



OPEN ACCESS

ORIGINAL ARTICLE

# BRCA1 Circos: a visualisation resource for functional analysis of missense variants

Ankita Jhuraney,<sup>1,2</sup> Aneliya Velkova,<sup>1,24</sup> Randall C Johnson,<sup>3</sup> Bailey Kessing,<sup>3</sup> Renato S Carvalho,<sup>4,5</sup> Phillip Whiley,<sup>6</sup> Amanda B Spurdle,<sup>6</sup> Maaïke P G Vreeswijk,<sup>7</sup> Sandrine M Caputo,<sup>8</sup> Gael A Millot,<sup>9</sup> Ana Vega,<sup>10</sup> Nicolas Coquelle,<sup>11</sup> Alvaro Galli,<sup>12</sup> Diana Eccles,<sup>13</sup> Marinus J Blok,<sup>14</sup> Tuya Pal,<sup>1</sup> Rob B van der Luijt,<sup>15</sup> Marta Santamariña Pena,<sup>16</sup> Susan L Neuhausen,<sup>17</sup> Talia Donenberg,<sup>18</sup> Eva Machackova,<sup>19</sup> Simon Thomas,<sup>20</sup> Maxime Vallée,<sup>21</sup> Fergus J Couch,<sup>22</sup> Sean V Tavtigian,<sup>23</sup> J N Mark Glover,<sup>11</sup> Marcelo A Carvalho,<sup>4,5</sup> Lawrence C Brody,<sup>24</sup> Shyam K Sharan,<sup>25</sup> Alvaro N Monteiro,<sup>1</sup> on behalf of the ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) Consortium

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/jmedgenet-2014-102766>).

For numbered affiliations see end of article.

## Correspondence to

Dr Alvaro N Monteiro, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Drive, Tampa, FL 33612, USA; [alvaro.monteiro@moffitt.org](mailto:alvaro.monteiro@moffitt.org)

Received 8 September 2014  
Revised 29 November 2014  
Accepted 5 December 2014  
Published Online First  
2 February 2015



Open Access  
Scan to access more  
free content

## ABSTRACT

**Background** Inactivating germline mutations in the tumour suppressor gene *BRCA1* are associated with a significantly increased risk of developing breast and ovarian cancer. A large number (>1500) of unique *BRCA1* variants have been identified in the population and can be classified as pathogenic, non-pathogenic or as variants of unknown significance (VUS). Many VUS are rare missense variants leading to single amino acid changes. Their impact on protein function cannot be directly inferred from sequence information, precluding assessment of their pathogenicity. Thus, functional assays are critical to assess the impact of these VUS on protein activity. *BRCA1* is a multifunctional protein and different assays have been used to assess the impact of variants on different biochemical activities and biological processes.

**Methods and results** To facilitate VUS analysis, we have developed a visualisation resource that compiles and displays functional data on all documented *BRCA1* missense variants. *BRCA1* Circos is a web-based visualisation tool based on the freely available Circos software package. The *BRCA1* Circos web tool (<http://research.nhgri.nih.gov/bic/circos/>) aggregates data from all published *BRCA1* missense variants for functional studies, harmonises their results and presents various functionalities to search and interpret individual-level functional information for each *BRCA1* missense variant.

**Conclusions** This research visualisation tool will serve as a quick one-stop publically available reference for all the *BRCA1* missense variants that have been functionally assessed. It will facilitate meta-analysis of functional data and improve assessment of pathogenicity of VUS.

## INTRODUCTION

Breast cancer is the most common cancer in women and approximately 5%–10% of breast cancer cases are hereditary, where a germline mutation is present within a known cancer susceptibility gene.<sup>1</sup> Individuals with inherited inactivating mutations in *BRCA1* have an increased risk of developing breast and ovarian cancers.<sup>2–4</sup> A recent

prospective study of mutation carriers estimates the cumulative risk of breast cancer to be 60% (95% CI 44% to 75%) and of ovarian cancer to be 59% (95% CI 43% to 76%) by the age of 70 years.<sup>5</sup> Inherited mutation in *BRCA1* accounts for 40%–45% of all hereditary breast cancer cases but for around 80% of cases in families with multiple cases of breast and ovarian cancers.<sup>6</sup> Thus, women with a strong family history are referred to genetic testing.

*BRCA1* genetic test is based on direct sequencing of coding exons<sup>7</sup> and can have the following outcomes: no sequence alteration, pathogenic variant, non-pathogenic variant or variants of unknown significance (VUS).<sup>8</sup> Despite early evidence to the contrary, it seems that individuals from *BRCA* mutation-positive families with no sequence alteration or with non-pathogenic variants are not at increased risk for breast and ovarian cancer.<sup>9–12</sup> Individuals testing positive for a pathogenic variant have a well-established elevated risk of developing breast and ovarian cancers.<sup>5</sup> However, for those that carry a VUS, test results cannot be used for risk assessment. Most of these VUS are alterations in intronic and regulatory regions or in-frame deletions/insertions and missense variants whose impact on function cannot be inferred from the sequence alone.<sup>13</sup>

Currently, a multifactorial statistical model classifies *BRCA* variants where each variant is assigned a prior probability of being pathogenic.<sup>14–17</sup> This model uses data from segregation analysis, co-occurrence, family history and tumour pathology and can be expanded to take into consideration other sources of data. To align variant classification to specific medical recommendations, the International Agency for Research on Cancer (IARC) Unclassified Genetic Variants Working Group defined a five-class system with specific probability thresholds.<sup>18</sup> Systematic use of this model has led to the classification of a large number of previously unclassified variants.<sup>16 19</sup>

However, despite a wealth of information from functional assays, these data have not yet been



CrossMark

**To cite:** Jhuraney A, Velkova A, Johnson RC, et al. *J Med Genet* 2015;**52**:224–230.

incorporated into the multifactorial model. Recently, a computational approach to infer disease relevance from functional data, called VarCall, was developed.<sup>20</sup> This latest development allows the systematic integration of functional data into the multifactorial model. To facilitate this analysis, we aggregated all data on functional assays for BRCA1 missense variants into one easy-to-use lookup tool to effectively determine the functional significance of the documented VUS.

