Meta-Analysis of *BRCA1* Polymorphisms and Breast Cancer Susceptibility

Metaanalýza polymorfizmů v *BRCA1* a náchylnost k nádorům prsu

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Summary

Background: *BRCA1* codes for a tumor suppressor protein involved in DNA repair. Based on the role of single nucleotide polymorphisms (SNPs) in the modification of gene expression and function and the existence of certain SNPs within 3'-untranslated region of *BRCA1* with the ability to change binding sites for mirRNAs, several association studies have been designed to explore the significance of SNPs within *BRCA1* gene in conferring breast cancer (BC) risk. This study aims to assess the relationship between *BRCA1* SNPs and BC using meta- analysis. ***Aim:*** To conduct a meta-analysis for retrieving case-control studies on the associations between the rs11655505, rs1799966, rs3737559, rs1799950, rs799917 and rs16941 *BRCA1* polymorphisms and BC. The pooled odds ratios and its 95% confidence intervals were measured using fixed and random model to define the association between these polymorphisms and BC risk. ***Conclusion:*** No significant association was found for any of these polymorphisms and BC risk in the allelic, homozygote, dominant or recessive models. Overall, our study implies that the mentioned polymorphisms are not associated with BC risk. However, our study did not exclude the possible contribution of other SNPs within this gene in BC nor substantial contribution of multiple variants within this gene in conferring BC risk.

Key words

BRCA1 – breast cancer – meta-analysis

Souhrn

Východiska: *BRCA1* kóduje nádorově supresorový protein, který je zapojen do DNA oprav. Na základě role jednonukleotidových polymorfizmů (SNPs) v modifikaci genové exprese a funkce a existence některých SNP v 3'-nepřekládané oblasti genu *BRCA1* se schopností změny vazebných míst pro miRNA bylo publikováno několik asociačních studií za účelem zjištění významu SNP v oblasti genu *BRCA1* ve srovnání s rizikem nádoru prsu (BC). Tato studie se zaměřila na zjištění vztahu mezi SNP v *BRCA1* a BC při použití metaanalýzy. ***Cíl:*** Provést metaanalýzu ze studií, které se zaměřily na asociaci mezi *BRCA1* polymorfizmy rs11655505, rs1799966, rs3737559, rs1799950, rs799917 a rs16941 a BC. Sloučené poměry pravděpodobnosti a intervaly spolehlivosti na hladině 95 % byly měřeny za použití pevného a náhodného modelu za účelem definovat souvislost mezi těmito polymorfizmy a rizikem BC. ***Závěr:*** U žádného z těchto polymorfizmů nebylo nalezeno žádné významné spojení v alelických, homozygotních, dominantních nebo recesivních modelech s rizikem BC. Celkově naše studie naznačuje, že zmíněné polymorfizmy nejsou spojeny s rizikem BC. Nicméně naše studie nevylučovala ani možný přínos jiných SNP v tomto genu v BC ani významný přínos více variant v rámci tohoto genu při určování rizik BC.

Klíčová slova

BRCA1 – nádory prsu – metaanalýza

The authors declare they have no potential conflicts of interest concerning drugs, products, or services used in the study.

Autoři deklarují, že v souvislosti s předmětem studie nemají žádné komerční zájmy.

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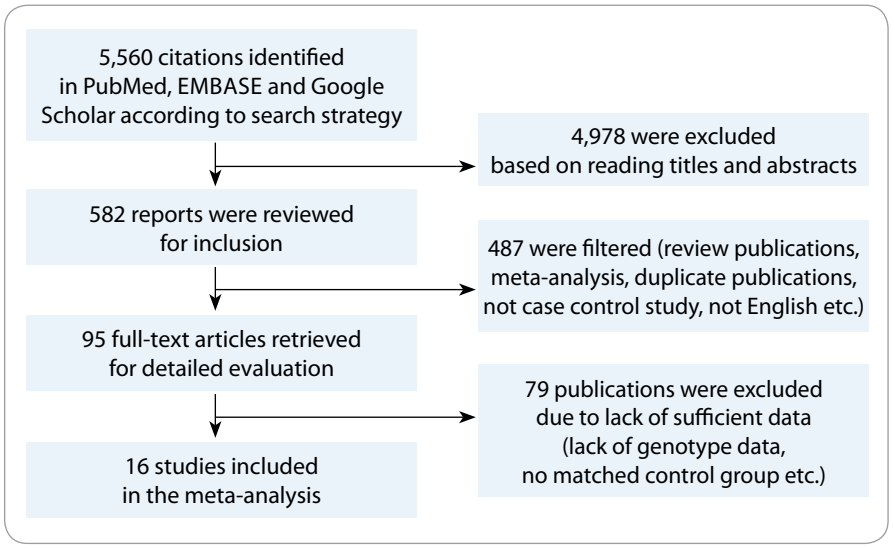
Introduction

