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Functional, Structural and Contextual Analysis of a Variant of Uncertain Clinical Significance in BRCA1: c.5434C->G (p.Pro1812Ala).

Rafael Morales[1,*], Raquel Serrano[2], Teresa Sardón[3], Carmen Román-Ortíz[4], Santiago González-Santiago[5], Roger Mulet[3], José Manuel Mas[3]

1. Genetic Counselling Unit, Medical Oncology Department, Hospital La Mancha Centro, Av La Constitución, Nº 3, 13600, Alcázar de San Juan, Ciudad Real (Spain)

2. Genetic Counselling Unit, Medical Oncology Department, Hospital Reina Sofía, Córdoba (Spain)

3. Anaxomics Biotech, Balmes 89, 4º-2ª, 08328, Barcelona (Spain)

4. Preventive Medicine Service, Hospital Virgen de la Salud, Toledo (Spain)

5. Genetic Counselling Unit, Medical Oncology Department, Hospital San Pedro de Alcántara, Cáceres (Spain)

Abstract

Interpreting variants of uncertain significance (VUS) for their effect on protein function, and therefore for the risk of developing cancer, has become a challenge in clinical practice for genetic counselling services. The present work combines structural bioinformatics and systems biology based mathematical modelling approaches with the aim of determining the pathogenicity of the mutation c.5434C->G (p.Pro1812Ala) in the BRCA1 gene (detected in a patient from a high risk family) and also to mechanistically understand the effect of this mutation in DNA damage response, a key process in cancer development. The results obtained showed that this mutation prevents the interaction of BRCA1 with key proteins of the cell cycle, subsequently impairing BRCA1-dependent induction of cell cycle arrest. The comparison of the molecular mechanisms associated with the native BRCA1 protein and the mutated variant function in DNA damage response showed that the latter undergoes a reduction in its ability to modulate pathways that are critical for DNA repair and cell cycle control. Therefore, this variant will not be able to exert its tumor suppressive action. Interestingly, these conclusions can be extrapolated to all mutations that, like c.5434C>G (p.Pro1812Ala) BRCA1, cause loss of BRCT domain activity.

Corresponding author: Rafael Morales, Genetic Counselling Unit, Medical Oncology Department, Hospital La Mancha Centro, Av La Constitución, Nº 3, 13600, Alcázar de San Juan, Ciudad Real (Spain), Telephone: +34 647 676 052, Mail: rmchamorro@sescam.jccm.es

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Introduction

Deleterious variants in the BRCA1 and BRCA2 genes account for approximately 20% of cases of hereditary breast and ovarian cancer. The BRCA1 gene is found on the long arm of chromosome 17 (17q21) and plays a crucial role in DNA damage response. BRCA1 inactivating mutations lead to genetic instability, indirectly causing tumours to occur as a result of an accumulation of mutations in cell cycle regulatory genes. In Spain, families that carry the deleterious mutation in the BRCA1 gene have a 52% cumulative risk of developing breast cancer and a 22% risk of developing ovarian cancer by the age of 70 (1).

The BRCA1 protein is a protein of 1863 amino acids. The two most important domains of BRCA1 are the RING domain and the BRCT domain. The RING domain is located at the amino terminus of BRCA1, between amino acids 1 and 109 (exons 2 - 7), and it is responsible for the E3·ubiquitin·ligase activity of BRCA1 (2) and binds to the BARD1 protein. The BRCT domain mediates binding with phosphorylated proteins such as: Abraxas (3,4), BACH1/BRIP1 (5), and CtIP (6), forming three mutually exclusive protein complexes named A, B and C complexes (see below). A number of other proteins also bind to the C-terminal region of BRCA1 and/or its central region (7) (Figure 1).

BRCA1 is involved in several steps of the DNA damage response process, a cell cycle control mechanisms that prevent the progression of the cell cycle while activating DNA repair routes. From a mechanistic point of view, it is known that BRCA1 forms part of 4 macrocomplexes (8,9) BRCA1A, BRCA1B BRCA1C and BRCC. The BRCA1A complex acts as a DNA damage sensor and regulates the G2/M cell cycle checkpoint. The BRCA1B complex participates in the G1/S and intra-S-phase checkpoints, ensuring genome integrity prior to DNA synthesis. The BRCA1C complex mediates DNA repair through homologous recombination, facilitating the formation of single-stranded DNA in the step prior to homologous recombination. The BRCC complex is capable of recognising the previously formed single-strand DNA and mediating strand invasion; it is considered the effector in homologous recombination.

