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A likelihood ratio approach for utilizing case-control data in the clinical classification of rare sequence variants: application to *BRCA1* and *BRCA2*

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Abstract

A large number of variants identified through clinical genetic testing in disease susceptibility genes, are of uncertain significance (VUS). Following the recommendations of the American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP), the frequency in case-control datasets (PS4 criterion), can inform their interpretation. We present a novel case-control likelihood ratio-based method that incorporates gene-specific age-related penetrance. We demonstrate the utility of this method in the analysis of simulated and real datasets. In the analyses of simulated data, the likelihood ratio method was more powerful compared to other methods. Likelihood ratios were calculated for a case-control dataset of *BRCA1* and *BRCA2* variants from the Breast Cancer Association Consortium (BCAC), and compared with logistic regression results. A larger number of variants reached evidence in favor of pathogenicity, and a substantial number of variants had evidence against pathogenicity - findings that would not have been reached using other case-control analysis methods. Our novel method provides greater power to classify rare variants compared to classical case-control methods. As an initiative from the ENIGMA Analytical Working Group, we provide user-friendly scripts and pre-formatted excel calculators for implementation of the method for rare variants in *BRCA1*, *BRCA2* and other high-risk genes with known penetrance.

Keywords

likelihood ratio; case-control; VUS; PS4; ACMG/AMP; variant classification; BRCA

Introduction

Clinical genetic testing of disease susceptibility genes often identifies variants of uncertain significance (VUS), complicating the clinical management of carriers and their families (Eccles et al., 2015). The assessment of the clinical significance of these rare sequence variants, including missense substitutions, in-frame deletions and insertions, and intronic variants, is essential to direct the clinical management of carriers and their relatives towards appropriate prevention, early detection and personalized treatments.

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The most widely used method for the interpretation of germline variants is via application of the standards and guidelines recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) (Richards et al., 2015). Strength levels (very strong, strong, moderate and supporting) are assigned to independent lines of evidence for or against variant pathogenicity. These strength levels are then combined and used in a scoring system to provide a clinical class, expressed as pathogenic, likely pathogenic, likely benign, benign, or VUS. These guidelines integrate various sources of information including the variant's nature and position (e.g., nonsense, frameshift, missense) and clinical data (e.g., prevalence in affected individuals and controls), and the combination of this information is interpreted to establish the significance of the variant under investigation with respect to risk. These criteria were recently reinterpreted in a quantitative Bayesian framework, which derived ranges of likelihood ratios (LRs) consistent with each of the evidence strength levels (Tavtigian et al., 2018). For case-control data, the specific criterion (PS4) states that a Relative Risk (RR) or Odds Ratio (OR) > 5.0 with nominal statistical significance (i.e., the confidence interval of the RR or OR does not include 1), provides strong evidence in favor of pathogenicity (Richards et al., 2015).

A significant advance in the classification of variants in cancer and other disease genes was the development of the multifactorial integrated likelihood ratio model (Goldgar et al., 2004); this model combines multiple features, under the assumption that each of them is an independent predictor of variant pathogenicity, in a Bayesian framework, thus providing a quantitative estimate of the pathogenicity of a variant (Goldgar et al., 2008). The ENIGMA consortium (Spurdle et al., 2012) has been applying and extending this multifactorial likelihood model. To date, application of this model has included clinically calibrated prior probabilities of pathogenicity derived from bioinformatic prediction of variant effect and location, along with a combined LR derived from clinical data (Goldgar et al., 2008), such as family history of cancer (Easton et al., 2007), breast cancer tumor pathology (Spurdle et al., 2014), variant co-segregation with disease (Belman, Parsons, Spurdle, Goldgar, & Feng, 2020; Thompson, Easton, & Goldgar, 2003), and variant co-occurrence in trans with a pathogenic variant (PV) in the same gene (Easton et al., 2007). This model can also incorporate LRs derived from variant frequency in cases and controls. Recently, case-control information derived from genotype data for 20 variants was incorporated into a comprehensive multifactorial likelihood analysis of *BRCA1* and *BRCA2* variants by ENIGMA (Parsons et al., 2019), using a method incorporating gene- and age-specific penetrance of PV carriers only. Such case-control LR calculations take into consideration gene- and age-specific penetrance values, and hence they might be expected to outperform the statistical measures currently recommended by ACMG/AMP for the analysis of case-control-data (i.e. OR or RR estimates).