We call this web tool 'BRCA1 Circos' and it currently represents ~700 documented missense variants (Breast Cancer Information Core (BIC), <http://research.nhgri.nih.gov/bic/>). Circos plots are visualisation tools widely used in the current literature to represent multidimensional genomic data<sup>21</sup> that were adapted by us to display the BRCA1 missense variants. Besides the visualisation resource, we have developed a single comprehensive database that incorporates all the present information on BRCA1 missense variants. We used PERL-based Circos modules to generate the BRCA1 Circos images and developed a webpage for the Circos to be used as an interactive tool along with a searchable database of all the variants (the webpage can be viewed at <http://research.nhgri.nih.gov/bic/circos/>).

## METHODS AND RESULTS

### Literature curation

We annotated all the missense variants that had been reported in (a) the literature or (b) the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project (ESP6500SI-V2 release; <http://evs.gs.washington.edu/EVS/>) or (c) the BIC database totalling 698 variants. We performed an extensive literature search for all functional assays. If the variants were present in the BIC, we have indicated the frequency of the variant in Circos. In addition to the traditional search tools and databases such as the National Center for Biotechnology Information's PubMed and Google Scholars, we also used publically available databases such as Align-GVGD (<http://brca.iarc.fr/PRIORS/BRCA1/index.php>) and the BRCA1 variant literature database (<http://chromium.liacs.nl/LOVD2/cancer/home.php>) to document the information available on missense variants in these databases. The information from these sources was used to develop a database of these variants and, when available, the impact on BRCA1 function as indicated in the original source. Online supplementary table S1 contains the complete BRCA1 Circos database. Updated versions will be available for download on the BRCA1 Circos webpage.

### Harmonising scores

Regardless of whether the data we obtained from the articles defined the results categorically or quantitatively, we have followed a controlled vocabulary<sup>16</sup> to define functional impact or no functional impact as mentioned by the authors in the respective papers. As a rule, we respected the authors' classification/score of individual variants. For example, in a quantitative test, we followed the authors' cut-off to determine which variant had a functional impact and which did not. In some cases, we might disagree with the final classification by the authors; however, without access to the raw data for each individual article to allow for reanalysis and without the benefit of the authors' expertise in the specific assays, we have decided to consistently follow the authors' determination.

To harmonise scores across all articles, we use controlled vocabulary previously described.<sup>8 16</sup> We classified variants as having a 'functional impact' or 'no functional impact' and intermediate phenotypes with 'mild functional impact' or 'intermediate functional impact'. Although many papers report a

categorical scoring system (eg, impact vs no impact), others report a continuous scoring system (eg, as percentage of wild-type activity) with a defined cut-off value. However, most articles with continuous data did not have readily accessible raw data, but rather figures with histograms that are not amenable to data extraction and reanalysis. Thus, we decided to use a categorical (and not quantitative) scoring system for consistency. Functional impact has been annotated as follows: N, no functional impact; F, functional impact; I, intermediate functional impact; M, mild functional impact and U, undetermined functional impact on the BRCA1 protein. The 'intermediate' and 'mild functional impact categories' were created to accommodate cases where intermediate values were reported. For example, in the study by Morris *et al*,<sup>22</sup> BRCA1 Ubiquitin ligase activity, binding to E2 and BARD1 binding was tested. The phenotype for each of these functional studies was assigned as (+++), (++), (+), (-) or (-/+). In this case, (+++) was defined as 'no functional impact', (++) as 'mild functional impact', (+) as 'intermediate functional impact', (-) as 'functional impact' and (-/+) as undetermined. As described above, we were always guided by the authors' interpretation.

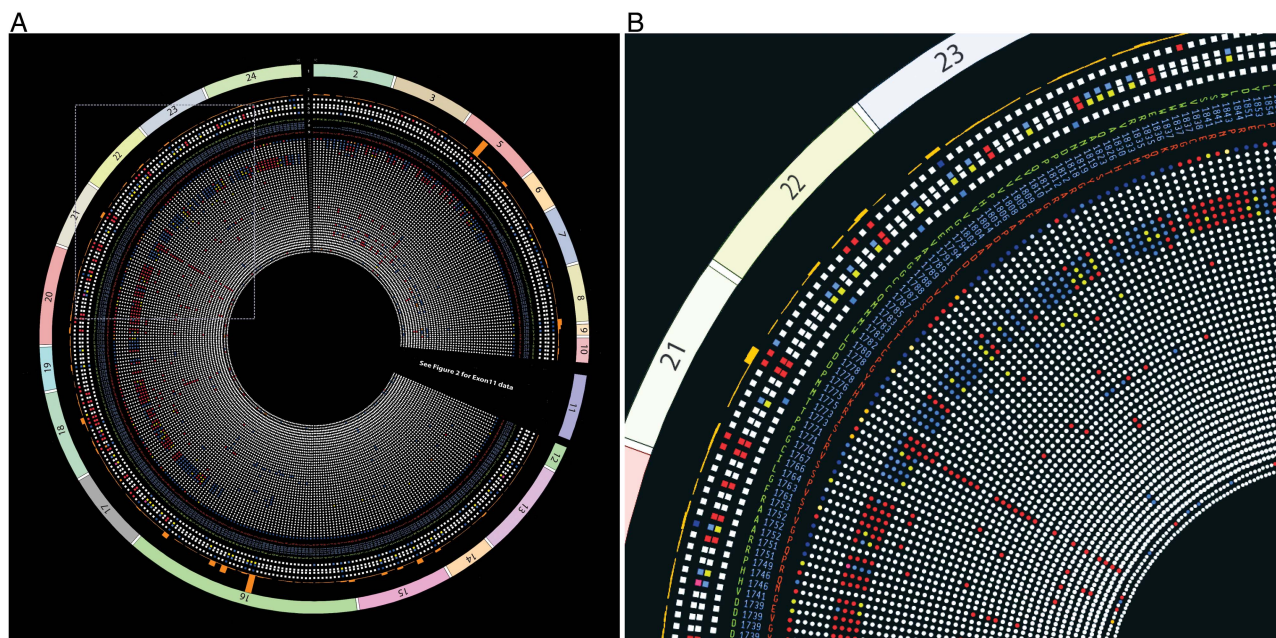
It is important to note that functional assays or predictions from protein sequences and structures can only assess a variant's impact on the protein function and not on pathogenicity, a clinical phenotype. Although there is a strong correlation between defects in protein function and pathogenicity, it is conceivable that not all variants that abrogate a certain function will lead to increased risk. The particular function being assayed may be unrelated to the protein tumour suppressor function. Conversely, a variant with no detectable impact on a specific function may have dramatic effect on a critical function of BRCA1 that is not the one being assessed.

