



Classification of 101 BRCA1 and BRCA2 variants of uncertain significance by cosegregation study: A powerful approach

Sandrine Caputo, Lisa Golmard, Mélanie Léone, Francesca Damiola, Marine Guillaud-Bataille, Françoise Revillion, Etienne Rouleau, Nicolas Derive, Adrien Buisson, Noémie Basset, et al.

► To cite this version:

Sandrine Caputo, Lisa Golmard, Mélanie Léone, Francesca Damiola, Marine Guillaud-Bataille, et al.. Classification of 101 BRCA1 and BRCA2 variants of uncertain significance by cosegregation study: A powerful approach. American Journal of Human Genetics, 2021, 108 (10), pp.1907-1923. 10.1016/j.ajhg.2021.09.003 . hal-03375857

HAL Id: hal-03375857

<https://hal.univ-reims.fr/hal-03375857>

Submitted on 16 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Classification of 101 *BRCA1* and *BRCA2* variants of uncertain significance by cosegregation study: a powerful approach

Sandrine M Caputo^{1, 2, \$, @}, Lisa Golmard^{1, 2, \$}, Mélanie Léone^{3, *, \$}, Francesca Damiola^{4, *, \$}, Marine Guillaud-Bataille^{5, \$}, Françoise Revillion^{6, \$}, Etienne Rouleau^{5, \$}, Nicolas Derive^{1, 2, \$}, Adrien Buisson^{3, \$}, Noémie Basset^{7, \$}, Mathias Schwartz^{1, 2, \$}, Paul Vilquin^{8, \$}, Celine Garrec^{9, \$}, Maud Privat^{10, \$}, Mathilde Gay-Bellile^{10, 11, \$}, Caroline Abadie¹², Khadija Abidallah^{1, 2}, Fabrice Airaud⁹, Anne-Sophie Allary¹³, Emmanuelle Barouk-Simonet¹⁴, Muriel Belotti^{1, 15}, Charlotte Benigni¹⁶, Patrick R. Benusiglio¹⁷, Christelle Berthemin^{1, 2}, Pascaline Berthet¹⁸, Ophelie Bertrand¹⁹, Stéphane Bézieau⁹, Marie Bidart^{20, 21}, Yves-Jean Bignon²², Anne-Marie Birot²³, Maud Blanluet^{1, 2}, Amelie Bloucard^{1, 2}, Johny Bombled⁵, Valerie Bonadona²⁴, Françoise Bonnet¹⁴, Marie-Noëlle Bonnet-Dupeyron²⁵, Manon Boulaire⁶, Flavie Boulouard²⁶, Ahmed Bouras³, Violaine Bourdon¹³, Afane Brahimi²⁷, Fanny Brayotel²⁸, Brigitte Bressac de Paillerets⁵, Noémie Bronnec²⁹, Virginie Bubien¹⁴, Bruno Buecher¹, Odile Cabaret⁵, Jennifer Carriere^{1, 2}, Jean Chiesa³⁰, Stephanie Chieze-Valéro³¹, Camille Cohen³², Odile Cohen-Haguenaue³², Chrystelle Colas^{1, 2}, Marie-Agnès Collonge-Rame³³, Anne-Laure Conoy⁶, Florence Coulet⁷, Isabelle Coupier⁸, Louise Crivelli³⁴, Véronica Cusin⁷, Antoine De Pauw^{1, 2}, Catherine Dehainault^{1, 2}, Hélène Delhomelle, MsC¹⁹, Capucine Delnatte^{9, 35}, Sophie Demontety¹, Philippe Denizeau³⁶, Pierre Devulder¹⁸, Helene Dreyfus³⁷, Catherine Dubois d'Enghein^{1, 2}, Anaïs Dupré¹⁴, Anne Durlach³⁸, Sophie Dussart³⁹, Anne Fajac⁴⁰, Samira Fekairi¹³, Sandra Fert-Ferrer⁴¹, Alice Fiévet⁵, Robin Fouillet²⁶, Emmanuelle Mouret-Fourme¹⁹, Marion Gauthier-Villars¹, Paul Gesta³¹, Sophie Giraud⁴², Laurence Gladieff, MD⁴³, Veronica Goldbarg⁴⁴, Vincent Goussot⁴⁵, Virginie Guibert⁹, Erell Guillerme⁷, Christophe Guy¹, Agnès Hardouin²⁶, Céline Heude¹⁸, Claude Houdayer^{1, 2}, Olivier Ingster⁴⁶, Caroline Jacquot-Sawka⁴⁷, Natalie Jones¹⁴, Sophie Krieger^{26, 48}, Sofiane Lacoste¹, Hakima Lallaoui⁴⁹, Helene Larbre²⁸, Anthony Laugé¹, Gabrielle Le Guyadec²⁰, Marine Le Mentec¹, Caroline Lecerf¹, Jessica Le Gall^{1, 2}, Bérengère Legendre⁶, Clémentine Legrand⁵⁰, Angéline Legros²⁶, Sophie Lejeune²⁷, Rosette Lidereau¹, Norbert Lignon⁴³, Jean-Marc Limacher⁵¹, Doriane Livon⁵², Sarab Lizard⁵³, Michel Longy¹⁴, Alain Lortholary⁵⁴, Pierre Macquere¹⁴, Audrey Mailliez⁶, Sarah Malsa⁵⁵, Henri Margot¹⁴, Véronique Mari⁵⁶, Christine Maugard⁵⁷, Cindy Meira⁶, Julie Menjard⁵⁸, Diane Molière²³, Virginie Moncoutier^{1, 2}, Jessica Moretta-Serra⁵², Etienne Muller^{26, 48}, Zoe Nevrière¹⁸, Thien-vu Nguyen Minh Tuan^{1, 2, \$}, Tetsuro Noguchi¹³, Catherine Noguès⁵², Florine Oca⁶, Cornel Popovici¹³, Fabienne Prieur⁵⁹, Sabine Raad¹⁴, Jean-Marc Rey⁸, Agathe Ricou^{26, 48}, Lucie Salle³¹, Claire Saule¹⁹, Nicolas Sevenet¹⁴, Fatoumata Simaga¹, Hagay Sobol¹³, Voreak Suybeng¹, Isabelle Tennevet^{60, 61}, Henrique Tenreiro¹, Julie Tinat²⁹, Christine Toulas⁶², Isabelle Turbiez¹⁵, Nancy Uhrhammer^{10, 11}, Pierre Vande Perre⁶², Dominique Vaur^{26, 48}, Laurence Venat⁶³, Nicolas Viellard¹, Marie-Charlotte Villy¹, Mathilde Warcoin¹, Alice Yvard⁵⁴, Helene Zattara⁶, Olivier Caron^{44, \$}, Christine Lasset^{24, \$}, Audrey Remenieras^{13, *, \$}, Nadia Boutry-Kryza^{3, *, \$}, Laurent Castéra^{26, 48, *, \$}, Dominique Stoppa-Lyonnet^{1, 64, 65}

1 Department of Genetics, Institut Curie, Paris, 75005, France

2 Paris Sciences Lettres Research University, Paris, 75005, France

3 Service de Génétique, plate-forme mixte des cancers fréquents, Hospices Civils de Lyon, Bron, 69229, France

4 Centre Léon Bérard, Lyon, 69373, France

5 Service de Génétique des Tumeurs, Gustave Roussy, Villejuif, F-94806, France

6 Department of Genetics, Centre Oscar Lambret, Lille, 59000, France

7 Département de génétique, AP-HP, Sorbonne université-Site Pitié-Salpêtrière, Paris, 75013, France

8 Département Pathologie et Oncobiologie, CHU Montpellier, Montpellier, 34070, France

9 Laboratoire de Génétique Moléculaire, Service de Génétique Médicale, Centre Hospitalier Universitaire (CHU) de Nantes, Nantes, 44093, France

10 Département d'Oncogénétique, Centre Jean Perrin, Clermont-Ferrand, 63000, France

11 INSERM U1240, Université Clermont Auvergne, Clermont-Ferrand, 63000, France

12 Unité d'Oncogénétique, Institut de Cancérologie de l'Ouest, Centre René Gauducheau, St Herbain-Nantes, 44805, France

13 Department of Cancer Biology, Laboratory of Molecular Oncogenetics, Institut Paoli Calmettes, Marseille, 13273, France
14 Unité d'Oncogénétique, Institut Bergonié, Bordeaux, 33076, France
15 Clinical research direction, Institut Curie, Paris, 75005, France
16 Hôpital d'Enfants, CHRU Nancy Hopitaux de Brabois, Vandoeuvre les Nancy, 54500, France
17 UF d'Oncogénétique, GH Pitié-Salpêtrière, AP-HP .Sorbonne Université, Paris, 75013, France
18 Oncogenetic, Department of Biopathology, Comprehensive Cancer Center François Baclesse, Caen, 14076, France
19 Department of Genetics, Institut Curie, Saint-Cloud, 92210, France
20 Laboratoire de Génétique Moléculaire : Maladies Héritaires et Oncologie, CHU Grenoble Alpes, Grenoble, 38700, France
21 INSERM 1209, Université Grenoble Alpes, Grenoble, 38700, France
22 Department of Oncogenetics, Centre Jean Perrin, UMR INSERM 1240, Université Clermont Auvergne, Clermont-Ferrand cedex, 63011, France
23 Service de Génétique, Hôpital Européen Georges Pompidou, Paris, 75015, France
24 Département Prévention Santé Publique, Centre Léon Bérard, Lyon, 69008, France
25 Consultations de Génétique, Centre Hospitalier de Valence, Valence, 26953, France
26 Laboratory of Cancer Biology and Genetics, Comprehensive Cancer Center François Baclesse, Caen, 14076, France
27 Department of Genetics, CHU Lille, Lille, 59000, France
28 Oncogenetics Laboratory, Institut Godinot, Reims, 51726, France
29 Génétique Médicale, CHU Bordeaux, Bordeaux, 33076, France
30 Department of Genetics, Centre Hospitalier Régional Universitaire, Nîmes, 30029, France
31 Service Oncogénétique Régional Poitou-Charentes, CH Georges Renon, Niort, 79000, France
32 Service de Génétique, CHI de Poissy-Saint Germain En Laye, Poissy, 78300, France
33 Oncobiologie Génétique Bioinformatique, CHRU Besançon, Besançon, 25030, France
34 Centre Eugène Marquis, Rennes, France
35 Unité d'Oncogénétique, Institut de Cancérologie de l'Ouest, Centre René Gauducheau, St Herbain-Nantes, 44805, France
36 Centre Hospitalo-Universitaire Rennes, Rennes, 35000, France
37 Institut Sainte Catherine, Avignon, 84082, France.
38 Consultation oncogénétique, CHU Reims, Reims, 51092, France
39 Département Prévention Santé Publique, Centre Léon Bérard, Lyon, 69008, France
40 Service d'Anatomie et Cytologie Pathologiques, Hôpital Tenon, AP-HP .Sorbonne Université, Paris, 75020, France
41 UF Consultation de génétique, Centre Hospitalier Métropole Savoie, Chambéry, 73011, France
42 Hospices Civils de Lyon, Lyon, 69229, France
43 Oncogenetics Department, Institut Claudius Regaud, Toulouse, 31059, France
44 Gustave Roussy, Département de médecine oncologique, Villejuif, F-94805, France
45 Département de Biologie et Pathologie des Tumeurs, Centre Georges François Leclerc, Dijon, 21000, France
46 Département de Génétique Médicale/oncogénétique, CHU ANGERS, Angers, 49000, France
47 Centre de Génétique, CHU de DIJON (hopital François Mitterrand) and CLCC de DIJON (Centre Georges François Leclerc), DIJON, 21000, France
48 Inserm U1245, Rouen University, Normandy Centre for Genomic and Personalized Medicine, Rouen, 76031, France
49 CH La rochelle, La Rochelle, 17000, France
50 Service de Génétique, CHU Grenoble Alpes, Grenoble, 38700, France
51 Génétique Oncologique Clinique, Hospices Civils de Colmar, COLMAR, 68000, France
52 Department of Clinical Cancer Genetics, Institut Paoli-Calmettes, Marseille, 13273, France
53 Department of Genetics, CHRU Nancy Hopitaux de Brabois, Vandoeuvre les Nancy, 54500, France
54 Department of Genetics, Hôpital Privé du Confluent, Nantes, 44202, France
55 Department of Genetics, CHU de Martinique , Fort de France, 97200, Martinique
56 Service oncogénétique, Centre Antoine Lacassagne, Nice, 06189, France
57 Génétique Oncologique Moléculaire, Hôpital de Hautepierre, Strasbourg, 67000, France
58 Department of Genetics, CHU BREST, Brest, 29600, France
59 Service de Génétique, CHU St Etienne, SAINT ETIENNE, 42055, France
60 Department of Genetics, Rouen University Hospital, Normandy Centre for Genomic and Personalized Medicine, Rouen, 76031, France.
61 Department of Oncology, Comprehensive Cancer Centre Henri Becquerel, Normandy Centre for Genomic and Personalized Medicine, Rouen, 76031, France
62 Oncogenetics Laboratory, Institut Claudius Regaud, Toulouse, 31059, France
63 Department of oncology, CHU Dupuytren, Limoges, 87042, France
64 Paris University, Paris, 75005, France
65 INSERM U830, Institut Curie, Paris, 75005, France