Breast cancer (BC) as the most common malignancy among women is associated with high mortality and morbidity [1]. Several susceptibility loci have been detected for this disorder [2–4]. *BRCA1*, located on chromosome 17q21, has been the first cancer susceptibility gene detected through a linkage study in families with early onset of the disease [5]. *BRCA1* gene as a prototype of tumor suppressor genes participates in protection of intact chromosome structure [6]. Highly penetrant variants of this gene explain less than 20% of the genetic risk of BC [7]. The polygenic model for BC emphasizes the existence of numerous low penetrance high risk alleles that totally confer BC risk [8]. Consequently, more common variants in *BRCA1* might also been associated with BC risk [8]. Considering the role of single nucleotide polymorphisms (SNPs) in modification of gene expression and function and the existence of certain SNPs within 3'-untranslated region of *BRCA1* with the ability to change binding sites for microRNAs (miRNAs), several association studies have been designed to explore the significance of SNPs within the *BRCA1* gene in conferring BC risk [9–11]. However, discrepancies have been detected in the results of such studies, which can be attributed to the heterogeneity of patients' samples, small sample sizes and ethnic origin of patients. Consequently, we conducted a systematic search and meta-analysis to reach a more accurate answer to the question regarding the extent of the contribution of *BRCA1* genomic variants in BC susceptibility.

Methods

Search for relevant articles

To find suitable studies for the present meta-analysis, we searched in the PubMed, Google Scholar, EMBASE and Web of Science databases until January 2018 using the following key words – 'breast cancer' or 'breast tumor' with '*BRCA1* gene polymorphism' or '*BRCA1* gene single nucleotide polymorphism' or '*BRCA1* gene SNPs' or '*BRCA1* gene SNP'. Besides, the articles were filtered with the terms 'rs11655505' or 'rs1799966' or 'rs3737559' or 'rs1799950' or 'rs799917' or 'rs16941'. We confined searches to full English articles. The collected studies were entered in the meta-analysis if a) the study provided association between the *BRCA1* genetic polymorphisms and the susceptibility to BC, b) the study was designed as a case-control study, c) genotype/allele data of the polymorphism(s) were provided in the study. Case reports, editorials and cell culture experiments were excluded from the meta-analysis. Schema 1 shows the process of selection of studies for inclusion in the meta-analysis.



Schema 1. A systematic flow chart demonstrating the course of selection of articles for this meta-analysis.

Data extraction

All manuscripts were evaluated by two authors (Dianatpour A. and Faramarzi S.) according to the inclusion and exclusion criteria. The first author's name, year of publication, ethnicity of study participants, source of DNA (blood or breast tissue) used for SNP genotyping, total number of cases and controls and genotype distribution were collected. The studies were scored based on the Newcastle-Ottawa scale (NOS), [12] and those with NOS scores more than 6 were chosen for Hardy-Weinberg equilibrium (HWE) analysis.

Statistical analysis

The analyses were carried out in SPSS version 20 (IBM Analytics, USA) and RevMan version 5.3. The association between *BRCA1* polymorphism (rs11 655505, rs1799966, rs3737559, rs1799 950, rs799917 and rs16941), and susceptibility of BC was assessed from the case-control studies through evaluation of odds ratios and 95% confidence intervals (CI). Moreover, we computed the pooled odds ratios and CIs for each SNP and assessed their significance of association with BC risk using P values in four genetic models including allelic (wild type (W) vs. minor (M)), homozygote (WW vs. MM), dominant (WW+WM vs. MM) and recessive (WW vs. WM+MM). Chi-square based Q statistic test and I^2 statistics were applied for assessment of the heterogeneity between the selected studies. Based on the value of I^2, the random-effects (DerSimonian and Laird's method) or fixed effects model were applied. The results were demonstrated as forest and funnel plots, which show the association of *BRCA1* genetic polymorphisms with BC and the possible existence of publication bias in the meta-analysis, respectively (Tab. 1).