In this paper, we present a novel case-control LR method, based on the same principle as used in the Parsons et al. (Parsons et al., 2019), that incorporates age information in both carriers and non-carriers in the dataset. The method can be used to obtain evidence in favor or against pathogenicity for rare variants in any gene for which there exist known age-specific penetrance estimates, based on data obtained from case-control studies. We illustrate the use of this method to calculate LRs for 24 *BRCA1* and 68 *BRCA2* variants from breast cancer case-control genotype data generated by the Breast Cancer Association

Consortium (BCAC) as part of the large-scale OncoArray project (Michailidou et al., 2017). We further demonstrate the utility of this case-control LR approach to aid interpretation of the clinical significance of variants using evidence aligned to ACMG/AMP code strengths or other classification methods.

Methods

Editorial Policies and Ethical Considerations

All participating studies were approved by the relevant ethics committees and informed consent was obtained from study participant (Michailidou et al., 2017). This research has been approved by the Cyprus National Bioethics Committee.

Case-control datasets

Simulated case-control dataset—Genotype data simulations were performed using the R (v3.6.1) (https://www.r-project.org/) statistical computing language. To create case-control datasets, genotypes for cases and controls were simulated using a Poisson distribution with *lambda* (λ) equal to the mean number of events (variant carriers) in the given interval, expressed as:

$$\lambda_{Cases} = N \times RR \times MAF$$

$$\lambda_{Controls} = N \times MAF$$

where N denotes the sample size, RR denotes the relative breast cancer risk of the causal variant and MAF denotes the minor allele frequency of the variant in the general population. Ages were simulated using normal distribution, with mean and standard deviation following the gene-specific age distribution in the CARRIERS population-based study (Hu et al., 2021).

Genotype data simulations were carried out for variants conferring a RR of 1 (indicating no increased risk), 2, 3, 4, 5, 6, 7, 8, 9 or 10, minor allele frequency in controls of 0.0001, 0.00005 or 0.00003 and sample size of N = 20,000 (20,000 breast cancer cases and 20,000 controls), 30,000 (30,000 breast cancer cases and 30,000 controls) or 50,000 (50,000 breast cancer cases and 50,000 controls). For each of these 90 scenarios, we simulated 10,000 replicates.

Additionally, in order to account for the possibility that age information is not available, we repeated the analysis using same age for all individuals.

BCAC OncoArray dataset—Genotype data were generated as part of the BCAC component of the OncoArray project (Michailidou et al., 2017) (studies included in the analysis are listed in Supplementary Table S1) and were available for 75,657 breast cancer cases and 52,987 controls of European ancestry. The majority of studies were population-based case–control studies, or case–control studies nested within population-based cohorts.

However, a subset of studies oversampled cases with a family history of breast cancer. Of these, 464 breast cancer cases and 1,347 controls had missing information regarding their age at diagnosis or interview, respectively and were excluded from the analyses. Another 1,445 cases and 858 controls were removed because their ages fell outside the interval of 21-80 years (the age range for which penetrance estimates were available). Cluster plots of 56 *BRCA1* and 127 *BRCA2* variants nominated by ENIGMA researchers for inclusion in the OncoArray project, were manually checked to review the automated calls. This was performed since automated genotype calling for rare variants from GWAS chips has been shown to be suboptimal (Coleman et al., 2016). Genotypes were adjusted for 41 *BRCA1* and 91 *BRCA2* variants, while 3 *BRCA1* and 2 *BRCA2* variants genotypes were determined to have been called correctly by automated clustering. Genotype recalling was not performed for 12 *BRCA1* and 34 *BRCA2* variants due to the low quality of the genotype data; these variants were not considered further.

After genotype cluster review and recalling, 16 *BRCA1* and 19 *BRCA2* variants were excluded from further analysis due to their high frequency (> 0.1%). Additionally, case-control LR calculations were not possible for four *BRCA1* and six *BRCA2* variants due to absence of variant carriers in the post filtering dataset. After these exclusions, case-control LR and logistic regression analysis was performed for 24 *BRCA1* and 68 *BRCA2* variants. It should be noted that some of the variants selected for the array have subsequently been classified or were those whose pathogenicity status were known and were included as positive or negative controls.