*: contributed equally to this work

\$: The variant classification working group

@: Corresponding author: Sandrine M. Caputo, Department of Genetics, Institut Curie, 26 rue d'Ulm, 75005 Paris, France, sandrine.caputo@curie.fr, Phone +33 (0)172389367

Abstract

Up to 80% of *BRCA1* and *BRCA2* genetic variants remain of uncertain clinical significance (VUS). Only variants classified as pathogenic or likely pathogenic can guide breast and ovarian cancer prevention measures and treatment by PARP-inhibitors. We report the first results of the ongoing French national COVAR (COsegregation VARiant) study the aim of which is to classify *BRCA1/2* VUS. The classification method was a multifactorial model combining different associations between VUS and cancer, including cosegregation data. At this time, among the 653 variants selected, 101 (15%) distinct variants shared by 1,624 families were classified as pathogenic/likely pathogenic or benign/likely benign by the COVAR study. Sixty-six of the 101 (65%) variants classified by COVAR would have remained VUS without cosegregation data. Of note, among the 34 variants classified as pathogenic by COVAR, 16 remained VUS or likely pathogenic when following the ACMG/AMP variant classification guidelines. Although the initiation and organization of cosegregation analyzes require a considerable effort, the growing number of available genetic tests results in an increasing number of families sharing a particular variant, increases their power. Here we demonstrate that variant cosegregation analyses are a powerful tool for the classification of variants in the *BRCA1/2* breast-ovarian cancer predisposition genes.

Introduction

Identification of the *BRCA1* (OMIM 113705) and *BRCA2* (OMIM 600185) genes 25 years ago opened up a new field in cancer genetics and *BRCA1/2* testing is now a paradigm in predictive medicine. Pathogenic germline *BRCA1/BRCA2* monoallelic variants are associated with a high risk of breast and ovarian cancer¹. They are classified as pathogenic when they have a putative loss of function effect, usually when a stop codon is introduced by nonsense substitutions, frameshift insertions/deletions (indels), complete splice defects or large gene rearrangements (LGR)²⁻⁶. However, many numerous identified variants, such as missense, synonymous, or variants surrounding coding exons even at canonical splicing positions, are difficult to classify without complementary analyzes and, in many cases, remain unclassified⁷⁻¹¹. An international effort to classify *BRCA1/2* variants was initiated in 2009 through “The Evidence-based Network for the Interpretation of Germline Mutant Alleles” (ENIGMA) and has recently been extended to other breast cancer predisposition genes¹².

In view of the increasing genetic testing capacity due to the use of next generation sequencing (NGS) and with the advent of PARP-inhibitors (PARPi) for the treatment of patients affected with a tumor driven by *BRCA1/BRCA2* inactivation, the number of patients tested and the number of variants detected are continuously increasing^{13,14}. In April 2021, about 40,000 and 25,000 *BRCA1/BRCA2* different germline variants have been reported worldwide in the BRCAexchange and ClinVar databases, respectively, of which nearly 80% and 50% are unclassified in term of their pathogenic effect¹⁵ (Table S1). However, due to the individual impacts of PARPi prescriptions and prophylactic decisions such as mastectomy and oophorectomy, a reliable classification of variants as either pathogenic or non-pathogenic is of utmost importance. In a survey of 3,672 women who underwent *BRCA1/BRCA2* testing in 2014-2015, Kurian *et al.* reported that 51% of women carrying a Variant of Uncertain Significance (VUS) underwent bilateral prophylactic mastectomy even in the absence of

family history of cancer¹⁶. In March 2016, the journalist Jeremy Lange reported in The New York Times the case of a woman whose doctors had conflicting information on her *BRCA1/BRCA2* test result. “The situation is ripe for overinterpretation and misinterpretation,” said a geneticist interviewed.

The large size of the *BRCA1* and *BRCA2* proteins, the complexity of their functions and the lack of reliable surrogate markers of their activity, such as pathological immunohistochemistry or a comprehensive functional assay, have led to the VUS classification approach based on a multifactorial model, which has been validated for *BRCA1/2* variant classification in previous studies^{11,17,18}. For each VUS, this model comprises a prior probability (PriorP) of pathogenicity based on Align-GVGD prediction score and combined likelihoods of pathogenicity derived from measurements of associations between VUS and cancer: personal and family cancer history, breast tumor pathology, co-occurrence of VUS with a known pathogenic variant of the same gene, and cosegregation data^{11,17,19–21}. Plon *et al.* proposed a classification of cancer predisposition gene variants based on 5 levels of likelihood of pathogenicity, known as the IARC-5-tier classes: (1) benign (BV), (2) likely benign (LBV), (3) of uncertain significance, (4) likely pathogenic (LPV), and (5) pathogenic (PV)²².

The COVAR (COsegregation VARIant) study has been set up within the French Unicancer Genetics Group (UGG) which brings together cancer genetics clinics and laboratories. Here, we report the first results of the COVAR study showing the value of cosegregation analyzes for classification of *BRCA1/BRCA2* variants. In addition, by comparing the COVAR results to the variant scoring obtained by following the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines, we demonstrate the power of the COVAR approach.

Subjects and methods

UGG cancer genetics clinic and laboratory network, FrOG (French OncoGenetics) Database

The French UGG was established in 1991 to contribute to the identification of cancer predisposing genes and estimation of cancer risks based on national (GENEPSO, GEMO, GENESIS) and international (IBCCS, CIMBA) genetic epidemiological studies and to define recommendations for genetic testing and management of individuals at risk^{1,2,5,23–29}. In 2020, the UGG consists of a network of 148 public/private cancer genetics clinics throughout France and 26 academic molecular genetics laboratories, 17 of which routinely perform breast-ovarian cancer genetic testing.

Between 1994 and 2018, about 260,000 index cases (ICs) were sequenced for *BRCA1/BRCA2* in the UGG cancer genetics clinic and laboratory network. The sequencing was performed by specific gene analyzes until 2017 and by multigene panel thereafter^{26,30}. ICs correspond to the first case to be tested in their families and are mostly women with a personal history of breast and/or ovarian cancer. Sequencing technologies evolved during this period, but genetic testing always included the search for substitutions and indels on all *BRCA1/2* coding exons and exon/intron boundaries, as well as the search for LGR. All identified variants (except for common polymorphisms) were reported in a dedicated *BRCA1/BRCA2* database initially called UMD-*BRCA1/BRCA2* (⁵, BRCAShare^{TM31}) and now called FrOG (French OncoGenetics) database. This database is a helpful everyday tool for molecular geneticists and an essential resource for the classification of variants. Patients are pseudonymously registered in the database by means of a family-ID assigned by the submitting laboratory⁵. Patients can therefore be mapped back through their family-ID for variant classification updates and research study invitations.

A working group composed of the UGG molecular geneticists is in charge of the classification of variants. The curation is under the responsibility of S.M.Caputo. Systematic classification criteria have been introduced in the framework of the IARC-5-classes of

pathogenicity²². The principles of classification are reported in Figure-1 and in Bérout *et al.*³¹.

In April 2021, of the 3,389 distinct *BRCA1* variants reported, 1,112 variants were classified as LPV/PV (class 4/5), 583 as BV/LBV (class 1/2) and 1,694 variants as class 3 (VUS), this last class representing 50% of all variants (Figure-2). A similar class distribution was observed for *BRCA2*: within 5,081 distinct *BRCA2* variants reported, 3,062 were classified as class 3 (60%).

The COVAR study and variant selection

The COVAR study was initiated in order to increase the proportion of classified variants by using cosegregation data within families. The study was designed to focus on sampling available relatives of the ICs and testing whether or not they carried the variant found in the IC with reference to their cancer phenotype. The model used here includes the absence/presence and age at diagnosis of *BRCA* associated cancers (breast, ovarian, pancreas for *BRCA1* and *BRCA2*, and prostate for *BRCA2* only)^{1,32-34}. The model compares the probability of observing the pedigree phenotypes and variant genotypes under the hypothesis that the variant is pathogenic with that under the hypothesis that the variant is benign with respect to risk^{17,19}. If this likelihood ratio (LR) is greater than 1, this indicates evidence in favor of a pathogenic effect of the variant and, conversely, if it is less than 1 this provides evidence that the variant is benign. For a given variant, combined cosegregation likelihood of pathogenicity is calculated by multiplying the LR obtained from families sharing the same variant (or different variants when the same impact at the mRNA and/or protein level has been demonstrated for each variant).

Variant selection is discussed by the variant classification working group according to the expected classification power. It is based on the number of families carrying the same variant, the number of potential participants among the relatives, and the level of PriorP, as a lower

level of evidence is required to reach a final classification of PV or LPV when PriorP is high (Figure-1). A variant may be selected in the COVAR study when it has been identified in at least three families, whatever its PriorP. A minimum PriorP of 0.66 is sufficient to select a variant, even if it has been identified in only one family; the threshold of 0.66 corresponds to the Align-GVGD C35 to C55 prediction classes. LPV are rare and systematically selected. Most selected variants are VUS. Splice variants with equivocal effect are also selected, *e.g.* the deep intronic variant *BRCA2*:c.6937+594T>G^{35,36}. Variant selection is an ongoing process; thus, a variant not initially selected may be subsequently selected based on identification of new families carrying the same variant.

Ethics Declaration

The COVAR study was authorized by Ethics Committee in 2011 (Comite de protection des personnes Ile de France III, Ref: Am5677-1-2940) and is registered in the international clinical trials platform (<https://clinicaltrials.gov/ct2/show/NCT01689584>). Informed consent for genetic testing was obtained from all individuals undergoing testing.

Patient inclusion and study participation

ICs carrying a variant selected in the COVAR study are invited to participate in the study by medical geneticists during the test result disclosure consultation, or later if the variant has been selected subsequently. Inclusion of relatives is prioritized as follows: individuals with a personal history of breast and/or ovarian cancer, older cancer-free women/males when they can be useful to infer genotypes. Potential participants receive invitation letters from the cancer genetics clinic that manage them. Relatives agreeing to participate in the study receive full information about the study by mail and can receive oral information from a genetic counselor upon request. After providing their written consent, they receive a salivary kit for DNA testing (Oragene®, DNA Genotek) and a short clinical questionnaire that includes sex, cancer status (site and age at diagnosis), age at interview for unaffected relatives and age at

possible prophylactic mastectomy and oophorectomy. They provide their permission to access medical files in order to retrieve pathology reports. Breast cancer details are collected³⁷. For relatives not included in the study and whose cancer status is useful for cosegregation analysis, efforts are made to obtain cancer validation by pathology report, medical record or *bona fide* clinical history. When a variant has been classified as BV or LBV, the ICs concerned are informed by mail and are invited to inform their relatives; when classified as LPV or PV, all study participants, regardless of individual test results, are invited to attend a cancer genetics clinic to perform diagnostic testing on a second sample.