### Circos plot

Circos (<http://circos.ca>) is a freely available web resource primarily used to represent genomic data.<sup>21</sup> Circos provides PERL-based modules that can be used as the basis to develop circular visualisation of data. We used these modules to install Circos and wrote our own script to represent the data present in our BRCA1 missense variant database. In Circos, each concentric ring is called a 'track'.

Figure 1 shows all the exons of the *BRCA1* gene in the outermost track (track 1). Tracks 2 and 3 represent the frequency of the variant in the BIC database and the IARC class, respectively. Tracks 4 and 5 depict results from the VarCall computational model, with track 4 representing the likelihood of being pathogenic and track 5 showing the associated Bayes' factor. Track 6 indicates whether the variant is present in the NHLBI Exome Sequencing Project.<sup>23</sup> A brief description for each track can be seen in table 1 and detailed descriptions and the key to interpreting them can be found in the online supplementary material 1 (<http://research.nhgri.nih.gov/bic/circos/trackdescriptions/>). Missense variants are shown in tracks 7–9 (reference amino acid: track 7, codon: track 8, variant amino acid: track 9). The remaining tracks show the functional impact on the variant as assessed by different functional assays (tracks 11–53). The functional assessment by each assay/prediction algorithm is colour coded as 'red' (functional impact), 'blue' (no functional impact), 'yellow' (mild/intermediate functional impact) or 'white' (no data available).

The script generates an image file that was used as the basis for the webpage. We generated two Circos figures: one with all variants in all coding exons (figure 2) and one removing data from exon 11 of BRCA1 (figure 1). This was done because a



**Figure 1** BRCA1 Circos excluding exon 11. (A) BRCA1 Circos with no data on display for exon 11 to facilitate data visualisation for the remaining exons (see [figure 2](#) for BRCA1 Circos including exon 11 data). All documented BRCA1 missense variants are represented in the Circos plot that contains 53 concentric tracks (left panel). (B) Magnified view of the region depicted in the inset (dashed lines) from (A). Refer to [table 1](#) for detailed descriptions of each track. BRCA1 Circos can be accessed via the website <http://research.nhgri.nih.gov/bic/circos/> with mouse-over and search functionalities.

large number of missense variants in exon 11 have been identified, but the functional data on these variants are sparse. Therefore, to better visualise the functional data for the other data-rich exons, we scaled the Circos plot to five times the radial size as compared with the figure ([figure 2](#)) where all exons are to scale.

### Interpreting BRCA1 Circos

The BRCA1 Circos plot should be read radially with track 1 being the outermost and track 53 being the innermost track. Each track is annotated by a track number and the corresponding data source ([table 1](#)). The data in each track are colour coded, and mouse-over on any data point on the figure on the website will allow the track number, track name and variant information to be displayed. As an example, we look at two variants: M1775K and M1775R ([figure 3](#)). The first track shows the exon; the variants lie in exon 21. Track 2 shows the BIC entries for each variant as a histogram (numerical values can be found in the functional database or can be seen using the mouse-over function on the website figure). In this case, there are no entries for M1775K but 31 entries in BIC for M1775R. Track 3 shows the IARC classification<sup>18</sup> for each variant and both variants are IARC class 5 represented as a red dot. Track 4 shows the computational classification of transcriptional assay data. The classification is performed based on the posterior probability that variant is protein damaging; both these variants have a value of 1, which is represented as a histogram. Track 5 represents the Bayes' factor and is calculated based on the transcriptional assay data; this is red for both the variants, indicating high probability of these variants being pathogenic.<sup>20</sup> Track 6 indicates whether the variants were found in the ESP Exome Viewer. Both variants are indicated as white because they were not found in the ESP Exome viewer. Tracks 7–9 show the variant M being the reference amino acid, 1775 being the amino acid position and R and K being the variant amino acids, respectively. Tracks 11–53 show the functional impact of the

variants using various functional assays and M1775K has lesser data than M1775R as shown by more white data points for M1775K. The majority of the functional assays show M1775R as having a functional impact on the protein. All the colour-coding information is shown in the database and in the track descriptions on the website for a consolidated viewing of all the information described here.

### Functional overlap

One of the goals of the BRCA1 Circos is to help in the meta-analysis of results from individual variants. For example, a variant 'A' that was tested multiple times and scored as of 'no functional impact' in every assay is considered to have stronger evidence against functional impact than a variant 'B' with only one assay reported in which it scored as no functional impact. But if all the assays performed for 'A' are functionally redundant (they assay the exact same function) and were performed in similar conditions, then the evidence against functional impact for 'A' is not stronger than the evidence for 'B'.

Data in tracks 11–53 represent results from several functional assays. Some tracks will represent data from the exact same assay reported in two or more separate papers. Others represent the same assays with differences in experimental conditions or design (eg, the transcriptional assay can be done in different cell types using the same constructs or in the same cell type but using constructs that span a different region of the protein). Finally, others can represent completely different experimental set up and assay a different function (eg, transcriptional activity in mammalian cells vs recombination in yeast). Thus, the user is cautioned to be aware of the functional overlap between assays when interpreting BRCA1 Circos data.

### Website development

Using the Circos program, we generated html image maps that represent the co-ordinates of every data point on the BRCA1



