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Functional variants in *NBS1* and cancer risk: evidence from a meta-analysis of 60 publications with 111 individual studies

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Several potentially functional variants of Nijmegen breakage syndrome 1 (*NBS1*) have been implicated in cancer risk, but individually studies showed inconclusive results. In this study, a meta-analysis based on 60 publications with a total of 39 731 cancer cases and 64 957 controls was performed. The multivariate method and the model-free method were adopted to determine the best genetic model. It was found that rs2735383 variant genotypes were associated with significantly increased overall risk of cancer under the recessive genetic model [odds ratio (OR) =1.12, 95% confidence interval (CI): 1.02–1.22, *P* = 0.013]. Similar results were found for rs1063054 under the dominant model effect (OR = 1.12, 95% CI: 1.01–1.23, *P* = 0.024). The I171V mutation, 657del5 mutation and R215W mutation also contribute to the development of cancer (for I171V, OR = 3.93, 95% CI: 1.68–9.20, *P* = 0.002; for 657del5, OR = 2.79, 95% CI: 2.17–3.68, *P* < 0.001; for R215W, OR = 1.77, 95% CI: 1.07–2.91, *P* = 0.025). From stratification analyses, an effect modification of cancer risks was found in the subgroups of tumour site and ethnicity for rs2735383, whereas the I171V, 657del5 and R215W showed a deleterious effect of cancer susceptibility in the subgroups of tumour site. However, rs1805794, D95N and P266L did not appear to have an effect on cancer risk. These results suggest that rs2735383, rs1063054, I171V, 657del5 and R215W are low-penetrance risk factors for cancer development.

Introduction

DNA double-strand breaks (DSBs) are a relatively dangerous form of DNA lesion, unrepaired or defectively repaired chromosomal irregularities may lead to cell apoptosis and probably cancer (1). There are two different pathways in the repair of DSBs in human cells: homologous recombination (HR) repair and non-homologous end-joining (NHEJ) pathways (2,3). Nijmegen breakage syndrome 1 (NBS1) protein plays decisive roles in HR repair pathway as a component of the MRN [a protein complex consisting of meiotic recombination 11 homologue (MRE11), human RAD50 homologue (RAD50) and NBS1] complex. It modulates the DNA damage signal sensing by recruiting phosphatidylinositol 3-kinase-like kinase family members, ataxia telangiectasia mutated kinase, ataxia telangiectasia and Rad3-related kinase, and probably DNA-dependent protein catalytic subunit to the DNA damage sites and activates them. It can also recruit MRE11 and RAD50 to the proximity of DSBs by an interaction with γ-H_2AX through the forkhead-associated [breast cancer terminal domain (BRCT)] domain at its C-terminus (4).

The human *NBS1* gene contains 16 exons spanning over 50 kb on human chromosome 8q21, which is highly polymorphic. Previous studies have mainly focussed on eight common *NBS1* variants, including two nucleotide substitutions in the 3′-untranslated region (3′-UTR) (rs2735383, rs1063054), E185Q (glutamic acid to glutamine, G>C; rs1805794), a five nucleotide deletion/insertion (657del5), I171V (isoleucine to valine, A>G; rs61754966), R215W (arginine to glutamine, G>A; rs61753718), P266L (proline to leucine, C>T; rs769420), D95N (aspartic acid to asparagine, G>A; rs61753720), because they are probably to be functional. These variants may alter its structure or expression and consequently influence the interaction of the NBS1 protein with other DSBs repair-related molecules.

Since reports of the association of *NBS1* variants with cancer susceptibility from individual studies are not consistent (5–7), and some recent meta-analysis analysed for an association between rs1805794 and several cancer types (8,9). In this study, the associations between eight functional variants (i.e. rs2735383, rs1063054, rs1805794, 657del5, I171V, R215W, D95N and P266L) and cancer risk were investigated.

Materials and Methods

Literature search strategy for identification of the studies

The relevant papers published before April 2013 were searched from the electronic databases Web of science, Embase, Medline, Chinese National Knowledge Infrastructure and China Biology Medicine disc. The search strategy for association between gene variants and cancer risk was used as follows: 'NBS1' or 'nibrin (NBN)', 'cancer', 'tumour' or 'carcinoma' and 'variant' or 'polymorphism'. No language limitations were used. Additional studies were identified *via* a manual review of the reference lists of identified studies and review articles.