Statistical model

Cosegregation LR is assessed using the statistical model developed by Thompson *et al.*¹⁹ and implemented in the COOL-webserver based on an improved penetrance model, developed by Belman *et al.*³⁸. In addition to the cosegregation LRs derived in this way, the multifactorial LR for each family includes LR co-occurrence¹⁷, personal and family cancer history^{39,40} and breast pathology LRs³⁷ as previously published¹¹. All these LR are multiplied to obtain the “combined-LR”. As previously mentioned by Goldgar *et al.*, since analysis of cosegregation is conditional on the phenotypes in the family, the data on cosegregation can be considered independent of the data on family history¹⁷. Combined LRs of families sharing the same variant are multiplied. The PriorP of each variant is based on the Align-GVGD prediction class score determined by phylogenetic conservation of the modified amino-acid, physicochemical change score for missense variants and the MaxEntScan splicing site prediction tool for suspected splicing defects²¹. PriorP and LRs are combined to calculate posterior probabilities using a Bayesian formula^{11,17,18}:

$$\text{Combined-LRs} = \text{LR}_{\text{coseg}} \times \text{LR}_{\text{co-occurrence}} \times \text{LR}_{\text{Fam-Hist}} \times \text{LR}_{\text{Pathology}}$$

$$\text{Posterior Odds} = \text{Combined-LRs} \times [\text{Prior Probability}/(1 - \text{Prior Probability})].$$

$$\text{Posterior Probability of pathogenicity} = \text{Posterior Odds}/(\text{Posterior Odds}+1).$$

For in-frame indels located in the same functional domain, the highest Align-GVGD PriorP is chosen¹⁸. Combined-LR (or LR-causality) thresholds used are those defined by Goldgar *et al.*: >1000:1 considered to be pathogenic (class 5) and <1:100 considered to be benign (class 1)¹⁷. It means that the Posterior Probability of pathogenicity is calculated only when the combined-LR is greater than 1000:1 or lower than 1:100. Variant classification is based on the Posterior Probability of pathogenicity, with values <0.001, between 0.001 and 0.049, between 0.05 and 0.99, or >0.99 corresponding to BV, LBV, LPV or PV, respectively²².

Results

Variant selection and patient inclusion

From December 2011 to April 2021, 645 VUS (214 *BRCA1* and 431 *BRCA2*) and 8 LPV variants (4 *BRCA1* and 4 *BRCA2*) were selected for the COVAR study (Figure-3A). These 653 variants included 518 missense, 53 intronic, 34 in-frame deletions/insertions, 22 synonymous, 14 nonsense or frameshift indels and 12 in-frame LGR.

By April 2021, 876 ICs carrying a selected variant (283 *BRCA1* and 593 *BRCA2*) and 1,525 relatives had been included (Figure-3A). Among the 876 participating ICs, no relative was included for 273 ICs, one relative was included for 205 ICs and more than one relative was included for 398 ICs (Figure-3B).

Variant classification

At the time of the study report, COVAR allowed the classification into clinically useful categories of 101 variants (15% of the selected variants) shared by 1,624 families registered in the UGG clinics network. Thirty-four variants (17 *BRCA1*, 17 *BRCA2*, 34%) were classified as PV; 4 *BRCA1* variants (4%) as LPV, and 63 variants (25 *BRCA1*, 38 *BRCA2*, 62%) were classified as BV or LBV (Tables 1, S2, S3, S4, S5). Table 1 reports LRs calculated for each component of each variant and its class with or without including cosegregation data. Of note, 29 of the 38 (76%) COVAR-classified PV/LPV and 37 of the 63 (59%) COVAR-classified

BV/LBV, overall 66 of the 101 (65%) COVAR-classified variants would have remained VUS without cosegregation data. Classified variant types were 74 missense, 11 intronic and 8 synonymous variants, 6 in-frame indels and 2 LGRs (Figure-4A). Each type contained variants classified either as pathogenic or benign, except for synonymous variants that were all classified as BV or LBV. Although 8 LPV variants were selected for analysis, none of them were re-classified.

Variant classification and pathogenicity prediction tools

A good concordance was observed between the Align-GVGD prediction tool for extreme classes and the COVAR study classification concerning amino-acid changes, *i.e.* most C65 variants were classified as pathogenic and C0 variants as benign (Figure-4B). However, six variants with a predicted pathogenic effect ranging from Align-GVGD class C35 to C65 were demonstrated to be benign or likely benign: *BRCA1*:c.92T>A/p.(Ile31Asn), c.5071A>G/p.(Thr1691Ala) and c.5117G>C/p.(Gly1706Ala) located in the RING and BRCT1 domains, respectively; *BRCA2*:c.7481G>A/p.(Arg2494Gln), c.9104A>C/p.(Tyr3035Ser) and c.9275A>G/p.(Tyr3092Cys) located in the helical and oligonucleotide binding 2 and 3 (OB2/3) domains, respectively. Conversely, 9 variants with Align-GVGD class C0 to C25 were demonstrated to be pathogenic: *BRCA1*:c.4963T>G/p.(Ser1655Ala) and c.5062G>T/p.(Val1688Phe) located in the BRCT1 domain, c.5254G>C/p.(Ala1752Pro) and c.5255C>A/p.(Ala1752Glu) located in the linker between BRCT1 and BRCT2 domains, c.5309G>T/p.(Gly1770Val), c.5309_5310delinsTT/p.(Gly1770Val) and c.5434C>G/p.(Pro1812Ala) located in the BRCT2 domain; *BRCA2*:c.8009C>T/p.(Ser2670Leu) and c.8009C>G/p.(Ser2670Trp) located in the OB1 domain. Poor concordance was observed between the COVAR study classification and SIFT and PolyPhen-2 prediction tools, especially for variants with a predicted pathogenic effect, almost half of which were classified by the COVAR study as BV or LBV (Figure-4B).

Specific variants

The *BRCA2*:c.6937+594T>G deep intronic variant previously reported to be associated with a cryptic exon inclusion between exons 12 and 13 was classified as a BV, like the *BRCA2*:c.9501+3A>T variant leading to partial exon 25 skipping.

Some variants were classified as pathogenic by combining pathogenicity likelihoods, as they have the same putative protein effect: *BRCA1*:c.5309G>T and c.5309_5310delinsTT that both result in Gly 1770 to Val substitution (a splice defect was excluded for both of these variants); *BRCA2*:c.68-?_316+?del (corresponding to exon 3 deletion), c.156_157insAlu, c.316+1G>T, c.316+2T>C, c.316+4del, c.316+5G>A and c.316+5G>G that all lead to the complete loss of the in-frame exon 3.

Some variants located in the same codon but resulting in different amino-acid changes were classified in different classes, *e.g.* three variants in *BRCA1* 1706 codon located in BRCT1 domain: *BRCA1*:c.5116G>A/p.(Gly1706Arg), c.5117G>A/p.(Gly1706Glu) and c.5117G>C/p.(Gly1706Ala) were classified as PV, LPV and BV, respectively.

Two *BRCA2* variants were systematically identified in *cis*: *BRCA2*:c.927A>G/p.(Ser309=) and c.7759C>T/p.(Leu2587Phe), classified as BV and LBV, respectively.

Comparison of COVAR and ACMG/AMP classification

We compared the COVAR-classification results with ACMG/AMP scoring by using two bioinformatics tools, Varsome and InterVar (Tables 1 and S3)^{41,42}. All variants could be compared with Varsome, but only 82 of the 101 COVAR classified variants could be assessed with InterVar, as InterVar does not process all variant types (indels, LGRs, intronic variants). Among the 34 variants classified as PV by the COVAR study, only 4 (11.8%) variants were classified in this category by Varsome and none by InterVar. As cosegregation data can be included in Varsome, we subsequently compared our results for the 34 COVAR-classified PVs with Varsome integrating cosegregation data. The number of Varsome-classified PVs

increased from 4 (11.8%) to 18 (52.9% of the COVAR-classified PVs). The other COVAR-classified PVs were classified as LPV by Varsome or InterVar or remained VUS. At the other extreme, among the 51 variants classified as benign by the COVAR study, two were classified similarly by Varsome, and only one was classified as benign by InterVar. The majority of the other COVAR-classified BVs remained VUS or LBV with both tools. In addition, it should be noted that nine COVAR-classified BVs were classified as LPV by Varsome or Intervar.

Discussion

COVAR classification or the power of cosegregation analysis

Among the 653 *BRCA1/2* variants selected for the COVAR study, 101 (15%) variants identified in 1,624 families had been classified at the time of this first report; 38% were classified as PV or LPV and 62% were classified as BV or LBV. Most of the selected and classified VUS were missense variants, but other variant types were also classified: in-frame indels, LGRs, synonymous and intronic variants. Among the 101 classified variants, 5 had never been previously reported, 13 were new for ClinVar and not reviewed in BRCAexchange, 79 (78.2%) were present but not reviewed in the BRCAexchange database. Twenty three (32.7%) and 59 (58.4%) variants were also reported in ClinVar as VUS or “conflicting interpretation”, respectively (Table-S3).

A very wide range of cosegregation LR values was observed ($1.4\text{E}+23$ to $9.4\text{E}-08$), illustrating the power of cosegregation analyzes in variant classification provided that sufficient data have been collected. No other measurement of association between VUS and cancer was more powerful in our dataset (Tables 1 and S3). Indeed, 66 of the 101 (65%) variants classified by COVAR would have remained VUS without cosegregation data.

Specific variants

Combining data for different genetic variants with a common protein impact allows the classification of a greater number of variants by increasing the statistical power of

cosegregation. However, mRNA analyzes must be performed before combining variant data in order to ensure that all variants have a similar splicing profile. We confirmed that the two *BRCA1* variants leading to the amino acid Gly1770 to Val substitution did not result in a splicing defect. Moreover, in order to classify the *BRCA2* exon 3 deletion, we combined only *BRCA2* variants leading to complete loss of exon 3⁴³.

Conversely, variants located on the same codon but leading to different amino acid changes were classified as pathogenic or benign, *e.g.* *BRCA1* variants located on codons 1691 or 1706. The class of each *BRCA1* 1691 or 1706 codon variant was concordant with previously published functional assays^{44–48}. Similarly, there was a good correlation between our classification of *BRCA2* missense variants and functional assay results when available^{49–51}.

Prior probability

Although cosegregation analysis is a powerful variant classification tool, the multifactorial model of pathogenicity that includes cosegregation data may have a number of limitations, especially the major influence of PriorP on posterior probability, as the better concordance observed between our classification and Align-GVGD prediction than SIFT or PolyPhen-2 prediction could be explained by the high weight of PriorP, because the Align-GVGD prediction score was used to determine PriorP. However, interestingly, some missense variants predicted as pathogenic by Align-GVGD were classified as benign and some variants predicted as benign were classified as pathogenic after cosegregation analysis. Lindor et al. pointed out that PriorP should not be used alone but combined with other arguments such as cosegregation, in a multifactorial model¹⁸. Re-evaluation of these PriorP is ongoing⁴⁰.

An example of the impact of PriorP on variant class is the observation for the two variants systematically identified in *cis*, *BRCA2*:c.927A>G/p.(Ser309=) and c.7759C>T/p.(Leu2587Phe), classified as BV and LBV, respectively. As all data and consequently all evidence of pathogenicity for these two variants are identical (since they

always occur together), the only difference in posterior pathogenicity probability calculation for these variants was their PriorP (C0 vs C15).

Combined-LR thresholds to estimate posterior probability

Combined-LR thresholds used for classification of variants were those defined by Goldgar *et al.* in 2004, a combined-LR greater than 1000:1 for PV and lower than 1:100 for BV. The calculation of posterior probability was not performed when combined-LR were between these thresholds, to ensure a sufficient level of evidence from observational data. Recent studies used the multifactorial model with prior probability truncated at 0.10 and 0.90 for minimum and maximum prior probability, respectively, which leads to combined-LR lower than 0.5 or greater than 2 to reach class 2 or class 4, respectively; in these studies the estimation of posterior probability was not performed only for combined-LR between 0.5 and 2^{11,21}. We could have classified 100 additional variants towards causality or neutrality if posterior probability had been calculated outside the range of 0.5-2 instead of the range of 1:100-1000:1. However, it may lead to variant misclassification because of a low level of observational data and an increased weight of prior probability. This misclassification, especially in the PV or LPV classes, can have clinical consequences leading to inappropriate prophylactic surgery decisions for carriers and unsafe surveillance discontinuation for non-carriers^{7,52}.