**Table 1** Track description for BRCA1 Circos

Track number	Description
Track 1	Exon number
Track 2	BIC entries (BIC; August 2012)— <a href="http://research.nhgri.nih.gov/bic/">http://research.nhgri.nih.gov/bic/</a>
Track 3	IARC class <sup>16</sup>
Track 4	Computational classification of transcriptional assay data <sup>20</sup>
Track 5	Bayes' factor <sup>20</sup>
Track 6	Exome viewer— <a href="http://evs.gs.washington.edu/EVS/">http://evs.gs.washington.edu/EVS/</a>
Track 7	Reference amino acid
Track 8	Codon
Track 9	Variant amino acid
Track 10	Align-GVGD ( <a href="http://brca.iarc.fr/PRIORS/BRCA1/index.php">http://brca.iarc.fr/PRIORS/BRCA1/index.php</a> )
Track 11	BARD binding <sup>22</sup>
Track 12	E2 binding <sup>22</sup>
Track 13	Ubiquitin ligase activity <sup>22</sup>
Track 14	Ubiquitin ligase activity <sup>24</sup>
Track 15	Bimolecular fluorescence complementation <sup>25</sup>
Track 16	Protease sensitivity <sup>26</sup>
Track 17	Phosphopeptide-binding activity <sup>26</sup>
Track 18	Phosphopeptide-binding specificity <sup>26</sup>
Track 19	Transcription assay <sup>26</sup>
Track 20	Transcription assay <sup>27</sup>
Track 21	Transcription assay <sup>17</sup>
Track 22	Transcription assay <sup>13</sup>
Track 23	Transcription assay <sup>28</sup>
Track 24	Transcription assay <sup>29</sup>
Track 25	Transcription assay <sup>30</sup>
Track 26	Transcription assay <sup>31</sup>
Track 27	Transcription assay <sup>32</sup>
Track 28	Transcription assay <sup>33</sup>
Track 29	Transcription assay <sup>34</sup>
Track 30	Transcription assay <sup>35</sup>
Track 31	Transcription assay <sup>36</sup>
Track 32	Transcription assay <sup>37</sup>
Track 33	Small colony phenotype <sup>38</sup>
Track 34	Small colony phenotype <sup>39</sup>
Track 35	Small colony phenotype <sup>40</sup>
Track 36	Small colony phenotype <sup>41</sup>
Track 37	Yeast localisation phenotype <sup>41</sup>
Track 38	ES viability <sup>42</sup>
Track 39	ES viability <sup>43</sup>
Track 40	Restoration of radiation resistance <sup>44</sup>
Track 41	Increase/restoration in radiation resistance <sup>24</sup>
Track 42	Homology-directed repair <sup>45</sup>
Track 43	Homology-directed repair <sup>46</sup>
Track 44	Homology-directed repair <sup>43</sup>
Track 45	Single-strand annealing <sup>46</sup>
Track 46	Centrosome number <sup>47</sup>
Track 47	Yeast intrachromosomal recombination <sup>40</sup>
Track 48	Yeast interchromosomal recombination <sup>40</sup>
Track 49	G2/M checkpoint proficiency <sup>24</sup>
Track 50	Subcellular localisation/formation of foci after IR <sup>48</sup>
Track 51	Subcellular localisation/cytoplasmic mislocalisation <sup>49</sup>
Track 52	Cisplatin response <sup>43</sup>
Track 53	PARP inhibitor sensitivity <sup>43</sup>

BIC, Breast Cancer Information Core; ES, Embryonic Stem Cell; GVGD, Grantham Variation-Grantham Deviation; IR, ionizing radiation; IARC, International Agency for Research on Cancer; PARP, Poly ADP Ribose Polymerase.

Circos image. The BRCA1 Circos website (<http://research.nhgri.nih.gov/bic/circos/>) also displays a searchable database with information on the functional assays and functional prediction algorithms for BRCA1 variants. The functionalities of the website are

- ▶ mouse-over 'magnifying glass' to zoom into any region of the Circos plot to visualise the data in that region;
- ▶ mouse-over on every data point on the tracks shows a label with 'track number, variant (amino acid change), track name and base change';
- ▶ track descriptions and key to interpret the functional data on every track;
- ▶ all tracks hyperlinked to the original article where the data were extracted from;
- ▶ double-clicking any part of the figure zooms into that region and allows the user to pan through the image;
- ▶ every data point in the Circos figure is hyperlinked to the information in the BRCA1 Missense variants functional database (<http://research.nhgri.nih.gov/bic/circos/allvariants/>).

All codes to develop the BRCA1 Circos and raw files are available on request. In the BRCA1 Circos, each track represents one set of functional assays or prediction algorithms that indicate the functional classification of a specific BRCA1 missense variant. Two papers reporting the same assay would be displayed in two separate tracks. Two separate assays reported in the same paper would also be displayed as two separate tracks.

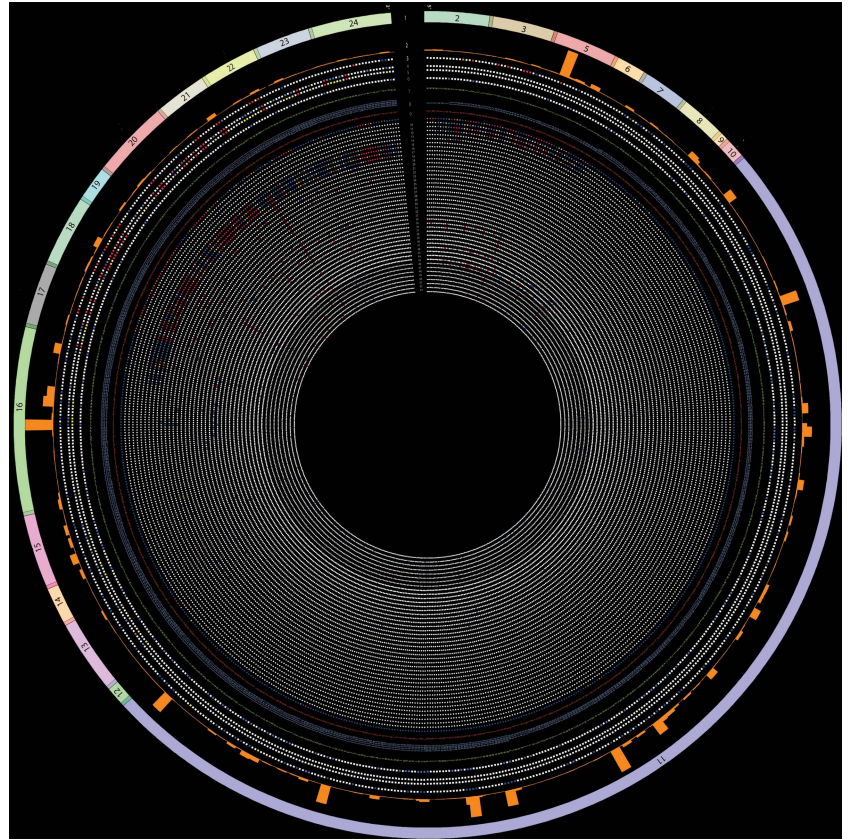
The current version of BRCA1 Circos depicts a specific functional assay per track. As more assays are published, the BRCA1 Circos can be updated by increasing the radius of a Circos plot which allows for more tracks to be incorporated; however, this may become unwieldy as the tool needs to fit in a standard computer screen. There are several options to solve to address this problem when it arises: (a) adding multiple BRCA1 Circos plots is a simple but the least preferable solution because we would lose the comprehensive view in one image; (b) taking advantage of our interactive interface (zooming in and mouse-over functions) and hide or show tracks (assays/studies) according to the user's choice and, finally, (c) tracks that have few variants (eg, <5) would be removed from the plot and the data would be available on the database but would not be depicted in the BRCA1 Circos.