Statistical analyses

Case-control likelihood ratio method—This method (detailed in Supplementary File 1) compares the likelihood of the distribution of the variant of interest among cases and controls, under the hypothesis that the variant is associated with similar risks of the disease in question, as the "average" pathogenic variant (H_p), compared to the likelihood under the hypothesis that it is a benign variant not associated with increased risk (H_b). These risks may be age-, sex- and/or country-specific. Thus:

$$LR = \frac{Pr\left(Data \mid H_p\right)}{Pr\left(Data \mid H_b\right)}$$

where *Data* denotes observed data on carrier status of a variant of interest, case-control status and age at diagnosis or interview, combined over all individuals in the dataset.

In order to calculate the above LR, we follow a survival analysis framework. We first determine the probability that an individual with genotype k remains unaffected at age t, $S_k(t)$; and the corresponding probability that an individual with genotype k is affected at age t, $f_k(t)$ (where $k = 0$ or 1 for non-carriers and carriers, respectively). These probabilities can be computed from the age-specific baseline incidence, $\lambda_0(t)$; and the age specific log-relative risk of an assumed pathogenic variant in the gene of interest, $\beta(t)$. These probabilities are given by:

$$S_k(t) = \exp(-\int_0^t \lambda_0(t)e^{\beta(t)k}dt)$$

$$f_k(t) = S_k(t)\, e^{\beta(t)k}$$

As detailed in Supplementary File 1, the likelihood ratio is, to close approximation, given by:

$$ccLR = \frac{\prod_{v_j=1} S_1(t_j)e^{\beta(t)d_j} \,/\, S_0(t_j)}{(\sum_j^N S_1(t_j)e^{\beta(t)d_j} \,/\, S_0(t_j))^K} \,\Big/\, \frac{1}{N^K}$$

where N is the total number of individuals, K is the number of variant carriers, v_j is the variant status (0 for non-carriers and 1 for variant carriers) and d_j is the disease status (0 for controls and 1 for cases) for individual *j*.

The baseline incidence rates $\lambda_0(t)$ were taken from the age-specific background rates for England and Wales (1998-2002) (https://ci5.iarc.fr/CI5I-X/Default.aspx) and the age-specific breast cancer relative risks for pathogenic variant carriers $\beta(t)$ were taken from the recent large-scale BRIDGES (Breast Cancer Risk after Diagnostic Gene Sequencing) project (Dorling et al., 2021). To allow for possible carrier frequency differences by country, stratified LR calculations were performed within each country and then multiplied to provide a final LR.

Likelihood ratios are further translated into ACMG/AMP code strength categories according to published recommendations (Tavtigian et al., 2018). Likelihood ratio estimates in favor of variant pathogenicity are scored as: very strong, LR 350; strong, 350 > LR 18.7; moderate, 18.70 > LR 4.33 and supporting, 4.33 > LR 2.08. Likelihood ratio evidence for benign variant status are scored as: very strong, LR 0.0029; strong, 0.0029 < LR 0.053; moderate, 0.053 < LR 0.231 and supporting, 0.231 < LR 0.48. No evidence strength corresponded to estimates of 0.48 LR < 2.08.

In a series of sensitivity analyses, the method was applied using three other published RR estimates: from case series unselected for family history of breast cancer (Antoniou et al., 2003), cohort series of *BRCA1* and *BRCA2* carriers (Kuchenbaecker et al., 2017) and breast cancer hazard ratio estimates for missense *BRCA1* and *BRCA2* variants (Li et al., 2022). In order to account for country-specific effects, the stratified analysis was also performed using age- and country-specific incidence rates, derived from the Cancer Incidence in Five Continents, volume 9, 1998-2002, (https://ci5.iarc.fr/CI5I-X/Default.aspx). Age-specific breast cancer incidences for Greece and North Macedonia were retrieved from the 2020 cancer registry (European cancer information system, ECIS, https://ecis.jrc.ec.europa.eu/) since cancer incidence data were not available for the years 1998-2019. Unstratified analyses were also performed for comparison.