Results

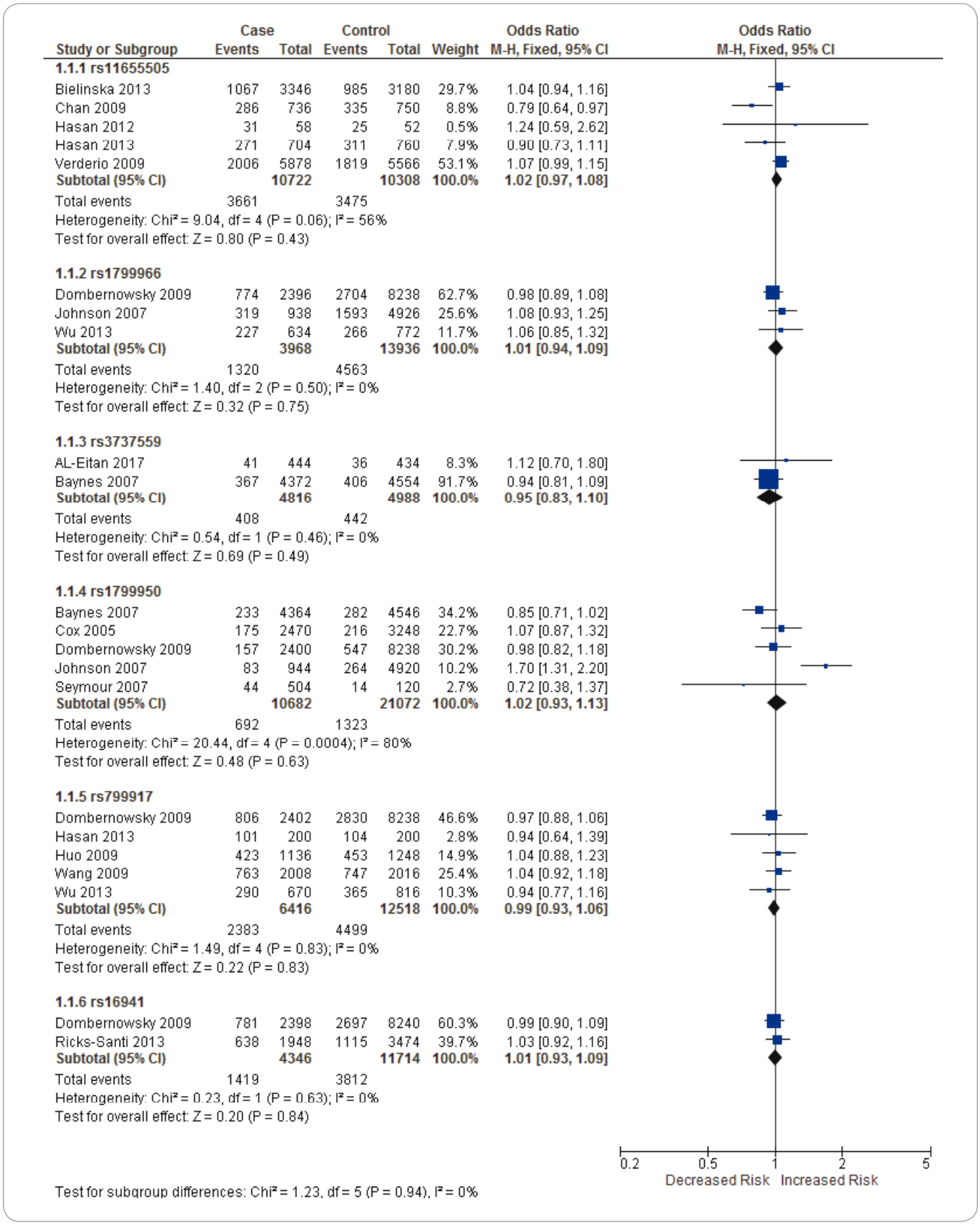
Features of studies included in the meta-analysis

After initial screening of relevant publications, 95 full text original researches were found. After exclusion of papers with insufficient genotype data and matched control groups, 16 studies remained.

