A Literature and Database Analysis of the Structure of BRCA1 and BRCA2 Proteins and Their Roles in Hereditary Breast Cancer

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Honors Thesis
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PASS WITH DISTINCTION
TO THE UNIVERSITY HONORS COLLEGE:

As thesis advisor for Jennifer L. Hughes,

I have read this paper and find it satisfactory.

Raymond Reeves
Thesis Advisor

Sept. 29, 2003
Date
Précis

Problem
This thesis analyzes the structures and functions of tumor suppressor proteins BRCA1 and BRCA2 in the hopes of understanding the processes that occur when BRCA1 and BRCA2 genes become mutated and predispose individuals to breast cancer. My work in Keith Dunker’s intrinsic disorder laboratory has provided me with a solid understanding of the subject. I was interested in this topic, not only as a female, but because I have a family history of breast cancer. I also wanted to find out what caused cells to mutate and develop cancer.

Context
By understanding the mechanism by which cancer develops, scientists can come up with anti-cancer drugs to help save the lives of hundreds of thousands of people. In addition, because disordered proteins have been implicated in diseases other than cancer, understanding the series of events that take place to cause these diseases will help in treating them.

Methods and Procedures
I used a predictive algorithm (PONDR) to learn about the three-dimensional structures of BRCA1 and BRCA2. PONDR is a relatively new technique for imaging structures, so I had some initial difficulty interpreting my results. The research was especially challenging because, except for the RING and BRCT domains in BRCA1 and the DBD in BRCA2, the three-dimensional structures of these proteins have not been
characterized. After conducting structural analysis, I researched literature and online databases to discover the functions of each of these proteins and their mutations.

**Findings**

I suggest that BRCA1 and BRCA2 are able to interact with many other proteins because their structures contain regions of disorder, which makes it easier to bind more molecules. I also suggest that a relationship exists between mutations and disordered regions, since mutations in each protein seem to be located in close proximity to disordered regions; however, I suggest further investigation to clarify this association.

**Conclusions**

It is difficult to compare my findings to other results in the field, especially because structural information about the proteins is scarce. Nevertheless, I use examples from proteins similar in one way or another to BRCA1 and BRCA2 to support my results. I think that in addition to further elucidating the connection between mutations and disorder, characterization of the full-length BRCA1 and BRCA2 proteins would be helpful in continuing this research.
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Introduction

Cancer is the second leading cause of death in United States, just behind heart disease. Each year, over one million new cases are diagnosed in the U.S. alone; each day, over 1,700 people die from the disease. Many factors influence the development and progression of cancer, including environment, nutrition, lifestyle, and genetics.

Breast cancer is one of the most common types of cancer in women, affecting 183,000 women each year. Over 20% of these women are expected to die of the disease. According to the National Cancer Institute, two percent of women younger than 50 years of age and ten percent of women younger than 80 years of age will develop breast cancer. Breast cancer also affects men, although its occurrence is much more rare, as women are 125 times more likely to have breast cancer than men.

While 90-95% of breast cancer cases result from sporadic mutations, five to ten percent are due to inherited germline mutations in BRCA1 and BRCA2, two tumor suppressor genes. BRCA1's role as a tumor suppressor was discovered after loss of heterozygosity on chromosome 17q21 was observed in many families with a history of the disease. BRCA2's role in tumor suppression was discovered in much the same way, with loss of heterozygosity on chromosome 13q12-q13.

Mutations in BRCA1 and BRCA2 are passed on to offspring via an autosomal dominant pattern of inheritance. Approximately 90% of the tumor suppressors' mutations delete the wild-type allele and cause expression of shortened or inoperative BRCA1 and BRCA2 proteins. The other ten percent of BRCA1 and BRCA2 mutations include missense mutations in tumor suppressor regions of the expressed BRCA1 and BRCA2 proteins. Taken together, these mutations cause the cell to lose control of the
cell cycle and DNA repair, two safety mechanisms designed to prevent progression of
cancer and other diseases\textsuperscript{2}.