Multifactorial model parameters

A last limitation is that the model does not consider the possibility of *de novo* variants and may therefore classify a variant as benign because of the absence of a family history of cancers although it is pathogenic. However, this constitutes a minor limitation, as the *BRCA1/2 de novo* variant rate is estimated to be only 0.3% [0.1%; 0.7%] and *de novo* status should be detected by genetic testing of both parents when available⁵³.

Note that the scoring for cosegregation data used in this study was designed for high penetrance variants. Some *BRCA1/2* PVs with moderate penetrance could remain classified as VUS. This class uncertainty for moderate penetrance variants has been previously reported for the *BRCA1*:c.5096G>A/p.(Arg1699Gln) PV, which was demonstrated to be associated with intermediate breast and ovarian cancer risks on the basis of the large volume of cosegregation data collected: using the standard multifactorial model for high penetrance variants, the *BRCA1*:c.5096G>A/p.(Arg1699Gln) would have remained classified as VUS; Spurdle et al. integrated in the model a lower level of *BRCA1* variant penetrance so this variant could be classified as pathogenic^{54,55}. Adaptation of the cosegregation statistical model developed by Thompson *et al.*, modifying the value of cancer risks and introducing Polygenic Risk Scores for breast cancer risk and subsequent ovarian cancer risk, should improve the classification of variants with moderate penetrance⁵⁶. Adaptation of the statistical model will be no doubt required for VUS of genes such as *CHEK2* and *ATM* that are associated with moderate risk. The multifactorial likelihood model of pathogenicity could also be improved by incorporating a complementary LR to the LR-pathology based on tumor status of a germline variant, as somatic loss of the wild-type allele would suggest pathogenicity and conversely loss of variant non-pathogenicity^{57,58}. A parameter called somatic to germline ratio (SGR) has recently been used for *TP53* variant classification⁵⁹. Increasing indications for *BRCA1/2* gene analyzes of tumors in order to guide PARPi therapy, thereby increasing the information regarding the wild-type allele, will allow the introduction of this information into the likelihood model.

ACMG/AMP classification

In 2015, the ACMG/AMP published general guidelines for gene variant classification based on various types of evidence, including population data, bioinformatics prediction tools, functional data and, when available, cosegregation data⁶⁰. We compared our results to the

ACMG/AMP classification using Varsome and Intervar, the bioinformatics tools that were developed to standardize scoring. Many discrepancies were observed between our classification and the ACMG/AMP classification and between variant scoring by the two tools. This observation was expected as recent studies already showed discrepancies between classification based on the multifactorial model used in our study and ACMG/AMP classification^{61,62}. Most variants classified as pathogenic in our study remained classified as LP or VUS while most BV remained LB or VUS. The greatest classification discrepancy concerned the 12 COVAR-classified BVs that were classified as LPV by Varsome or InterVar. Three of these 12 variants were in-frame amino-acid deletions classified as LPV by Varsome. The weight applied to this variant type may be excessive in the ACMG/AMP scoring. Six other COVAR-classified BV were also classified as LPV by InterVar because it took into account reporting of the variant as pathogenic in public databases with no available evidence of pathogenicity as a supporting element for pathogenicity. Uncurated public database classification should not be used as a classification criterion to avoid misclassification based on insufficient data. Counting meiosis has been proposed for ranking of supporting evidence of pathogenicity based on cosegregation data in the ACMG/AMP classification^{60,63}. It allowed us to input cosegregation data in the Varsome-tool after counting meiosis, resulting in better concordance for our 34 classified-PVs, increasing the number of Varsome-classified PVs from 4 (11.8%) to 18 (52.9%). We used the same levels of cosegregation as proposed by Jarvik and Browning in 2016 to consider cosegregation data as supporting, moderate or strong evidence of pathogenicity for ACMG/AMP classification. We could not include cosegregation data for comparison of BV classification, as counting meiosis is unable to generate any evidence in favor of a benign effect⁶⁴. The ACMG/AMP scoring system is globally based on discrete variables with somewhat subjective ranking of evidence.

Cosegregation data computed in a quantitative multifactorial model, as performed in this study, allows more robust classification.

Functional assays

A large-scale functional assay based on cell survival after BRCA1 disruption has been recently developed to classify nearly 4,000 known or possible *BRCA1* variants located in RING and BRCT-domains⁴⁶. All variants were generated by saturation genome editing with CRISPR/Cas9-system in human haploid cell-lines, allowing an identical genomic background for all tested variants. This functional assay is very promising: it gives accurate results for a large number of variants. However, because the reporting test is cell survival, a surrogate marker of the BRCA1 role in DNA repair by homologous recombination, other BRCA1-altered functions could be missed. Moreover, *in vitro* data alone should not be used as the basis for medical advice until they have been clinically validated⁶⁵. Such functional assays can be used to prioritize variants selected for clinical and genetic studies such as the COVAR study. A “LR functional assay” would be helpful to implement functional data in the multifactorial model but there is no international consensus yet on which assays and which weights should be used.

Artificial Intelligence

Finally, Artificial Intelligence (AI) is being developed for more and more clinical applications including genetic variant classification. At the present time, algorithms are being developed to integrate literature data, gene and protein structure, species alignment (Corona-AI, REVEL,...). Using machine-learning on the ClinVar *BRCA1/2* variant database, Favalli et al developed an interesting variant classification algorithm. However, AI variant classification still requires critical examination by geneticists, especially with regard to the quality of the data on which AI tools are trained ⁶⁶.

Conclusion

In conclusion, the first series of results of the COVAR study show that cosegregation is a highly powerful tool for *BRCA1/2* variant classification. Cosegregation studies, *i.e.* linkage studies, for VUS classification may be as useful as they were 25 years ago for the identification of a large of disease-related genes, including *BRCA1/2*. However, in order to be feasible, such studies must be organized by clinical and laboratory networks at a nationwide or even worldwide level such as ENIGMA. Although the initiation and organization of these studies requires considerable effort, the growing number of available genetic tests results in an increasing number of families sharing a particular variant, thereby increasing their power. Here we demonstrate that variant cosegregation studies, *i.e.* “linkage studies 2.0” for reference to the linkage studies which led to the localization of the *BRCA1* and *BRCA2* genes thirty years ago^{67,68}, are a powerful tool for the classification of variants in the *BRCA1/2* breast-ovarian cancer predisposition genes.

Data Availability

The dataset in support of the current study has not been deposited in a public repository because it is composed of patient data including pedigrees which are indirectly identifying. The data are available on request from the corresponding author. A large part of the variants have been deposited in BRCAShare (<http://www.umd.be/>), and all variants will be annotated in the upcoming FrOG (French OncoGenetics) database, as listed in Table S4 and all material and data supporting this study is included within the manuscript (or by request to the authors if this is the case).

Acknowledgements

This article is dedicated to the memory of Olga Sinilnikova.

The authors thank the clinicians and biologists (Table S6) involved in the UGG. This work was funded by grants from the French National Cancer Institute (2013-1-BCB-01-531_ICH-

1) and the French Ligue contre le cancer 92. The COVAR study is supported by the GENETICANCER and “Cercle Olympe” associations and AstraZeneca.

Declaration of Interests

Dominique Stoppa-Lyonnet and the Institut Curie have received honoraria for her participation in education meetings organized by AstraZeneca or Tesaro®. The remaining authors declare no conflict of interest.

References

1. Kuchenbaecker, K.B., Hopper, J.L., Barnes, D.R., Phillips, K.-A., Mooij, T.M., Roos-Blom, M.-J., Jervis, S., van Leeuwen, F.E., Milne, R.L., Andrieu, N., et al. (2017). Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *JAMA* 317, 2402–2416.
2. Lesueur, F., Mebirouk, N., Jiao, Y., Barjhoux, L., Belotti, M., Laurent, M., Léone, M., Houdayer, C., Bressac-de Paillerets, B., Vaur, D., et al. (2018). GEMO, a National Resource to Study Genetic Modifiers of Breast and Ovarian Cancer Risk in BRCA1 and BRCA2 Pathogenic Variant Carriers. *Front. Oncol.* 8, 490.
3. Rebbeck, T.R., Friebel, T.M., Friedman, E., Hamann, U., Huo, D., Kwong, A., Olah, E., Olopade, O.I., Solano, A.R., Teo, S.-H., et al. (2018). Mutational spectrum in a worldwide study of 29,700 families with BRCA1 or BRCA2 mutations. *Hum. Mutat.* 39, 593–620.
4. Rouleau, E., Jesson, B., Briaux, A., Nogues, C., Chabaud, V., Demange, L., Sokolowska, J., Coulet, F., Barouk-Simonet, E., Bignon, Y.J., et al. (2012). Rare germline large rearrangements in the BRCA1/2 genes and eight candidate genes in 472 patients with breast cancer predisposition. *Breast Cancer Res. Treat.* 133, 1179–1190.
5. Caputo, S., Benboudjema, L., Sinilnikova, O., Rouleau, E., Bérout, C., and Lidereau, R. (2012). Description and analysis of genetic variants in French hereditary breast and ovarian cancer families recorded in the UMD-BRCA1/BRCA2 databases. *Nucleic Acids Res.* 40, D992–D1002.
6. Caputo, S.M., Telly, D., Briaux, A., Sesen, J., Ceppi, M., Bonnet, F., Bourdon, V., Coulet, F., Castera, L., Delnatte, C., et al. (2021). 5' Region Large Genomic Rearrangements in the BRCA1 Gene in French Families: Identification of a Tandem Triplication and Nine Distinct Deletions with Five Recurrent Breakpoints. *Cancers* 13, 3171.
7. de la Hoya, M., Soukarieh, O., López-Perolio, I., Vega, A., Walker, L.C., van Ierland, Y., Baralle, D., Santamariña, M., Lattimore, V., Wijnen, J., et al. (2016). Combined genetic and splicing analysis of BRCA1 c.[594-2A>C; 641A>G] highlights the relevance of naturally occurring in-frame transcripts for developing disease gene variant classification algorithms. *Hum. Mol. Genet.* 25, 2256–2268.
8. Meulemans, L., Mesman, R.L.S., Caputo, S.M., Krieger, S., Guillaud-Bataille, M., Caux-Moncoutier, V., Léone, M., Boutry-Kryza, N., Sokolowska, J., Révillion, F., et al. (2020). Skipping nonsense to maintain function: the paradigm of BRCA2 exon 12. *Cancer Res.*
9. Tubeuf, H., Caputo, S.M., Sullivan, T., Rondeaux, J., Krieger, S., Caux-Moncoutier, V., Hauchard, J., Castelain, G., Fiévet, A., Meulemans, L., et al. (2020). Calibration of Pathogenicity Due to Variant-Induced Leaky Splicing Defects by Using BRCA2 Exon 3 as a Model System. *Cancer Res.* 80, 3593–3605.
10. Muller, D., Rouleau, E., Schultz, I., Caputo, S., Lefol, C., Bièche, I., Caron, O., Noguès, C., Limacher, J.M., Demange, L., et al. (2011). An entire exon 3 germ-line rearrangement in the BRCA2 gene: pathogenic relevance of exon 3 deletion in breast cancer predisposition. *BMC Med. Genet.* 12, 121.