Tab. 1. Association between the individual study characteristics and *BRCA1* polymorphisms.

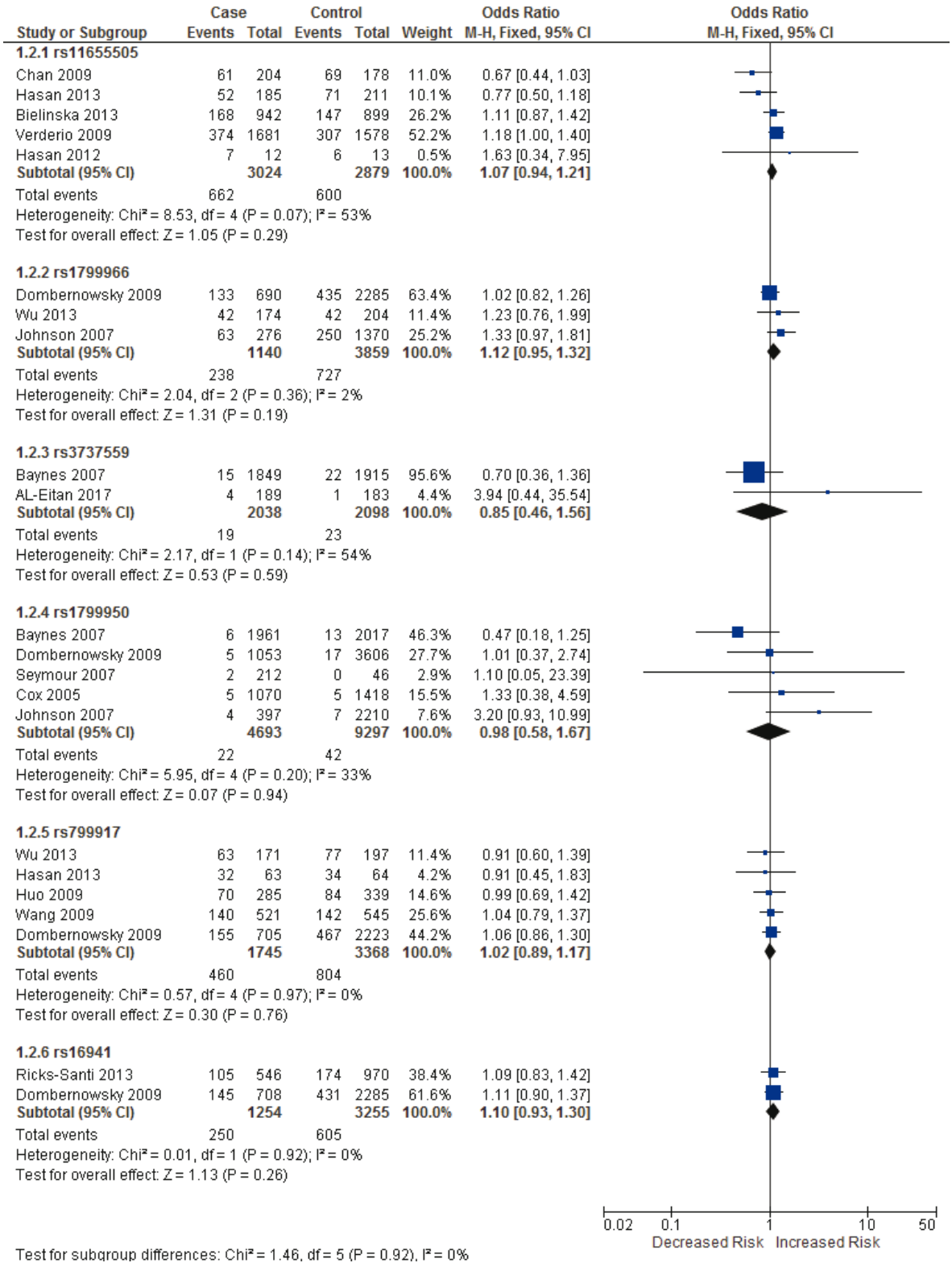
First author's name	Year	Country	Source of DNA	Ethnicity	No. of cases	No. of controls	Genotype frequency in controls			Genotype frequency in cases			NOS Score	HWE/ Chi-Square
							WW	WM	MM	WW	WM	MM		
rs11655505														
Chan et al.	2009	China	blood	Asian	368	375	109	197	69	143	164	61	7	0.2242/1.48
Verderio et al.	2009	several	blood	several	2,939	2,783	1,271	1,205	307	1,307	1,258	374	8	0.3999/0.71
Hasan et al.	2012	India	tissue	Indo-American	29	26	7	13	6	5	17	7	6	0.9939/5.71
Bielinska et al.	2013	Poland	blood	Polish Caucasian	1,673	1,590	752	691	147	774	731	168	7	0.5149/0.42
Hasan et al.	2013	India	blood	Indo-American	352	380	140	169	71	133	167	52	7	0.1179/2.44
rs1799966														
Johnson et al.	2007	UK	blood	Caucasian	469	2,463	1,120	1,093	250	213	193	63	7	0.4853/0.49
Dombernowsky et al.	2009	Denmark	blood	Caucasian	1,198	4,119	1,850	1,834	435	557	508	133	7	0.5353/0.38
Wu et al.	2013	several	blood	several	317	386	162	182	42	132	143	42	8	0.3885/0.74
rs3737559														
Baynes et al.	2007	UK	blood	–	2,186	2,277	1,893	362	22	1,834	337	15	7	0.3139/1.01