Interpreting variants of uncertain significance for the effect of protein function, and therefore for the risk of developing cancer, has become a challenge in clinical practice for genetic counselling services. It is necessary to develop tools that will help us reduce the high percentage of variants of uncertain significance, therefore facilitating genetic counselling process.

The variant of uncertain significance c.5434C->G (p.Pro1812Ala = Pro1812Ala) has been found in a high-risk family, where the proband is a woman diagnosed with papillary serous cystadenocarcinoma of the ovary, and her mother and maternal aunt are diagnosed with breast cancer. The c.5434C>G variant is found at position 28 of exon 23 of the BRCA1 gene. In this work we apply systems biology based mathematical modelling approaches to mechanistically determine the pathogenic effect of this variant.

Methods

The assessment of the physiological consequences of the c.5434C->G (p.Pro1812Ala) mutation with regard to its predisposition to develop cancer was approached by two complementary computational perspectives: a structural approach and a systems biology based functional approach.

Structural bioinformatics analysis

A three-dimensional structure of the BRCA1 protein was modelled after experimentally obtained structures contained in PDB, taking into account the changes induced by the mutation according to published literature (10,11). Then, a molecular dynamics simulation in a system of interacting particles was run to simulate the variability of the BRCA1 in the cytoplasm

(Continued on page 3)

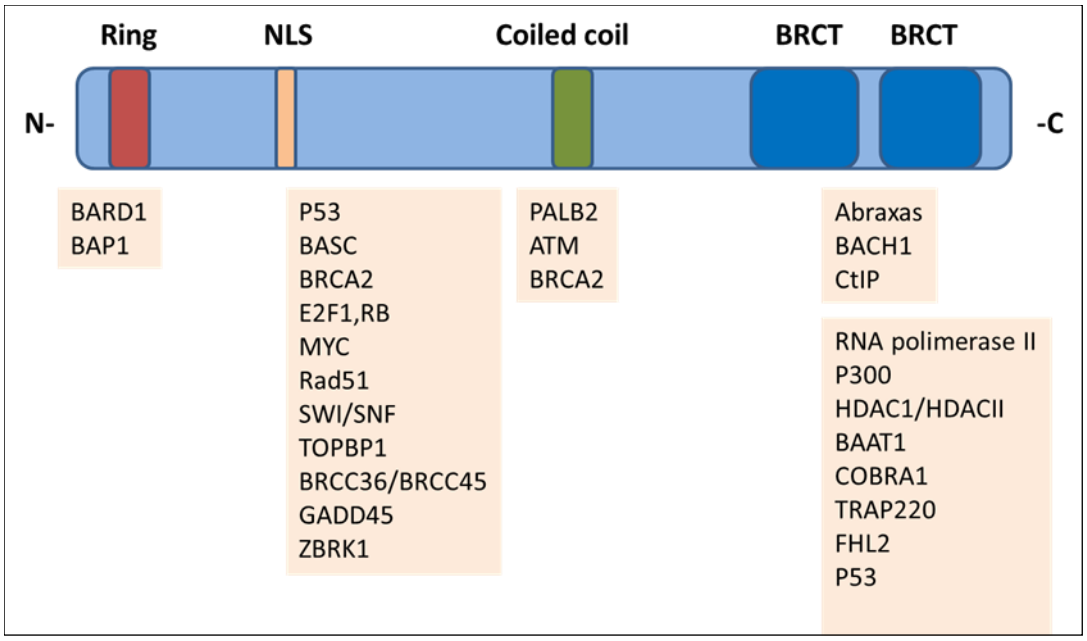

Figure 1: BRCA1 domains and interacting proteins: BRCA1 contains a RING domain at its N-terminus, two BRCT domains at the C-terminus and a coiled-coil domain upstream of BRCT domains. The interacting proteins are shown under the region of BRCA1 required for their association.

and predict its time evolution. The resulting structures with the lowest values of free energy were selected, under the assumption that they are more stable and, hence, may be found in vivo. This exercise was conducted using CHARMM22 (12) force field and calculating the partial charges through the Gasteiger method.

The presence of significant structural changes in the BRCA1 protein was detected through statistical parameters and subjective assessment after visualization and the stability of the mutated protein was determined based on certain key values, including potential energy, RMSD, and Ramachandran outliers. The predictions were compared with the conclusions of published studies.