Mutations in \textit{BRCA1} and \textit{BRCA2} predispose individuals to both breast and
ovarian cancers\textsuperscript{10,11}. Because 60-80\% of women with \textit{BRCA1} or \textit{BRCA2} mutations
develop breast cancer\textsuperscript{2}, my investigation will focus on mutations that increase
susceptibility to this disease. It is necessary to examine the functions and structures of
wild-type \textit{BRCA1} and \textit{BRCA2} proteins to uncover the roles that each plays and to
understand how these roles are altered in the aftermath of \textit{BRCA1} and \textit{BRCA2} mutations
and in the development of cancer.

\textit{The Structure-Function Paradigm}

For over a century, the concept that a protein’s three-dimensional structure
determined its function prevailed\textsuperscript{12}. Indeed, a protein’s fold confers a specific shape
upon it that allows it to interact with other molecules\textsuperscript{13}. Nevertheless, the discovery of
proteins that did not require three-dimensional structure to function has prompted a re­
examination of this view\textsuperscript{12}. In fact, 35-51\% of eukaryotic proteins are predicted to
contain disordered regions\textsuperscript{14}. Over 90 unstructured proteins have already been
catalogued, including many involved in DNA and protein binding, phosphorylation, and
cell regulation\textsuperscript{14,15}.

Dunker \textit{et al.} (2002) report that unstructured proteins are more flexible in binding
and can therefore interact with numerous molecules\textsuperscript{14}. For example, the tumor
suppressor protein p53 is able to bind numerous molecules through its intrinsically
disordered domains\textsuperscript{15}. Upon binding, the protein adopts a more stable, ordered
conformation\textsuperscript{16}. This energy change is not uncommon among disordered proteins, as many become more structured following interaction with their partners\textsuperscript{14}.

BRCA1 and BRCA2 interact with numerous molecules, including p53, RAD51, CBP/p300, CtIP, P/CAF, histone deacetylases, and other proteins\textsuperscript{17}. I suggest that, like p53, BRCA1 and BRCA2 contain regions of disorder that mediate multiple molecular interactions. Here I use the latest data from literature and databases, in conjunction with PONDR, to predict such structure and provide for a better understanding of the proteins' mechanisms for binding and other interactions. Williams \textit{et al.} (2001) propose that mutations interfering with DNA repair or transcriptional activity weaken or completely unfold the structures in these regions\textsuperscript{18}. I use data from PONDR to clarify the relationship between cancer-predisposing mutations in BRCA1 and BRCA2 and the proteins' three-dimensional structures.

\textbf{Experimental Procedures}

\textit{Materials}

Published literature and online databases were searched for information about BRCA1 and BRCA2 proteins, including sequence, structure, and function. Full-length sequences for BRCA1 and BRCA2 were obtained from the Swiss-Prot database\textsuperscript{19} (BRC1\_HUMAN and BRC2\_HUMAN, respectively) and entered into PONDR VL-XT, a \textit{Predictor of Natural Disordered Regions}, which produced textual and graphical predictions of disorder.

\textit{Predictor Training}

To predict the likelihood of disorder based on amino acid sequence data, PONDR VL-XT required the incorporation of three overlapping neural network predictors. Long,
internal regions of disorder characterized by X-ray crystallography or NMR were used to train the VL1 predictor; N- and C-terminal regions of disorder characterized by X-ray crystallography were used to train the XT predictors. Overlapping predictions were averaged and smoothed lengthwise along the entire prediction region.

**Predictor Accuracy**

VL-XT demonstrated 78% accuracy when predicting order on a per-residue basis. An investigation into the exactitude of PONDR’s predictions indicated that the algorithm was more effective when predicting regions with 39 or more consecutively disordered residues. VL-XT’s error rate for falsely predicting order for a single disordered residue was 20.1%; however, this rate dropped to 1.7% for regions of 40 or more consecutively disordered residues.

**Disorder Predictions**

Each residue subjected to VL-XT was assigned a prediction of disorder from 0 to 1, with 0 being ordered and 1 being disordered. Although most predictions fell between 0 and 1, residues with predictions at or above 0.5 were considered disordered.

