UNITED STATES DISTRICT COURT
FOR THE SOUTHERN DISTRICT OF NEW YORK

ASSOCIATION FOR MOLECULAR PATHOLOGY; AMERICAN COLLEGE OF MEDICAL GENETICS; AMERICAN SOCIETY FOR CLINICAL PATHOLOGY; COLLEGE OF AMERICAN PATHOLOGISTS; HAIG KAZAZIAN, MD; ARUPA GANGULY, PhD; WENDY CHUNG, MD, PhD; HARRY OSTRER, MD; DAVID LEDBETTER, PhD; STEPHEN WARREN, PhD; ELLEN MATLOFF, M.S.; ELSA REICH, M.S.; BREAST CANCER ACTION; BOSTON WOMEN'S HEALTH BOOK COLLECTIVE; LISBETH CERIANI; RUNI LIMARY; GENAE GIRARD; PATRICE FORTUNE; VICKY THOMASON; KATHLEEN RAKER,

Plaintiffs,

-against-

UNITED STATES PATENT AND TRADEMARK OFFICE; MYRIAD GENETICS; LORRIS BETZ, ROGER BOYER, JACK BRITTAIN, ARNOLD B. COMBE, RAYMOND GESTELAND, JAMES U. JENSEN, JOHN KENDALL MORRIS, THOMAS PARKS, DAVID W. PERSHING, and MICHAEL K. YOUNG, in their official capacity as Directors of the University of Utah Research Foundation,

Defendants.

No. 09 Civ. 4515 (RWS)

ECF Case

DECLARATION OF DR. DONNA SHATTUCK

I, Donna Shattuck, declare under penalty of perjury:

1. I received my B.A. in Biology from SUNY Binghamton, my Ph.D. in Microbiology in 1982 from the University of Virginia, and performed postdoctoral training at Washington University in St. Louis. A copy of my curriculum vitae and a list of my research publications are attached as Exhibits 1 & 2, respectively.

2. I was employed by Myriad Genetics, Inc. between July 1, 1993 and July 1, 2009. At Myriad I held several senior positions, including Vice President of Population Genetics at the time of my departure.

3. I was personally involved in the identification of the *BRCA1* gene as a breast and ovarian cancer susceptibility gene. In that project, I led the mutation screening effort, resequencing the newly isolated candidate genes to detect mutations that co-segregate with breast cancer cases. I am one of the named inventors in United States Patent Nos. 5,693,473, 5,709,999, 5,710,001, 5,747,282, & 5,753,441.

4. The identification of *BRCA1* required the positional cloning approach. In positional cloning of the *BRCA1* gene, the following steps were necessary:

(a) obtaining DNA samples from large, well-documented families with inherited breast cancer;

(b) discovering appropriate polymorphic markers in the *BRCA1* region;

(c) typing individuals from suitable families with suitable polymorphic markers to yield a sufficiently small chromosomal region containing the *BRCA1* gene;

(d) identifying gene structures within that small chromosomal region; and

(e) identifying causal mutations in the gene structures that segregate with breast cancer in a statistically significant manner, but not with control or non-cancer patients.

5. Each of the above steps required substantial effort and technical difficulties had to be overcome in each. Indeed, the *BRCA1* gene would not have been identified had failure occurred in any one of the identified steps.

THE ART TAUGHT SEVERAL DIFFERENT GENOMIC REGIONS FOR *BRCA1*

6. Even in September of 1994, when Myriad and its collaborators (the University of Utah and the NIEHS, an institute within the NIH) had identified the *BRCA1* gene, there was still a great deal of puzzling information in the public domain with regard to the chromosomal location of the *BRCA1* gene. Such puzzling information led to a general state of confusion and frustration within the field.