Studies were included if they met the following inclusion criteria: (i) studies used a case–control study design, (ii) studies investigated the association between *NBS1* variants and cancer risk with genotyping data for at least one of eight variants, rs2735383, rs1063054, rs1805794, 657del5, I171V, R215W, D95N and P266L, (iii) abstracts, unpublished reports were not considered, (iv) there were sufficient results for extraction of data, i.e. number of subjects for each genotype in cancer and control groups. Two reviewers (P.G., M.L.) independently assessed eligible articles for inclusion. Disagreements were resolved by discussion.

Data extraction

Data from each manuscript were extracted: author, year of publication, country of origin, ethnicity, cancer type, source of control groups (population-based, hospital-based or mixed controls), genotype method, the number of cases and controls and allele frequency. For studies including subjects of different ethnicities or countries, data were extracted separately.

Statistical analysis

Crude odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the strength of the association between respective *NBS1* variants and cancer risk.

For rs2735383, rs1063054 and rs1805794, data analyses were performed as follows. First, the goodness-of-fit chi-square test is used to test deviation from Hardy–Weinberg equilibrium for each study, and only in control groups.

Second, a *Q*-test for heterogeneity was performed separately for three ORs, i.e. CC vs. GG (OR_1), CG vs. GG (OR_2) and CC vs. CG (OR_3) for rs2735383, rs1805794, and CC vs. AA (OR_1), CA vs. AA (OR_2) and CC vs. CA (OR_3) for rs1063054. If heterogeneity was found in at least one of the three ORs, meta-regression model was used to explore the cause by fitting a covariable such as ethnicity, cancer type or source of control groups. If there was no heterogeneity, the fixed-effect model was used to determine the ORs and their 95% CIs; otherwise, the random-effect model was used to pool. If the overall gene effect was statistically significant, further comparisons of OR_1, OR_2 and OR_3 were explored. These pairwise differences can be used to indicate the most appropriate genetic model as follows (10).

(i) If $OR_1 = OR_3 \neq 1$ and $OR_2 = 1$, then a recessive model is suggested.
(ii) If $OR_1 = OR_2 \neq 1$ and $OR_3 = 1$, then a dominant model is suggested.
(iii) If $OR_2 = 1/OR_3 = 1$ and $OR_1 = 1$, then a complete overdominant model is suggested.
(iv) If $OR_1 > OR_2 > 1$ and $OR_1 > OR_3 > 1$ (or $OR_1 < OR_2 < 1$ and $OR_1 < OR_3 < 1$), then a codominant model is suggested.

Third, the gene effect was estimated by using the genetic model-free approach (11). This model is based on a simple reparameterization and uses the OR between the homozygous genotypes to capture the magnitude of the genetic effect, and lambda (λ), the ratio of log (OR_1) and log (OR_2), to capture the genetic mode of inheritance. The value of λ is not restricted, but values equal to 0, 0.5 and 1 correspond to the recessive, codominant and dominant genetic model, respectively, and values >1 or <0 would suggest overdominant genetic model. The two log ORs could be modelled as a fixed effect or as a random effect, as described in the second statistical analysis enumerated above.

Once the data have indicated the best genetic model, this model is used to collapse the three genotypes into two groups (except in the case of a codominant model), and the pooled gene effect is then estimated. Sensitivity analyses were performed by excluding studies not in Hardy–Weinberg equilibrium. Begger's funnel plots and the Egger's test were used to estimate publication bias (12).

For 657del5, I171V, R215W, D95N and P266L, because only one OR (carriers with missense alterations vs. non-carriers) was calculated, it is not necessary to determine the best genetic model. The statistic method was similar with that described in the previous paragraph. The meta-analysis was conducted using Stata software (version 12.0; StataCorp LP, College Station, TX, USA). A *P* value of <0.05 was considered statistically significant. All the *P* values were two sided.