11. Parsons, M.T., Tudini, E., Li, H., Hahnen, E., Wappenschmidt, B., Feliubadaló, L., Aalfs, C.M., Agata, S., Aittomäki, K., Alducci, E., et al. (2019). Large scale multifactorial likelihood quantitative analysis of BRCA1 and BRCA2 variants: An ENIGMA resource to support clinical variant classification. *Hum. Mutat.*
12. Spurdle, A.B., Healey, S., Devereau, A., Hogervorst, F.B., Monteiro, A.N., Nathanson, K.L., Radice, P., Stoppa-Lyonnet, D., Tavtigian, S., Wappenschmidt, B., et al. (2012). ENIGMA - Evidence-based Network for the Interpretation of Germline Mutant Alleles: An international initiative to evaluate risk and clinical significance associated with sequence variation in BRCA1 and BRCA2 genes. *Hum. Mutat.* 33, 2–7.
13. Couch, F.J., Nathanson, K.L., and Offit, K. (2014). Two decades after BRCA: setting paradigms in personalized cancer care and prevention. *Science* 343, 1466–1470.
14. Colas, C., Golmard, L., de Pauw, A., Caputo, S.M., and Stoppa-Lyonnet, D. (2019). “Decoding hereditary breast cancer” benefits and questions from multigene panel testing. *Breast Edinb. Scotl.* 45, 29–35.
15. Cline, M.S., Liao, R.G., Parsons, M.T., Paten, B., Alquaddoomi, F., Antoniou, A., Baxter, S., Brody, L., Cook-Deegan, R., Coffin, A., et al. (2018). BRCA Challenge: BRCA Exchange as a global resource for variants in BRCA1 and BRCA2. *PLoS Genet.* 14, e1007752.
16. Kurian, A.W., Li, Y., Hamilton, A.S., Ward, K.C., Hawley, S.T., Morrow, M., McLeod, M.C., Jagsi, R., and Katz, S.J. (2017). Gaps in Incorporating Germline Genetic Testing Into Treatment Decision-Making for Early-Stage Breast Cancer. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* 35, 2232–2239.
17. Goldgar, D.E., Easton, D.F., Deffenbaugh, A.M., Monteiro, A.N.A., Tavtigian, S.V., Couch, F.J., and Breast Cancer Information Core (BIC) Steering Committee (2004). Integrated evaluation of DNA sequence variants of unknown clinical significance: application to BRCA1 and BRCA2. *Am. J. Hum. Genet.* 75, 535–544.
18. Lindor, N.M., Guidugli, L., Wang, X., Vallée, M.P., Monteiro, A.N.A., Tavtigian, S., Goldgar, D.E., and Couch, F.J. (2012). A review of a multifactorial probability-based model for classification of BRCA1 and BRCA2 variants of uncertain significance (VUS). *Hum. Mutat.* 33, 8–21.
19. Thompson, D., Easton, D.F., and Goldgar, D.E. (2003). A full-likelihood method for the evaluation of causality of sequence variants from family data. *Am. J. Hum. Genet.* 73, 652–655.
20. Tavtigian, S.V., Byrnes, G.B., Goldgar, D.E., and Thomas, A. (2008). Classification of rare missense substitutions, using risk surfaces, with genetic- and molecular-epidemiology applications. *Hum. Mutat.* 29, 1342–1354.
21. Vallée, M.P., Sera, T.L.D., Nix, D.A., Paquette, A.M., Parsons, M.T., Bell, R., Hoffman, A., Hogervorst, F.B.L., Goldgar, D.E., Spurdle, A.B., et al. (2016). Adding In Silico Assessment of Potential Splice Aberration to the Integrated Evaluation of BRCA Gene Unclassified Variants. *Hum. Mutat.*
22. Plon, S.E., Eccles, D.M., Easton, D., Foulkes, W.D., Genuardi, M., Greenblatt, M.S., Hogervorst, F.B.L., Hoogerbrugge, N., Spurdle, A.B., Tavtigian, S.V., et al. (2008). Sequence

variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum. Mutat.* 29, 1282–1291.

23. Mazoyer, S., Lalle, P., Narod, S.A., Bignon, Y.J., Courjal, F., Jamot, B., Dutrillaux, B., Stoppa-Lyonnet, D., and Sobol, H. (1993). Linkage analysis of 19 French breast cancer families, with five chromosome 17q markers. *Am. J. Hum. Genet.* 52, 754–760.

24. Eisinger, F., Bressac, B., Castaigne, D., Cottu, P.-H., Lansac, J., Lefranc, J.-P., Lesur, A., Noguès, C., Pierret, J., Puy-Pernias, S., et al. (2006). [Identification and management of hereditary breast-ovarian cancers (2004 update)]. *Pathol. Biol. (Paris)* 54, 230–250.

25. Sinilnikova, O.M., Dondon, M.-G., Eon-Marchais, S., Damiola, F., Barjhoux, L., Marcou, M., Verny-Pierre, C., Sornin, V., Toulemonde, L., Beauvallet, J., et al. (2016). GENESIS: a French national resource to study the missing heritability of breast cancer. *BMC Cancer* 16, 13.

26. Moretta, J., Berthet, P., Bonadona, V., Caron, O., Cohen-Haguenauer, O., Colas, C., Corsini, C., Cusin, V., De Pauw, A., Delnatte, C., et al. (2018). [The French Genetic and Cancer Consortium guidelines for multigene panel analysis in hereditary breast and ovarian cancer predisposition]. *Bull. Cancer (Paris)*.

27. Girard, E., Eon-Marchais, S., Olasso, R., Renault, A.-L., Damiola, F., Dondon, M.-G., Barjhoux, L., Goidin, D., Meyer, V., Le Gal, D., et al. (2019). Familial breast cancer and DNA repair genes: Insights into known and novel susceptibility genes from the GENESIS study, and implications for multigene panel testing. *Int. J. Cancer* 144, 1962–1974.

28. Jiao, Y., Lesueur, F., Azencott, C.-A., Laurent, M., Mebirouk, N., Laborde, L., Beauvallet, J., Dondon, M.-G., Eon-Marchais, S., Laugé, A., et al. (In press). A new hybrid record linkage process to render epidemiological databases interoperable: application to the GEMO and GENEPSO studies involving BRCA1 and BRCA2 mutation carriers. *BMC Med. Res. Methodol.*

29. French Unicancer Genetics Group (UGG) of cancer genetics clinic and laboratory network <http://www.unicancer.fr/la-recherche-unicancer/les-groupes-transversaux/groupe-genetique-cancer-ggc>.

30. Institut National Du Cancer (2020). Oncogénétique en 2018 - Consultations, laboratoires et suivi (2017 et 2018).

31. Bérout, C., Letovsky, S.I., Braastad, C.D., Caputo, S.M., Beaudoux, O., Bignon, Y.J., Bressac-De Paillerets, B., Bronner, M., Buell, C.M., Collod-Bérout, G., et al. (2016). BRCA Share: A Collection of Clinical BRCA Gene Variants. *Hum. Mutat.*

32. Easton, D.F., Pharoah, P.D.P., Antoniou, A.C., Tischkowitz, M., Tavtigian, S.V., Nathanson, K.L., Devilee, P., Meindl, A., Couch, F.J., Southey, M., et al. (2015). Gene-panel sequencing and the prediction of breast-cancer risk. *N. Engl. J. Med.* 372, 2243–2257.

33. Mocci, E., Milne, R.L., Méndez-Villamil, E.Y., Hopper, J.L., John, E.M., Andrulis, I.L., Chung, W.K., Daly, M., Buys, S.S., Malats, N., et al. (2013). Risk of pancreatic cancer in breast cancer families from the breast cancer family registry. *Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol.* 22, 803–811.

34. Antoniou, A.C., Cunningham, A.P., Peto, J., Evans, D.G., Lalloo, F., Narod, S.A., Risch, H.A., Eyfjord, J.E., Hopper, J.L., Southey, M.C., et al. (2008). The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. *Br. J. Cancer* 98, 1457–1466.
35. Gaildrat, P., Krieger, S., Théry, J.-C., Killian, A., Rousselin, A., Berthet, P., Frébourg, T., Hardouin, A., Martins, A., and Tosi, M. (2010). The BRCA1 c.5434C->G (p.Pro1812Ala) variant induces a deleterious exon 23 skipping by affecting exonic splicing regulatory elements. *J. Med. Genet.* 47, 398–403.
36. Anczuków, O., Buisson, M., Léoné, M., Coutanson, C., Lasset, C., Calender, A., Sinilnikova, O.M., and Mazoyer, S. (2012). BRCA2 deep intronic mutation causing activation of a cryptic exon: opening toward a new preventive therapeutic strategy. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 18, 4903–4909.
37. Spurdle, A.B., Couch, F.J., Parsons, M.T., McGuffog, L., Barrowdale, D., Bolla, M.K., Wang, Q., Healey, S., Schmutzler, R., Wappenschmidt, B., et al. (2014). Refined histopathological predictors of BRCA1 and BRCA2 mutation status: a large-scale analysis of breast cancer characteristics from the BCAC, CIMBA, and ENIGMA consortia. *Breast Cancer Res. BCR* 16, 3419.
38. Belman, S., Parsons, M.T., Spurdle, A.B., Goldgar, D.E., and Feng, B.-J. (2020). Considerations in assessing germline variant pathogenicity using cosegregation analysis. *Genet. Med.* 1–8.
39. Easton, D.F., Deffenbaugh, A.M., Pruss, D., Frye, C., Wenstrup, R.J., Allen-Brady, K., Tavtigian, S.V., Monteiro, A.N.A., Iversen, E.S., Couch, F.J., et al. (2007). 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.* 81, 873–883.
40. Li, H., LaDuca, H., Pesaran, T., Chao, E.C., Dolinsky, J.S., Parsons, M., Spurdle, A.B., Polley, E.C., Shimelis, H., Hart, S.N., et al. (2019). Classification of variants of uncertain significance in BRCA1 and BRCA2 using personal and family history of cancer from individuals in a large hereditary cancer multigene panel testing cohort. *Genet. Med.*
41. Li, Q., and Wang, K. (2017). InterVar: Clinical Interpretation of Genetic Variants by the 2015 ACMG-AMP Guidelines. *Am. J. Hum. Genet.* 100, 267–280.
42. Kopanos, C., Tsiolkas, V., Kouris, A., Chapple, C.E., Albarca Aguilera, M., Meyer, R., and Massouras, A. (2019). VarSome: the human genomic variant search engine. *Bioinformatics* 35, 1978–1980.
43. Caputo, S.M., Léone, M., Damiola, F., Ehlen, A., Carreira, A., Gaidrat, P., Martins, A., Brandão, R.D., Peixoto, A., Vega, A., et al. (2018). Full in-frame exon 3 skipping of BRCA2 confers high risk of breast and/or ovarian cancer. *Oncotarget* 9, 17334–17348.
44. Millot, G., Carvalho, M.A., Caputo, S.M., Vreeswijk, M.P.G., Brown, M.A., Webb, M., Rouleau, E., Neuhausen, S.L., Hansen, T. v. O., Galli, A., et al. (2012). A Guide for Functional Analysis of BRCA1 Variants of Uncertain Significance (VUS). *Hum. Mutat.* 33, 1526–1537.