Systems biology based functional approach

Therapeutic Performance Mapping System (TPMS) technology (Anaxomics Biotech, Barcelona) (13) was applied to generate and analyze mathematical models able to simulate in silico DNA damage response in the context of a native (wt) BRCA1 or the studied variant. Briefly, DNA damage response was first characterized at a molecular level through hand curated bibliography search. Then, a protein interaction map based on the human protein-protein interaction network was generated. The map was extended by adding knowledge-oriented connectivity layers, i.e., protein-to-protein interactions, including physical interactions and modulations, signalling, metabolic relationships, and gene expression regulation. Data was obtained from public and private external databases (Binding Database (14), BioGRID (15), IntAct (16), REACTOME (17,18)…) and the manual curation of scientific literature. In the case of the mutated BRCA1 model the impaired protein interactions due to the mutation were restricted when constructing the protein network.

This static map was transformed into a dynamic mathematical model with predictive capacity through training them with functional information collected in Anaxomics proprietary Truth Table database. In brief, to train the mathematical models a collection of known input-output physiological signals was used. The molecular description of these input-output physiological

signals mainly derives from literature mining and a compendium of databases that accumulate biological and clinical knowledge, such as microarray databases (e.g. GEO (19,20)) and drug databases (e.g. DrugBank (21)). The methods applied rely within the field of Artificial Intelligence, including artificial intelligence graph theory and statistical pattern recognition techniques; genetic algorithms, artificial neural networks, dimensionality reduction techniques; and stochastic methods like Simulated Annealing, Monte Carlo among others (22).

Once the models had been generated, they were analysed in two sequential steps: First the mechanism of action of functional protein and the mutated protein in DNA damage response was evaluated independently, as detailed in the literature (23), and secondly the two individual mechanisms of action obtained were compared in order to assess the impact of the mutation on DNA damage response activity.

Results

In order to determine the physiological consequences of BRCA1 c.5434C->G (p.Pro1812Ala) mutation, this variant was analysed from two complementary perspectives to determine, first, the protein structural changes caused by the mutation in BRCA1 and then, the impact of these changes in the protein functionally in the context of the DNA damage response.

Structural analysis of BRCA1 c.5434C->G

It has been previously reported that the c.5434C->G (p.Pro1812Ala) mutation causes an important splicing defect in BRCA1, resulting in the omission of exon 23, which in turn leads to the appearance of a premature stop codon (p.Gly1803GlnfsX11) (10). The resulting protein is truncated and lacks the second BRCT domain. The mutated protein BRCA1 is therefore shorter than the wild-type protein due to interruption of the BRCT domain. However, according to the results obtained in our structural analysis, comparison of the wild-type protein and the mutated protein shows that both variants of the protein seem to have the same secondary and tertiary structure. Deletion of the second BRCT domain does not appear to bring about changes in the secondary and tertiary structures of the rest of the protein (Figure 2).

Both proteins have the same number of α helices, β strands and turns (24). The spatial distribution of the secondary structure is essentially the same, with slight differences that could be attributed to the molecular dynamics simulation. Domains other than BRCT, such as RING, NLS and Coiled Coil domains, do not seem to be altered.

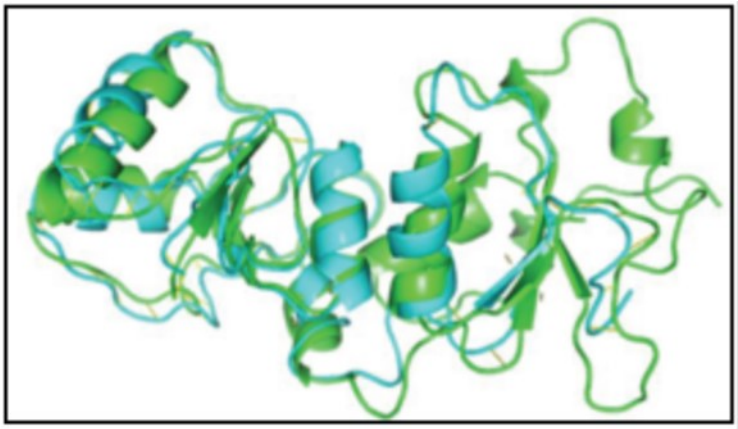

Figure 2: Comparison between the 3D protein structure of functional BRCA1 (in green) and mutated BRCA1 models (in blue)

Additionally, in order to evaluate whether the structural similarity between the variants remains invariable over time (i.e. if they are equally stable), we tracked the conformational changes through molecular dynamics during 100 picoseconds (Figure 3). The simulation confirmed that the variant remains stable through time. The values of free energy remained low along the simulation and the RMSD, which measures the similarity in the 3D structure, did not vary. That is, despite the disruption of the second BRCT domain, the