Results

Characteristics of studies

On the basis of the described search strategy, a total of 127 epidemiological studies after initial screening (as of April 2013) was found. Among these, 73 publications appeared to have met the inclusion criteria and were subjected to further examination. Thirteen studies were excluded because they were not case–control studies (13–21), letters to the editor (22,23) or variants not included in the eight variants (24,25). Finally, a total of 60 eligible publications (5–7,26–82) with 111 case–control studies met the present inclusion criteria (Figure 1 and Table I), in which 39 731 cases and 64 957 controls were included for the pooled analysis. All the studies were published in English except for three (80–82).

Quantitative synthesis

NBS1 *rs2735383*. Thirteen eligible studies included 7561 cases and 8432 control subjects were analysed. The C allele frequency was 38.1% with 95% CI between 35.8 and 40.5%. Heterogeneity was checked for OR_1 (CC vs. GG), OR_2 (CG vs. GG) and OR_3 (CC vs. CG). Results indicated no heterogeneity for OR_1, OR_2 or OR_3 (for OR_1: $\chi^2 = 20.49$, $P = 0.058$; for OR_2: $\chi^2 = 11.36$, $P = 0.499$; for OR_3: $\chi^2 = 11.44$, $P = 0.492$). Hence, these studies were pooled by use of logistic regression with the fixed-effects model. The estimated OR_1, OR_2 and OR_3 were 1.12 (95% CI: 1.02–1.23), 1.02 (95% CI: 0.95–1.09) and 1.11 (95% CI: 1.01–1.21). By using the genetic model-free approach, the estimated λ was 0.13 (95% CI: −0.56 to 0.83), close to 0. These estimates suggest a recessive model effect of the C allele, and therefore GG and CG were combined and compared with CC (CC vs. CG+GG). The pooled OR was 1.12 (95% CI: 1.02–1.22, $P = 0.013$), $P = 0.170$ for heterogeneity (Figure 2).

Although there was no substantial between-study heterogeneity among the 13 studies, subgroup analysis was also carried out. Nine of 13 studies were conducted in Chinese, 1 in Caucasians and 3 in mixed population. Only the result for Chinese population was analysed, the OR was 1.14 (95% CI: 1.03–1.25, $P = 0.009$), $P = 0.067$ for heterogeneity. In order to avoid the result bias caused from multiple different cancers, the subgroup analysis was performed by cancer type, individuals with CC genotype had a significantly higher risk of lung cancer, the OR was 1.28 (95% CI: 1.21–1.46, $P < 0.001$),

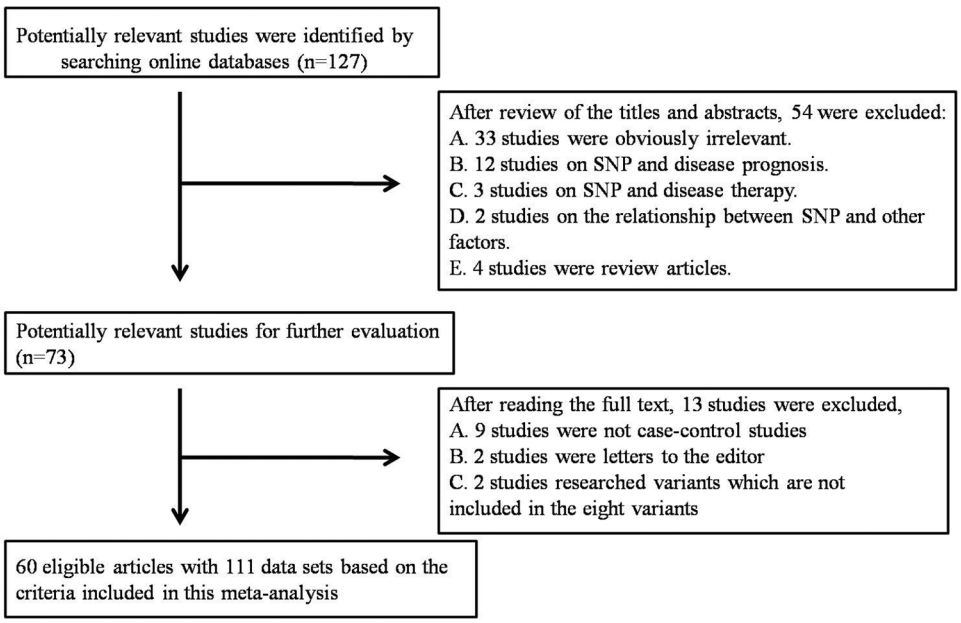


Fig. 1. Study flow chart for the process of selecting the final 60 publications.

