RESEARCH BRIEF

Biallelic Deleterious *BRCA1* Mutations in a Woman with Early-Onset Ovarian Cancer

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ABSTRACT

BRCA1 and *BRCA2* are the most important breast and ovarian cancer susceptibility genes. Biallelic mutations in *BRCA2* can lead to Fanconi Anemia and predisposition to cancers, while biallelic *BRCA1* mutations have not been confirmed, presumably because one wild-type *BRCA1* allele is required during embryogenesis. This study describes an individual who was diagnosed with ovarian carcinoma at age 28 and found to have one allele with a deleterious mutation in *BRCA1*, c.2457delC (p.Asp821Ilefs*25), and a second allele with a variant of unknown significance (VUS) in *BRCA1*, c.5207T>C (p.Val1736Ala). Medical records revealed short stature, microcephaly, developmental delay and significant toxicity from chemotherapy. *BRCA1* p.Val1736Ala co-segregated with cancer in multiple families, associated tumors demonstrated loss of wild-type *BRCA1*, and *BRCA1* p.Val1736Ala showed reduced DNA damage localization. These findings represent the first validated example of biallelic deleterious human *BRCA1* mutations, and have implications for the interpretation of genetic test results.

SIGNIFICANCE: Accurate assessment of genetic testing data for *BRCA1* mutations is essential for clinical monitoring and treatment strategies. Here we report the first validated example of an individual with biallelic *BRCA1* mutations, early onset ovarian cancer, and clinically significant hypersensitivity to chemotherapy.

INTRODUCTION

Hereditary breast and ovarian cancer syndrome is predominantly caused by heterozygous, germline mutation in the *BRCA1* or *BRCA2* genes(1). Several forms of Fanconi Anemia, characterized by bone marrow failure and malignancy, can be a consequence of biallelic mutations in *BRCA2(2)* or biallelic mutations in genes encoding *BRCA2* and *BRCA1* associated proteins PaLB2 and BRIP1(3-7). Despite a frequency of approximately 1.5% in the Ashkenazi Jewish population for the *BRCA1* mutations, 185delAG [Human Genome Variation Society (HGVS) c.68_69delAG] and 5382insC (HGVS c.5266dupC), no homozygous or compound heterozygote carriers of these mutations have been reported. Although *Brca1* nullizygosity results in embryonic lethality in mice(8), genetically engineered mice harboring biallelic mutations that correspond to human cancer-associated missense mutations within the *BRCA1* BRCT domain are viable through adulthood and display highly penetrant cancer susceptibility(9). These findings raise the possibility that a partially functional *BRCA1* allele *in trans* with a deleterious truncating mutation (or with a similarly partially functioning mutation) in *BRCA1* could be present within the same individual, and could contribute to familial cancer susceptibility in humans. Herein we document the presence of a functionally deleterious *BRCA1* BRCT domain missense alteration *in trans* with a pathogenic *BRCA1* alteration in a woman with dysmorphic features and early onset ovarian carcinoma.

RESULTS

The proband (Fig. 1A, ego 28, arrow) presented at age 28 with stage IV papillary serous ovarian carcinoma. Medical records revealed a history of microcephaly, short stature (adult height of 150 cm) and developmental delay with limited speech at age 4 years. Review of pictures provided by the family demonstrates coarse features with low anterior hairline, macrognathia, a prominent nasal bridge and small alae nasi. She did not have obvious abnormalities of her thumbs and had a normal complete blood count at the time of her cancer diagnosis. Neither ataxia nor telangiectasias were documented. This individual was found to have a known deleterious mutation in *BRCA1* reported as 2576delC (HGVS c.2457delC; p.Asp821Ilefs*25) and a variant of unknown significance (VUS) in *BRCA1* (HGVS c.5209T>C) p.Val1736Ala, as well as a VUS in *BRCA2* (HGVS, c.971G>C; p.Arg324Thr). Treatment with carboplatin (target area under the concentration versus time curve in mg/mL•min (AUC) of 5) and paclitaxel (175 mg/m2) resulted in significant toxicity requiring hospitalization due to fever and grade 4 neutropenia (absolute neutrophil count (ANC) nadir of 160 per cubic millimeter), as well as grade 3 anemia (nadir hemoglobin 7.8g/dl) and grade 4 thrombocytopenia (nadir 3000 per cubic millimeter), for which she received red blood cell and platelet transfusions. She also developed grade $\geq$3 nausea, diarrhea and mucositis. As a result of the excess toxicity, carboplatin and paclitaxel were discontinued after two cycles. She received no further therapy and died six months following her diagnosis. Extreme sensitivity to the interstrand crosslinking agent carboplatin is not typically observed in heterozygous *BRCA1* mutation carriers(10-12), but is seen in biallelic *BRCA1* mutant cells and mice, suggesting that both *BRCA1* alleles were compromised for DNA repair function.