## DISCUSSION

Mutations that disrupt BRCA1 lead to a substantial increase in risk of breast and ovarian cancers.<sup>2–5</sup> Thus, genetic testing for BRCA1 is critical to identify individuals at high risk and can be used to inform clinical decisions. For example, mutation carriers might consider prophylactic surgery or increased surveillance. Current testing for BRCA1 is primarily based on sequencing exons and exon-intron borders,<sup>7</sup> but other approaches that sequence non-coding regions such as introns and 5' and 3' untranslated region are starting to be used.<sup>50</sup>

Sequence-based methods can identify DNA variations that can be used to infer the outcome on the protein product. Thus, variants that cause premature or incorrect termination of the protein can, for the purposes of clinical management, be inferred to be pathogenic. However, DNA variants whose effect on the protein cannot be easily inferred from changes in the genetic code present an enormous clinical challenge.<sup>16</sup> These variants are usually called VUS and their number is bound to increase with wide spread sequencing of clinical samples. These

**Figure 2** BRCA1 Circos including exon 11. All documented BRCA1 missense variants are represented in the Circos plot that contains 53 concentric tracks. Refer to [table 1](#) for detailed descriptions of each track. BRCA1 Circos can be accessed via the website <http://research.nhgri.nih.gov/bic/circos/> with mouse-over and search functionalities.

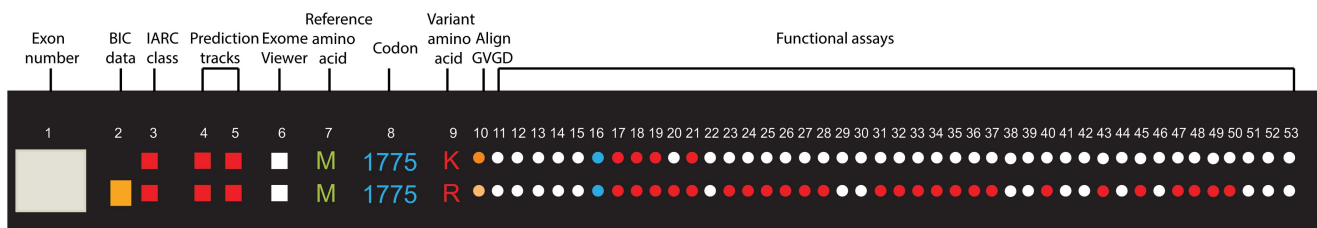


VUS are primarily missense variants that replace one amino acid for another and variants that may affect splicing of the transcript.

Due to the relative rarity of individual variants in the population, classical approaches such as family studies or case-control studies are difficult to conduct. Thus, in the past few years, several methods have been developed to interrogate the potential functional impact of a certain variant on the function of BRCA1.<sup>8</sup> These methods are not without their caveats and caution is warranted when interpreting their results because BRCA1 is a multifunctional protein but it is still unclear which functions contribute to its tumour-suppressive actions. Nevertheless, results from functional assay show a strong correlation with pathogenicity and provide an important source of information to help in the classification of VUS.<sup>26</sup> Recently, a computational model, called VarCall, was developed to convert results of functional assays into likelihood ratios of pathogenicity, allowing for functional data to be incorporated into multifactorial models of variant classification.<sup>20</sup> Thus, we believe that using functional information will improve and accelerate the classification of variants for BRCA1.

To facilitate the process of analysing functional data, we developed a database and a visualisation tool that can display all the available functional data for individual BRCA1 variants. This tool allows for the direct comparison of results from different functional tests and provides a global picture of the functional coverage in BRCA1. It also provides a resource in which non-specialists can navigate around different functional assays and weigh information from several sources. There have been multiple efforts to facilitate systematic integration of all the data available on these variants such as the BIC (<http://research.nhgri.nih.gov/bic/>) and the LOVD BRCA1/2 literature (<http://chromium.liacs.nl/LOVD2/cancer/home.php>) database. However, there is no comprehensive resource to help interpret and visualise data available on these variants in the current literature along with the information from multiple databases. We have developed a novel web-based resource for the interpretation and visualisation of BRCA1 missense variants.

BRCA1 Circos is the first interactive tool that combines data for all documented BRCA1 missense variants with an easy-to-interpret visualisation structure. It combines information from multiple functional assays and databases in one comprehensive layout. Besides the detailed information on every



**Figure 3** Zooming in on an individual variant. A sample section of the BRCA1 Circos representing two missense variants, M1775K and M1775R, is shown. Each variant should be read radially to get information on all the tracks for the each variant. Refer to [table 1](#) for detailed descriptions of each track. BIC, Breast Cancer Information Core; GVG, Grantham Variation-Grantham Deviation; IARC, International Agency for Research on Cancer.

variant, this tool is also useful to obtain a global view at the current data available for missense variants of BRCA1 to guide future efforts on VUS characterisation.

The data in the BRCA1 missense variant database and, consequently, BRCA1 Circos use controlled vocabulary as discussed by Lindor *et al*<sup>16</sup> classifying the variants as having a ‘functional impact’, ‘no functional impact’ or ‘mild/intermediate functional impact’. This makes it easier to consolidate data from multiple studies regardless of whether the results were quantitative or qualitative. These data have been colour coded and represented in an easy-to-use manner with many different features such as zooming into a particular exon, variant and obtaining information on multiple variants at a glance. We also provide the original articles for every track in the Circos and it is advisable to read the original publications to obtain any experimental data.

Several limitations apply to the data in the BRCA1 Circos. In particular, because most assays described here have not been validated extensively nor were conducted with specific standard operating procedures in a clinical environment, results should be taken with caution. In addition, individual-level data could suffer from clerical errors, swapped samples, mistakes in constructs and other problems that are inherent to research.<sup>13</sup> As the gold standard for VUS classification<sup>14–16</sup> requires that more than one independent source of data to classify a variant, functional data represented in the BRCA1 Circos should not, at the present stage, be considered as a single source for the purposes of clinical decisions. It is expected that as assays are validated and reported as likelihood ratios (eg, as in VarCall), the functional data will be incorporated in the multifactorial model. For rare variants for which genetic data may not be available, it is possible that properly validated assays could be used as a single source for clinical decisions but this approach needs to be further assessed in future studies.