The number of disordered residues in each predicted disordered segment was calculated; subsequent analysis concentrated on regions containing more than 40 disordered predictions in a row; short segments of ordered predictions found between long predictions of disorder; and disordered predictions shown elsewhere to be ordered.

PONDR’s predictions for each protein were analyzed against literature and database data to determine a potential structure-function relationship and link to breast cancer predisposition.
Results and Discussion

Published literature and online databases were principal sources of information about the sequences, structures, and functions of BRCA1 and BRCA2. Comparisons between human BRCA1 and BRCA2 proteins and their murine counterparts, Brca1 and Brca2, supplied additional clues about structure and function.

PONDOR VL-XT was used to predict disordered regions in BRCA1 and BRCA2 proteins. Short regions of disorder predicted for each protein, including those with fewer than 40 consecutively predicted disordered residues, were eliminated from this analysis, as longer disorder regions predicted with more accuracy.

Functional Interactions of BRCA1

The BRCA1 gene encodes a tumor suppressor protein of 1,863 residues with a RING finger motif at its N-terminus and a repeating BRCT domain at its C-terminus. These domains are highly conserved despite relatively low conservation of the rest of the protein, as human and mouse forms share just 60% sequence homology.

![Fig. 1. Structure of the BRCA1 protein.](Graphic from Trends in Genetics, 2000.)
The RING finger motif (residues 24-65) of BRCA1 contains four pairs of Cys-His residues that bind zinc and regulate protein and DNA interactions. Although not exclusive to BRCA1, this motif is one of few that participates in dimerization: it interacts with the RING motif of BARD1 (BRCA-associated RING domain 1) to form a heterodimeric complex. BRCA1's RING domain interacts with many proteins besides BARD1, including transcription factors (ATF1), cell cycle regulators (E2F), and deubiquitinating enzymes (BAP1). In addition to binding numerous proteins, the RING domain demonstrates high-affinity DNA binding, although this binding does not seem to be very sequence-specific.

The BRCT domain of BRCA1 contains two BRCT repeats (BRCT1, residues 1642-1736; BRCT2, residues 1756-1855) involved in DNA repair and tumor suppressor functions. This region binds to other BRCT regions and proteins involved in transcription-related functions, such as p53, CtIP, p300, and BACH1.

Between the RING and BRCT regions of BRCA1 lie two nuclear localization signals (NLS), which bind importin-α. Binding sites for retinoblastoma protein (RB), RAD50, and MYC surround the NLS.

BRCA1 has been found in BASC (BRCA1 associated genome surveillance complex) along with proteins from Bloom's syndrome and ataxia telangiectasia (ATM). Many of these proteins, like BRCA1, exhibit DNA repair activity, performing double-strand break repair and mismatch repair. Other proteins in the complex, such as ATR, are involved in cell cycle control. Both ATM and ATR phosphorylate BRCA1 at three sites (residues 1387, 1423, and 1524) following DNA damage, which allows BRCA1 to interact with BRCA2, RAD51, and BARD1 to move to sites of destruction.
BRCA1 also interacts with FANCD2, a protein from the *FANCD2* gene, following DNA damage. Both proteins localize to nuclear foci after a single ubiquitin residue is added to *FANCD2*, a process that further implicates BRCA1’s involvement in ubiquination activity. Like *BRCA1* or *BRCA2* mutations, mutations in *FANCD2* lead to Fanconi’s anemia, a condition that increases the risk of cancer, birth defects, and bone marrow inefficacy\(^\text{17}\).

**PONDR for BRCA1**

PONDR predicted 36 disordered regions in BRCA1, comprising 986 of its 3,418 residues. Seven of these disordered predictions contained long (>40 amino acids) regions of disorder, which were examined in subsequent analyses.