AL-Eitan et al.	2017	Jordan	blood	Arabs	222	217	182	34	1	185	33	4	6	0.6599/0.19
rs1799950														
Cox et al.	2005	USA	blood	–	1,235	1,624	1,413	206	5	1,065	165	5	7	0.3830/0.76
Baynes et al.	2007	UK	blood	–	2,182	2,273	2,004	256	13	1,955	221	6	7	0.1252/2.35
Seymour et al.	2007	Italy	blood	Caucasian	252	60	46	14	0	210	40	2	6	0.3063/1.05
Johnson et al.	2007	UK	blood	Caucasian	472	2,460	2,203	250	7	393	75	4	7	0.9737/0.001
Dombernowsky et al.	2009	Denmark	blood	Caucasian	1,200	4,119	3,589	513	17	1,048	147	5	7	0.7706/0.09
rs799917														
Huo et al.	2009	China	blood	Asian	568	624	255	285	84	215	283	70	7	0.7573/0.09
Dombernowsky et al.	2009	Denmark	blood	Caucasian	1,201	4,119	1,756	1,896	467	550	496	155	7	0.1871/1.74
Wang et al.	2009	China	blood	Asian	1,004	1,008	403	463	142	381	483	140	8	0.6264/0.24
Wu et al.	2013	several	blood	several	335	408	120	211	77	108	164	63	8	0.3535/0.86
Hasan et al.	2013	Saudi Arabia	blood	Arabs	100	100	30	36	34	31	37	32	7	0.0053/7.77
rs16941														
Dombernowsky et al.	2009	Denmark	blood	Caucasian	1,199	4,120	1,854	1,835	431	563	491	145	7	0.4630/0.54
Ricks-Santi et al.	2013	USA	blood	Caucasian	974	1,737	796	767	174	441	428	105	6	0.5871/0.29