In conclusion, we have developed a web-based tool to visualise complex data on BRCA1 variants using an open-source resource—Circos.<sup>21</sup> This study can be used as a platform for the development of a comprehensive functional database for other cancer susceptibility genes such as BRCA2 and can be used by researchers and genetic counsellors to better interpret the function of VUS.

#### Author affiliations

<sup>1</sup>Cancer Epidemiology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA

<sup>2</sup>University of South Florida Cancer Biology PhD Program, Tampa, Florida, USA

<sup>3</sup>Frederick National Laboratory for Cancer Research, National Cancer Institute, Frederick, Maryland, USA

<sup>4</sup>Instituto Federal de Educação, Ciência e Tecnologia, Rio de Janeiro, RJ, Brazil

<sup>5</sup>Instituto Nacional de Câncer, Divisão de Farmacologia, Rio de Janeiro, Brazil

<sup>6</sup>Genetics and Population Health Division, QIMR, BNE, Brisbane, Queensland, Australia

<sup>7</sup>Department of Human Genetics, Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands

<sup>8</sup>Service de Génétique, Institut Curie, Hôpital René Huguenin, Paris, France

<sup>9</sup>Institut Curie, Université Pierre et Marie Curie, Paris, France

<sup>10</sup>Fundación Pública Galega de Medicina Xenómica, Santiago, Spain

<sup>11</sup>Department of Biochemistry, University of Alberta, Alberta, Canada

<sup>12</sup>Instituto di Fisiologia Clinica, Consiglio Nazionale delle Ricerche, Pisa, Italy

<sup>13</sup>University of Southampton, Southampton, UK

<sup>14</sup>Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, the Netherlands

<sup>15</sup>Department of Medical Genetics, University Medical Center Utrecht, Utrecht, the Netherlands

<sup>16</sup>CIBERER, Madrid, Spain

<sup>17</sup>Department of Population Sciences, Beckman Research Institute of the City of Hope, Duarte, California, USA

<sup>18</sup>University of Miami Medical School, Miami, Florida, USA

<sup>19</sup>Department of Cancer Epidemiology and Genetics, Masaryk Memorial Cancer Institute, Brno, Czech Republic

<sup>20</sup>Salisbury District Hospital, Salisbury, Wiltshire, UK

<sup>21</sup>International Agency for Research on Cancer, Lyon, France

<sup>22</sup>Department of Laboratory Medicine and Pathology, and Health Sciences Research, Mayo Clinic, Rochester, Minnesota, USA

<sup>23</sup>Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, Utah, USA

<sup>24</sup>Genome Technology Branch, National Human Genome Research Institute, Bethesda, Maryland, USA

<sup>25</sup>Center for Cancer Research, National Cancer Institute, Frederick, Maryland, USA

**Acknowledgements** The authors would like to sincerely thank Tyra Wolfsberg and Suiyuan Zhang for helping with webpage development and all individuals and families who have generously donated their time, samples and information to facilitate research on the predisposition factors of breast and ovarian cancer.

**Collaborators** ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) Consortium.

**Contributors** AJ, AV and ANM conceived the project. AV, RSC, GAM, MV and SVT contributed to the development of the database. AJ designed and developed the Circos images. AJ, RCJ and BK designed and developed the web tool. PW, ABS, MGPV, SMC, GAM, AV, NC, AG, DE, MJB, TP, RBvdL, MSP, SLN, TD, EM, ST, MV, FJC, SVT, JNMG, MAC, LCB and SKS contributed to the curation of the database and the testing and refinement of the web tool. AJ, AV and ANM wrote the paper. All authors contributed to the discussion and overall data interpretation and approved the final manuscript.

**Funding** This work was funded by H. Lee Moffitt Cancer Center Foundation (Miles for Moffitt), and the US National Cancer Institute. The ENIGMA consortium is funded by a supplement to NIH RO1 award CA116167.

**Competing interests** None.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** All data will be available for public use and download at the website for the BRCA1 Circos (NHGRI).

**Open Access** This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

#### REFERENCES

- Claus EB, Risch N, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. *Am J Hum Genet* 1991;48:232–42.
- Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994;266:66–71.
- Tavtigian SV, Simard J, Rommens J, Couch F, Shattuck-Eidens D, Neuhausen S, Merajver S, Thorlacius S, Offit K, Stoppa-Lyonnet D, Belanger C, Bell R, Berry S, Bogden R, Chen Q, Davis T, Dumont M, Frye C, Hattier T, Jammulapati S, Janecki T, Jiang P, Kehrer R, Leblanc JF, Mitchell JT, McArthur-Morrison J, Nguyen K, Peng Y, Samson C, Schroeder M, Snyder SC, Steele L, Stringfellow M, Stroup C, Swedlund B, Swense J, Teng D, Thomas A, Tran T, Tranchant M, Weaver-Feldhaus J, Wong AK, Shizuya H, Eyfjord JE, Cannon-Albright L, Labrie F, Skolnick MH, Weber B, Kamb A, Goldgar DE. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. *Nat Genet* 1996;12:333–7.
- Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. Identification of the breast cancer susceptibility gene BRCA2. *Nature* 1995;378:789–92.
- Mavaddat N, Peock S, Frost D, Ellis S, Platte R, Fineberg E, Evans DG, Izatt L, Eeles RA, Adlard J, Davidson R, Eccles D, Cole T, Cook J, Brewer C, Tischkowitz M, Douglas F, Hodgson S, Walker L, Porteous ME, Morrison PJ, Side LE, Kennedy MJ, Houghton C, Donaldson A, Rogers MT, Dorkins H, Miedzybrodzka Z, Gregory H, Eason J, Barwell J, McCann E, Murray A, Antoniou AC, Easton DF, EMBRACE. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. *J Natl Cancer Inst* 2013;105:812–22.
- Easton DF, Bishop DT, Ford D, Crockford GP. Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. *Am J Hum Genet* 1993;52:678–701.
- Frank TS, Manley SA, Olopade OI, Cummings S, Garber JE, Bernhardt B, Antman K, Russo D, Wood ME, Mullineau L, Isaacs C, Peshkin B, Buys S, Venne V, Rowley PT, Loader S, Offit K, Robson M, Hampel H, Brenner D, Winer EP, Clark S, Weber B, Strong LC, Thomas A. Sequence analysis of BRCA1 and BRCA2: correlation of mutations with family history and ovarian cancer risk. *J Clin Oncol* 1998;16:2417–25.
- Millot GA, Carvalho MA, Caputo SM, Vreeswijk MP, Brown MA, Webb M, Rouleau E, Neuhausen SL, Hansen TV, Galli A, Brandão RD, Blok MJ, Velkova A, Couch FJ,