The mother of ego 28 was diagnosed with ovarian cancer at age 53 and died at 55. A maternal great aunt (Fig. 1A, Family A, Ego 1) was diagnosed with breast and ovarian cancers at ages 59 and 69, respectively, and a contralateral breast cancer at age 76. A second maternal great-aunt (Ego 9, sister of Ego 1) was diagnosed with primary peritoneal cancer at age 67 and died at 68. Notably, both carried the *BRCA1* p.Val1736Ala variant of uncertain significance (VUS) but not the known pathogenic mutation *BRCA1* 2576delC (HGVS c.2457delC). Additional genetic testing in the family revealed that the brother of the proband (ego 27) carries the *BRCA1* c.2457delC mutation and the paternal lineage also had multiple cases of early onset breast cancer. To investigate this variant further, we were able to obtain pedigrees on 11 additional families with the *BRCA1* p.Val1736Ala VUS. A total of 9 of these pedigrees which had additional genotyping of family members were used to assess co-segregation using methods described in Thompson et al(13) (a representative pedigree is shown in Fig. 1B and characteristics of the families are detailed in Supplementary table 1). The combined odds ratio in favor of p.Val1736Ala being pathogenic was 234:1 assuming the age-specific penetrance estimated in Antoniou et al.(14) Loss of heterozygosity (LOH) analysis was performed on genomic DNA extracted from *BRCA1* p.Val1736Ala mutation positive tumors using a custom designed Taqman assay (Table 1 and Supplementary Fig. S1). Ovarian/primary peritoneal cancer tumor blocks from Family A egos 1 and 9 demonstrated LOH at the wild-type *BRCA1* allele with retention of the p.Val1736Ala allele. Conversely, in ego 28, who carried germ-line *BRCA1* c.2457delC and p.Val1736Ala alterations *in trans*, the ovarian tumor did not display LOH at either allele suggesting that p.Val1736Ala expression is not selected against in tumors.

The BRCA1 BRCT residue p.Val1736 is conserved across 18 different vertebrate species (Fig. 2A). In contrast with other BRCT residues that exhibit cancer-associated point mutations, structural models predict that p.Val1736 does not make direct contact with phospho-peptide ligands (Fig. 2B). Rather, Val1736 resides in a hydrophobic pocket, which may affect the stability of residues Pro1749 and Cys1697, both of which are required for BRCT function in DNA repair and tumor suppression. Transfection of a DNA double strand break (DSB) reporter cell line (Supplementary Fig. S2)(15) with an epitope tagged carboxy-terminal region of BRCA1 revealed that a wild-type (WT) BRCT fragment was observed at greater than 80% of DSBs, while the fragment containing p.Val1736Ala was reduced to below 40% (P = 0.0029), intermediate to the WT protein and known BRCT mutant p.Pro1749Arg, which was present at less than 20% of DSBs (P = 0.0017) (Fig. 2C, D). Similarly, co-immunoprecipitation experiments with the same epitope tagged BRCA1 fragments demonstrated significantly diminished interaction between p.Val1736Ala and RAP80, a BRCA1 BRCT interacting protein, in comparison to WT BRCT containing fragments (Fig. 2E). Consistent with these results, overexpression of the WT BRCT fragment acted as a dominant negative allele by reducing IR induced Rad51 foci formation and homology directed DSB repair by a significantly greater extent than overexpression of BRCA1 fragments containing either the BRCT mutations p.Pro1749Arg or p.Val1736Ala (Supplementary Fig. S3).

