characterizing the breast tumours in non-*BRCA1*/*BRCA2* linked families by a combination of comparative genome hybridization, loss of heterozygosity, immunohistochemistry and microarray analysis. In this way, specific somatic genetic changes might be exploited to further subgroup the non-*BRCA1*/*BRCA2* families, thereby decreasing genetic heterogeneity among them. Thus, this collaboration has now collected 78 families for a linkage search, and several preliminary hints for linkage are currently being pursued further.

Pathology of *BRCA*-associated breast and ovarian cancer

The BCLC resources have recently been extended with sections and paraffin-embedded tumour tissues from over 300 breast and ovarian cancer patients from BCLC fami-

lies. Many of these patients are known carriers of *BRCA1* or *BRCA2* mutations, whereas others are from families that probably harbour mutations in other genes. Sunil Lakhani (University College London Medical School, London, UK) is maintaining this resource, which has already led to an accurate description of the *BRCA1*- and *BRCA2*-linked morphology of breast cancer [12,13]. He presented the results of immunophenotyping these cancers, showing that *BRCA1*-, but not *BRCA2*-linked tumours are often negative for oestrogen receptor and progesterone receptor, relative to age-matched, apparently sporadic cases. Approximately 40% of *BRCA1*-related tumours showed positive p53-staining, which was confirmed by two Italian studies (by Paolo Radice, Istituto Nazionale, Tumori, Milan, Italy, and Maria Caligo, University of Pisa, Pisa, Italy) that examined the presence of *p53* gene mutations in *BRCA1*-related tumours. Non-*BRCA1*/*BRCA2* familial breast tumours are more often of invasive lobular type (but only significantly so when compared with the *BRCA1*-related cancers), and are overall of lower grade than are sporadic tumours.

Low risk genes

The notion that certain polymorphisms in genes that are involved in hormone or carcinogen metabolism, or in DNA repair processes, might confer low risks to breast cancer has attracted much attention. Although the identification of such factors is not facilitated by the BCLC database, they could explain major proportions of breast cancer in our population. Paul Pharoah presented a meta-analysis of 46 published studies on this topic, indicating that most have analyzed limited numbers of cases and that none of the 12 positive associations had been replicated by more than one study [14]. After pooled analysis, only four associations remained, all with RRs below 2: *GSTM1* (homozygous null), *TP53* (72Pro), *GSTP1* (105Val) and *CYP19* ($TTTA_{10}$). None of these could be replicated in his own population-based study of almost 1200 cases (incident cases diagnosed at age below 65 years, and prevalent cases diagnosed at age below 55 years) from East Anglia, UK.

Clinical dilemmas

Finally, a clinical research session illustrated how quickly genetic knowledge on hereditary breast cancer has moved to the bedside, but not without posing serious dilemmas. All over the world, women attend cancer family clinics to learn about their inherited cancer risks. Many of the preventive options currently offered to a woman who has been presymptomatically diagnosed as being a carrier of a BRCA mutation remain without formal supporting evidence, however. Difficulty in recruiting unbiased cohorts of individuals or patients, or in performing proper prospective case–control studies hampers progress in this area. Malcolm Pike (University of Southern California, Los Angeles, USA), Mitch Dowsett (Royal Marsden Hospital, London, UK), and Steven Narod (Women's College Hospital, Toronto, Canada) discussed chemoprevention options. The choice of preventive agent is hotly debated, and good prospective data on *BRCA* mutation carriers is lacking. Timothy Rebbeck discussed prophylactic surgery, which is intuitively the most secure way to reduce breast cancer risk to below population levels. Although data on its efficacy are emerging [15], this option is socially ill accepted in many parts of the world, and formal proof of its preventive effect in *BRCA* carriers is again still lacking. For those who opt for a wait-and-see policy there are uncertainties regarding treatment outcome as well. William Foulkes (McGill University, Montreal, Quebec, Canada) presented a summary of several studies to show that some indicate no survival difference, whereas others find a poorer survival in *BRCA* mutation carriers than in noncarriers. Finally, Flora van Leeuwen (Netherlands Cancer Institute, Amsterdam, The Netherlands) moderated a discussion on how oral contraceptives might interact with the breast and ovarian cancer risks conferred by *BRCA1* and *BRCA2* mutations.

Conclusion

The field of inherited susceptibility to breast cancer has made a giant leap forward during the past decade, but new challenges lie ahead. The BCLC has been extremely successful in collating linkage and clinical data from families with inherited breast cancer, enabling it to establish cancer risks, genetic heterogeneity estimates and prevalence estimates for *BRCA1* and *BRCA2* mutations, and other estimates. The search for additional susceptibility genes will require very large numbers of highly selected groups of multiple case families. Likewise, the search for genetic modifiers of risk will require large numbers of samples, as will the establishment of typical tumour characteristics of cancers linked to *BRCA1* or *BRCA2* mutations. The BCLC continues to provide an excellent platform for such studies to be organized.

References

1. Hall J, Lee M, Newman B, *et al*: **Linkage of early-onset familial breast cancer to chromosome 17q21.** *Science* 1990, **250**:1684–1689.
2. Easton D, Bishop D, Ford D, *et al*: **Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families.** *Am J Hum Genet* 1993, **52**:678–701.
3. Ford D, Easton D, Bishop D, *et al*: **Risks of cancer in BRCA1-mutation carriers.** *Lancet* 1994, **343**:692–695.
4. Ford D, Easton DF, Stratton M, *et al*: **Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families.** *Am J Hum Genet* 1998, **62**:676–689.
5. Breast Cancer Linkage Consortium: **Cancer risks in BRCA2 mutation carriers.** *J Natl Cancer Inst* 1999, **91**:1310–1316.
6. Gayther SA, Warren W, Mazoyer S, *et al*: **Germline mutations of the *BRCA* 1 gene in breast and ovarian cancer families provide evidence for a genotype–phenotype correlation.** *Nature Genet* 1995, **11**:428–433.
7. Gayther SA, Mangion J, Russell P, *et al*: **Variation of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene.** *Nature Genet* 1997, **15**:103–105.
8. Devilee P: **BRCA1 and BRCA2 testing: weighing the demand against the benefits.** *Am J Hum Genet* 1999, **64**:943–948.
9. Phelan CM, Rebbeck TR, Weber BL, *et al*: **Ovarian cancer risk in *BRCA1* carriers is modified by the *HRAS1* variable number of tandem repeat (VNTR) locus.** *Nature Genet* 1996, **12**:309–311.

10. Rebbeck TR, Kantoff PN, Krithivas K, *et al*: **Modification of BRCA1-associated breast cancer risk by the polymorphic androgen-receptor CAG repeat.** *Am J Hum Genet* 1999, **64**:1371–1377.
11. Peto J, Collins N, Barfoot R, *et al*: **Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer.** *J Natl Cancer Inst* 1999, **91**:943–949.
12. Breast Cancer Linkage Consortium: **Pathology of familial breast cancer: differences between breast cancers in carriers of BRCA1 or BRCA2 mutations and sporadic cases.** *Lancet* 1997, **349**:1505–1510.
13. Lakhani SR, Jacquemier J, Sloane JP, *et al*: **Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations.** *J Natl Cancer Inst* 1998, **90**: 1138–1145.
14. Dunning AM, Healey CS, Pharoah PD, *et al*: **A systematic review of genetic polymorphisms and breast cancer risk.** *Cancer Epidemiol Biomarkers Prev* 1999, **8**:843–854.
15. Hartmann LC, Schaid DJ, Woods JE, *et al*: **Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer.** *N Engl J Med* 1999, **340**:77–84.

Appendix

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