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Prevalence of pathogenic variants and variants of unknown significance in patients at high risk of breast cancer: A systematic review and meta-analysis of gene-panel data

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ABSTRACT

Background: Gene-panels are used to assess predisposition to breast cancer by simultaneous testing of multiple susceptibility genes. This approach increases the identification of variants of unknown significance (VUS) that cannot be used in clinical decision-making. We performed a systematic review of published studies to calculate the prevalence of VUS and pathogenic variants (PV) in routinely tested breast cancer susceptibility genes in patients at high risk of breast cancer.
Methods: We comprehensively searched the literature using Medline through May 23, 2017 for studies conducting gene-panel testing on germline DNA of women with familial breast cancer and reporting on both PVs and VUSs. A meta-analysis of the collected data was carried out to obtain pooled VUS and PV prevalence estimates per gene using a generalized linear mixed model with logit link for binomial distribution.
Results: Of 602 publications, 4 were eligible and included 1870 patients. The panels encompassed 4–27 considered genes. Overall, the estimated probability per gene of a PV and VUS was 55% (95% confidence interval (CI) 26%–81%) and 91% (95% CI 78%–97%), respectively ($p = 0.0066$). The estimated probability per patient of a PV and VUS was 8% (95% CI 1%–34%) and 23% (95% CI 7%–52%), respectively ($p = 0.0052$). The ratio of VUS to PV was highest in the mismatch repair genes *MLH1*, *MSH2*, *MSH6*, *PMS2* (18.7), *CDH1* (13.4) and *ATM* (9.5). Amongst the 1468 patients tested for *BRCA1* and *BRCA2*, only these two genes had a VUS to PV ratio of less than one (0.2 and 0.6, respectively).
Conclusion: With the current panels, the probability of detecting a VUS is significantly higher than the probability of detecting a PV. Better classification of VUSs is therefore critical and requires gene-specific VUS-assessment in every future study of gene-panel testing in patients at high risk of breast cancer.

1. Introduction

Next-generation sequencing technologies allow for the sequencing of multiple genes simultaneously in a cost-effective way, increasing the likelihood of detecting variants (Walsh et al., 2010). This technological breakthrough allowed commercial gene-panel testing for breast cancer predisposition to enter clinical practice, with a promise towards personalized care (Desmond et al., 2015). Since the discovery two decades ago that mutations in *BRCA1* and *BRCA2* cause hereditary breast and ovarian cancer syndrome with an autosomal dominant pattern of inheritance, a huge amount of research has been dedicated to these genes. Evidence now demonstrates that screening and prevention measures adapted to this high-risk population reduce cancer-related mortality (NCCN, 2018).

Several breast cancer susceptibility genes have since been discovered. Some of them have been validated as increasing the risk of breast cancer, thus warranting a wide range of clinical measures. These genes form with *BRCA1* and *BRCA2* the core of all gene-panels. Mutations in *TP53*, *STK11*, *PTEN* and *CDH1* are well studied and predispose to Li Fraumeni syndrome, Peutz-Jeghers syndrome, Cowden disease and hereditary diffuse gastric cancer, respectively. *PALB2* variants have also been determined to be of high penetrance using robust

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life-time risk of breast cancer estimates (Antoniou et al., 2014). *ATM*, *CHEK2*, *NBN* and *NF1* are considered to only moderately increase the risk, with only *CHEK2* presenting acceptable confidence in the inherent risk estimates (Schmidt et al., 2016). Numerous others are suspected to raise the risk of breast and/or ovarian cancer, but still need confirmation, given their low penetrance, very rare prevalence, and/or divergent results between studies (Nielsen et al., 2016). Genes belonging to this category, such as *RAD51C*, *RAD51D*, *BRIP1*, *BARD1*, *MRE11A*, *FANCM*, *RECQL*, *MLH1*, *MSH2*, *MSH6* and *PMS2*, are generally included in the panels (Easton et al., 2015). Yet, the clinical yield of adding such genes to a gene-panel needs improvement, for two main reasons. The cancer risk estimation associated with these genes is unknown or at best vague. This was recently highlighted in a meta-analysis deemed to obtain more robust estimates of the cancer risk associated with loss-of-function mutations in *ATM*, *PALB2*, *CHEK2*, *NBN*, *BRIP1* and *RAD50* (Aloraifi et al., 2015). Aloraifi et al calculated pooled odds ratios for protein-truncating variants of 3.2 (95% CI 2.0–5.0) for *ATM*, 3.3 (95% CI 2.6–4.1) for *CHEK2* and 21.4 (95% CI 10.1–45.3) for *PALB2*. They however lacked sufficient data concerning *NBN*, *BRIP1* and *RAD50*. Secondly, these approaches generate far more variants of unknown significance (VUS) than pathogenic variants (PV), the ratio being correlated to the size of the panel (Kurian et al., 2014).

The issue of VUSs is not systematically addressed by studies reporting on the results of gene-panel testing in patients at high risk of breast cancer. The main objective of this systematic review is to assess the prevalence of PVs and of VUSs when performing gene-panel testing in a population considered at high risk for breast cancer. This will give an insight of the magnitude of this issue and of the benefit of routinely testing for the presence of mutations outside *BRCA1* and *BRCA2*.