We are aware of only one previous report of biallelic deleterious mutations in *BRCA1* in humans. In this report, a Scottish woman was found to be homozygous for *BRCA1*2800delAA(HGVS c. 2681_2682delAA, p.Lys894Thrfs*8)(16). This individual was diagnosed with breast cancer at age 32, and subsequently developed a contralateral breast

cancer. Homozygosity for this mutation was plausible particularly because it is a founder mutation in the studied population(17). Nevertheless, this report has long been questioned because potential primer bias in PCR-based genotyping could have led to preferential amplification of the putative mutant allele and hence masking of true heterozygosity(18). Because of the importance of this single report for the interpretation of our own results, we re-sequenced peripheral blood lymphocyte DNA from the reported biallelic carrier and found that only one *BRCA1* allele harbored the designated mutation c. 2681_2682delAA, while the other allele was found to be wild-type at this position (Fig. 3A, B). Therefore, the purported homozygous carrier was in actuality heterozygous for a *BRCA1* mutation.

DISCUSSION

Here we report the first individual with validated biallelic mutations in *BRCA1*. Compelling evidence is presented that *BRCA1* p.Val1736Ala is both pathogenic and can support viability through adulthood *in trans* to a deleterious mutation in exon 11 of *BRCA1* (*BRCA1* 2576delC). *BRCA1* p.Val1736Ala diminishes protein-protein interaction with RAP80, localization to DSBs, and imparts cancer susceptibility independent of other *BRCA1* or *BRCA2* alterations. LOH analysis was also consistent with pathogenicity. Loss of the wild type allele occurred in both tumors that carried the p.Val1736Ala VUS *in trans* to wild type *BRCA1*, however LOH did not occur in the ovarian cancer of the proband (ego 28), which was compound heterozygous for p.Val1736Ala and 2576delC, indicative of a scenario in which selective pressure did not exist to delete either pathogenic allele.

Several features of the index patient were uncharacteristic for monoallelic *BRCA1* mutation carriers. In addition to the aforementioned developmental delay, microcephaly and short stature, ovarian cancer was diagnosed below the age of 30, which is unusual for *BRCA1* mutation carriers(19). She also had extreme sensitivity to the interstrand crosslinking agent carboplatin, a characteristic not typically displayed in heterozygous *BRCA1* mutation carriers *in vivo*(10-12, 20).

While complete BRCA1 deficiency results in early embryonic lethality in mice, it should be considered that certain biallelic *BRCA1* mutations that mimic human cancer associated mutations can support viability through adulthood in mice(9). Genetically engineered mice harboring biallelic BRCT mutations (p.Ser1598Phe) that corresponds to a known cancer-causing allele in humans (p.Ser1655Phe) were viable through adulthood and displayed similar cancer susceptibility to mice completely lacking *BRCA1* gene function in the mammary gland(9). Moreover, complete deletion of exon 11 or introduction of a mutation that produces a stop codon in the *BRCA1* exon 11 region, as predicted in the 2576delC allele, disrupts full length BRCA1 protein leaving intact an evolutionarily conserved exon 10 to exon 12 splice variant. The *BRCA1* delta 11 splice product contains the BRCA1 RING domain and BRCT repeats, localizes to DNA damage sites, and can support viability in certain mouse backgrounds, yet still confers cancer susceptibility(21-23). The BRCA1 delta 11 splice isoform is expressed at both the RNA and protein levels in human cells(24), however, it is not known if this is the case in the context of the 2576delC mutation. It is therefore likely that partial DNA repair function of p.Val1736Ala (c.5209T>C), and possibly 2576delC (c.2457delC), or both of these mutant alleles is permissive for viability in humans.

Structural modeling suggests p.Val1736Ala is unique when compared to other BRCT missense mutations, in that it lies distal to the phosphopeptide-binding pocket. Prior studies have shown that p.Val1736Ala exhibits thermal instability and partial loss of function in transcriptional reporter assays(25, 26). Our results are also consistent that p.Val1736Ala is a hypomorphic alteration with respect to biochemical and cellular function. While BRCA1 p.Val1736Ala is predicted to be hypomorphic in terms of DNA repair function, it is not evident that it has reduced penetrance with respect to cancer susceptibility. Collectively, these findings together with observations from genetically engineered mouse models(9) are strongly suggestive that viability and tumor suppression phenotypes are not completely concordant among *BRCA1* mutant alleles.