45. Thouvenot, P., Ben Yamin, B., Fourrière, L., Lescure, A., Boudier, T., Del Nery, E., Chauchereau, A., Goldgar, D.E., Houdayer, C., Stoppa-Lyonnet, D., et al. (2016). Functional Assessment of Genetic Variants with Outcomes Adapted to Clinical Decision-Making. *PLoS Genet.* *12*, e1006096.
46. Findlay, G.M., Daza, R.M., Martin, B., Zhang, M.D., Leith, A.P., Gasperini, M., Janizek, J.D., Huang, X., Starita, L.M., and Shendure, J. (2018). Accurate classification of BRCA1 variants with saturation genome editing. *Nature* *562*, 217–222.
47. Fernandes, V.C., Golubeva, V.A., Pietro, G.D., Shields, C., Amankwah, K., Nepomuceno, T.C., Gregoriis, G. de, Abreu, R.B.V., Harro, C., Gomes, T.T., et al. (2019). Impact of amino acid substitutions at secondary structures in the BRCT domains of the tumor suppressor BRCA1: Implications for clinical annotation. *J. Biol. Chem.* jbc.RA118.005274.
48. Petitalot, A., Dardillac, E., Jacquet, E., Nhiri, N., Guirouilh-Barbat, J., Julien, P., Bouazzaoui, I., Bonte, D., Feunteun, J., Schnell, J.A., et al. (2019). Combining Homologous Recombination and Phosphopeptide-binding Data to Predict the Impact of BRCA1 BRCT Variants on Cancer Risk. *Mol. Cancer Res. MCR* *17*, 54–69.
49. Biswas, K., Das, R., Eggington, J.M., Qiao, H., North, S.L., Stauffer, S., Burkett, S.S., Martin, B.K., Southon, E., Sizemore, S.C., et al. (2012). Functional evaluation of BRCA2 variants mapping to the PALB2-binding and C-terminal DNA-binding domains using a mouse ES cell-based assay. *Hum. Mol. Genet.* *21*, 3993–4006.
50. Mesman, R.L.S., Calléja, F.M.G.R., Hendriks, G., Morolli, B., Misovic, B., Devilee, P., van Asperen, C.J., Vrieling, H., and Vreeswijk, M.P.G. (2019). The functional impact of variants of uncertain significance in BRCA2. *Genet. Med. Off. J. Am. Coll. Med. Genet.* *21*, 293–302.
51. Richardson, M.E., Hu, C., Lee, K.Y., LaDuca, H., Fulk, K., Durda, K.M., Deckman, A.M., Goldgar, D.E., Monteiro, A.N.A., Gnanaolivu, R., et al. (2021). Strong functional data for pathogenicity or neutrality classify BRCA2 DNA-binding-domain variants of uncertain significance. *Am. J. Hum. Genet.* *108*, 458–468.
52. Mighton, C., Charames, G.S., Wang, M., Zakoor, K.-R., Wong, A., Shickh, S., Watkins, N., Lebo, M.S., Bombard, Y., and Lerner-Ellis, J. (2019). Variant classification changes over time in BRCA1 and BRCA2. *Genet. Med. Off. J. Am. Coll. Med. Genet.* *21*, 2248–2254.
53. Golmard, L., Delnatte, C., Laugé, A., Moncoutier, V., Lefol, C., Abidallah, K., Tenreiro, H., Copigny, F., Giraudeau, M., Guy, C., et al. (2016). Breast and ovarian cancer predisposition due to de novo BRCA1 and BRCA2 mutations. *Oncogene* *35*, 1324–1327.
54. Spurdle, A.B., Whaley, P.J., Thompson, B., Feng, B., Healey, S., Brown, M.A., Pettigrew, C., kConFab, Van Asperen, C.J., Ausems, M.G.E.M., et al. (2012). BRCA1 R1699Q variant displaying ambiguous functional abrogation confers intermediate breast and ovarian cancer risk. *J. Med. Genet.* *49*,.
55. Moghadasi, S., Meeks, H.D., Vreeswijk, M.P., Janssen, L.A., Borg, Å., Ehrencrona, H., Paulsson-Karlsson, Y., Wappenschmidt, B., Engel, C., Gehrig, A., et al. (2018). The BRCA1 c. 5096G>A p.Arg1699Gln (R1699Q) intermediate risk variant: breast and ovarian cancer risk estimation and recommendations for clinical management from the ENIGMA consortium. *J. Med. Genet.* *55*, 15–20.

56. Mavaddat, N., Michailidou, K., Dennis, J., Lush, M., Fachal, L., Lee, A., Tyrer, J.P., Chen, T.-H., Wang, Q., Bolla, M.K., et al. (2019). Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *Am. J. Hum. Genet.* *104*, 21–34.
57. Spearman, A.D., Sweet, K., Zhou, X.-P., McLennan, J., Couch, F.J., and Toland, A.E. (2008). Clinically applicable models to characterize BRCA1 and BRCA2 variants of uncertain significance. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* *26*, 5393–5400.
58. Santana dos Santos, E., Spurdle, A.B., Carraro, D.M., Briault, A., Southey, M., Torrezan, G.T., Petitalot, A., Leman, R., Lafitte, P., kConFab, I., et al. (Accepted). Value of the loss of heterozygosity to BRCA1 variant classification. *NPJ Breast Cancer*.
59. Fortuno, C., Cipponi, A., Ballinger, M.L., Tavtigian, S.V., Olivier, M., Ruparel, V., Haupt, Y., Haupt, S., Study, I.S.K., Tucker, K., et al. (2019). A quantitative model to predict pathogenicity of missense variants in the TP53 gene. *Hum. Mutat.* *40*, 788–800.
60. Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W.W., Hegde, M., Lyon, E., Spector, E., et al. (2015). Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med. Off. J. Am. Coll. Med. Genet.* *17*, 405–424.
61. Lee, J.-S., Oh, S., Park, S.K., Lee, M.-H., Lee, J.W., Kim, S.-W., Son, B.H., Noh, D.-Y., Lee, J.E., Park, H.-L., et al. (2018). Reclassification of BRCA1 and BRCA2 variants of uncertain significance: a multifactorial analysis of multicentre prospective cohort. *J. Med. Genet.* *55*, 794–802.
62. Dines, J.N., Shirts, B.H., Slavin, T.P., Walsh, T., King, M.-C., Fowler, D.M., and Pritchard, C.C. (2020). Systematic misclassification of missense variants in BRCA1 and BRCA2 “coldspots.” *Genet. Med.* 1–6.
63. Jarvik, G.P., and Browning, B.L. (2016). Consideration of Cosegregation in the Pathogenicity Classification of Genomic Variants. *Am. J. Hum. Genet.* *98*, 1077–1081.
64. Rañola, J.M.O., Liu, Q., Rosenthal, E.A., and Shirts, B.H. (2018). A comparison of cosegregation analysis methods for the clinical setting. *Fam. Cancer* *17*, 295–302.
65. Chanock, S.J. (2018). The paradox of mutations and cancer. *Science* *362*, 893–894.
66. Favalli, V., Tini, G., Bonetti, E., Voza, G., Guida, A., Gandini, S., Pelicci, P.G., and Mazzarella, L. (2021). Machine learning-based reclassification of germline variants of unknown significance: The RENOVO algorithm. *Am. J. Hum. Genet.* *108*, 682–695.
67. Hall, J.M., Lee, M.K., Newman, B., Morrow, J.E., Anderson, L.A., Huey, B., and King, M.C. (1990). Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* *250*, 1684–1689.
68. Wooster, R., Neuhausen, S.L., Mangion, J., Quirk, Y., Ford, D., Collins, N., Nguyen, K., Seal, S., Tran, T., and Averill, D. (1994). Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science* *265*, 2088–2090.

Figure Legends

Figure 1: Variant classification criteria in the framework of the 5 classes of likelihood of pathogenicity and COVAR study variant selection criteria.

The variant classification or selection for COVAR study relies on the presence of at least one of the listed criteria.

(1) For large duplications, tandem status disrupting the gene must be demonstrated by mRNA analysis or breakpoint sequencing, otherwise this variant should be considered to be as VUS

(2) Multifactorial likelihood model proposed by Goldgar et al., 2004

(3) This classification criterion has been used since January 2019. Before 2019, these splice variants were classified in class 5 and were confirmed by mRNA analysis.

(4) Total gnomAD cohort or subpopulations except for Finnish, Ashkenazi Jewish and “Other” groups due to the possibility of a founder effect and/or the small number of subjects.

gnomAD started to be used as the control database for classification criteria in July 2017. Between December 2014 and June 2017, allele frequency was determined from the ExAC database, with the same thresholds and population groups for classification. Between 2012 and November 2014, allele frequency was determined from the 1000 Genomes Project and Exome Sequencing Project databases, with only one threshold, allele frequency > 1% for classification in class 1 variant, and only the total cohort of both projects

(5) With no clinical or biological signs of Fanconi anemia

(6) Align-GVGD prediction algorithm classifies variants from C0 to C65 classes; C0 corresponds to the least likely functional variants and C65 corresponds to the most likely functional variants (Tavtigian *et al.*, 2006). The last species of IARC alignment, purple sea urchin, is removed for Align-GVGD prediction when it is the only species with an amino acid change; in this case, the inclusion criterion corresponds to Align-GVGD class C35 to C65 AND deleterious prediction by SIFT algorithm (Sim *et al.*, 2012).

IARC: International Agency for Research on Cancer

Figure 2: Increase of the number of *BRCA1* and *BRCA2* variants in the FrOG database since 2012, not including the result of the COVAR study classification.

Figure 3: Patients and variants in the COVAR study from December 2011 to November 2019

A/ Number of patients and variants included and number of variants classified

B/ Number of families participating in the COVAR study according to the number of relatives included.

Figure 4: A/ Classified variants according to the type of variation. B/ Classified variants according to the *in silico* predicted protein effect: Align-GVGD, SIFT, or PolyPhen-2.

Table 1: Variants classified by the COVAR study.

Tables

Table 1: Variants classified by the COVAR study.