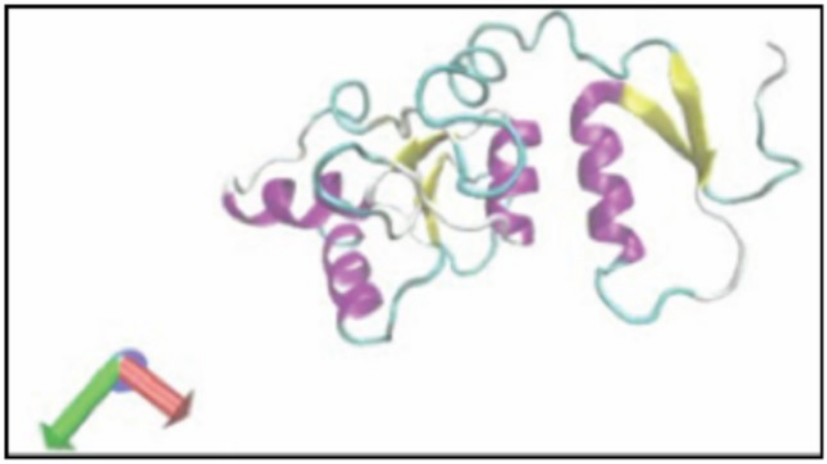

Figure 3: Dynamic Protein Model of the BRCA1 Pro1812Ala Variant

rest of the protein (including RING, NLS and coiled coil domains) remains functional.

Mechanistic comparison between Wild-type and Mutated BRCA1

Mathematical models simulating DNA damage response related mechanisms were used to compare two molecular scenarios: expression of the wild type and the c.5434C->G (Pro1812Ala) variant. Working on the basis that loss of the second BRCT domain disturbs the whole BRCT region (25), all interactions associated with this domain were removed from the protein-protein interaction network. The full list of protein-protein interactions that have been deleted can be found in Supplemental data 1. The model corresponding to the mutated variant has been defined with restrictions that eliminate functional associations that depend on the BRCT domain. Interestingly, the formation of the BRCA1 complexes A, B and C was impaired in the mutated variant model, since the interaction with Abraxas/ FAM175A, Bach1/BRIP1 and CTIP proteins require the BRCT domain of BRCA1.

According to our results, DNA damage response is severely affected by the mutation (Figure 4), being the most relevant consequences:

Mismatch repair (MMR): Since the mutated BRCA1 cannot activate MLH1, MSH2 or MSH6, which are key mediators of this repair mechanism, it is expected that MMR becomes severely impaired. As a result, mismatch mutations that are not repaired might persist and promote the apparition of tumors.

Homologous recombination (HR): BRCA2, COM1 and PALB3 are central mediators of the HR process, so their loss might result in the complete impairment of

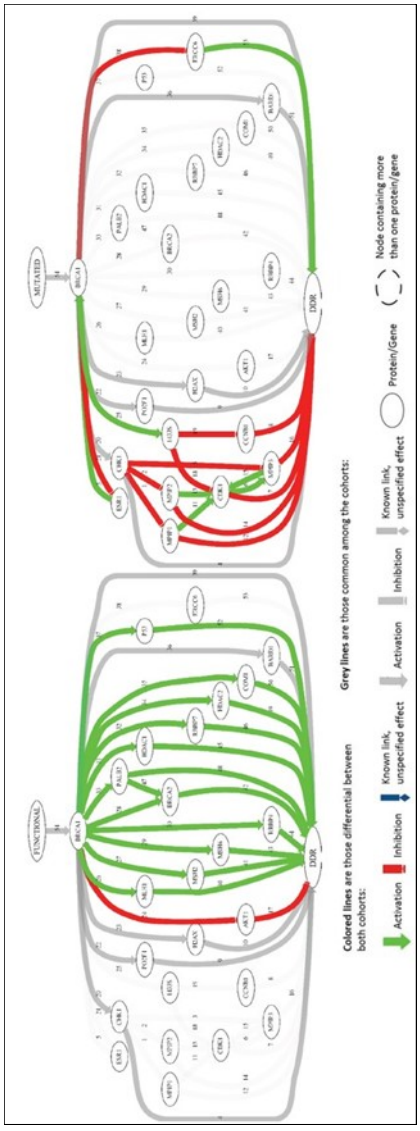

Figure 4: Mechanistic comparison of the functional BRCA1 protein and the mutated BRCA1 protein. The description of the nodes and interactions is available as Supplementary data.

this pathway. Consequently, mutations that should be repaired by means of this process will not be fixed, potentially leading to cancer. HR participates in the repair of DNA double-stranded breaks (DSB) and interstrand crosslinks (CLS), as well as in providing support for DNA replication (26).