Apart from the biological implications, the findings in this study have importance to the interpretation of genetic variants. Variants of uncertain significance (VUS) are a common finding in genetic testing for inherited cancer syndromes and pose challenges in counseling and management(27). Co-occurrence of a VUS *in trans* with a known deleterious *BRCA1* mutation is felt to be a strong indication that the VUS is not clinically important(28). Our findings suggest the presence of a *BRCA1* VUS *in trans* with an established deleterious *BRCA1* mutation should not be considered as definitive evidence against pathogenicity. This work also highlights the importance of examining multiple distinct lines of evidence when interpreting a VUS, including clinical phenotype. This lesson is particularly pertinent in the era of massively parallel DNA sequencing, as a large number of VUS will be identified using this methodology and caution will be needed in interpreting these for clinical use.

METHODS

Loss of heterozygosity analysis

DNA was extracted from either cell lines or from tumors following microdissection of cancer tissue to over 70% tumor (Supplementary Fig. S3). Loss of heterozygosity (LOH) was assessed by the University of Pennsylvania Genomics Facility using a custom designed Taqman assay to distinguish a single nucleotide alteration at nucleotide position 5207, codon 1736 from the wildtype (WT) allele (Table 1 and Supplementary Fig. S1).

BRCA1 c.2681_2682delAA re-sequencing

Lymphocyte DNA from the patient was amplified by PCR using the primers F1: 5'-AACCACAGTCGGGAAACAAG-3'and R2: 5'- TGATGGGAAAAAGTGGTGGT-3' and the QIAGEN (Toronto, Canada) HotStar Plus PCR system according to the manufacturer's instructions. PCR products were sequenced by Sanger sequencing using the Applied Biosystem's 3730xl DNA Analyzer technology and the traces were visualized using the software Chromas (Technelysium).

Immunofluorescence

Immunofluorescence was performed in the DNA double-strand break reporter cells as described previously (15). No additional authentication on cell lines was performed. All analyses were carried out on unmodified images that were captured with a QImaging RETIGA-SRV camera connected to a Nikon Eclipse 80i miscroscope.

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Table 1

Sample ID	Description	Germline BRCA1	Taqman Result	Interpretation
S2504	Cell line	WT/WT	A/A	No LOH
S2366	Tumor (breast)	WT/WT	A/A	No LOH
Ego 1	Lymphoblasts	WT/ p.Val1736Ala	A/G	No LOH
Ego 10	Lymphoblasts	WT/ p.Val1736Ala	A/G	No LOH
Ego 1	Tumor (Breast)	WT/ p.Val1736Ala	G/G	LOH at WT
Ego 9	Tumor (Ovarian)	WT/ p.Val1736Ala	G/G	LOH at WT
Ego 28	Tumor (Ovarian)	p.2576delC/ p.Val1736Ala	A/G	No LOH

Table 1. Loss of heterozygosity (LOH) from the index family. Ovarian/primary peritoneal cancer tumor blocks from Family A egos 1 and 9 demonstrated LOH had occurred at the wildtype BRCA1 allele with retention of the p.Val1736Ala allele. “A” at position 5207 represents the wildtype allele and “G” at position 5207 represents the V1736A allele. Conversely, in ego 28, who carried germline *BRCA1* 2576delC and p.Val1736Ala alterations *in trans*, the ovarian tumor did not display LOH at either allele.