Detailed R scripts and pre-formatted excel calculators (user can either input individual-level data or tabulated by age groups) for the calculation of case-control LRs can be found using the following GitHub link: (https://github.com/BiostatUnitCING/ccLR). The files provided can be used to derive estimates based on the RR from Dorling et al. (Dorling et al., 2021), Kuchenbaecker et al. (Kuchenbaecker et al., 2017) or Antoniou et al. (Antoniou et al., 2003). In addition, this method can also be used to compute case-control LRs for variants in other disease susceptibility genes, by using age-specific penetrance estimates for the gene of interest (indicated by "Custom" gene in the pre-formatted excel calculators and R script). Furthermore, to allow for the possibility that age information is not available (or is only available for a subset of the dataset) user can incorporate individuals with unknown age at diagnosis or interview in any of the age groups specified in the tabulated calculator.

Odds ratio analysis—Odds ratio analysis was performed using logistic regression adjusted by age and country (if applicable) and Fisher's exact test (corrected using Haldane's method when simulations resulted in zero variant carriers in cases or controls (Agresti, 2003)). Logistic regression p-values were estimated using the likelihood ratio test. Based on the original ACMG/AMP recommendations (Richards et al., 2015), an OR estimate greater than 5.0, with the confidence interval not including 1.0, was used to define strong evidence of pathogenicity (PS4).

Evaluation and application of the case-control analyses methods—The simulated datasets were analyzed using the novel case-control LR method, logistic regression (adjusted by age), and Fisher's exact test. The case-control LR method was applied using age-specific breast cancer ORs for *BRCA1* and *BRCA2* PVs (Dorling et al., 2021). For causal variants with relative risk of 2 to 10, the power of the case-control LR method was estimated either as the probability of reaching at least supporting (LR ≥ 2.08) or at least strong pathogenic (LR ≥ 18.7) evidence. For benign variants with relative risk of 1, the power of the case-control LR method was estimated either as the probability of reaching at least supporting (LR ≤ 0.48) or at least strong (LR ≤ 0.053) benign ACMG/AMP evidence. Correspondingly, type I error for pathogenicity was calculated as the probability of obtaining at least supporting or at least strong pathogenic ACMG/AMP evidence when the relative risk was set to 1. Equivalently, type I error for evidence against pathogenicity was calculated as the probability of obtaining at least supporting or at least strong benign ACMG/AMP evidence when the relative risk was greater than one. The power of the OR methods was estimated as the probability of reaching the ACMG/AMP PS4 criterion (OR > 5.0, CI not including 1.0, p-value < 0.05). Following the analyses results of the simulated datasets, optimal LR cut-offs (to maximize power and minimize type I error) are used to define ACMG/AMP evidence strengths for the 92 variants included in the BCAC OncoArray dataset.

Results

Simulated datasets

Based on the simulation results for high-risk *BRCA1* (RR > 9) and *BRCA2* (RR > 5) variants, LR of strong and very strong evidence in favor of pathogenicity (LR ≥ 18.7) and

of at least supporting evidence against pathogenicity (LR 0.48), should be used in order to maintain a high power (> 80%) and low type I error (< 0.05) (Supplementary Table S2).

Results for all measures in all simulated datasets show that the power to achieve strong evidence in favour of pathogenicity is consistently greater for the case-control LR method using age-specific breast cancer risks compared to standard OR analysis methods (Figure 1, Supplementary Table S2). The power to correctly categorize variants with a RR comparable to a typical *BRCA1* PV was >80% in all scenarios except for small datasets (N 30,000) with causal variants present at the lower frequency (MAF = 0.00003) (Figure 1A).

In addition, the case-control LR method can also be used to obtain evidence against pathogenicity, something that cannot be achieved using standard OR analysis methods. Results from simulated case-control datasets of benign variants (RR of 1, Figure 2), show that the case-control LR method using the age-specific RRs of the "average" *BRCA1* PV, exhibits adequate power (> 80%) to identify variants with evidence against pathogenicity (LR 0.48) for larger datasets (N 30,000) and MAF of 0.0001.

The implementation of the method to account for datasets with missing information, assuming the same age for all individuals, demonstrated reduced power and increased type I error in all simulations. However, the type I error was still less than 0.05 in all cases (Supplementary Figures S1 and S2, Supplementary Table S3).