NOS – Newcastle-Ottawa scale, HWE – Hardy-Weinberg equilibrium



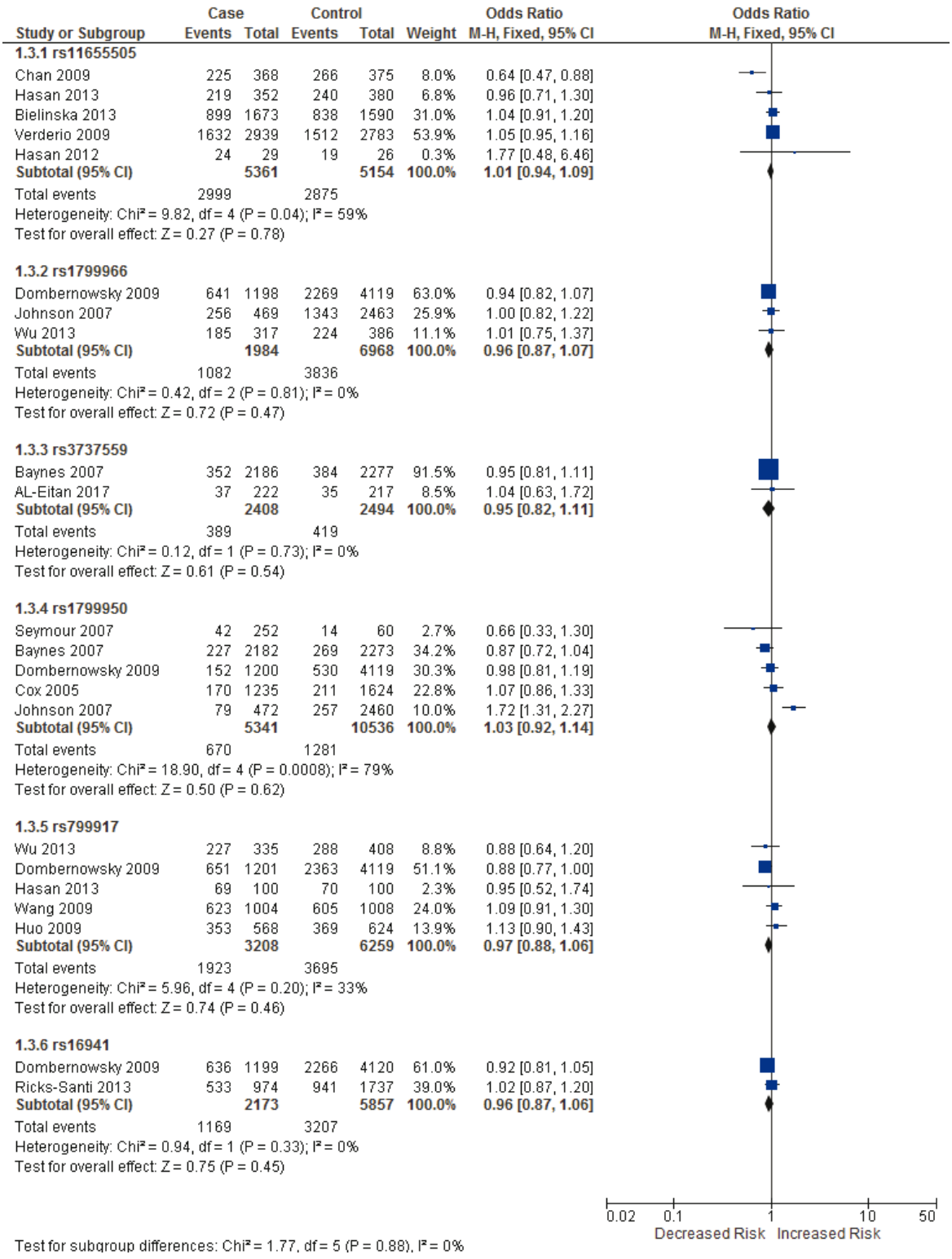
Graph 1. Forest plot of the risk for *BRCA1* polymorphisms in allelic model. The error bars indicate 95% CI. Solid squares represent each study in the meta-analysis. Solid diamonds represent pooled OR.