Nucleotide excision repair: Although the BRCA1-P53 interaction is lost, the inhibition of ERCC6, also known as Cockayne syndrome group (CSB) (27), appears to acquire a more prominent role. ERCC6 is known to promote the ubiquitination of P53 (28), which is considered by some authors as a "nucleotide excision repair master-switch". Hence, by inhibiting ERCC6, the BRCA1 variant may stabilize the activity of P53 induced by alternative routes. ERCC6 is crucial for transcription-coupled DNA repair (TCR), which removes the cytotoxic transcription-blocking DNA lesions (29), by recruiting the components of the NER machinery such as RNAPIIo or XAB2 (30).

Non-homologous end joining (NHEJ): With BRCA1 unable to activate MSH6, a certain impact on this process is expected. However, H2AX is still active, as well as other proteins that are important for this mechanism. Hence, little real change in this mechanism is expected.

Cell cycle arrest: Despite the disruption of the interactions with key proteins of the cell cycle, namely AKT, P53, HDAC 1/2 and RBBP 4/7, the Pro1821Ala variant is still capable of binding and affecting other mediators of cell cycle arrest. Thus, albeit this process will be much more fragile and sensitive to future mutations, it is expected that BRCA1 retains a certain ability to stop the cell cycle.

The different biological processes and protein interaction altered by the studied mutation are summarized in Table 1.

Table 1: Summary of the differences in DNA damage repair related processes between wild-type and mutated BRCA1.

Process	Affected	Not affected	New
MMR	MLH1, MSHS, MSH6	None	-
HR	COMI, BRCA2, PALB2	BARDI, H2AX	-
NER	P53	None	ERRC6
NHEJ	MSH6	H2AX	-
Cell cycle	P53, AKT1, HDAC1, HDAC2, RBBP4, RBBP7	COMI	MPIP1, MPIP2, MPIP3, 1433S, CDK1, CCNB1

MMR = Mismatch Repair

HR = Homologous Recombination

NER = Nucleotide Excision Repair

NHEJ = Non-Homologous End Join

Overall, the comparison between the MoAs of the full-length BRCA1 and its mutated variant shows that the latter undergoes a drastic reduction in its ability to modulate a number of proteins and pathways which are critical for DNA repair and cell cycle control.

Discussion

In order to investigate the hypothetically pathogenic role of the BRCA1 c.5434C->G (p.Pro1812Ala) mutation, a study was carried out using computational methods. A structural model was created and it was assessed from a functional and systems biology point of view.

The c.5434C->G (p.Pro1812Ala) mutation causes an important splicing defect in BRCA1, resulting in the omission of exon 23, which in turn leads to the appearance of a premature stop codon (p.Gly1803GlnfsX11) (10). However, and according to our results, it does not lead to secondary or tertiary structural changes. The structural configuration of the wild-type protein is preserved, which coincides with the findings of Drikos et al.: the c.5434C->G (p.Pro1812Ala)

mutation preserves the ability to interact through the BRCT domain with proteins such as BRIP1 and RBBP8. But binding affinity and stability were substantially modified, which could alter the conformational equilibrium and therefore affect the protein's function in vivo (11).

As a result of a dysfunctional BRCT domain, the mutated protein cannot carry out its role in DNA damage response and cell cycle regulation.

BRCA1 activity that depends on proteins that interact with other domains, such as interaction of the RING domain with the BARD1 protein, is not expected to be affected by this mutation (31).

In the c.5434C->G (p.Pro1812Ala) variant, p53 is not activated by the altered BRCT domain. The initiation of the apoptotic response may therefore be compromised (24). Although this process will be much more fragile and sensitive to future mutations, it is expected that BRCA1 will retain a certain ability to stop the cell cycle. The activation of p53 and other key mediators of the cell cycle and apoptosis does not depend on BRCA1 alone.

The results of structural and mechanical analyses mean that the c.5434C->G (p.Pro1812Ala) variant can be reclassified from a variant of unknown significance to a pathogenic variant. The following evidence supports this statement:

Segregation: the c.5434C->G (p.Pro1812Ala) variant was suspected of being harmful in at least one family (32).

Population frequency: The c.5434C->G (p.Pro1812Ala) mutation has been identified in patients with breast cancer or ovarian cancer, in both Spanish and non-Ashkenazi Jewish women (32).