- Monteiro AN; ENIGMA Consortium Functional Assay Working Group. A guide for functional analysis of BRCA1 variants of uncertain significance. *Hum Mutat* 2012;33:1526–37.
- 9 Gronwald J, Huzarski T, Byrski T, Debniak T, Metcalfe K, Narod SA, Lubinski J. Direct-to-patient BRCA1 testing: the Twoj Styl experience. *Breast Cancer Res Treat* 2006;100:239–45.
- 10 Rowan E, Poll A, Narod SA. A prospective study of breast cancer risk in relatives of BRCA1/BRCA2 mutation carriers. *J Med Genet* 2007;44:e89; author reply e88.
- 11 Korde LA, Mueller CM, Loud JT, Struwing JP, Nichols K, Greene MH, Mai PL. No evidence of excess breast cancer risk among mutation-negative women from BRCA mutation-positive families. *Breast Cancer Res Treat* 2011;125:169–73.
- 12 Harvey SL, Milne RL, McLachlan SA, Friedlander ML, Birch KE, Weideman P, Goldgar D, Hopper JL, Phillips KA; kConFab Investigators. Prospective study of breast cancer risk for mutation negative women from BRCA1 or BRCA2 mutation positive families. *Breast Cancer Res Treat* 2011;130:1057–61.
- 13 Carvalho MA, Marsillac SM, Karchin R, Manoukian S, Grist S, Swaby RF, Urmenyi TP, Rondinelli E, Silva R, Gayol L, Baumbach L, Sutphen R, Pickard-Brzosowicz JL, Nathanson KL, Sali A, Goldgar D, Couch FJ, Radice P, Monteiro AN. Determination of cancer risk associated with germ line BRCA1 missense variants by functional analysis. *Cancer Res* 2007;67:1494–501.
- 14 Goldgar DE, Easton DF, Deffenbaugh AM, Monteiro AN, Tavtigian SV, Couch FJ; Breast Cancer Information Core (BIC) Steering Committee. Integrated evaluation of DNA sequence variants of uncertain clinical significance: application to BRCA1 and BRCA2. *Am J Hum Genet* 2004;75:535–44.
- 15 Easton DF, Deffenbaugh AM, Pruss D, Frye C, Wenstrup RJ, Allen-Brady K, Tavtigian SV, Monteiro AN, Iversen ES, Couch FJ, Goldgar DE. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. *Am J Hum Genet* 2007;81:873–83.
- 16 Lindor NM, Guidugli L, Wang X, Vallee MP, Monteiro AN, Tavtigian S, Goldgar DE, Couch FJ. A review of a multifactorial probability-based model for classification of BRCA1 and BRCA2 variants of uncertain significance (VUS). *Hum Mutat* 2012;33:8–21.
- 17 Tischkowitz M, Hamel N, Carvalho MA, Birrane G, Soni A, van Beers EH, Joosse SA, Wong N, Novak D, Quenneville LA, Grist SA, Nederlof PM, Goldgar DE, Tavtigian SV, Monteiro AN, Ladias JA, Foulkes WD; kConFab. Pathogenicity of the BRCA1 missense variant M1775K is determined by the disruption of the BRCT phosphopeptide-binding pocket: a multi-modal approach. *Eur J Hum Genet* 2008;16:820–32.
- 18 Plon SE, Eccles DM, Easton D, Foulkes WD, Genuardi M, Greenblatt MS, Hogervorst FB, Hoogerbrugge N, Spurdle AB, Tavtigian SV; IARC Unclassified Genetic Variants Working Group. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat* 2008;29:1282–91.
- 19 Vallée MP, Francy TC, Judkins MK, Babikyan D, Lesueur F, Gammon A, Goldgar DE, Couch FJ, Tavtigian SV. Classification of missense substitutions in the BRCA genes: a database dedicated to Ex-UVs. *Hum Mutat* 2012;33:22–8.
- 20 Iversen ES, Couch FJ, Goldgar DE, Tavtigian SV, Monteiro AN. A computational method to classify variants of uncertain significance using functional assay data with application to BRCA1. *Cancer Epidemiol Biomarkers Prev* 2011;20:1078–88.
- 21 Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. Circos: an information aesthetic for comparative genomics. *Genome Res* 2009;19:1639–45.
- 22 Morris JR, Pangon L, Boutell C, Katagiri T, Keep NH, Solomon E. Genetic analysis of BRCA1 ubiquitin ligase activity and its relationship to breast cancer susceptibility. *Hum Mol Genet* 2006;15:599–606.
- 23 Tennesen JA, Bigham AW, O'Connor TD, Fu W, Kenny EE, Gravel S, McGee S, Do R, Liu X, Jun G, Kang HM, Jordan D, Leal SM, Gabriel S, Rieder MJ, Abecasis G, Altshuler D, Nickerson DA, Boerwinkle E, Sunyaev S, Bustamante CD, Bamshad MJ, Akey JM, Broad GO, Seattle GO; NHLBI Exome Sequencing Project. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. *Science* 2012;337:64–9.
- 24 Ruffner H, Joazeiro CA, Hemmati D, Hunter T, Verma IM. Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proc Natl Acad Sci USA* 2001;98:5134–9.
- 25 Sarkar M, Magliery TJ. Re-engineering a split-GFP reassembly screen to examine RING-domain interactions between BARD1 and BRCA1 mutants observed in cancer patients. *Mol Biosyst* 2008;4:599–605.
- 26 Lee MS, Green R, Marsillac SM, Coquelle N, Williams RS, Yeung T, Foo D, Hau DD, Hui B, Monteiro AN, Glover JN. Comprehensive analysis of missense variations in the BRCT domain of BRCA1 by structural and functional assays. *Cancer Res* 2010;70:4880–90.
- 27 Carvalho M, Pino MA, Karchin R, Beddor J, Godinho-Netto M, Mesquita RD, Rodarte RS, Vaz DC, Monteiro VA, Manoukian S, Colombo M, Ripamonti CB, Rosenquist R, Suthers G, Borg A, Radice P, Grist SA, Monteiro AN, Billack B. Analysis of a set of missense, frameshift, and in-frame deletion variants of BRCA1. *Mutat Res* 2009;660:1–11.