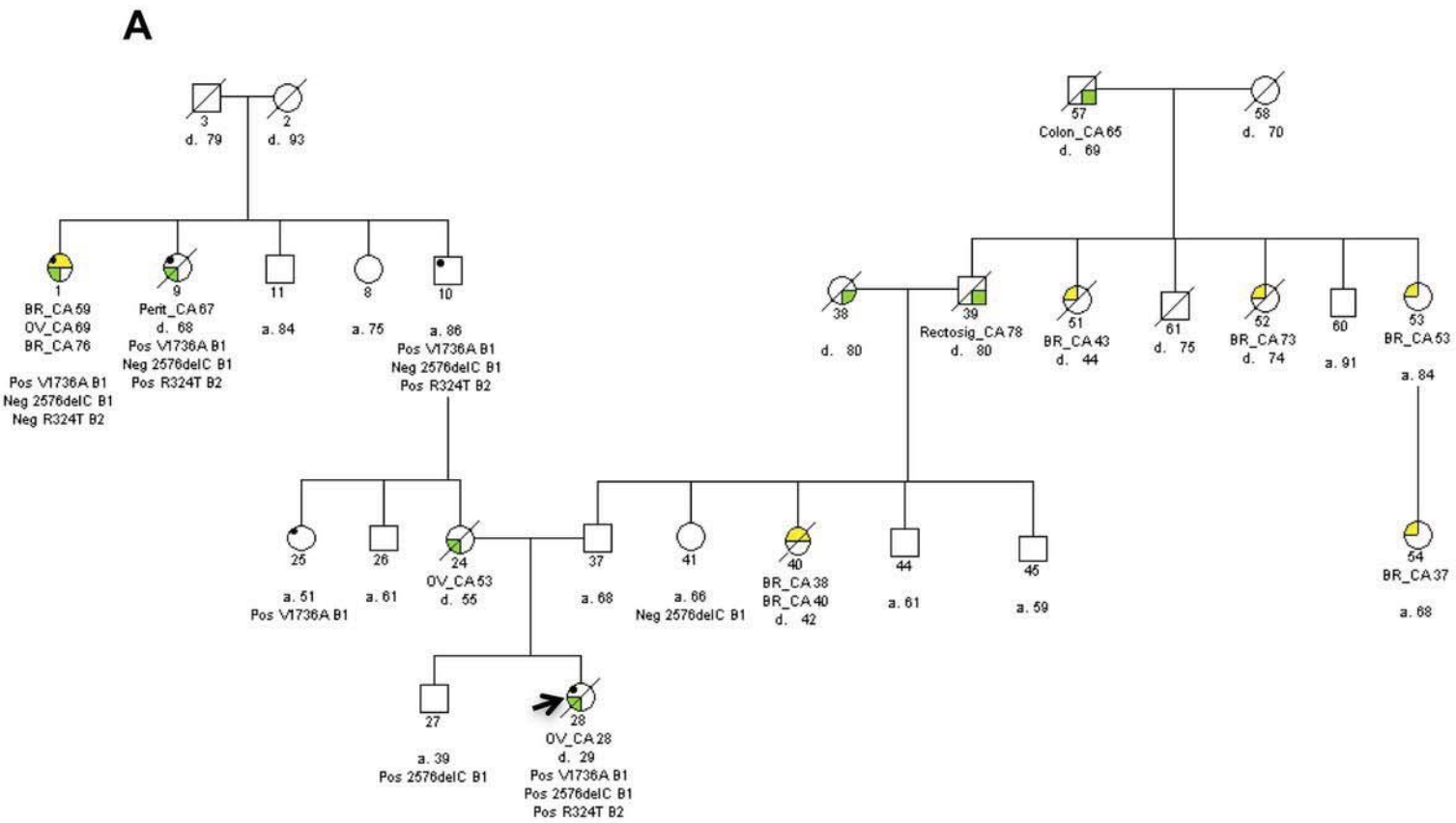
Figure 1. Pedigrees of families with BRCA1 p.Val1736Ala**. A,** pedigree of the index family is shown. Circles indicate females and squares indicate males. Slashes indicate death. The proband is indicated by an arrow. Shading in the left lower quadrant indicates ovarian cancer. Shading in the left upper quadrant indicates unilateral breast cancer, and in both left and right upper quadrants, bilateral breast cancer. Shading in the right lower quadrant indicates cancer which is not breast or ovarian. Current ages or age at death, and age at cancer diagnosis are listed below each individual, as is genetic status if known. UNK is unknown. **B**, a second representative pedigree with the BRCA1 p.Val1736Ala alteration is shown.

Figure 2. Analysis of the BRCA1 p.Val1736Ala Mutation. **A**, partial sequence alignment of a BRCA1 BRCT domain from different species showing that BRCA1 V1736 and P1749 residues (highlighted in red) are completely conserved across all vertebrate species. Numbers on top of the alignment indicate amino acid positions of the human BRCA1 protein. Conservation below describes sequence conservation (*, identical; :, ≥ 80% conservation; ., ≥ 60% conservation). **B,** modeling (based on pdb code 1t15) of the interaction between the BRCA1 BRCT domains and a peptide of BACH1. BRCA1 is colored in grey with disease causing mutants of the conserved residues in red. The BACH1 peptide is colored in purple. **C**, WT BRCA1 (shown as a green focus) but not the p.Val1736Ala or p.Pro1749Arg mutant efficiently colocalized with mcherryLacIFokI fusion endonuclease induced DNA double-strand breaks. **D**, percentage of cells with BRCA1 (WT or mutant) colocalizing to FokI was quantified. At least 100 cells were assessed for each data point (n > 100). Measurements were obtained in triplicates and reported as means of three

replicates. P values were calculated using student t-test, with $P < 0.05$ for all comparisons. Error bars indicate standard error of the mean (S.E.M). **E,** Co-immunoprecipitation of Epitope tagged BRCA1 (Myc-BRCA1), WT or mutants from 293T cells at room temperature for 2 hours followed by immunoblot for RAP80.