Evolutionary preservation: At position 1812 of the BRCA1 protein the amino acid proline is highly conserved between species, suggesting that the normal function of the protein plays an essential role. The c.5434C->G (p.Pro1812Ala) variant causes a change in the splicing pattern of BRCA1 (10). Transcriptions expressed from the mutant c.5434C->G (p.Pro1812Ala) exhibit an omission of exon 23 in 75% of cases.

Functional assay: Our study did not indicate any significant change in structure. However, Drikos et al. observed a lower thermal stability that could influence conformational equilibrium. Assays of c.5434C->G (p.Pro1812Ala) mutation activity showed slightly reduced gene activity (11). An in vivo reporter gene assay showed that in c.5434C->G (p.Pro1812Ala) mutation activity clearly affects function (32). This assay assessed the BRCT domain's ability to induce transcriptional activation (10).

BRCA1 mutations with a similar splicing effect, including the neighbouring variants c.4987-3C>G (33) and c.5075-1G>C (34), were classified as pathogenic. Another study that examined a missense variant of unknown significance in the C-terminus region, close to variant c.5434C->G (p.Pro1812Ala), revealed significant alterations in BRCT structure and function (35).

Systems biology tools were used to evaluate the impact of the BRCA1 c.5434C->G (p.Pro1812Ala) mutation on DNA damage response from a mechanistic perspective. The results of this analysis support the findings of structural and functional methods explaining how the c.5434C->G (p.Pro1812Ala) mutation leads to impaired DNA repair and cell cycle control. This conclusion can be extrapolated to all mutations that cause loss of BRCT domain activity.

As regards DNA repair pathways, Mismatch Repair (MMR) and homologous recombination can be strongly altered in cells that harbour the c.5434C->G (p.Pro1812Ala) variant, whereas the effect on non-homologous end joining (NHEJ) and nucleotide excision repair (NER) seem to be less significant. Each of these mechanisms plays an independent and irreplaceable role

Supplemental data 1: Restrictions used in the construction of the mutated BRCA1 model

Uniprot A	Gene A	Restriction	Uniprot B	
P35869	AHR	does not activate	P38398	BRCA1
P38398	BRCA1	does not activate	Q92793	CREBBP
P38398	BRCA1	does not activate	Q96RL1	UIMC1
P38398	BRCA1	does not activate	P15311	EZR
P78347	GTF21	does not activate	P38398	BRCA1
Q9NWV8	BABAM1	does not activate	P38398	BRCA1
P38398	BRCA1	does not activate	Q86YC2	PALB2
Q8N163	CCAR2	does not inhibit	P51948	BRCA1
P38398	BRCA1	does not inhibit	P38398	MNAT1
P51587	BRCA2	does not activate	Q06609	RAD51
P38398	BRCA1	does not activate	Q13287	NM1
P67870	CSNK2B	does not activate	P38398	BRCA1
Q9Y4A5	TRRAP	does not activate	P38398	BRCA1
P38398	BRCA1	does not activate	P26038	MSN
P38398	BRCA1	does not activate	Q99708	RBBP8
P38398	BRCA1	does not activate	Q09028	RBBP4
P38398	BRCA1	does not activate	Q9Y216	NINL
P38398	BRCA1	does not activate	Q16576	RBBP7
Q15648	MED1	does not activate	P38398	BRCA1
P38398	BRCA1	does not activate	Q92769	HDAC2
P38398	BRCA1	does not inhibit	P31749	AKT1
P38398	BRCA1	does not activate with	60934	NBN
Q86YC2	PALB2	does not interact	P51587	BRCA2
P38398	BRCA1	does not activate	P84022	SMAD3
P38398	BRCA1	does not inhibit	Q8WXE1	ATRIP
P38398	BRCA1	does not activate	Q14032	BAAT
P38398	BRCA1	does not activate	Q6R327	RICTOR
P38398	BRCA1	does not activate	P31749	AKT1
P23771	GATA3	does not activate	P38398	BRCA1
P00519	ABL1	does not activate	P38398	BRCA1
P38398	BRCA1	does not activate	P52701	MSH6
P38398	BRCA1	does not activate	P51587	BRCA2
P38398	BRCA1	does not activate	P49959	MRE11A
P38398	BRCA1	does not activate	Q92878	RAD50
P38398	BRCA1	does not activate	P40692	MLH1
P38398	BRCA1	does not activate	P43246	MSH2
P38398	BRCA1	does not activate	P35241	RDX
Q08211	DHX9	does not inhibit	P38398	BRCA1
P84022	SMAD3	does not inhibit	P38398	BRCA1
P38398	BRCA1	does not activate	P35222	CTNNB1
P38398	BRCA1	does not activate	Q9BX63	BRIP1
P38398	BRCA1	does not activate	Q13547	HDAC1

Uniprot A	Gene A	Restriction	Uniprot B	
P38398	BRCA1	does not activate	P04637	TP53
Q86YC2	PALB2	does not activate	P51587	BRCA2
P09651	HNRNPA1	does not inhibit	P38398	BRCA1
P38398	BRCA1	does not activate	Q6UWZ7	FAM175A
Q92830	KAT2A	does not activate	P38398	BRCA1
P38398	BRCA1	does not activate	Q99759	MAP3K3
P38398	BRCA1	does not inhibit	Q86YC2	PALB2

Supplemental data 2: Comparison in the mechanism of action: Bibliographic references, node and link information.