Table I. Characteristics of studies included in the meta-analysis

Author (year)	Publication year	Country	Ethnicity	Cancer type	Case control	Source of control	Genotype method
rs2735383							
Choudhury *et al.* (40)	2008	UK	Mixed	Bladder cancer	736-770	Mixed	Taqman real-time PCR
Fan *et al.* (82)	2010	China	Chinese	Lung cancer	575-575	Population	PCR-RFLP
Han *et al.* (74)	2009	USA	Mixed	Breast cancer	237-474	Population	Illumina
Huang *et al.* (27)	2011	China	Chinese	Hepatocellular carcinoma	865-900	Population	PCR-RFLP
Jiang *et al.* (30)	2010	China	Chinese	ALL	175-350	Hospital	PCR-RFLP
Li *et al.* (62)	2013	China	Chinese	Acute myeloid leukemia	428-600	Hospital	PCR-RFLP
Qiu *et al.* (81)	2011	China	Chinese	Lung cancer	781-781	Population	PCR-RFLP
Rollinson *et al.* (68)	2006	UK	Caucasian	Lymphoid malignancies	442-445	Population	Taqman real-time PCR
Teo *et al.* (75)	2011	UK	Mixed	Bladder cancer	711-680	Mixed	Taqman real-time PCR
Yang[a] *et al.* (26)	2012	China	Chinese	Lung cancer	1056-1056	Hospital	PCR-RFLP
Yang[b] *et al.*	2012	China	Chinese	Lung cancer	503-623	Hospital	PCR-RFLP
Zheng[a] *et al.* (28)	2011	China	Chinese	Nasopharyngeal carcinoma	700-758	Hospital	PCR-RFLP
Zheng[b] *et al.*	2011	China	Chinese	Nasopharyngeal carcinoma	352-400	Hospital	PCR-RFLP
rs1063054							
Choudhury *et al.* (40)	2008	UK	Mixed	Bladder cancer	743-783	Mixed	Taqman real-time PCR
Park[a] *et al.* (32)	2010	USA	Mixed	Lung cancer	539-918	Population	Taqman real-time PCR
Park[b] *et al.*	2010	USA	Mixed	UADT cancer	403-927	Population	Taqman real-time PCR
Park[c] *et al.*	2010	USA	Mixed	Oropharynx cancer	240-927	Population	Taqman real-time PCR
Park[d] *et al.*	2010	USA	Mixed	Laryngeal cancer	77-927	Population	Taqman real-time PCR
Park[e] *et al.*	2010	USA	Mixed	Bladder cancer	176-168	Hospital	Taqman real-time PCR
Park[f] *et al.*	2010	China	Chinese	Oesophagus cancer	201-382	Population	Taqman real-time PCR
Park[g] *et al.*	2010	China	Chinese	Stomach cancer	189-382	Population	Taqman real-time PCR
Park[h] *et al.*	2010	China	Chinese	Hepatocellular carcinoma	189-382	Population	Taqman real-time PCR
rs1805794							
Auranen[a] *et al.* (47)	2005	UK	Caucasian	Ovarian cancer	721-848	Population	Taqman real-time PCR
Auranen[b] *et al.*	2005	USA	Caucasian	Ovarian cancer	308-383	Population	Taqman real-time PCR
Auranen[c] *et al.*	2005	Denmark	Caucasian	Ovarian cancer	299-827	Population	Taqman real-time PCR
Auranen[d] *et al.*	2005	UK	Caucasian	Ovarian cancer	258-734	Population	Taqman real-time PCR
Bastos *et al.* (35)	2009	Portugal	Caucasian	Thyroid cancer	109-217	Hospital	Taqman real-time PCR
Broberg *et al.* (60)	2005	Sweden	Caucasian	Bladder cancer	61-154	Population	MALDI-TOF MS
Choudhury *et al.* (40)	2008	UK	Mixed	Bladder cancer	748-788	Mixed	Taqman real-time PCR
Desjardins *et al.* (37)	2009	Canada	Caucasian	Breast cancer	97-73	Population	PCR-SSCP and direct sequencing
Fan *et al.* (82)	2010	China	Chinese	Lung cancer	575-575	Population	PCR-RFLP
Festa *et al.* (49)	2005	Sweden	Caucasian	Basal cell carcinoma	241-574	Hospital	PCR-RFLP
Figueroa *et al.* (43)	2007	USA	Caucasian	Bladder cancer	1086-1020	Hospital	Taqman real-time PCR
Gil *et al.* (29)	2012	Poland	Caucasian	Colorectal cancer	133-100	Hospital	PCR-RFLP
Han *et al.* (74)	2009	USA	Mixed	Breast cancer	238-470	Population	Illumina
Hebbring *et al.* (45)	2006	USA	Caucasian	Prostate cancer	321-200	Mixed	Direct sequencing
Huang *et al.* (27)	2011	China	Chinese	Hepatocellular carcinoma	865-900	Population	PCR-RFLP
Huang *et al.* (80)	2012	China	Chinese	Hepatocellular carcinoma	119-95	Population	PCR-SSCP
Jelonek[a] *et al.* (31)	2010	Poland	Caucasian	Colon cancer	132-153	Population	PCR-RFLP