7. For example, as shown in Exhibit 3, while Albertsen et al.[1] disclosed that BRCA1 was located between markers D17S776 and D17S78, Smith et al.[2] taught that *BRCA1* should be between D17S702 and EDH17D, which region does not actually contain the *BRCA1* gene. Moreover, Jones et al.[3] suggested that the *BRCA1* gene was in the region between 1A1.3B and D17S78, which is actually distal to the *BRCA1* gene. In addition, Cropp et al.[4] incorrectly localized *BRCA1* to a small region between D17S846 and D17S746.

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[1] Albertsen *et al.*, *A physical map and candidate genes in the* BRCA1 *region on chromosome 17q12-21*, NAT. GENET., 8:387-91 (1994).
[2] Smith *et al.*, *Localisation of the breast-ovarian cancer susceptibility gene* (BRCA1) *on 17q12-21 to an interval of < or = 1 cM*, GENES CHROM. CANCER, 10:71-6 (1994).
[3] Jones *et al.*, *The detailed characterisation of a 400 kb cosmid walk in the* BRCA1 *region: identification and localisation of 10 genes including a dual-specificity phosphatase*, HUM. MOL. GENET., 11:1927-34 (1994).
[4] Cropp *et al.*, *Evidence for involvement of BRCA1 in sporadic breast carcinomas*, CANCER RES., 54:2548-51 (1994).

8. Given the confusion, a skilled artisan would not have known in which chromosomal region to look for the *BRCA1* gene. Indeed, it would have required a great deal of effort to analyze and identify genes in each of the suggested regions and to then determine if the genes harbor mutations that co-segregate with breast cancer in individual members of breast cancer families (kindreds).

EXTENSIVE FAMILY PEDIGREE ANALYSIS AND SAMPLE COLLECTION WERE CRITICAL THROUGHOUT THE SEARCH FOR *BRCA1*

9. One of the keys to the Myriad Collaboration's success in characterizing *BRCA1* was the large, well documented, and highly informative collection of family pedigrees (breast and ovarian cancer kindreds) it had in its possession, and the numerous DNA samples obtained from many members of these kindreds. This was particularly important because during the period of the discovery of *BRCA1*, competition and rivalries between research groups led to the competing groups fervently protecting their samples and kindreds, and not sharing kindreds and samples with each other.

10. Large and informative kindreds are essential for successful linkage analysis. The families need be large in order to provide sufficient statistical power for the analysis. The families need be informative, i.e., exhibiting clear co-inheritance of mutations within the *BRCA1* gene with the disease, such that segregation of a *BRCA1* susceptibility allele can be deciphered by statistical means. As a matter of fact, even some large families with a large number of individuals afflicted with breast cancer were more confusing than helpful in the search for *BRCA1*. This is because the cancer running through these families could have been due to susceptibility genes other than *BRCA1*. For example, Exhibit 4 illustrates

that Kindreds 1911 and 1927 both have over 9 affected individuals, yet the LOD scores derived from these families were not useful in identifying *BRCA1* by linkage analysis.

11. It was well-recognized in the field that the Myriad Collaboration had gathered and characterized the largest and best breast cancer kindred collections. This was largely due to decades of extensive effort by Dr. Mark Skolnick, a pioneer of family collection and pedigree analysis for linkage analysis. *See* D. Skolnick. Dr. Skolnick was the leader of the Myriad Collaboration and is a named inventor in United States Patent Nos. 5,710,001, 5,747,282, & 5,753,441.

12. Indeed, our Kindred 2082 was the largest and best breast cancer kindred known. As shown in Exhibits 4 and 5, Kindred 2082 has 51 breast cancer cases and 22 ovarian cases. With kindreds such as Kindred 2082, as well as other inventive approaches, we were able to narrow the putative *BRCA1* region to about 600 kb by linkage analysis, thereby making the isolation and of the *BRCA1* gene possible.

13. The large and informative family collections in the Myriad Collaboration's possession were also critical in identifying mutations in the *BRCA1* gene that co-segregated with disease in the breast and ovarian cancer pedigrees.