BCAC OncoArray dataset

Logistic regression results—Using logistic regression, two *BRCA2* variants (2%) (Table 1) reached strong pathogenic evidence following the ACMG/AMP classification criterion (PS4 criterion, OR > 5, p-value < 0.05, CI not including 1.0) (Richards et al., 2015). Detailed logistic regression results for all variants are shown in Supplementary Table S4.

Case-control LRs and ACMG/AMP Code Strengths—In the country-stratified baseline analysis (using the breast cancer ORs estimated from BRIDGES (Dorling et al., 2021)), evidence in favor of pathogenicity (defined as LR 18.70 following the simulation cut-offs) was achieved for 6 variants (6.5%) (Table 2), of which 3 variants were assigned very strong and another 3 strong strengths. Evidence against pathogenicity (defined as LR 0.48) was observed for 59 variants (64.1%), of which 26 were assigned very strong, 14 strong, 7 moderate and 12 supporting strengths. The results for the remaining 27 variants (29.3%) were uninformative. Case-control LRs and corresponding ACMG/AMP code strengths for all 92 *BRCA1* and *BRCA2* variants are shown in Supplementary Table S4. The different sensitivity analyses did not show any major discrepancies in the estimated LRs (Supplementary Table S5).

Discussion

This study provides a detailed description of the methodology to calculate case-control LRs for rare variants using case-control data, based on age- and gene-specific relative risks and age information for non-carriers. The LRs are calculated by comparing the likelihood

of the distribution of the variant of interest in cases and controls, under the hypothesis that the variant has similar age-specific relative risks as the "average" pathogenic variant, compared to the hypothesis that it is not associated with increased (or decreased) disease risk. We evaluated the method using simulated datasets and further applied it to derive LRs for pathogenicity for individual variants from analysis of genotype data from a large case-control study. These can now be used in combination with other evidence to inform variant classification - either according to ACMG/AMP classification standards and guidelines (Richards et al., 2015; Tavtigian et al., 2018), or using multifactorial likelihood modelling approaches (Goldgar et al., 2004; Parsons et al., 2019). Further, we provide user-friendly scripts and a pre-formatted excel calculator to facilitate the future implementation of this method for the calculation of case-control LRs. These resources may be readily applied for the calculation of LRs to be used in classification of VUS in the *BRCA1* and *BRCA2*, and other disease susceptibility genes with known penetrance values.

Notably, our results demonstrate the improved performance of our LR-based method for assessing variant pathogenicity as it considers gene- and age-specific penetrance for carriers and age information for non-carriers. Using simulated case-control datasets we show that the case-control LR method using age-specific breast cancer ORs from high-penetrance genes (e.g. *BRCA1* and *BRCA2*) outperforms other OR analysis methods. These observations reflect the fact that the method presented here is more suitable for the analysis of rare variants in a case-control setting. We further provide cut-offs of LRs in favor or against pathogenicity, to be used in a real setting.

Analysis of the BCAC OncoArray data using our proposed method provided informative pathogenic ACMG/AMP classification evidence for six out of 92 variants analyzed. Furthermore, 59 variants reached evidence against pathogenicity, something that is not directly measured as a code strength through classical calculations of ORs. Given that *a priori*, the vast majority of rare sequence variants (e.g., *BRCA1* and *BRCA2*) will be neutral with respect to risk, this is a key advantage of our approach. In contrast, using logistic regression analysis, informative ACMG/AMP classification criterion PS4 (OR > 5.0, p-value < 0.05, CI not including 1.0) was reached only for two variants.

There are possible caveats that should be recognized. Selection of cases or controls for a family history of cancer would affect the carrier probabilities. The likelihood ratios would then be inaccurate, but in principle this could be considered by incorporating family history in the likelihoods, if known. Depletion of cases with known pathogenic variants by prior clinical sequencing could also bias the likelihood ratios, therefore the method is best applied to population-based case-control studies. For these reasons we highlight the ACMG/AMP recommendation to review all available evidence for/against pathogenicity for a given variant, and to denote obviously conflicting findings for different evidence types, before assigning a final classification. A conservative approach may be to assign case-control weight with a cap, for example at moderate strength for or against pathogenicity.