Gene	Exon/ Intron	Nucleotide nomenclature	Protein nomenclature	Prior Probability of pathogenici ty	Nb of familie s	Family history LR	Co- occurrenc e LR	Pathology LR	Combined LR without cosegregation	IARC 5-tier class without co- segregation data ^d	Nb of families with co- segregation data	Vars ome Class s	Cosegregation LR	Combined LR	Posterior Probability of pathogenicity	IARC 5-tier class
PATHOGENIC																
BRCA1	3	c.121C>T	p.(His41Tyr)	0.81	4	4.88	-	73.42	358.04	-	3	P	128.396	45970.26	0.9999949	5
BRCA1	08-09	c.547+1G>T	-	0.97	4	1.87	-	703.797	1313.41	-	4	P	204.08743	268050.27	0.9999988	5'
BRCA1	16	c.4963T>G	p.Ser1655Ala	0.03	9	1.23	-	19.78	24.36	3	5	LP	93.2268842	2271.10	0.98596299	4
BRCA1	17	c.4994T>A	p.(Val1665Glu)	0.81	15	5.89	-	41.24	243.08	-	3	LP	53.95	13114.14	0.99998211	5
BRCA1	17	c.5017_5019del	p.(His1673del)	0.81	6	0.49	-	7.98	3.92	3	3	LP	488.41099	1914.77	0.99987751	5
BRCA1	17	c.5062G>T	p.(Val1688Phe)	0.03	1	11.69	-	11.79	137.84	3	1	LP	24.025	3311.52	0.9903305	5
BRCA1	17	c.5072C>T	p.(Thr1691Ils)	0.81	4	3.87	-	23.23	89.99	-	2	P	22.24	2001.76	0.999882832	5
BRCA1	18	c.5116G>A	p.(Gly1706Arg)	0.81	17	3.17	-	11.79	37.34	-	2	P	61.93	2312.62	0.99989858	5
BRCA1	18	c.5117G>A	p.(Gly1706Glu)	0.81	10	2.61	-	3.12	8.14	3	4	LP	7.37	59.97	0.99610374	4'
BRCA1	18	c.5144G>A	p.(Ser1715Asn)	0.66	4	6.42	-	-	6.42	3	2	P	167.97	1078.99	0.99952279	5 ^e
BRCA1	18	c.5145C>G	p.(Ser1715Arg)	0.81	3	6.78	-	13.91	94.28	-	2	LP	28.80	2715.44	0.99991362	5 ^e
BRCA1	20	c.5213G>T	p.(Gly1738Val)	0.81	2	6.05	-	6.21	37.55	-	1	LP	44.08	1655.39	0.99985832	5
BRCA1	20	c.5216A>G	p.(Asp1739Gly)	0.81	9	0.75	-	1009.26	754.45	-	2	LP	10.57	7976.71	0.99997059	5
BRCA1	20	c.5216A>T	p.(Asp1739Val)	0.81	5	5.39	-	71.42	384.68	-	3	LP	3.34	1285.88	0.99981762	5
BRCA1	20	c.5254G>C	p.(Ala1752Pro)	0.03	6	3.80	-	26.97	102.45	3	3	LP	5.88794143	603.24	0.94912744	4
BRCA1	20	c.5255C>A	p.(Ala1752Glu)	0.03	2	10.98	-	10.61	116.44	3	1	LP	14.53517	1692.47	0.98125391	4
BRCA1	21	c.5309G>T ^a	p.(Gly1770Val)	0.03	33	2.48	-	5.599E+10	4.66E+12	5	11	P	3668.96	1.71E+16	0.999999999	5 ^e
BRCA1	21	c.5309_5310delinsTT ^a	p.(Gly1770Val)	0.03	2	-	-	-	-	5	1	LP	-	-	-	5
BRCA1	23	c.5426T>G	p.(Val1809Gly)	0.66	16	2.36	-	129.20	304.56	-	8	P	60.2	18334.43	0.9999719	5
BRCA1	23	c.5434C>G	p.(Pro1812Ala)	0.03	12	5.94	-	34.64	205.76	3	5	P	79.1602119	16288.35	0.99801887	5
BRCA1	24	c.5509T>C	p.(Trp1837Arg)	0.81	3	6.81	-	-	6.81	-	2	LP	297.01	2021.43	0.99988397	5 ^e
BRCA2	3	c.92G>C	p.(Trp31Ser)	0.81	4	2.99	-	3.12	9.31	-	2	P	355.52	3309.48	0.99992913	5
BRCA2	3	c.68-?_316+?del ^b	p.(Asp23_Leu105del)	0.5	4	1381.48	-	15.01	20737.45	5	1	P	1.42701E+23	2.96E+27	1	5
BRCA2	3	c.156_157insAlu ^b	-	0.5	6						0	P				5
BRCA2	I3	c.316+1G>T ^b	-	0.5	1						0	P				5
BRCA2	I3	c.316+2T>C ^b	-	0.5	2						0	P				5 ^e
BRCA2	I3	c.316+4del ^b	-	0.5	2						1	LP				5
BRCA2	I3	c.316+5G>A ^b	-	0.5	2						1	LP				5
BRCA2	I3	c.316+5G>C ^b	-	0.5	28						10	LP				5
BRCA2	16	c.7787G>T	p.(Gly2596Val)	0.5	4	0.27	-	3.35	0.89	3	3	VUS	556.66	497.61	0.99799442	5
BRCA2	16	c.7795_7797delGAA ^c	p.(Glu2599del)	0.81	101	229.56	-	0.93	212.46	-	14	P	10545472.1	2240517687	1	5
BRCA2	17	c.7975A>G	p.(Arg2659Gly)	0.81	22	3.58	-	0.56	2.02	3	14	P	1114.89	2253.70	0.99989593	5
BRCA2	18	c.8009C>G	p.(Ser2670Trp)	0.29	12	0.87	-	10.19	8.88	3	5	LP	819.97	7283.30	0.99966396	5
BRCA2	18	c.8009C>T	p.(Ser2670Leu)	0.29	15	8.47	-	0.87	7.33	3	5	LP	271.63	1990.34	0.99877143	5
BRCA2	18	c.8057T>C	p.(Leu2686Pro)	0.66	18	2.40	-	54.91	131.96	-	6	VUS	27.92	3684.35	0.9998602	5
BRCA2	23	c.9004G>A	p.(Glu3002Lys)	0.66	25	1.16	-	7.20	8.36	3	11	P	301.09	2518.20	0.999795471	5
BRCA2	24	c.9154C>T	p.(Arg3052Trp)	0.81	16	0.99	-	12.07	11.996	-	6	P	102.26	1820.69	0.99987118	5'
BRCA2	25	c.9371A>T	p.(Asn3124Ile)	0.81	16	1.67	-	8.32	5.40	-	6	P	207.49	2876.22	0.99991845	5'

BENIGN																
BRCA1	3	c.92T>A	p.(Ile31Asn)	0.66	8	0.23	-	0.004	0.0008	2	3	VUS	0.12	9.78E-5	0.000189741	1
BRCA1	06	c.243A>G	p.(Gln81=)	0.02	18	0.03	-	0.3906892	0.0102	-	3	B	2.05213421	0.0209869	0.00042812	1 ^{e,f}
BRCA1	06-07	c.301+6T>C	-	0.34	5	1.94	0.001776	0.30	0.01	-	2	VUS	1.6324848	0.00167665	0.00086298	1
BRCA1	10	c.670+8C>T	-	0.5	11	0.32	-	0.0006	0.0002	1	4	LB	2.39	0.0005	0.000450112	1
BRCA1	11	c.693G>A	p.(Thr231=)	0.02	20	0.45	-	0.35	0.16	-	3	LB	0.002	0.0003	5.8992E-06	1 ^f
BRCA1	11	c.1065G>A	p.(Lys355=)	0.02	40	1.27	0.006087	0.0008	0.001	1	4	LB	0.09	5.31E-07	1.0831E-08	1 ^f
BRCA1	11	c.1242_1262del	p.(Asp414_Asp420del)	0.02	3	1.82	-	0.08	0.15	3	2	LP	0.09	0.01	0.00027235	1
BRCA1	11	c.1384G>A	p.(Gly462Arg)	0.02	9	0.22	-	0.002	0.0005	1	5	VUS	0.95	0.0005	9.5128E-06	1
BRCA1	11	c.1844C>T	p.(Ser615Phe)	0.03	4	0.19	-	0.21	0.04	-	2	VUS	0.03	0.001	3.735E-05	1
BRCA1	11	c.2083G>T	p.(Asp695Tyr)	0.03	45	0.31	-	0.0003	8.03E-05	1	13	VUS	9.36E-08	7.52E-12	2.325E-13	1
BRCA1	11	c.2662C>T	p.(His888Tyr)	0.03	19	0.55	-	0.0004	0.0002	1	4	VUS	0.008	1.87E-06	5.7872E-08	1
BRCA1	11	c.2798G>A	p.(Gly933Asp)	0.03	9	0.22	-	0.01	0.003	1	2	VUS	3.47	0.01	0.00034753	1
BRCA1	11	c.2884G>A	p.(Glu962Lys)	0.03	9	0.07	-	0.05	0.003	1	2	LB	0.39	0.001	4.1619E-05	1 ^f
BRCA1	11	c.2935C>T	p.(Arg979Cys)	0.02	16	0.31	-	0.0004	0.0001	1	3	VUS	3.22	0.0004	7.6066E-06	1
BRCA1	11	c.3327_3329del	p.(Lys111del)	0.02	5	0.06	-	0.21	0.012	-	2	LP	0.49	0.006	0.000122496	1
BRCA1	11	c.3708T>G	p.(Asn1236Lys)	0.03	49	0.94	0.008	5.07E-05	4.78E-05	1	9	VUS	0.12	4.51E-08	1.3946E-09	1
BRCA1	11	c.3891_3893delTTC	p.(Ser1298del)	0.02	9	0.24	-	0.05	0.01	1	2	LP	1.28	0.01	0.00028881	1
BRCA1	14	c.4417T>C	p.(Ser1473Pro)	0.03	20	0.14	-	0.003	0.000459893	1	2	VUS	1.195	0.00054971	1.7001E-05	1
BRCA1	14-15	c.4485-96A>G	-	0.5	4	0.29	-	0.009	0.003	2	3	VUS	0.017	4.26E-05	4.2602E-05	1
BRCA1	17	c.4993G>A	p.(Val1665Met)	0.29	20	0.42	-	0.16	0.07	-	5	LB	0.007	0.00050058	0.00020442	1
BRCA1	17	c.5005G>T	p.(Ala1669Ser)	0.03	7	1.09	-	0.04	0.04	-	3	VUS	0.25	0.01	0.00033277	1
BRCA1	17	c.5071A>G	p.(Thr1691Ala)	0.66	16	1.36	-	0.02	0.02	-	4	LP	0.13	0.003	0.006215	2
BRCA1	18	c.5117G>C	p.(Gly1706Ala)	0.66	76	0.35	-	1.19E-05	4.14E-06	1	12	VUS	1.47E-06	6.09E-12	1.1816E-11	1 ^f
BRCA1	24	c.5531T>C	p.(Leu1844Pro)	0.03	3	0.14	-	1.07	0.15	-	2	VUS	0.0001	1.91E-05	5.9194E-07	1
BRCA1	24	c.5531T>G	p.(Leu1844Arg)	0.03	3	0.15	-	0.002	0.0003	1	2	LB	0.15	4.1067E-05	1.2701E-06	1 ^f
BRCA2	3	c.122C>T	p.(Pro41Leu)	0.03	16	0.40	-	2.77	1.11	3	2	VUS	0.05	0.06	0.001881892	2
BRCA2	3	c.231T>G	p.(Thr77=)	0.01	51	0.49	0.00002	0.79	0.38	1	5	LB	26.95	0.0002	1.7357E-06	1 ^f
BRCA2	05	c.433_435del	p.(Val145del)	0.5	12	0.18	-	0.20	0.04	-	4	VUS	0.02835067	0.0010593	0.00105818	2
BRCA2	10	c.800G>A	p.(Gly267Glu)	0.03	22	0.53	0.006	0.05	0.02	1	6	VUS	5.05	0.0007	2.1713E-05	1
BRCA2	10	c.831T>G	p.(Asn277Lys)	0.02	21	0.42	0.006	0.81	0.34	1	4	VUS	0.03	5.83E-05	1.1898E-06	1
BRCA2	10	c.927A>G ^h	p.(Ser309Ser)	0.02	13	0.12	-	0.33	0.04	-	5	LB	0.18	0.007	0.000150187	1 ^f
BRCA2	10	c.1012G>A	p.(Ala338Thr)	0.03	8	0.02	-	0.58	0.012	-	2	LB	0.33877369	0.00418389	0.00012938	1
BRCA2	10	c.1244A>G	p.(His415Arg)	0.02	7	0.67	-	0.36	0.24	-	2	VUS	0.23	0.06	0.00115238	2
BRCA2	10	c.1564G>C	p.(Gly522Arg)	0.03	9	0.32	-	0.44	0.14040988	-	3	LB	0.04183382	0.00587388	0.00018163	1
BRCA2	10	c.1798T>C	p.(Tyr600His)	0.03	28	0.59	-	0.09	0.05	-	2	LB	0.13	0.007	0.00021097	1
BRCA2	11	c.2771A>T	p.(Asn924Ile)	0.02	29	0.75	-	0.01	0.01	1	9	VUS	0.42	0.004	8.8819E-05	1
BRCA2	11	c.2944A>C	p.(Ile982Leu)	0.03	18	0.50	-	0.099	0.05	-	3	LB	0.01205085	0.00059572	1.8424E-05	1
BRCA2	11	c.3262C>T	p.(Pro1088Ser)	0.02	7	0.83	-	-	0.83	-	1	VUS	0.001	0.0008	1.6999E-05	1
BRCA2	11	c.5552T>G	p.(Ile1851Ser)	0.03	13	1.24	-	-	1.24	-	7	VUS	0.02	0.02	0.00067659	1
BRCA2	11	c.5634C>G	p.(Asn1878Lys)	0.03	22	0.62	-	0.31	0.19	-	4	VUS	0.03	0.006	0.00017755	1
BRCA2	11	c.5975C>T	p.Ser1992Leu	0.29	21	0.28	-	0.53	0.15	3	3	VUS	0.16817314	0.02496567	0.01009431	2
BRCA2	11	c.6322C>T	p.(Arg2108Cys)	0.02	41	0.97	0.00001	0.57	0.55	-	3	LB	5.96	0.03	0.00058336	1
BRCA2	12-13	c.6937+5947>G	-	0.5	31	0.92	1.78E-8	0.06	1.06E-09	1	12	LB	115.71	1.23E-7	1.2263E-07	1 ^f
BRCA2	14	c.7057G>C	p.(Gly2353Arg)	0.03	11	0.69	-	0.27	0.19	-	4	VUS	0.02	0.003	0.00010507	1
BRCA2	14	c.7219G>C	p.(Val2407Leu)	0.03	5	0.16	-	1.44	0.23	-	3	VUS	0.20491405	0.04770388	0.0014732	2
BRCA2	15	c.7481G>A	p.(Arg2494Gln)	0.66	7	0.19	0.0022	0.51	0.098	-	4	VUS	0.61	0.0001	0.00025681	1