14. Again as illustrated by Kindreds 1925, 1911 and 1927 in Exhibit 4, some families don't harbor *BRCA1* mutations, even though they have a large number of apparently inherited breast cancer cases. If a skilled person only had such families in their collection, and even if he or she were able to isolate a small portion of the chromosome harboring a breast or ovarian cancer risk gene through linkage analysis and candidate gene isolation, he or she would still have not been

able to recognize that that gene is indeed the *BRCA1* gene. Unlike the process of isolating genes based on biochemical functions (e.g., erythropoietin gene), a disease gene is identified via positional cloning only when a causal mutation that exists within that gene is found to co-segregate with the disease in question.

SUITABLE FAMILIES WITH USEFUL POLYMORPHIC MARKERS HELPED NARROW DOWN A WORKABLE CHROMOSOMAL REGION CONTAINING THE *BRCA1* GENE

15. In this respect, it is worth noting that after we isolated a candidate gene which corresponded to the *BRCA1* gene, we selected 8 priority families for mutation screening or resequencing. 5 of these families were found to harbor segregating *BRCA1* mutations. In three of these families, the mutations were either a nonsense change (i.e., a stop codon) or frameshift mutations resulting in premature stop codons. Such mutations immediately informed us that they are deleterious mutations that result in alterations in the structure and functions of the protein encoded by the gene, which is consistent with a tumor suppressor mutation resulting in the phenotype of breast cancer. In fact, at the time this work was being conducted *BRCA1* was suspected to be a classic tumor suppressor gene like the p53 gene.

16. With the technology available in 1994, only a limited number of families could be comprehensively screened for mutations. Having large and informative families and correctly choosing the families for resequencing were extremely important technical decisions that lead to the Myriad Collaboration's success.

THE MYRIAD COLLABORATION DEFIED CONVENTION IN ITS SEARCH FOR *BRCA1*

17. Another step we undertook that, in hindsight, appeared to be critical to our success was that in addition to yeast artificial chromosomes (YACs), we used P1 and BAC clones in candidate gene identification. This was in contrast to both the general custom in the art of using only YACs in positional cloning,[5] and to our competitors' touting the use of YACs and cosmids specifically in the *BRCA1* effort.[6]

18. YACs were commonly used in the art because they can accommodate large genomic DNA fragments (100-1000 kb), thereby making genomic cloning and gene identification and analysis within a large chromosomal region more convenient due to their sheer size. It was also thought that the size of YACs would make it more likely that an entire gene would be contained within one or very few YAC clones.

19. BACs (bacteria artificial chromosomes) are bacteria-based and can accommodate, on average, inserts of about 150kb, while bacteriophage-based P1 clones typically have inserts of about 50-85 kb.

20. We used P1 and BAC clones to physically map the *BRCA1* region, to partially sequence the genomic DNA, and to isolate cDNAs, primarily because we recognized that they were more stable and more manageable in size. Indeed, at least three of the nine YACs isolated in our *BRCA1* physical mapping studies

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[5] Watson, James D., Gilman, M., Witkowski, J.A., and Zoller, M. 1992. *Recombinant DNA,* 2nd edition. pp. 590-4. Scientific American Books, New York.

[6] *See* Weber *et al.*, *Familial breast cancer. Approaching the isolation of a susceptibility gene,* CANCER, 74:1013-20 (1994); Friedman *et al.*, *The search for* BRCA1, CANCER RES., 54:6374-82 (1994); Jones *et al.*, *supra* note 3.

contained deletions.[7] More illustrative is the fact that the group led by Mary-Claire King recognized after our publication of the *BRCA1* gene that the YAC-derived genomic clone they were analyzing actually had a deletion at the site of the BRCA1 gene. Indeed, Dr. Mary-Claire King proclaimed in one scientific conference after our discovery of the *BRCA1* gene that "P1 and BACs are the winners of the day."