2. Methods

2.1. Study approach

The methods used for this systematic review and meta-analysis follow the guidelines established by PRISMA-P (Shamseer et al., 2015). Cochrane's handbook (handbook.cochrane.org) was used during the preparation of the protocol.

2.2. Search criteria

We first broadly searched for publications addressing the presence of PVs in patients at high risk of breast cancer. This search was conducted on Medline retaining English literature. Queried terms were combinations of MeSH and free terms : ((inherited OR familial OR hereditary OR family OR bilateral OR high risk) AND (mutation OR mutated OR variant) AND (truncating OR pathogenic) AND ("breast cancer" OR "breast neoplasm") AND gene).

We subsequently focused on studies that specifically conducted gene-panel testing on germline DNA of women diagnosed with breast cancer who had a family history of breast cancer or had presented bilateral breast cancer, and reported the presence of PVs as defined by precise criteria (variants affecting splice-sites, creating a premature stop codon, creating a frameshift or proven by functional testing to be pathogenic) and the presence of VUSs. We then went through the references of all selected studies and relevant reviews to identify missing studies. To avoid missing data, conference abstracts referenced by the American Society of Medical Oncology (meetinglibrary.asco.org) or European Society for Medical Oncology (oncologypro.esmo.org) were also considered, but only retained if also published in the literature.

A review of the studies was first performed on the basis of the title and abstract, and subsequently more precisely by full text reading, with the following preplanned criteria : regarding the population, we excluded studies conducted on male breast cancer only, sporadic breast cancer, early onset breast cancer without presence of a family history, or bilateral breast cancer. Regarding the intervention, we excluded studies testing only *BRCA1* or *BRCA2*, analyzing somatic DNA only, not defining the variants considered pathogenic, not reporting VUSs or considered case reports of a specific variant.

The studies were independently evaluated by two reviewers (CVM, FPD). Disagreements between reviewers were resolved by discussion.

Table 1
List of considered genes and their inclusion (0 : gene not present ; 1 : gene present) in the gene-panel used in each publication.

Gene	Kim (Kim et al., 2017)	Kraus (Kraus et al., 2017)	Maxwell (Maxwell et al., 2016)	Tung, cohort 1 (Tung et al., 2016)	Tung, cohort 2 (Tung et al., 2016)
ATM	0	1	1	1	1
BAP1	0	0	1	0	0
BARD1	0	0	1	1	1
BLM	0	0	1	0	0
BRCA1	0	1	1	1	0
BRCA2	0	1	1	1	0
BRIP1	0	0	1	1	1
CDH1	0	1	1	1	1
CHEK2	1	1	1	1	1
EPCAM	0	0	0	1	1
FANCC	0	0	1	0	0
FANCM	0	0	1	0	0
MLH1	0	1	1	1	1
MRE11A	1	0	1	0	0
MSH2	0	1	1	1	1
MSH6	0	1	1	1	1
NBN	0	1	1	1	1
NF1	0	0	1	0	0
PALB2	1	1	1	1	1
PMS2	0	1	1	1	1
PPM1D	0	0	1	0	0
PTEN	0	0	1	1	1
RAD50	1	0	1	0	0
RAD51C	0	1	1	1	1
RAD51D	0	1	1	1	1
RECQL	0	0	0	0	0
STK11	0	0	1	1	1
TP53	0	1	1	1	1
XRCC2	0	0	1	0	0
Total	4	14	27	18	16

2.3. Data extraction

Data was independently extracted by two reviewers (CVM, AC). Disagreements between reviewers were resolved by discussion. Preplanned extraction compiled following information for each study: 1) regarding the population : criteria used for selection of the subjects, total number of cases, age, ethnicity, subtype of breast cancer, previous genetic testing results if relevant, and 2) regarding the intervention : number and names of sequenced genes, PV count (global, per gene and per patient), VUS count (global, per gene and per patient), type of database used to classify the variant, and type of study (academic vs involvement of the sequencing company in the authorship). In each selected study, only the known or strongly suspected susceptibility genes were considered. This gene list was designed before data extraction (Table 1). Study data were collected and managed using REDCap electronic data capture tools, allowing double data entry to ensure high data quality (Harris et al., 2009).

2.4. Statistical analysis

The database was extracted from REDCap for analysis in SAS software (version 9.4). All statistical analyses were preplanned. Standard methods were used, recommended by the PRISMA-P guidelines (Shamseer et al., 2015). Raw data were used to calculate percentage of presence of PVs and VUSs in each publication and globally, both per gene and per patient. 95% confidence intervals by publication were

estimated based on logit approximation. Likewise, global presence of PVs and VUSs was estimated using a generalized linear model with a logit link for binomially distributed data. The proportions of genes or patients with either PVs or VUSs were compared based on a generalized linear model with the publication as random effect, and the nature of mutation (PV or VUS) as fixed effect. For each considered gene, heterogeneity among the selected studies was detected with chi-squared tests ($p < 0.05$ indicating significant heterogeneity) and if present quantified with the I2 statistic (I2 value > 50% indicating substantial heterogeneity).