CI – confidence interval, OR – odds ratio, M-H – Mantel-Haenszel method



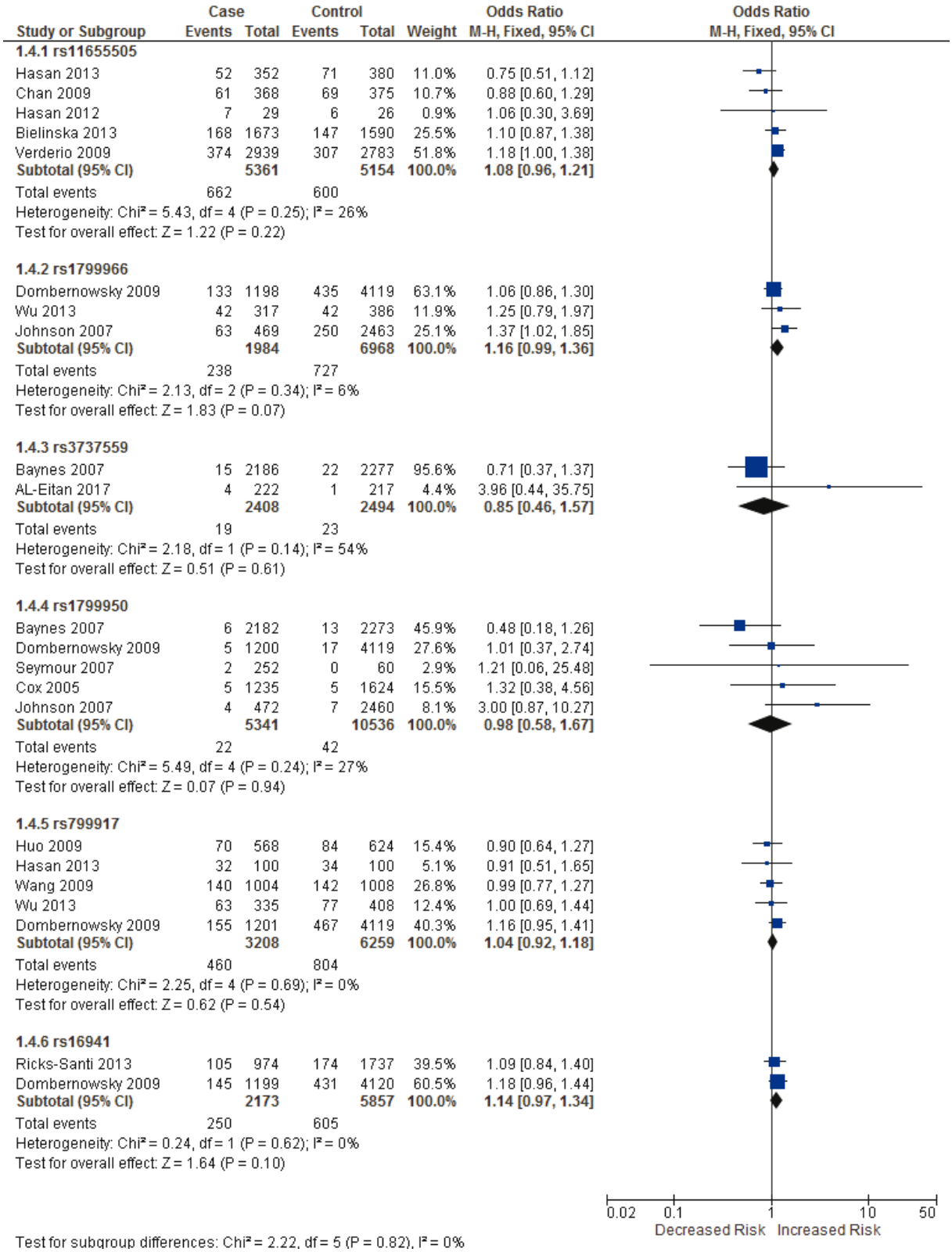
Graph 2. Forest plot of the risk for *BRCA1* polymorphisms in homozygote model. The error bars indicate 95% CI. Solid squares represent each study in the meta-analysis. Solid diamonds represent pooled OR.

CI – confidence interval, OR – odds ratio, M-H – Mantel-Haenszel method



Graph 3. Forest plot of the risk for *BRCA1* polymorphisms in dominant model. The error bars indicate 95% CI. Solid squares represent each study in the meta-analysis. Solid diamonds represent pooled OR.

CI – confidence interval, OR – odds ratio, M-H – Mantel-Haenszel method



Graph 4. Forest plot of the risk for *BRCA1* polymorphisms in recessive model. The error bars indicate 95% CI. Solid squares represent each study in the meta-analysis. Solid diamonds represent pooled OR.

CI – confidence interval, OR – odds ratio, M-H – Mantel-Haenszel method.