21. It was later recognized that the genomic DNA in the *BRCA1* region has one of the highest densities of Alu repeats ever reported. Smith et al.[8] examined 326 loci in GenBank for repeat structures. Only three genes have Alu densities greater than *BRCA1*.[9] Alu repeats are well known to cause large DNA rearrangements such as deletions and duplications. Apparently, the high density of Alu repeats in the *BRCA1* region exacerbated the problem of instability and insert rearrangements associated with YACs, but all of this is based on hindsight (i.e., the Alu repeats were only discovered because we provided the structure of the gene). At the time we selected P1 and BACs, it was by no means obvious to a skilled person to avoid YACs and use P1 and BACs. Our heavy reliance on P1 and BACs may even have seemed a mistake to most others in the field since it required analyzing many more smaller clones, which was regarded as being much less efficient than analyzing a few large YAC clones.

22. Another step we took that contributed to our success was our adoption of a novel hybrid selection technique, i.e., a modified version of hybrid selection.[10]

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[7] *See* Harshman *et al.*, *Comparison of the positional cloning methods used to isolate the BRCA1 gene*, HUM. MOL. GENET., 4:1259-66 (1995).
[8] Smith *et al.*, *Complete genomic sequence and analysis of 117 kb of human DNA containing the gene* BRCA1, GENOME RES., 6:1029-49 (1996).
[9] *Id.* at 1044.
[10] *See* Harshman *et al.*, *supra* note 7; Hattier *et al.*, *Monitoring the efficacy of hybrid selection during positional cloning: the search for* BRCA1, MAMM. GENOME, 6:873-9 (1995).

23. Generally, in hybrid selection (also called solution hybrid capture), biotinylated cloned, genomic DNA is used as a probe for hybridization in a solution of target cDNA. The hybridized probe with cDNA annealed to it is then captured by avidin on magnetic beads and the hybridized cDNA can subsequently be washed off from the beads. The cDNA is then PCR-amplified. The amplified, enriched cDNA can then be cloned and re-screened with genomic DNA to confirm the hybridization and the positive clones sequenced.

24. Specifically, in the Myriad team's novel modified version of this technique of hybrid selection, the probe was first rendered single stranded by treatment of restriction fragments of cloned genomic DNA (from the P1, BAC and cosmid clones) with exonuclease III. The largely single-stranded probe DNA was then reacted with photo-activatable biotin and UV light to generate the final probe for hybridization. This modification minimized the competitive renaturation of probe structures during hybridization, thereby increasing hybridization efficiency and sensitivity.[11]

25. The second modification relates to target cDNA synthesis. "First, mRNA was treated with DNase prior to cDNA synthesis. This process diminished the possibility of contamination from genomic DNA. Second, cDNA synthesis involved the use of two separate primers for first and second strand synthesis. This improved the cloning efficiency of the amplified products and allowed some types of artefactual products to be identified easily." In addition, the primers

[11] *See* Hattier *et al.*, *supra* note 10.

incorporated into the cDNAs at the 5' and 3' ends are different, allowing immediate recognition of the orientation of the captured cDNAs.[12]

26. Overall, a total of 39 independent candidate gene fragments (CGFs) were isolated by this modified hybrid selection technique. Our modified hybrid selection proved to be the most effective method used in the search for candidate genes.[13] In fact, Dr. Roger Wiseman, a major Myriad collaborator excitedly declared that the method is "the closest thing to magic" he has seen.[14]

MYRIAD COLLABORATION PIECED TOGETHER THE EVIDENCE TO ARRIVE AT THE TRUE STRUCTURE OF *BRCA1*

27. The first assembly of the *BRCA1* gene from segments of cDNA identified also presented great challenges to us. It is now known – because of our discovery – that the *BRCA1* gene has 24 exons (22 of which are coding) with a long open reading frame of 5,592 nucleotides distributed over 100 kb. Completion, for the first time, of a gene of so many exons and containing such large exons (exon 11 being more than 3.4 kb) certainly required some ingenuity and avoidance of sequence errors. As discussed above, the *BRCA1* gene could only be identified by finding mutations present in affected members of certain kindreds. If an incomplete open reading frame was mistakenly believed to be complete, segregating mutations located in the missing portion of the coding frame might have been missed and the *BRCA1* gene might have not been identified. Of course, only after the complete gene is known can its structure can be easily analyzed.