![PONDR Analysis for BRCA1](image)

**Fig. 2. PONDR analysis for BRCA1.**

A total of 36 disordered regions were predicted in BRCA1; however, only seven regions with long (>40 amino acids) regions of disorder were characterized in this analysis. Predictions below the 0.5 threshold are considered to be ordered; predictions above this threshold are considered to be disordered.
Six of the seven disorder predictions (residues 206-272, 743-785, 1003-1077, 1127-1169, 1325-1391, and 1404-1501) could not be associated with a previously characterized domain of BRCA1, so it was difficult to compare PONDR’s predictions with experimental data. The remaining disorder prediction (residues 1520-1655) overlapped with BRCT1 (residues 1642-1736), so a comparison between PONDR’s outputs and experimental data was possible.

Data from literature and databases do not equate disorder predictions of residues 206-272 and 743-785 with any of BRCA1’s functional activities; therefore, the significance of these regions in the protein remains uncertain. Given the numerous binding partners of BRCA1, however, it seems likely that the two disorder predictions are located within or near regions that require conformational freedom to interact with many different molecules. The tumor suppressor protein p53 serves as an excellent example to illustrate this point because it binds a variety of molecules via its disordered regions\(^{14}\). I suggest that the disorder predictions of residues 206-272 and 743-785 allow BRCA1 to interact with a variety of partners, similar to the way in which p53 can bind multiple partners through its disordered regions. The existence of BRCA1’s various binding partners supports this idea.

Short predictions of order were found separating long predictions of disorder for residues 1003-1077 and 1127-1169. Such short regions of order located between long regions of disorder are indicative of conformational transitions that occur upon the region’s interactions with other molecules\(^ {14,15,26}\). For example, an ordered prediction was assigned to a region in the center of 4EBP1, a protein that has been shown by NMR to be disordered. This region of predicted order partially overlapped the site at which 4EBP1
interacted with its binding partner, eTF4E. Thus, 4EBP1 is thought to adopt an ordered conformation upon binding to eTF4E. I predict that residues 1003-1077 and 1127-1169 are binding regions in BRCA1 that undergo disorder-to-order transformations upon binding to their partners, similar to the way in which 4EBP1 becomes ordered upon binding to eTF4E. Given the number of molecules that BRCA1 interacts with and the arrangement of PONDR's predictions, it is likely that this is the case.

Three remaining predictions of disordered residues (residues 1325-1391, 1404-1501, and 1520-1655) are also separated by short predictions of ordered residues. I predict that these regions are additional binding regions in BRCA1 that undergo disorder-to-order transitions upon binding. Moreover, each of these regions contains a phosphorylation site (at residues 1387, 1423, and 1524). Scully and Puget (2002) report that phosphorylation allows BRCA1 to bind BRCA2, RAD51, and BARD1 before relocating to the site of DNA damage. I suggest that phosphorylation causes the region to adopt a disordered conformation, which allows BRCA1 to interact with BRCA2, RAD51, and BARD1 and reposition itself near sites of DNA damage. Other instances of the structural effects of phosphorylation are in agreement with this suggestion (see refs. 5&6 for review).

Only one disorder prediction (residues 1520-1655) overlapped with one of BRCA1's previously characterized domains, BRCT1 (residues 1642-1736). Williams et al. (2003) used X-ray crystallography to determine the structure of BRCA1's BRCT repeats. The authors reported the wild-type repeats to be stably folded under experimental conditions. Although the findings of Williams et al. seem to disagree with PONDR's output, disorder predictions shown elsewhere to be ordered are indicative of
conformational changes within the protein. For example, the Protein Data Bank classifies tumor suppressor p53’s oligomerization region as ordered, yet PONDR predicts disorder in this very region. To explain this discrepancy, Li et al. (2000) have proposed that p53 undergoes a disorder-to-order transition upon interaction with its binding partner. As previously mentioned, the BRCT repeats interact with multiple partners. Perhaps, as I suggest, the BRCT region is able to bind numerous molecules because of its disordered state. That this region has been implicated previously in such binding, based on its separation from another disordered prediction by a short ordered prediction and by its possession of a phosphorylation site, further confirms this idea and supports PONDR’s output.