Uniprot KB	Name	Displayed Name*
O14757	Serine/threonine-protein kinase Chk1	CHK1
P03372	Estrogen receptor	ESR1
P06493	Cyclin-dependent kinase 1	CDK1
P14635	G2/mitotic-specific cyclin-B1	CCNB1
P14859	POU domain, class 2, transcription factor 1	PO2F1
P16104	Histone H2AX	H2AX
P30304	M-phase inducer phosphatase 1	MPIP1
P30305	M-phase inducer phosphatase 2	MPIP2
P30307	M-phase inducer phosphatase 3	MPIP3
P31749	RAC-alpha serine/threonine-protein kinase	AKT1
P31947	14-3-3 protein sigma	1433S
P38398	Breast cancer type 1 susceptibility protein	BRCA1
P40692	DNA mismatch repair protein Mlh1	MLH1
P43246	DNA mismatch repair protein Msh2	MSH2
P51587	Breast cancer type 2 susceptibility protein	BRCA2
P52701	DNA mismatch repair protein Msh6	MSH6
Q09028	Histone-binding protein RBBP4	RBBP4
Q13547	Histone deacetylase 1	HDAC1
Q16576	Histone-binding protein RBBP7	RBBP7
Q86YC2	Partner and localizer of BRCA2	PALB2
Q92769	Histone deacetylase 2	HDAC2
Q99708	DNA endonuclease RBBP8	COM1
Q99728	BRCA1-associated RING domain protein 1	BARD1
P04637	Cellular tumor antigen p53	P53
Q03468	DNA excision repair protein ERCC-6	ERCC6

Link Information:

Link 1 (CHK1 → MPIP1)	PubMed ID: 12399544, 12676583, 12759351, 12963847, 15657099, 17292828, 18480045, 9278511
Link 2 (CHK1 → MPIP2)	PubMed ID: 15657099, 9278511
Link 3 (CHK1 → MPIP3)	PubMed ID: 10681541, 11016625, 11390642, 11836499, 12660173, 15657099, 15705874
Link 4 (CHK1 → DDR)	PubMed ID: 17292828
Link 5 (ESR1 → BRCA1)	PubMed ID: 10334989, 11244506, 11493692, 11782371, 12400015, 15674350, 16061635, 16260778
Link 6 (CDK1 → MPIP3)	PubMed ID: 10864927, 11836499, 15657099, 17349584
Link 7 (CDK1 → DDR)	PubMed ID: 22962268
Link 8 (CCNB1 → DDR)	PubMed ID: 23890385
Link 9 (PO2F1 → DDR)	PubMed ID: 15563594
Link 10 (H2AX → DDR)	PubMed ID: 19273588
Link 11 (MPIP1 → CDK1)	PubMed ID: 12411508
Link 12 (MPIP1 → DDR)	PubMed ID: 10740819
Link 13 (MPIP2 → CDK1)	PubMed ID: 11516829, 9585407
Link 14 (MPIP2 → DDR)	PubMed ID: 10740819
Link 15 (MPIP3 → CDK1)	PubMed ID: 10864927, 11836499, 15657099, 17349584
Link 16 (MPIP3 → DDR)	PubMed ID: 10740819
Link 17 (AKT → DDR)	PubMed ID: 22481935
Link 18 (1433S → CDK1)	PubMed ID: 10524633, 15657099
Link 19 (1433S → CCNB1)	Kegg: 04110-Cell cycle, 04115-p53 signaling pathway
Link 20 (BRCA1 → CHK1)	PubMed ID: 11836499, 17380128
Link 21 (BRCA1 → ESR1)	PubMed ID: 10334989, 11244506, 11493692, 11782371, 12400015
Link 22 (BRCA1 → PO2F1)	PubMed ID: 11777930, 15657099, 18000219
Link 23 (BRCA1 → H2AX)	PubMed ID: 10959836, 11927591, 12419185, 12485996, 18001824, 18001825, 18344987, 19766185,19805320, 20681793
Link 24 (BRCA1 → AKT1)	PubMed ID: 10542266, 19074868, 21242970
Link 25 (BRCA1 → 1433S)	PubMed ID: 11384963
Link 26 (BRCA1 → MLH1)	PubMed ID: 10783165, 15657099, 17148452, 21240188
Link 27 (BRCA1 → MSH2)	PubMed ID: 10783165, 11498787, 15657099, 15886699, 16260778
Link 28 (BRCA1 → BRCA2)	PubMed ID: 11477095, 14499622, 14636569, 15657099, 17664283, 19369211, 21135251, 9774970
Link 29 (BRCA1 → MSH6)	PubMed ID: 10783165, 11498787, 15657099, 16260778
Link 30 (BRCA1 → RBBP4)	PubMed ID: 10220405, 15657099
Link 31 (BRCA1 → HDAC1)	PubMed ID: 10220405, 15657099
Link 32 (BRCA1 → RBBP7)	PubMed ID: 10220405, 11394910, 11746496, 15657099
Link 33 (BRCA1 → PALB2)	PubMed ID: 19268590, 19369211, 19553677, 19584259, 23038782
Link 34 (BRCA1 → HDAC2)	PubMed ID: 10220405, 15657099, 21946536, 22318606
Link 35 (BRCA1 → COM1)	PubMed ID: 10196224, 10764811, 10910365, 11689934, 11739404, 14578343, 15657099, 16818604,16843262, 17525340, 17525342, 18171670, 18285836, 19452558, 20351172, 21908405, 9738006, 9811458
Link 36 (BRCA1 → BARD1)	PubMed ID: 10026184, 10477523, 10635334, 11257228, 11278247, 11301010, 11498787, 11504724,11526114, 11573085, 11773071, 11925436, 11927591, 12154023
Link 37 (BRCA1 → P53)	PubMed ID: 14636569, 14710355, 15571721, 15657099, 16677609, 16969499, 19509300, 21742769,9482880
Link 38 (BRCA1 → ERCC6)	PubMed ID: 21756275
Link 39 (BRCA1 → DDR)	PubMed ID: 21203981
Link 40 (MLH1 → DDR)	PubMed ID: 12939400
Link 41 (MSH2 → DDR)	PubMed ID: 23391514
Link 42 (BRCA2 → DDR)	PubMed ID: 11707511
Link 43 (MSH6 → DDR)	PubMed ID: 21075794
Link 44 (RBBP4 → DDR)	PubMed ID: 16522651
Link 45 (HDAC1 → DDR)	PubMed ID: 16522651
Link 46 (RBBP7 → DDR)	PubMed ID: 16522651
Link 47 (PALB2 → BRCA2)	PubMed ID: 16793542, 17200672, 19268590, 19369211, 19553677, 19584259, 19609323, 20332121, 23038782
Link 48 (PALB2 → DDR)	PubMed ID: 24998779
Link 49 (HDAC2 → DDR)	PubMed ID: 0802485
Link 50 (COM1 → DDR)	PubMed ID: 24403251
Link 51 (BARD1 → DDR)	PubMed ID: 16391231
Link 52 (P53 → DDR)	PubMed ID: 12195423, 12052432
Link 53 (ERCC6 → DDR)	PubMed ID: 5340056

STIMULUS: Activation of BRCA1 A ☐B: Activation relationship

RESPONSE: DDR (DNA Damage Response) A ☐B: Inhibition relationship

in DNA repair. Failure of any one of them makes it impossible to repair a certain type of damage, which will eventually lead to an accumulation of mutations.

Together with structural and functional analysis, mechanistic insight provides a sound basis for defining the c.5434C->G (p.Pro1812Ala) mutation as a pathogenic variant. The BRCA1 c.5434C->G (Pro1812Ala) mutation causes deletion of the second BRCT domain, which plays a key role in critical processes, such as DNA repair and cell cycle control. It is therefore to be expected that this variant will not be able to exert its tumour suppressor action.

In conclusion, the c.5434C->G (p.Pro1812Ala) mutation creates an environment with increased risk of binding and tolerance to damage, which promotes the accumulation of mutations. Despite some compensation mechanisms, DNA damage whose repair is not induced, cell cycle arrest or stimulation of the apoptotic machinery may go unnoticed. This phenomenon can lead to mutations in other tumour suppressor genes or oncogenes, which causes cancer to develop. This hypothesis is consistent with Knudson's two-hit hypothesis (36) and with increased cancer susceptibility associated with BRCA1 (37).

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