BRCA2	15	c.7504C>T	p.(Arg2502Cys)	0.03	22	0.61	-	0.19	0.12	-	3	LB	0.09	0.01	0.00031486	1
BRCA2	16	c.7759C>T ^h	p.(Leu2587Phe)	0.29	13	0.12	-	0.33	0.04	-	5	VUS	0.18	0.007	0.0029973	2
BRCA2	17	c.7915C>G	p.(Pro2639Ala)	0.29	6	0.15	-	0.61	0.09	-	3	VUS	0.03	0.003	0.00123378	2
BRCA2	17	c.7928C>G	p.(Ala2643Gly)	0.64	33	0.58	0.0076	0.03	0.02	-	9	VUS	0.01	1.68E-06	2.9926E-06	1
BRCA2	17	c.7933A>G	p.(Arg2645Gly)	0.29	16	0.24	-	0.05	0.01	2	7	VUS	2.93	0.03	0.01329559	2
BRCA2	17	c.7954G>A	p.(Val2652Met)	0.29	27	0.52	0.005	0.76	0.002	1	4	VUS	1.29609452	0.00251463	0.00102605	2
BRCA2	18	c.7992T>A	p.(Ile2664=)	0.02	8	0.82	-	0.35	0.28	-	3	LB	0.00753887	0.00214733	4.3821E-05	1^f
BRCA2	18	c.8010G>A	p.(Ser2670=)	0.02	13	0.33	-	1.91	0.62	-	2	LB	0.097	0.06	0.00123121	2^f
BRCA2	18	c.8084C>T	p.(Ser2695Leu)	0.03	14	0.43	0.003	0.10	0.0001	-	4	VUS	0.11852903	1.6682E-05	5.1595E-07	1
BRCA2	18	c.8111C>T	p.(Ser2704Phe)	0.03	7	0.64	-	0.13	0.08	-	3	VUS	0.04636364	0.00387602	0.00011986	1
BRCA2	23	c.9104A>C	p.(Tyr3035Ser)	0.66	31	0.64	0.007	0.32	0.20	2	9	VUS	0.33	0.0005	0.00092147	1
BRCA2	23	c.9116C>T	p.(Pro3039Leu)	0.03	24	0.4	-	0.02	0.00754	1	7	VUS	0.55	0.004	0.00012765	1
BRCA2	24	c.9206G>T	p.Cys3069Phe	0.03	3	0.07	-	0.59	0.04	-	3	VUS	0.03255207	0.00143349	4.4333E-05	1
BRCA2	25	c.9275A>G	p.(Tyr3092Cys)	0.81	30	0.36	0.008	2.001	0.0056	2	9	VUS	1.44	0.008	0.03490992	2
BRCA2	25	c.9501+3A>T	-	0.34	30	0.49	-	0.20	0.09920669	-	7	VUS	0.001	0.0001	7.3072E-05	1
BRCA2	26	c.9583A>G	p.(Thr3195Ala)	0.03	26	0.42	0.005	0.16	0.0003	1	5	LB	0.26	7.6865E-05	2.3773E-06	1
BRCA2	26	c.9606G>C	p.(Pro3202=)	0.02	21	0.40	-	0.04	0.015	-	3	B	0.26	0.0037923	7.7388E-05	1^f

a. *BRCA1* variants leading to p.(Gly1770Val) were combined

b. *BRCA2* variants leading to complete loss of exon were combined

c. This pathogenic variant probably has a French origin because we find it in many families, particularly in the North of France

d. “-” means that the posterior probability of pathogenicity was not calculated because the combined LR was not <0.01 or >1000

e. This variant was also classified by ClinVar

f. This variant was also classified by BRCAexchange

g. This variant was also classified by ACMG using Varsome

h. Both variants were always observed altogether. Their cosegregation in 4 meioses supports that they are in cis.

See Table S3 for details of ClinVar, BRCAexchange and ACMG classification.

Class 5 – Pathogenic

- Nonsense or frameshift truncating variant (located before *BRCA2* p.Lys3326ter polymorphism)
- Large rearrangement ⁽¹⁾ of one or several exons, out of frame or in-frame in a functional domain
- Splice variant, out of frame or in-frame defect in a functional domain, complete splice defect demonstrated by mRNA analysis
- Missense variant affecting Cys residue in *BRCA1* RING domain
- Pathogenicity probability > 0.99 using multifactorial likelihood model ⁽²⁾

Class 4 – Likely Pathogenic

- Splice variant in +1/+2/-1/-2 with predicted abolition of consensus splice site, out of frame or in-frame defect in a functional domain, without predicted rescue by a cryptic splice site ⁽³⁾
- Pathogenicity probability 0.95 to 0.99 using multifactorial likelihood model ⁽²⁾

Class 3 – Unknown significance

- Insufficient evidence to classify variant
- Pathogenicity probability 0.05 to 0.949 using multifactorial likelihood model ⁽²⁾

Class 2 – Likely Benign

- Allele frequency in gnomAD ⁽⁴⁾ controls 0.1 to 1%
- Intronic or synonymous variant with splice negative prediction
- Truncating variant after *BRCA2* Lys3326ter polymorphism
- Pathogenicity probability 0.001 to 0.049 using multifactorial likelihood model ⁽²⁾

Class 1 – Benign

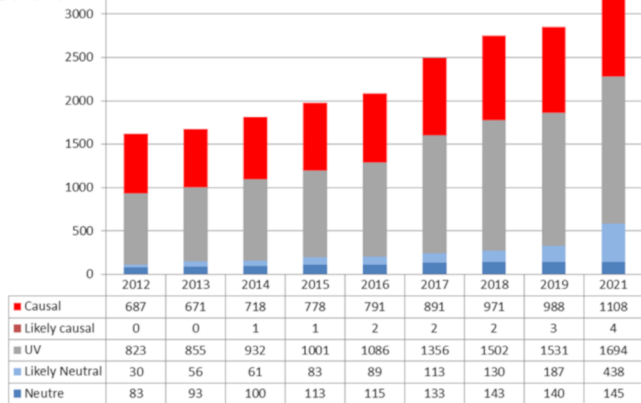
- Allele frequency in gnomAD ⁽⁴⁾ controls > 1%
- Co-occurrence in *trans* with pathogenic variant in the same gene ⁽⁵⁾
- Co-occurrence with two different pathogenic variants in the same gene ⁽⁵⁾, unknown *cis/trans* status
- Pathogenicity probability < 0.001 using multifactorial likelihood model ⁽²⁾



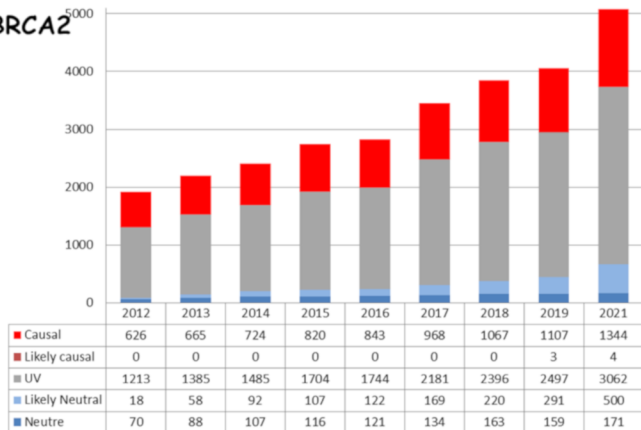
COVAR study variant selection criteria

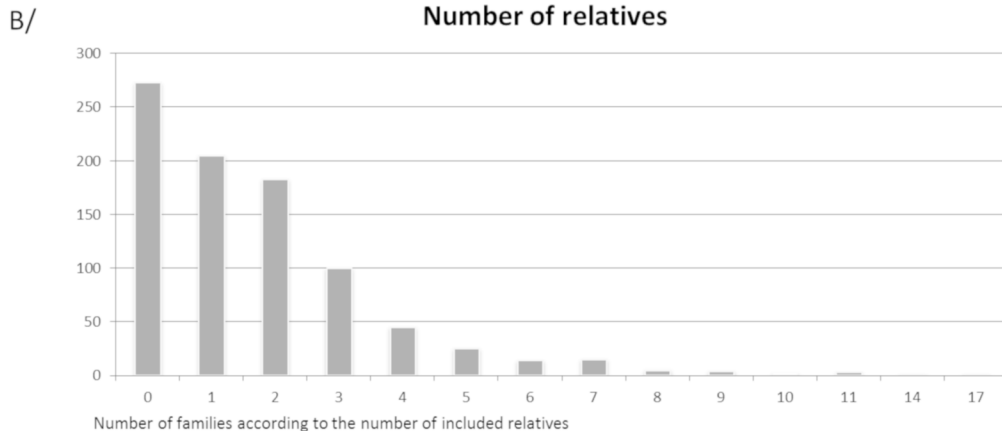
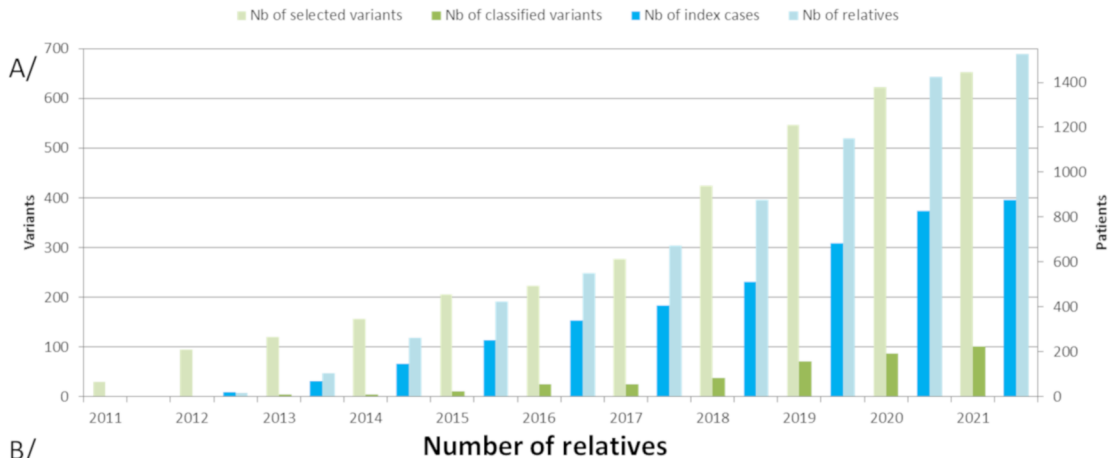
- Variant identified in at least three families
- Missense variant with AGVGD ⁽⁶⁾ class C35 to C65 using IARC alignment
- Splice variant leading to in-frame exon skipping outside a functional domain
- Splice variant with partial effect, *i.e.* resulting in both altered and normal transcript
- In-frame amino acid deletion in a functional domain
- Functional assay suggesting deleterious effect
- Literature data suggesting deleterious effect
- *BRCA1/2* variant database suggesting deleterious effect
- Other variant leading to same effect of amino acid change increasing family number at three

BRCA1

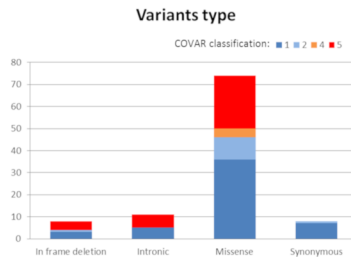


BRCA2

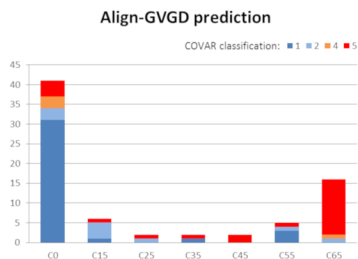




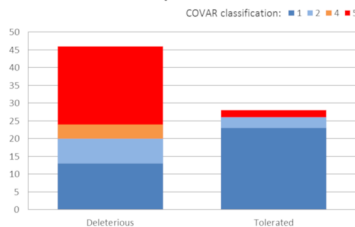
A/



B/



SIFT prediction



PolyPhen-2 prediction