- 28 Kaufman B, Laitman Y, Carvalho MA, Edelman L, Menachem TD, Zidan J, Monteiro AN, Friedman E. The P1812A and P25T BRCA1 and the 5164del4 BRCA2 mutations: occurrence in high-risk non-Ashkenazi Jews. *Genet Test* 2006;10:200–7.
- 29 Phelan CM, Dapic V, Tice B, Favis R, Kwan E, Barany F, Manoukian S, Radice P, van der Luijt RB, van Nesselrooij BP, Chenevix-Trench G, Caldes T, de la Hoya M, Lindquist S, Tavtigian SV, Goldgar D, Borg A, Narod SA, Monteiro AN; kConFab. Classification of BRCA1 missense variants of unknown clinical significance. *J Med Genet* 2005;42:138–46.
- 30 Mirkovic N, Marti-Renom MA, Weber BL, Sali A, Monteiro AN. Structure-based assessment of missense mutations in human BRCA1: implications for breast and ovarian cancer predisposition. *Cancer Res* 2004;64:3790–7.
- 31 Worley T, Vallon-Christersson J, Billack B, Borg A, Monteiro AN. A naturally occurring allele of BRCA1 coding for a temperature-sensitive mutant protein. *Cancer Biol Ther* 2002;1:497–501.
- 32 Vallon-Christersson J, Cayan C, Haraldsson K, Loman N, Bergthorsson JT, Brøndum-Nielsen K, Gerdes AM, Møller P, Kristofferson U, Olsson H, Borg A, Monteiro AN. Functional analysis of BRCA1 C-terminal missense mutations identified in breast and ovarian cancer families. *Hum Mol Genet* 2001;10:353–60.
- 33 Monteiro AN, August A, Hanafusa H. Evidence for a transcriptional activation function of BRCA1 C-terminal region. *Proc Natl Acad Sci USA* 1996;93:13595–9.
- 34 Lovelock PK, Healey S, Au W, Sum EY, Tesoriero A, Wong EM, Hinson S, Brinkworth R, Bekesy A, Diez O, Izatt L, Solomon E, Jenkins M, Renard H, Hopper J, Waring P, Tavtigian SV, Goldgar D, Lindeman GJ, Visvader JE, Couch FJ, Henderson BR, Southey M, Chenevix-Trench G, Spurdle AB, Brown MA, kConFab Investigators. Genetic, functional, and histopathological evaluation of two C-terminal BRCA1 missense variants. *J Med Genet* 2006;43:74–83.
- 35 Ostrow KL, McGuire V, Whittemore AS, DiCioccio RA. The effects of BRCA1 missense variants V1804D and M1628T on transcriptional activity. *Cancer Genet Cytogenet* 2004;153:177–80.
- 36 Quiles F, Fernandez-Rodriguez J, Mosca R, Feliubadalo L, Tornero E, Brunet J, Blanco I, Capella G, Pujana MA, Aloy P, Monteiro A, Lázaro C. Functional and structural analysis of C-terminal BRCA1 missense variants. *PLoS one* 2013;8:e61302.
- 37 Kawaku S, Sato R, Song H, Bando Y, Arinami T, Noguchi E. Functional analysis of BRCA1 missense variants of uncertain significance in Japanese breast cancer families. *J Hum Genet* 2013;58:618–21.
- 38 Coyne RS, McDonald HB, Edgemon K, Brody LC. Functional characterization of BRCA1 sequence variants using a yeast small colony phenotype assay. *Cancer Biol Ther* 2004;3:453–7.
- 39 Humphrey JS, Salim A, Erdos MR, Collins FS, Brody LC, Klausner RD. Human BRCA1 inhibits growth in yeast: potential use in diagnostic testing. *Proc Natl Acad Sci USA* 1997;94:5820–5.
- 40 Caligo MA, Bonatti F, Guidugli L, Aretini P, Galli A. A yeast recombination assay to characterize human BRCA1 missense variants of unknown pathological significance. *Hum Mutat* 2009;30:123–33.
- 41 Millot GA, Berger A, Lejour V, Boule JB, Bobo C, Cullin C, Lopes J, Stoppa-Lyonnet D, Nicolas A. Assessment of human Nter and Cter BRCA1 mutations using growth and localization assays in yeast. *Hum Mutat* 2011;32:1470–80.
- 42 Chang S, Biswas K, Martin BK, Stauffer S, Sharan SK. Expression of human BRCA1 variants in mouse ES cells allows functional analysis of BRCA1 mutations. *J Clin Invest* 2009;119:3160–71.
- 43 Bouwman P, van der Gulden H, van der Heijden I, Drost R, Klijn CN, Prasetyanti P, Pieterse M, Wientjens E, Seibler J, Hogervorst FB, Jonkers J. A high-throughput functional complementation assay for classification of BRCA1 missense variants. *Cancer Discov* 2013;3:1142–55.
- 44 Scully R, Ganesan S, Vlasakova K, Chen J, Socolovsky M, Livingston DM. Genetic analysis of BRCA1 function in a defined tumor cell line. *Mol Cell* 1999;4:1093–9.
- 45 Ransburgh DJ, Chiba N, Ishioka C, Toland AE, Parvin JD. Identification of breast tumor mutations in BRCA1 that abolish its function in homologous DNA recombination. *Cancer Res* 2010;70:988–95.
- 46 Towler WI, Zhang J, Ransburgh DJ, Toland AE, Ishioka C, Chiba N, Parvin JD. Analysis of BRCA1 variants in double-strand break repair by homologous recombination and single-strand annealing. *Hum Mutat* 2013;34:439–45.
- 47 Kais Z, Chiba N, Ishioka C, Parvin JD. Functional differences among BRCA1 missense mutations in the control of centrosome duplication. *Oncogene* 2012;31:799–804.
- 48 Au WW, Henderson BR. The BRCA1 RING and BRCT domains cooperate in targeting BRCA1 to ionizing radiation-induced nuclear foci. *J Biol Chem* 2005;280:6993–7001.
- 49 Rodriguez JA, Au WW, Henderson BR. Cytoplasmic mislocalization of BRCA1 caused by cancer-associated mutations in the BRCT domain. *Exp Cell Res* 2004;293:14–21.
- 50 Walsh T, Lee MK, Casadei S, Thornton AM, Stray SM, Pennil C, Nord AS, Mandell JB, Swisher EM, King MC. Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. *Proc Natl Acad Sci USA* 2010;107:12629–33.