Our method gains power in part because it leverages data on individual-level age, but we have to acknowledge that age is not always available. The method can be implemented more approximately by assuming that individuals with unknown information are of the same age,

but this reduces power, because the expectation that carriers of risk variants develop the disease at a younger age is then not utilised. It may also increase type I error, because the likelihood ratio may be calculated for an age that is not appropriate for the dataset (for example, if the dataset consists predominantly of older individuals), although the type I error was still low in the simulations we considered. In the tabulated pre-formatted calculator, we allow user to incorporate individuals with unknown age at diagnosis or interview, in any of the age groups specified. A conservative approach would be to include individuals with unknown age in the oldest age-group. In this way, case-control genotypes from both existing data and new series, with and without age data, can be incorporated. However, we would like to emphasize that pooling series, particularly from different populations with different age/ethnicity structures or with different genotyping technologies can lead to biased results. Ideally, datasets should be analysed separately and the overall likelihood ratio generated by multiplying the study-specific likelihood ratios.

Conclusions

This manuscript describes in detail a novel method used for the calculation of the case-control LR to provide evidence of variant pathogenicity. This LR method is more informative compared to logistic regression analysis (or an OR calculation based on contingency tables and Fisher's exact test). It improves power as it considers age- and gene-specific penetrance values and age information for non-carriers, and can provide both evidence in favor and against pathogenicity. In addition, this method can be also implemented towards the classification of VUS in any disease susceptibility gene for which disease-penetrance has been reliably estimated. Open access scripts and pre-formatted excel calculators with code and instructions on how to use the method, are available at the following address: https://github.com/BiostatUnitCING/ccLR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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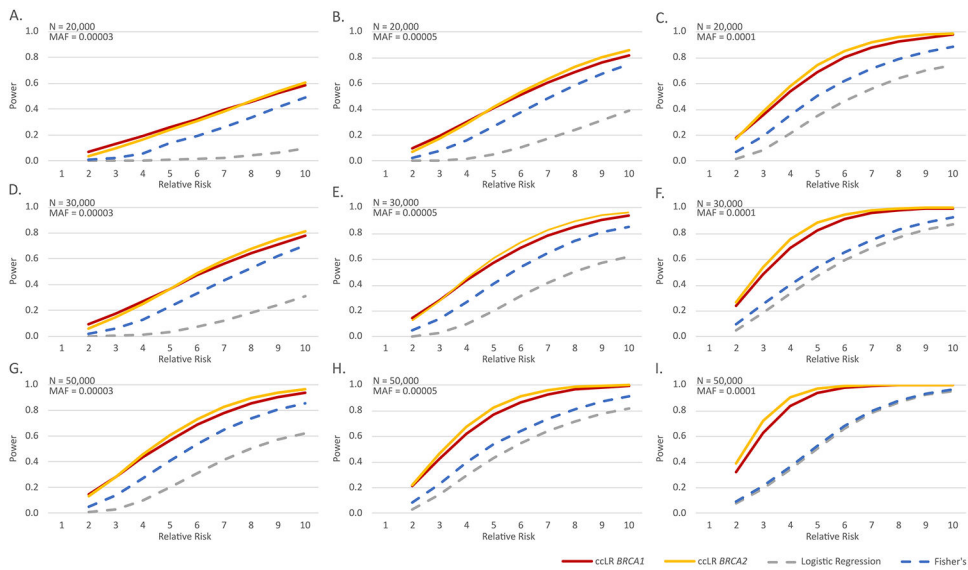
Figure 1.
Performance of the case-control likelihood ratio method and odds ratio analysis in providing at least strong ACMG/AMP evidence in favor of pathogenicity (LR 18.7), using simulated datasets. Power equals to the probability of reaching at least strong pathogenic ACMG/AMP evidence. Genotype data simulations were carried out for causal variants conferring disease relative risk between 2-10. We performed 10,000 simulations for each case scenario. Results represent simulated case-control data for 20,000 (panels A,B,C) or 30,000 (panels D,E,F) or 50,000 (G,H,I) breast cancer cases and controls and minor allele frequency of 0.00003 (panels A,D,G), 0.00005 (panels B,E,H) or 0.0001 (panels C,F,I). ccLR, case-control likelihood ratio; MAF, minor allele frequency; N, sample size.
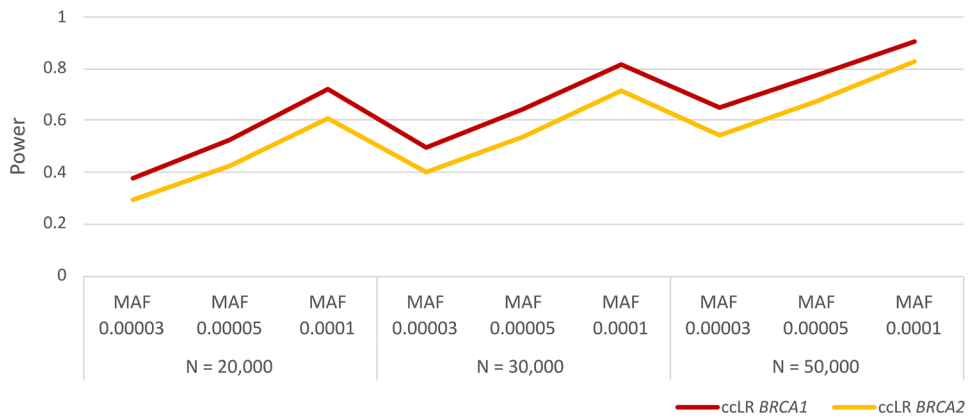
Figure 2.
Performance of the case-control likelihood ratio method in providing ACMG/AMP evidence against pathogenicity, using simulated datasets. Power equals to the probability of reaching at least supporting benign ACMG/AMP evidence (LR   0.48) when the relative risk was set to 1. We performed 10,000 simulations for each case scenario. Results represent simulated case-control data for 20,000, 30,000 or 50,000 breast cancer cases and controls and minor allele frequency of 0.00003, 0.00005 or 0.0001, ccLR, case-control likelihood ratio; MAF, minor allele frequency; N, sample size.