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[12] *See id.*
[13] *See* Harshman *et al.*, *supra* note 7.
[14] *See The Glittering Prize*, NAT. GENET., 8:105-6 (1994).

28. Indeed, four of the CGFs were alternative splice forms of *BRCA1* missing various exons. The alternative splice forms invariably resulted in truncated proteins after translation. If the Myriad Collaboration had stopped there – believing such CGFs to be represent complete open reading frames – the full length cDNA would not have been assembled, and important causal mutations might have been missed in the mutation screening of the kindreds.

29. Because of its sheer size, a full-length *BRCA1* cDNA was never identified in our screens. The full coding structure was deduced by piecing together non-overlapping cDNA fragments. As shown in Exhibit 6, many cDNA fragments were isolated. Even minor sequence errors could have led to the obliteration of an open reading frame, and the *BRCA1* gene would not have been recognized and causal mutations would not have been discovered even in the largest and most informative kindreds. Again, only after the complete cDNA structure is known can it be reproduced easily by follow-on researchers.

BRCA1 WAS SURPRISINGLY FOUND TO NOT FOLLOW THE TRADITIONAL TUMOR SUPPRESSOR MODEL

30. Throughout the search for *BRCA1* it was widely assumed in the field that the gene we sought encoded a classic tumor suppressor. Typical tumor suppressor genes such as the previously known APC and p53 genes are involved in both familial and sporadic cancers. The following is quoted from a news report on our findings in the journal *Science*:

31. "As the name implies, tumor suppressors act as "brakes" on the conversion of a normal healthy cell into a cancerous one, and their loss or inactivation leads to cancer. Breast cancer had seemed to follow the classic model

of a tumor suppressor at work: In about one half of sporadic, and all familial, cancers a stretch of chromosome 17 where researchers had been searching for *BRCA1* is lost from tumor cells, suggesting that *BRCA1* mutations play a role in both types of cancer. But the apparent absence of *BRCA1* defects in sporadic tumors suggests otherwise."[15]

32. The article went on to quote preeminent genetic epidemiologist Neil Risch as arguing "that because the gene fails to show all the predicted features of *BRCA1*, the evidence that it really is the long-sought gene is not completely watertight."[16]

33. That no somatic mutations were found in the breast or ovarian tumor cells we initially analyzed was reported in Futreal et al.[17] Although unexpected, the lack of somatic mutations in breast or ovarian tumor cells did not detract from our evidence from kindred analysis nor our conviction that the gene we had found was indeed *BRCA1*. After the publication of the gene we called BRCA1, the scientific community went on to find mutations in this gene in many hereditary breast and ovarian cancer families, confirming our belief that is the gene we had identified was, indeed, the *BRCA1* gene.

34. This again demonstrates that the discovery of the *BRCA1* gene was by no means a trivial exercise, but a scientific accomplishment that required many inventive steps, not the least of which was to contradict the scientific dogma of the time.

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[15] Rachel Nowak, *Breast Cancer Gene Offers Surprises*, Science, 265:1796-9 (1994).
[16] *See id.*
[17] Futreal *et al.*, BRCA1 *mutations in primary breast and ovarian carcinomas*, Science, 266:120-2 (1994).

Pursuant to 28 USC § 1746, I declare under penalty of perjury that the foregoing is true and correct.

Donna M Shattuck

Donna Shattuck, Ph.D

Executed on December 21, 2009

CERTIFICATE OF SERVICE

This is to certify that on December 23, 2009, a true and correct copy of the foregoing document has been served on all counsel of record via the court's ECF system.

/s/ Brian M. Poissant
Brian M. Poissant