The methodological quality of observational studies can be difficult to assess. We used the validated Downs & Black checklist for non-randomized studies (Downs and Black, 1998). We also used a personal version of a validated quality appraisal tool for case series, adapted in advance to this systematic review (Moga et al., 2012). This personalized version consisted of 16 preselected items pertaining to seven different areas, with criteria regarding study objectives, study population, intervention, outcome measures, statistical analyses, results, conclusions, competing interests and sources of support.

3. Results

3.1. Characteristics of the studies

Out of the 602 publications considered, only 4 met our inclusion criteria (Fig. 1) (Kim et al., 2017; Kraus et al., 2017; Maxwell et al., 2016; Tung et al., 2016). These publications are further referenced by the first author name, with in addition the cohort for the Tung publication (Tung et al. (2016)), as it includes two independent cohorts published in one single manuscript. The studies included 1870 breast cancer patients from families at high risk, 402 of whom had previously tested negative for *BRCA1* and *BRCA2* (Table 2). Supplementary material in Tung et al., (2016) permitted to derive the necessary data regarding the subgroup of patients with a personal history of breast cancer and from a family at high risk. Information about age of the patients, although planned to be collected initially, could not be retrieved in the selected publications. Globally, the Caucasian ethnicity was the most frequent and formed the main origin of patients in the publications of Kraus (Kraus et al., 2017) and Tung (both cohorts (Tung et al. (2016))). Kim's cohort was solely made of Asian (Korean) patients. Information regarding Ashkenazi Jewish heritage was present in only one publication (excluded from Tung cohort 1, 25% of the patients in Tung cohort 2 (Tung et al. (2016))) (Table S1). Each panel encompassed 4 to 27 of the 29 genes we considered in this study. Only one gene (*RECQL*) was not present in any panel. Sequencing methods were globally similar between the four publications : all were performed on HiSeq or MiSeq sequencers (Illumina Inc., San Diego, USA) using paired-end sequencing reads aligned to the GRCh37/Hg19 version of the human genome. Two publications (Kim et al., 2017; Tung et al., 2016) were collaborations between academic and commercial

Table 2
Number of subjects and breast cancer characteristics in each publication.

Publication	Number of subjects tested	Number of subjects with a personal history of breast cancer	Number of subjects with triple negative breast cancer	Number of subjects previously tested for *BRCA1 / BRCA2*
Kim	235	235	NA	235
Kraus	581	581	179	0
Maxwell	253	253	NA	0
Tung, cohort 1	634[a]	634	NA	0
Tung, cohort 2	167[a]	167	NA	167

NA: not available.
[a] Representing the subgroup of patients with a personal history of breast cancer and from a family at high risk.

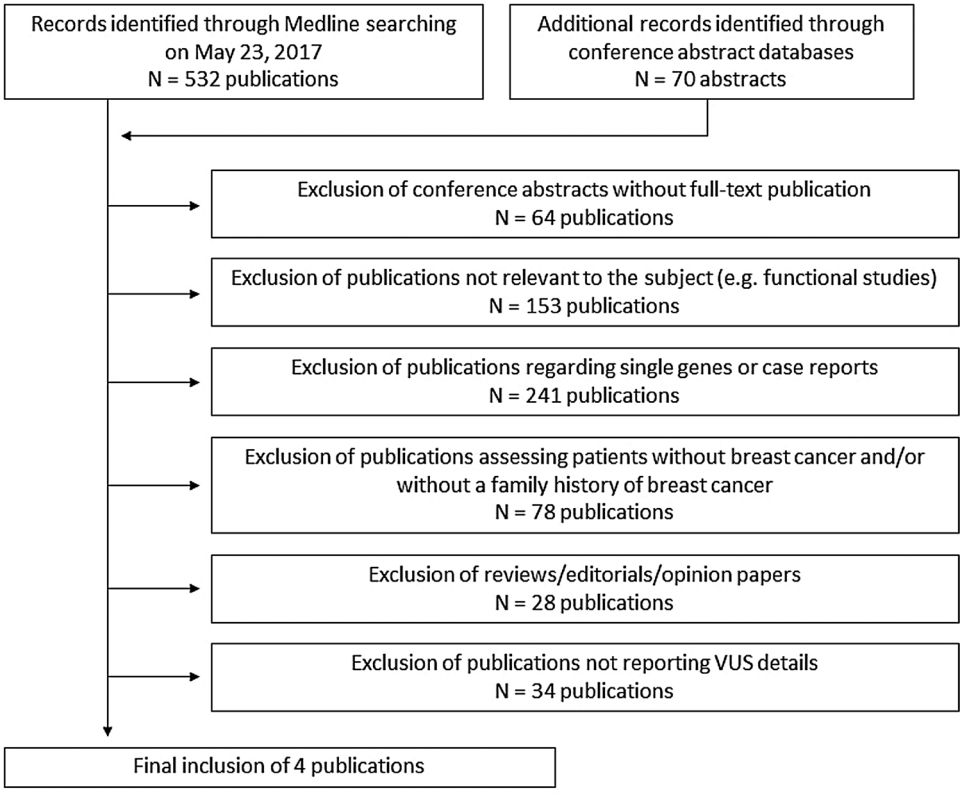

Fig. 1. PRISMA flow-diagram depicting the two-staged search-strategy, number of considered publications, included and excluded studies and main reasons for exclusion.