These studies had NOS scores of 6 to 9 and assessed rs11655505 [9,13–16], rs1799966 [4,8,17], rs3737559 [10,18], rs1799950 [8,10,17,19,20], rs799917 [4,17,21–23] or rs16941 [17,24]. Assessment of genotype distribution in control groups of all included researches showed their compliance with HWE. Tab. 1 shows the detailed information of included studies, such as first author's name, year of publication, ethnicity and genotype in the cases and controls in addition to the NOS score, chi-square and HWE P values.

Assessment of association between SNPs and BC risk

None of the assessed SNPs were associated with BC risk in any of allelic, homozygote, dominant or recessive models (Graph 1–4). Besides, the funnel plots were depicted to evaluate the presence of publication bias in the meta-analysis of the mentioned SNPs in 4 genetic models. The overall results of the funnel plot showed fairly symmetrical shapes implying low probability of publication bias.

Discussion

Up to now, several BC predisposition factors including SNPs have been identified with either single-locus or epistatic effects which might be applied for BC risk assessment. Numerous strategies have been suggested to enhance accuracy of risk prediction programs with the hope of inclusion of informative SNPs in population-based risk screening programs [5]. Among putative informative SNPs for such programs are SNPs located in genes with significant role in BC. BRCA1 as the most significant genetic risk factor for BC is a tumor suppressor gene involved in various cellular processes, such as maintenance of X chromosome inactivation as well as the DNA damage response [25]. Other genes implicated in the repair of DNA double strand breaks are also associated with BC risk [4]. Previous studies have assessed the association of BRCA1 SNPs with BC risk in distinct ethnic groups. In the present meta-analysis, we evaluated associations between six *BRCA1* SNPs (rs11655505, rs1799966, rs3737559, rs1799950, rs799917 and rs16941) and BC risk using the pooled data of these publications. None of these SNPs resides in a known functional domain of BRCA1. Moreover, online tools for evaluation of the functional importance of missense variants, such as Align-GVGD and SIFT, have predicted them to be benign variants according to the low degree of conservation during evolution and minor biochemical alterations between the reference and variant amino acid [26]. The rs11655505 (c.-2265C>T) is located in the promoter region of *BRCA1* gene and enhances promoter activity [15]. The CT genotype of this SNP has been associated with decreased expression of BRCA1 [16]. The rs1799966 (c.4837A>G (p.Ser1613Gly)) is a missense variant classified as a benign variant by expert panel. The rs3737559 (c.4357+117G>A) is an intronic variant located in IVS13. The rs1799950 (c.1067A>G (p.Gln356Arg)) and the rs799917 (c.2612C>G (p.Pro871 Arg)) are other missense variants classified as benign by expert panel. The rs16941 (c.3113A>G (p.Glu1038Gly)) is a missense variant with conflicting interpretations of pathogenicity.

We demonstrated lack of association between 6 SNPs within *BRCA1* gene and BC risk. The heterogeneity analysis showed the I^2 values very small for rs1799966, rs799917 and rs16941 in all the genetic models, implying lack heterogeneity in these SNPs with BC risk. In addition, I^2 values for rs3737559 were zero in allelic and dominant models. However, for the other two SNPs (rs11655505 and rs1799950), I^2 values show the inconsistency between studies included in the meta-analysis. In addition to such heterogeneity, there were some other limitations in our study, including lack of ethnicity-based analysis and exclusion of studies written in other languages. In addition, we did not assess BC patients based on the molecular subtypes or environmental factors. However, the stringent quality check of included studies enhanced the reliability of our results.

Our study did not exclude the possible contribution of other SNPs within this gene in BC nor substantial contribution of multiple variants within this gene in conferring BC risk. Evidence supporting the second possibility originated from a genome-wide association study in BC patients, which showed that single influence of most of these risk alleles were imperceptibly small. However, the risk conferred by multiple variants was significant suggesting consideration of a risk score through integration of a properly weighted calculation of all possibly functional variants within *BRCA1* and other genes [8].

Conclusion

The assessed SNPs within *BRCA1* gene are not associated with BC risk. Future studies with larger sample sizes in different ethnic populations and in distinct molecular subtypes of BC are needed to define the association of other SNPs in *BRCA1* gene with BC risk.

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