Figure 3. Re-sequencing of *BRCA1* c.2681_2682delAA mutation carrier**. A,** chromatogram showing the wild-type *BRCA1* sequence in lymphocyte DNA from a non-carrier individual. **B**, chromatogram from lymphocyte DNA showing the heterozygous status of the Scottish woman previously reported as homozygous for the *BRCA1* c.2681_2682delAA mutation. Both the mutant and wild-type alleles are clearly present.

Figure 1



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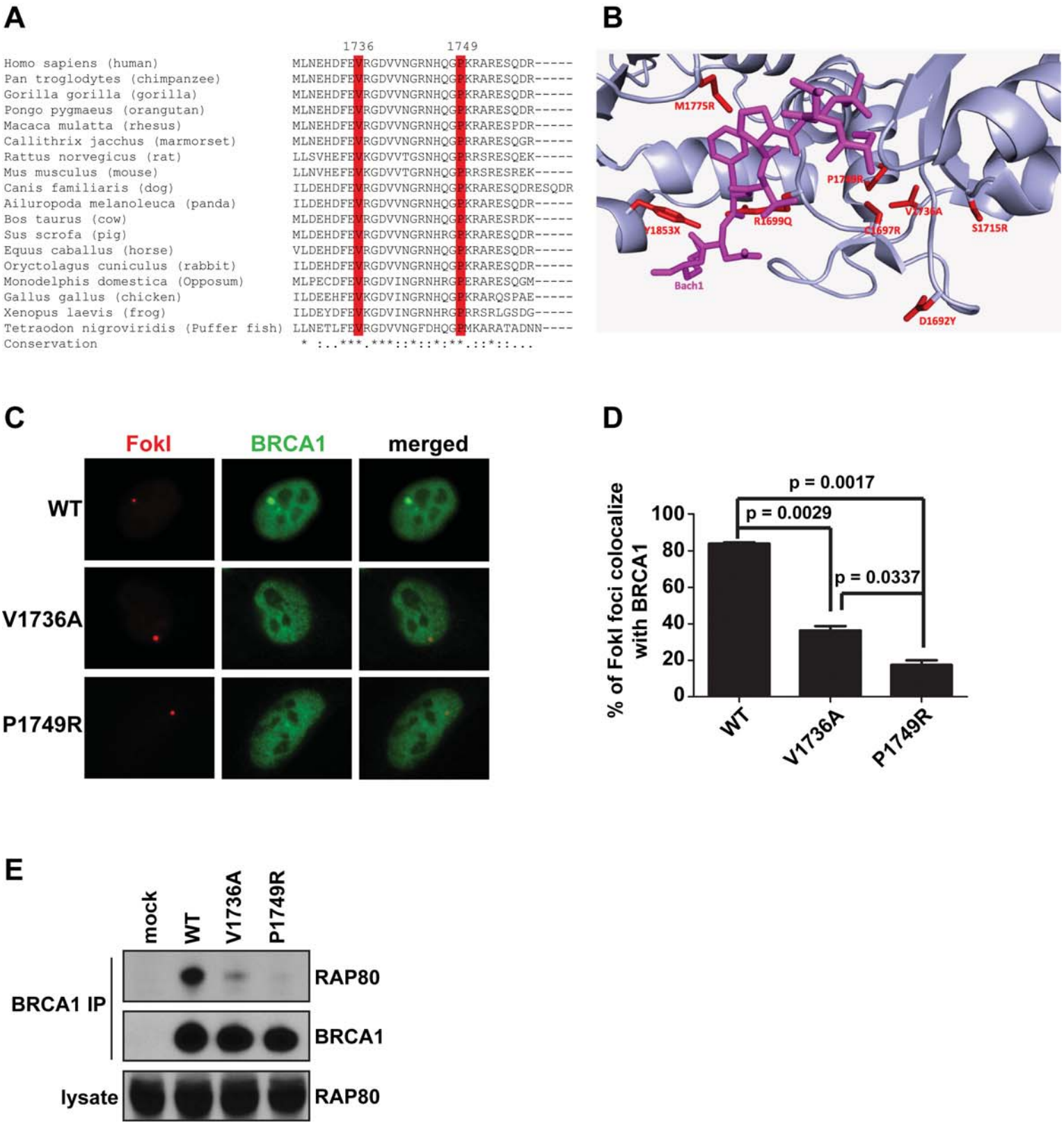
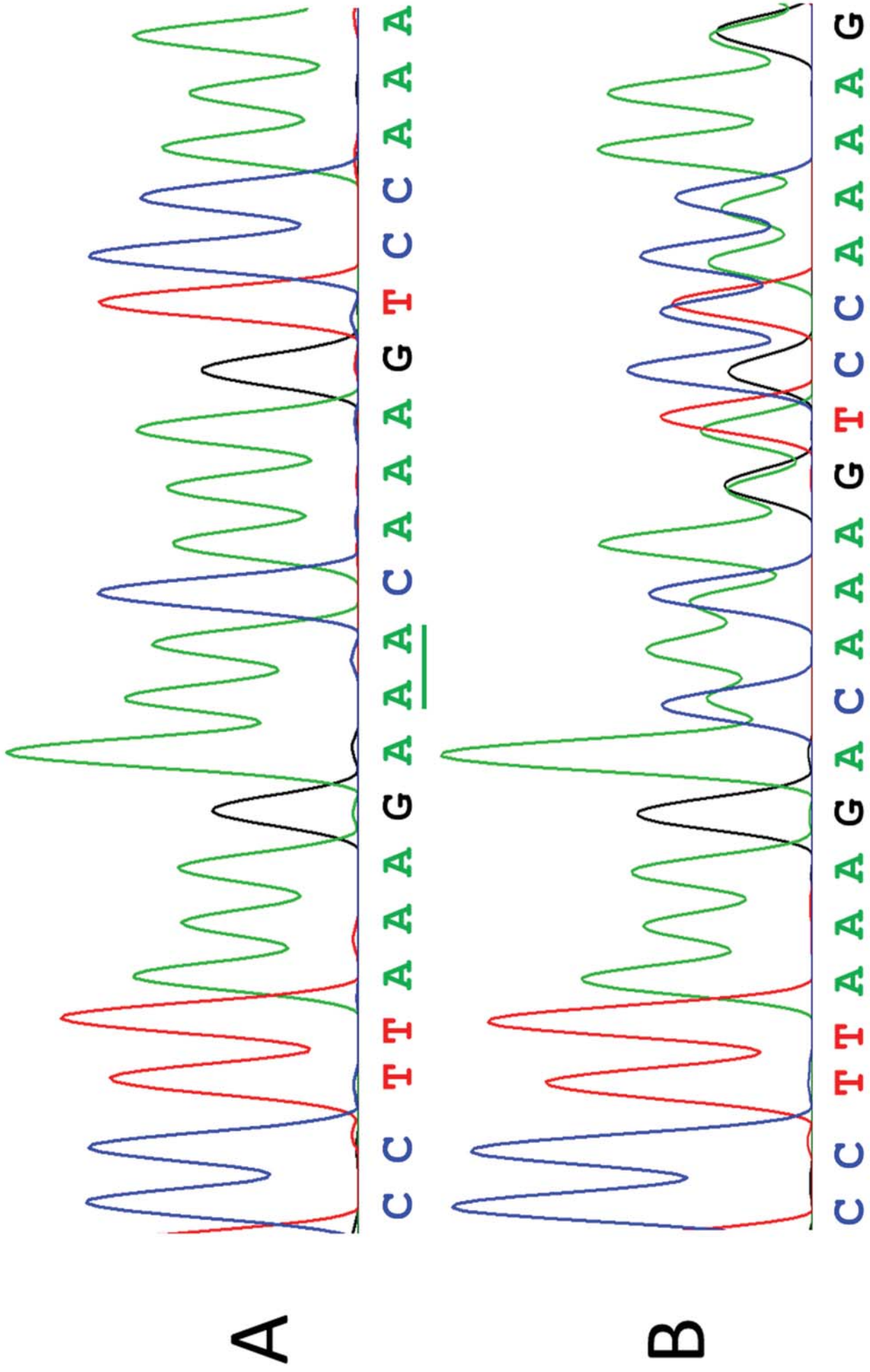
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Figure 2





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CANCER DISCOVERY

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