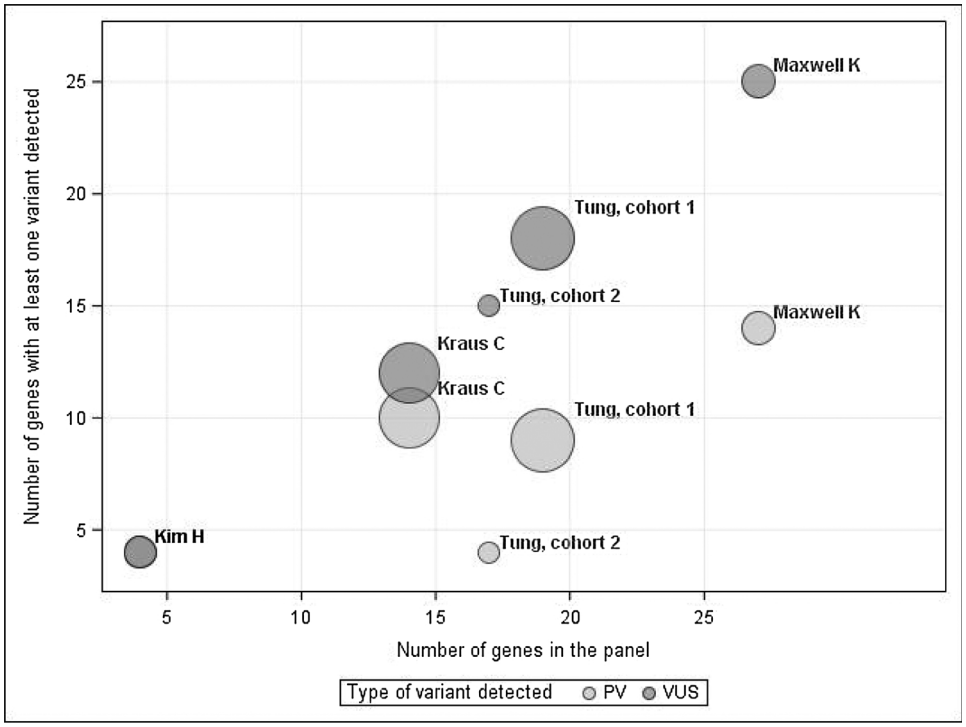

Fig. 2. Number of pathogenic variants (PV) or variants of unknown significance (VUS) detected according to the number of genes in the panel used. Bubble size reflects the number of patients tested. Bubbles superimpose for the publication of Kim.

laboratories. Of the two remaining (Kraus et al., 2017; Maxwell et al., 2016), all authors were academics.

3.2. Global PV and VUS data

The extracted raw data of PVs and VUSs in each publication are summarized in table S2. Based on these, we estimated the probability of carrying a PV at 55% (95% confidence interval (CI) 26%–81%) and of carrying a VUS at 91% (95% CI 78%–97%) per gene (Table S3). This difference was statistically significant ($p = 0.0066$). At the exception of the publication of Kim et al., (2017) (only 4 genes), genes that carried a VUS outnumbered those that carried a PV. This difference increased with the size of the panel (Fig. 2).

One publication (Maxwell et al., 2016) did not provide information to extract the number of patients with at least one PV or one VUS. Considering the patients of the remaining publications, the estimated probability of carrying a PV or a VUS was 8% (95% CI 1%–34%) and 23% (95% CI 7%–52%) for each patient, respectively (Table S4). This difference was also statistically significant ($p = 0.0052$). However, compared to the analysis by gene, the size of the panel did not appear to be related to the magnitude of the difference (Fig. 3).

3.3. PVs and VUSs data per gene

For downstream analyses, only the genes carrying at least one PV and/or VUS were considered. To increase the effect size, the mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, *PMS2* were considered as one entity. Besides, MMR-deficient patients are generally offered uniform recommendations for cancer surveillance and prevention (NCCN, 2017). The list of genes was thus reduced to *ATM*, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, MMR-genes, *MRE11A*, *PALB2*, *PTEN* and *TP53*. Data regarding each publication can be found in Table S5. The publication of Maxwell (Maxwell et al., 2016) lacked patient information allowing part of these analyses. Data were thus pooled for each gene (Table 3, graphically depicted in Fig. 4). As expected, *BRCA1*, *BRCA2*, *CHEK2* and *PALB2* were the genes most frequently carrying a PV, with *BRCA1* and *BRCA2* accounting for 72% of the PVs. This underscores the rarity of PVs in the remaining genes, found in < 0.7% for each within the tested patients. For each gene, the number of VUSs per patient was more evenly distributed, with only *MRE11A* presenting a VUS in < 1% of the tested patients.

To get a more robust, pooled estimate for the probability of a patient to be detected with ≥ 1 PV or VUS, we modeled this information by taking into account only the genes (*ATM*, *PALB2*, *CHEK2*, *TP53*, *CDH1* and the MMR-genes) present in at least three gene-panels (Table 4). This confirmed that all of them had a higher probability of being detected with a VUS than with a PV.