**Mutations in BRCA1**

The Swiss-Prot database lists seventeen mutations consistently found in breast cancer that occur within BRCA1. Three occur within the BRCA1-BARD1 complex, specifically at Lys 22, Cys 61, and Cys 64. Brzovic et al. (2001) report that the first 100 N-terminal residues of BRCA1, including the RING domain, give rise to 20% of all BRCA1 disease-related mutations. Interestingly, some mutations within this region interfere with BRCA1’s structure. For example, mutations at Cys 24 or Cys 44 cause BRCA1 to fold incorrectly, and mutations throughout BRCA1’s N-terminus weaken the RING structure or interfere with protein binding or tumor suppressor activity.

Additional mutations have been shown to interfere with BCRA1’s structure. Early studies on the BRCT region, which has also been studied for its associations with breast cancer, revealed that a mutation at Tyr 1853 weakened the fold of the region by deleting the last 11 residues of BRCT2. Mutations at Ala 1708 and Met 1775 were
shown to interfere with DNA repair functions and transcriptional activity. During characterization of the BRCT domain, Williams et al. (2003) confirmed that mutant BRCT repeats failed to fold stably under experimental conditions and proposed that this disruption in structure likely inhibited tumor suppressor activity.

Although mutations in BRCA1’s N-terminus and BRCT repeats failed to appear in regions containing long disordered predictions, disorder predictions of shorter length were nonetheless found in these same areas (J.H., PONDR output for BRCA1). These results, when combined with previous findings, lead me to suggest that mutations occurring in these regions cause local conformational changes, which may disrupt the normal functioning of BRCA1 and lead to the development of breast cancer. Detection of five breast cancer mutations within three disorder predictions (residues 206-272, 1003-1077, and 1127-1169) provides additional evidence of the relationship between mutations in breast cancer and regions of disorder.

**Functional Interactions of BRCA2**

The BRCA2 gene encodes a tumor suppressor protein of 3,418 residues with eight centrally located BRC repeats (BRC1 – BRC8, residues 1002-2085) and a recently characterized C-terminal DBD (approximately residues 2400-3200). These regions are two of the only identifiable domains in the protein, as two-thirds of the structure is yet to be resolved.

Like the BRCT repeats in BRCA1, the BRC repeats in BRCA2 are highly conserved. Six of the eight repeats regulate BRCA2’s interactions with RAD51 to promote resistance to radiation among other protective functions. BRCA2, RAD51, and PCNA (proliferating cell nuclear antigen) localize to nuclear foci following
Fig. 3. Structure of the BRCA2 protein.
BRCA2, a protein of 3,418 residues, is characterized by eight central BRC repeats and a C-terminal nuclear localization signal (NLS), similar to the two in BRCA1. Although the C-terminal DBD is not shown, it lies N-terminal to the NLS. The BRC repeats bind to RAD50 and various other proteins.

Graphic from Trends in Genetics, 2000.

DNA damage; RAD51’s involvement in homologous recombination and PCNA’s involvement in DNA synthesis and repair indicate BRCA2’s functional role in these processes.32

The C-terminal region of BRCA2, also known as the DBD (DNA/DSS1-binding domain), is the most conserved region in BRCA2.30 It consists of three oligonucleotide/oligosaccharide-binding fold domains, which mediate protein interactions, bind ssDNA, and interact with RAD51. These functions provide further evidence for BRCA2’s role in DNA repair and homologous recombination.31,32

BRCA2 contains a nuclear localization signal (NLS), similar to the two in BRCA1, C-terminal to the oligonucleotide/oligosaccharide-binding fold domains. A P/CAF binding region is located within BRCA2’s N-terminus, which has been implicated in regulation of transcription.11 BRCA2 also interacts with BRAF35 (BRCA2-associated factor 35), a DNA-binding protein. Because BRAF35 binds to cruciform DNA, it is
thought to assist BRCA2 in DNA repair and cell cycle control. In addition, high levels of BRAF35 and BRCA2 are found in proliferating cells during embryogenesis\textsuperscript{17}.