Table 1.

Statistically significant associated variants with breast cancer risk estimated by logistic regression (based on the ACMG/AMP PS4 criterion)

Gene	Variant_ID (GRCh37/hg19)	HGVS nucleotide	HGVS protein	Variant Carriers		OR (95%CI)	P-value
				Cases	Controls		
				N (Frequency)	N (Frequency)		
BRCA2	chr13_32937506_C_G	c.8167G>C	p. (Asp2723His)	18/72392 (2.49×10^{-4})	1/50680 (1.97×10^{-5})	12.30 (1.66-91.23)	0.014
	chr13_32954180_C_T	c.9154C>T	p. (Arg3052Trp)	10/72563 (1.38×10^{-4})	1/50779 (1.97×10^{-5})	8.32 (1.04-66.48)	0.045

Variant nomenclature according to: *BRCA2* (NM_000059.3, NP_000050.2)

Table 2.

Variants with informative LRs in favor of pathogenicity, estimated by the baseline analysis

Gene	Variant_ID (GRCh37/hg19)	HGVS nucleotide	HGVS protein	Variant Carriers		LR
				Cases	Controls	
				N (Frequency)	N (Frequency)	
BRCA1	chr17_41234451_A_G	c.4327C>T	p.(Arg1443*)	11/72558 (1.52×10^{-4})	3/50781 (5.91×10^{-5})	526.71
	chr17_41215947_T_G	c.5096G>T	p.(Arg1699Leu)	17/72560 (2.34×10^{-4})	3/50780 (5.91×10^{-5})	307.47
BRCA2	chr13_32937506_C_G	c.8167G>C	p.(Asp2723His)	18/72392 (2.49×10^{-4})	1/50680 (1.97×10^{-5})	8193.33
	chr13_32953453_A_G	c.8755-1G>A	p.?	3/72562 (4.13×10^{-5})	-	41.18
	chr13_32954180_C_T	c.9154C>T	p.(Arg3052Trp)	10/72563 (1.38×10^{-4})	1/50779 (1.97×10^{-5})	86.82
	chr13_32968940_A_T	c.9371A>T	p.(Asn3124Ile)	16/72548 (2.21×10^{-4})	-	3530.99

Variant nomenclature according to: *BRCA1* (NM_007294.4, NP_009225.1), *BRCA2* (NM_000059.3, NP_000050.2)

LR, Likelihood ratio.