We then calculated for each gene the pooled ratio of the number of patients with ≥ 1 VUS to the number of patients with ≥ 1 PV (Fig. 5). This VUS/PV ratio is highest in the pooled MMR-genes (18.7), *CDH1* (13.4) and *ATM* (9.5). *BRCA1* and *BRCA2* were the only genes with a ratio < 1 (0.2 and 0.6, respectively). *PALB2* and *CHEK2*, the other two frequently sequenced genes in this population, had a VUS/PV ratio of 2.5 and 2.2, respectively.

Of the 836 considered VUSs, 565 (67.6%) were found only once. Eighty-four VUSs (10.1%) were reported more than once (Table S6). Of these, 43 (5.1%) were found in 2 or 3 cohorts. No VUS was present in more than 3 cohorts.

3.4. Assessment of heterogeneity

We assessed heterogeneity among the publications based on a Chi-square test. Statistical tests revealed heterogeneity among the different gene-panels regarding the percentage of genes carrying a PV ($p = 0.022$, $I2 = 65\%$). However, no heterogeneity was demonstrated regarding the percentage of genes detected with a VUS ($p = 0.83$) (Table S7). Differences between the considered genes were observed regarding the percentage of patients with ≥ 1 VUS or PV (Table S8). For both PV and VUS, no heterogeneity was observed for the core genes *BRCA1*, *BRCA2* and *CHEK2*. Significant heterogeneity was present regarding the VUS assessment, but not the PV assessment, for *ATM*, *PALB2*, *TP53* and the MMR-genes. In line with recommendations, publication bias was

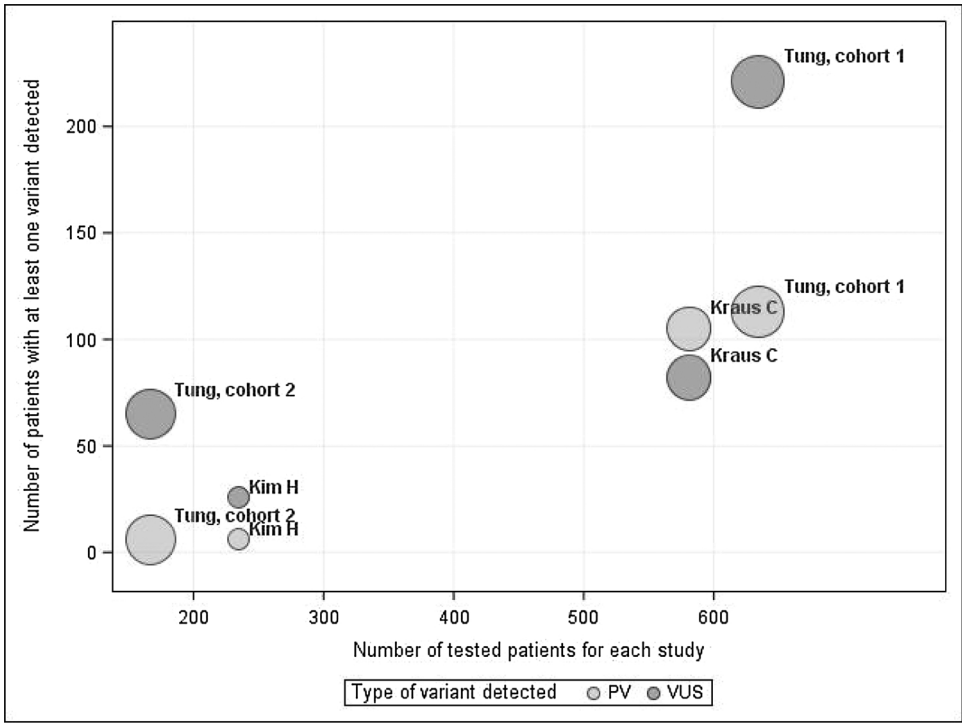

Fig. 3. Number of pathogenic variants (PV) or variants of unknown significance (VUS) detected according to the number of patients in the panel used. Bubble size reflects the number of genes in each panel.

not explored as less than 10 publications were considered (Sterne et al., 2011).

3.5. Risk of bias

Table S9 summarizes the risk of bias assessment according to the validated Downs and Black checklist (Downs and Black, 1998). The different studies were homogeneous and presented good quality of reporting and external validity profile. Due to the type of studies, internal validity could not be fully assessed or appeared weak.

Using our personally adapted version of the Moga quality appraisal tool for case series studies (Moga et al., 2012), 16 pre-selected quality criteria pertaining to seven different areas were assessed for the quality of the selected manuscripts (Table S9). For study objective, the quality criterion was met by all publications. For study population, all quality criteria but one were met by all publications. For intervention, outcome measures and statistical analysis, all quality criteria were met by all publications. For "results and conclusion", the criterion "reference population used as control" was not met (for all publications), but the other quality criterion was met. For "competing interests and sources of support", either information about competing interest and sources of support was not reported, or a commercial company participated to the study. Only one study met both criteria for quality in this respect.