**PONDR for BRCA2**

PONDR predicted 53 disordered regions in BRCA2, comprising 899 of its 3,418 residues. Four of these disordered predictions contained long (>40 amino acids) regions of disorder, which were examined in subsequent analyses.

Fig. 4. **PONDR analysis for BRCA2.**
A total of 53 disordered regions were predicted in BRCA1; however, only four regions with long (>40 amino acids) regions of disorder were characterized in this analysis. Predictions below the 0.5 threshold are considered to be ordered; predictions above this threshold are considered to be disordered.

Three of the four disordered predictions (residues 180-222, 424-477, and 2232-2315) could not be associated with a previously characterized domain of BRCA2, so it was difficult to compare PONDR’s predictions with experimental data. The remaining disorder prediction (residues 3360-3406) was positioned just C-terminal to the NLS,
although comparisons between PONDR’s outputs and experimental data proved difficult as well.

Because two-thirds of BRCA2 remains to be characterized, data from literature and databases did not implicate residues 180-222, 424-477, or 2232-2315 in any of BRCA2’s functional activities, and the significance of these regions in the protein remains uncertain. As in BRCA1, however, the number of BRCA2’s binding partners increases the likelihood that these disorder predictions are located within or near sites of interaction that require conformational freedom to facilitate various instances of molecular binding. I suggest that the disorder predictions of residues 180-222, 424-477, and 2232-2315 allow BRCA1 to interact with numerous partners, just as p53 is able to bind multiple partners via its disordered regions.

The remaining disorder prediction (residues 3360-3406) lies just C-terminal to the NLS. Based on the proximity of this disorder prediction to the NLS, I suggest that disorder in this region may mediate the interactions of the NLS and its surrounding regions. The lack of structural information about the NLS, however, makes evaluation of this proposal challenging.

**Mutations in BRCA2**

The Swiss-Prot database lists 20 mutations consistently found in breast cancer that occur within BRCA2. According to Shamoo (2003), cancer-related mutations scattered throughout the DBD account for 27% of mutations found in the Breast Cancer Information Database.

Like BRCA1, mutations in BRCA2’s DBD did not appear in regions containing disordered predictions of significant length; however, disorder predictions of shorter
length were nonetheless found in this area (J.H., PONDR output for BRCA2). Again, I suggest that mutations occurring within this region result in local conformational changes, which may disrupt BRCA2's normal functioning and lead to the formation of breast cancer. Detection of four breast cancer mutations within two disordered predictions (residues 180-222 and 2232-2315)\textsuperscript{19} offers further evidence of the relationship between mutations in breast cancer and regions of disorder.

**Implications in Cancer Research, Treatment, and Drug Design**

PONDR outputs predict the existence of disordered regions in tumor suppressor proteins BRCA1 and BRCA2. The BRCA proteins' involvement in functions commonly performed by disordered proteins, such as cell regulation and DNA and protein binding, increases the likelihood that these proteins mediate such activities through regions of disorder\textsuperscript{14,15}.

Cancer is just one of many diseases caused by a protein’s inability to fold correctly\textsuperscript{14}. It has been suggested that recognition of unstructured binding regions by disorder predictors like PONDR could aid in the development of novel mechanisms by which anti-cancer drugs interact with these sites. For example, the anti-cancer drug taxol is thought to interact with its target site via a disordered region\textsuperscript{15}.

Clarification of the relationship between protein disorder and cancer mutations could offer additional insights into future cancer treatments. Current research on tumor suppressor protein p53 suggests that the introduction of molecules with high affinities for the protein's native state causes equilibrium to shift away from the protein's disordered state. This shift is accompanied by restoration of the protein's ordered conformation and re-establishment of any lost functions\textsuperscript{16}. Perhaps similar mechanisms could be used to
reinstate the loss of cell cycle control and DNA repair seen in mutated BRCA1 and BRCA2 proteins. Mutations listed in the Swiss-Prot database would be ideal targets for this research, as their relationship to breast cancer formation has already been established\textsuperscript{19}.

References


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