4. Discussion

We performed a systematic review to comprehensively evaluate the prevalence of PVs and VUSs when performing gene-panel testing in a population considered at high risk for breast cancer, and to give an insight about the underreporting of VUS results in the clinical literature. Out of the 602 identified records, only four studied well-defined high-risk breast cancer cases and reported both PV and VUS data. Less than

Table 3
Pooled information regarding : number of pathogenic variants (PVs), patients with at least one PV, number of variants of unknown significance (VUS), and patients with at least one VUS, according to the selected genes. Includes information collected by Maxwell (Maxwell et al., 2016) for the number of PV/VUS, without taking into account the patients tested in Maxwell for number of patients with at least one PV/VUS.

Gene	Number of PV	Number of patients with at least one PV					Number of VUS	Number of patients with at least one VUS				
		N	*n*	%	LL	UL		*N*	*n*	%	LL	UL
BRCA1	90	1215	86	7.08	5.76	8.66	15	1215	15	1.23	0.75	2.04
BRCA2	76	1215	70	5.76	4.58	7.22	47	1215	40	3.29	2.42	4.46
CHEK2	27	1617	25	1.55	1.05	2.28	61	1617	54	3.34	2.57	4.34
PALB2	13	1617	12	0.74	0.42	1.30	28	1617	30	1.86	1.30	2.64
ATM	14	1382	9	0.65	0.34	1.25	110	1382	85	6.15	5.00	7.55
MRE11A	2	235	1	0.43	0.06	2.96	4	235	2	0.85	0.21	3.34
MMR-genes	3	1382	3	0.22	0.07	0.67	74	1382	57	4.12	3.19	5.31
CDH1	3	1382	2	0.14	0.04	0.58	30	1382	26	1.88	1.28	2.75
TP53	6	1382	2	0.14	0.04	0.58	18	1382	16	1.16	0.71	1.88
PTEN	0	801	0	0	0	–	10	801	10	1.25	0.67	2.30

N = total number of patients tested. *n* (%) = number (and percentage) of patients with PV/VUS detected for the corresponding gene. LL, UL = lower and upper limits of the 95% Confidence Interval for the percentage. MMR: mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2* pooled together.

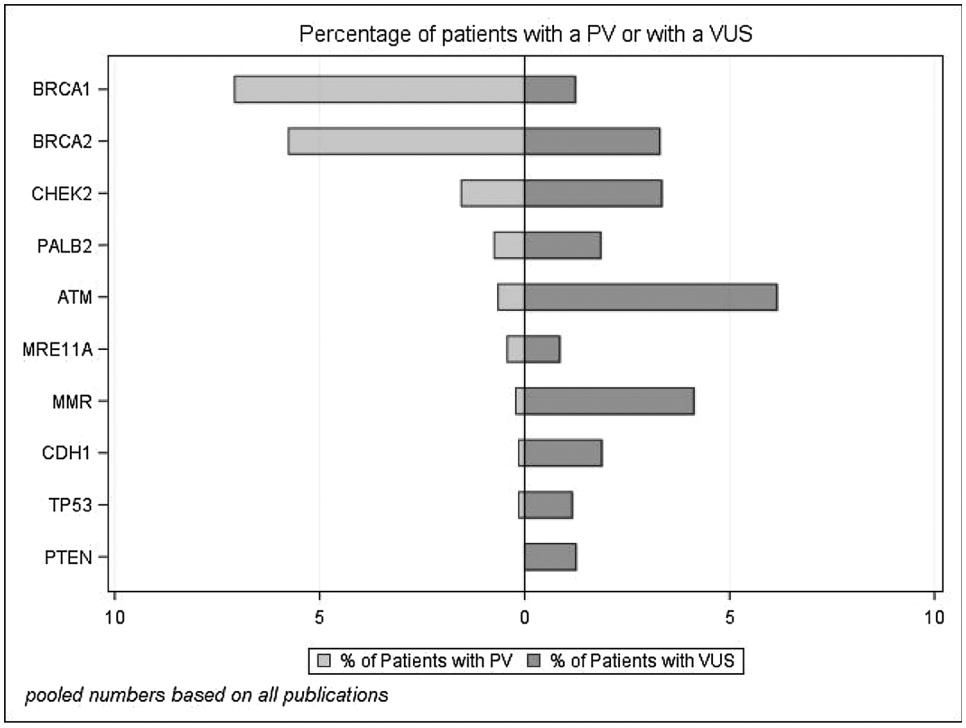

Fig. 4. Pooled number of pathogenic variants (PV) and variants of unknown significance (VUS) for each selected gene.

Table 4
Pooled estimate for probability of a patient to be detected with at least one pathogenic variant (PV) and -separately- with at least one variant of unknown significance (VUS). Only genes with information available from at least 3 panels included in this model.

Gene	Number of publications including this gene	Percentage of patients with ≥ 1 PV			Percentage of patients with ≥ 1 VUS		
			95% CI			95% CI	
		estimate	LL	UL	estimate	LL	UL
ATM	3	0.65	0.16	2.69	4.98	0.13	68.21
PALB2	4	0.74	0.30	1.84	2.12	0.59	7.32
CHEK2	4	1.55	0.82	2.90	3.34	2.18	5.09
TP53	3	0.11	0.00	10.49	1.05	0.05	18.97
MMR- genes	3	0.22	0.02	2.55	3.66	0.27	34.37
CDH1	3	0.10	0.00	67.96	2.07	0.52	7.85

10% of the publications satisfying the population criteria published VUS data in addition to the PVs. By data aggregation, we estimated that presently, per gene, the probability of carrying a VUS is significantly higher than the probability of carrying a PV (91 vs 55%). The main reason for this is that only a few genes historically benefited from widespread sequencing in this population. Most of the variants in the suspected susceptibility genes lack confirmation of their pathogenicity and are thus presently considered of unknown significance. Altogether, a VUS was found in 23% of the patients, whereas only 8% carried a PV. These differences appear more pronounced as the number of genes included in the panel increases. Only a minority of the VUSs (5.1%) were reported in more than one cohort. For these variants, pooling of the data of several cohorts could help assess their true clinical significance.

BRCA1 and *BRCA2* were the genes carrying more than 70% of the PVs. *CHEK2* and *PALB2*, the other two well-studied breast cancer susceptibility genes, account for another 15%. This clear delineation between the genes cannot be found anymore when considering VUSs. Subsequently, the VUS/PV ratio was < 1 only for *BRCA1* and *BRCA2*, and the closest to one for *PALB2* and *CHEK2*. Thus, genes outside the ones previously screened in single-gene approaches, but currently included in the panels, carry a higher probability of being detected with a VUS than with a PV. This translates into a negative risk-benefit balance regarding the probability of helping clinical decision-making.

Heterogeneity among the publications is a limitation regarding the calculation of the observed effect sizes of our systematic review. It also demonstrates what is known and what still needs to be studied in more detail. Variant classification appears more homogeneous regarding the historically well-known genes *BRCA1*, *BRCA2* and *CHEK2*, studied across wide populations. Pathogenic variants in *PALB2*, *TP53*, *ATM* and the MMR-genes are well known to predispose to breast and pancreatic cancer, Li-Fraumeni syndrome, Ataxia-Telangiectasia syndrome and Lynch syndrome, respectively. Percentage of PVs in these genes appear homogeneous among the publications. This points towards an equivalent classification of a variant as a PV by the different genetic centers. Conversely, heterogeneity of the VUS results in these genes, and the global results in the remaining genes, are arguments towards a blur still surrounding their classification. Differences in the screened populations also explain part of the heterogeneity, as rare variants are prone to be clustered in specific populations.

Gene-panels should be seen as a formidable opportunity to collect ever increasing amounts of genetic data. Given the difficulty to correctly interpret and classify the majority of the variants in recently discovered genes, only the well-established breast cancer susceptibility genes should be mentioned on routine clinical reports. All variants in the remaining genes, whether PVs or VUSs, should be aggregated in large research open access databases and joined to the list of clinically useful genes only when both risk estimates and variant classification become obvious (Thompson et al., 2016). This is becoming even more important as the gene-panels are now not only used for detecting genetic predisposition to a disease, but increasingly also with the aim of unraveling somatic mutations predictive of a response to a personalized treatment. Virtually all of these panels contain genes with an impact at the germline level. We can suspect the number of VUSs discovered in these genes to dramatically increase, corresponding to the passenger mutations arising somatically due to the genomic instability that is

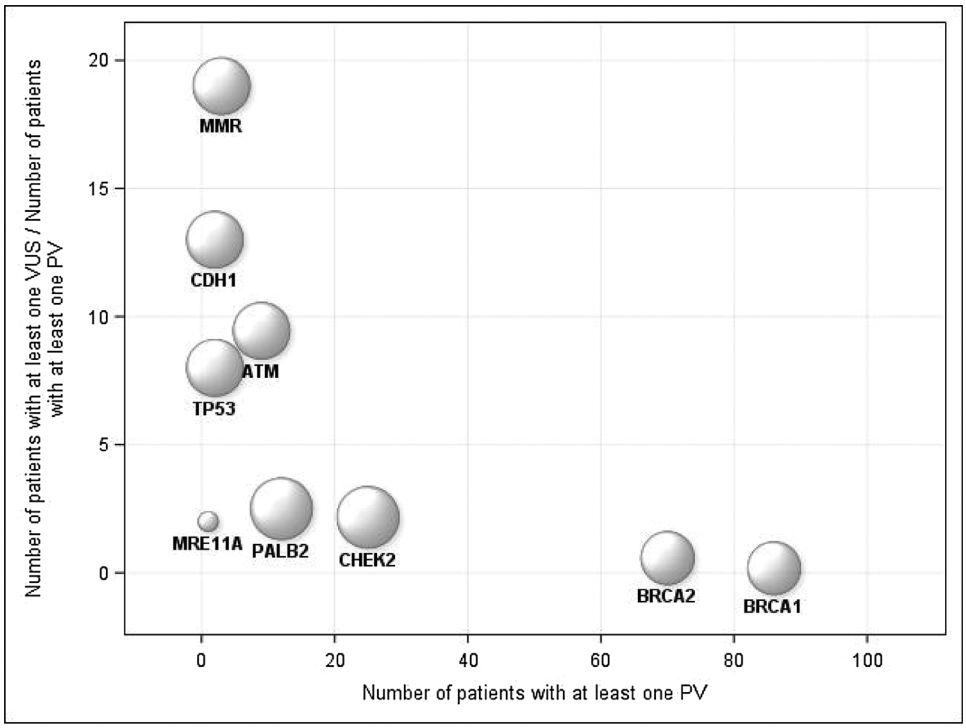

Fig. 5. Pooled data for each gene regarding the ratio between the number of patients with at least one variant of unknown significance (VUS) to the number of patients with at least one pathogenic variant (PV). Data from Maxwell lacked this information. MMR : mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2* pooled together. Bubble size represents the number of patients tested.

driving tumorigenesis.

This systematic review highlights the lack of VUS reporting in the majority of gene-panel studies for high-risk breast cancer families. In the panels used for patients at high risk of breast cancer, the probability of detecting a VUS is significantly higher than the probability of detecting a PV. As exemplified by the calculated VUS/PV ratios, only *BRCA1* and *BRCA2* appear to demonstrate a positive risk-benefit balance regarding the probability of helping clinical decision-making. Systematic VUS assessment and inclusion in open access databases in upcoming studies could gradually turn the tide for the remaining breast cancer susceptibility genes.

Conflicts of interest

The authors have declared no conflicts of interest.

Authorship contribution

CVM and FD performed the study query and evaluated the studies for inclusion. CVM and AC extracted the data, performed the statistical analyses and interpretation. CVM and FD drafted the article. All authors revised it critically and approved the final submitted version.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.critrevonc.2018.09.009.

References

Aloraifi, F., McCartan, D., McDevitt, T., et al., 2015. Protein-truncating variants in moderate-risk breast cancer susceptibility genes: a meta-analysis of high-risk case-control screening studies. Cancer Genet. 208, 455–463.
Antoniou, A.C., Casadei, S., Heikkinen, T., et al., 2014. Breast-cancer risk in families with mutations in PALB2. N. Engl. J Med. 371, 497–506.
Desmond, A., Kurian, A.W., Gabree, M., et al., 2015. Clinical actionability of multigene panel testing for hereditary breast and ovarian cancer risk assessment. JAMA Oncol. 1, 943–951.
Downs, S.H., Black, N., 1998. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J. Epidemiol. Commun. Health 52, 377–384.
Easton, D.F., Pharoah, P.D., Antoniou, A.C., et al., 2015. Gene-panel sequencing and the prediction of breast-cancer risk. N. Engl. J. Med. 372, 2243–2257.
Harris, P.A., Taylor, R., Thielke, R., et al., 2009. Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. J. Biomed. Inf. 42, 377–381.
Kim, H., Cho, D.Y., Choi, D.H., et al., 2017. Frequency of pathogenic germline mutation in CHEK2, PALB2, MRE11, and RAD50 in patients at high risk for hereditary breast cancer. Breast Cancer Res. Treat. 161, 95–102.
Kraus, C., Hoyer, J., Vasileiou, G., et al., 2017. Gene panel sequencing in familial breast/ovarian cancer patients identifies multiple novel mutations also in genes others than BRCA1/2. Int. J. Cancer 140, 95–102.
Kurian, A.W., Hare, E.E., Mills, M.A., et al., 2014. Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk assessment. J. Clin. Oncol. 32, 2001–2009.
Maxwell, K.N., Hart, S.N., Vijai, J., et al., 2016. Evaluation of ACMG-guideline-based variant classification of cancer susceptibility and Non-cancer-associated genes in families affected by breast cancer. Am. J. Hum. Genet. 98, 801–817.
Moga, C., Guo, B., Schopflocher, D., Harstall, C., 2012. Development of a Quality Appraisal Tool for Case Series Studies Using a Modified Delphi Technique. http://www.ihe.ca/publications/development-of-a-quality-appraisal-tool-for-case-series-studies-using-a-modified-delphi-technique.
NCCN, 2017. Genetic/familial High-Risk Assessment: Colorectal (Version 3.2017).
NCCN, 2018. Genetic/Familial High-Risk Assessment: Breast and Ovarian (Version 1.2018).
Nielsen, F.C., van Overeem Hansen, T., Sorensen, C.S., 2016. Hereditary breast and ovarian cancer: new genes in confined pathways. Nat. Rev. Cancer 16, 599–612.
Schmidt, M.K., Hogervorst, F., van Hien, R., et al., 2016. Age- and tumor subtype-specific breast cancer risk estimates for CHEK2*1100delC carriers. J. Clin. Oncol. 34 (23), 2750–2760.
Shamseer, L., Moher, D., Clarke, M., et al., 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ 349, g7647.
Sterne, J.A., Sutton, A.J., Ioannidis, J.P., et al., 2011. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ 343, d4002.
Thompson, E.R., Rowley, S.M., Li, N., et al., 2016. Panel testing for familial breast cancer: calibrating the tension between research and clinical care. J. Clin. Oncol. 34 (13), 1455–1459.
Tung, N., Lin, N.U., Kidd, J., et al., 2016. Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer. J. Clin. Oncol. 34 (13), 1460–1468.
Walsh, T., Lee, M.K., Casadei, S., et al., 2010. Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. Proc. Natl. Acad. Sci. U. S. A. 107, 12629–12633.