Table I. *Continued*

Author (year)	Publication year	Country	Ethnicity	Cancer type	Case control	Source of control	Genotype method
Jelonek[b] *et al.*	2010	Poland	Caucasian	Head and neck cancer	104-110	Population	PCR-RFLP
Jelonek[c] *et al.*	2010	Poland	Caucasian	Breast cancer	93-425	Population	PCR-RFLP
Jiang *et al.* (30)	2010	China	Chinese	ALL	175-350	Hospital	PCR-RFLP
Kuschel *et al.* (51)	2002	UK	Caucasian	Breast cancer	1694-734	Population	Taqman real-time PCR
Lan *et al.* (48)	2005	China	Chinese	Lung cancer	118-111	Population	Taqman real-time PCR
Li *et al.* (62)	2013	China	Chinese	Acute myeloid leukemia	428-600	Hospital	PCR-RFLP
Loizidou *et al.* (36)	2010	Cyprus	Caucasian	Breast cancer	1104-1154	Population	MALDI-TOF MS
Lu *et al.* (44)	2006	USA	Caucasian	Breast cancer	421-423	Hospital	PCR-RFLP
Margulis *et al.* (38)	2008	USA	Caucasian	Renal cell carcinoma	322-333	Population	Taqman real-time PCR
Millikan[a] *et al.* (5)	2005	USA	African American	Breast cancer	766-681	Population	Taqman real-time PCR
Millikan[b] *et al.*	2005	USA	Caucasian	Breast cancer	1273-1136	Population	Direct sequencing
Mosor *et al.* (78)	2008	Poland	Caucasian	Lymphoid malignancies	157-275	Population	PCR-SSCP
Pardini *et al.* (61)	2008	Czech	Caucasian	Colorectal cancer	532-530	Hospital	Taqman real-time PCR
Qiu *et al.* (81)	2011	China	Chinese	Lung cancer	781-781	Population	PCR-RFLP
Sanyal *et al.* (50)	2004	Sweden	Caucasian	Bladder cancer	299-278	Hospital	Taqman real-time PCR
Silva *et al.* (33)	2010	Portugal	Caucasian	Breast cancer	289-548	Hospital	Taqman real-time PCR
Smith[a] *et al.* (39)	2008	USA	Caucasian	Breast cancer	318-407	Hospital	MALDI-TOF MS
Smith[b] *et al.*	2008	USA	African American	Breast cancer	53-74	Hospital	MALDI-TOF MS
Thirumaran *et al.* (6)	2006	Germany	Caucasian	Basal cell carcinoma	529-533	Hospital	Taqman real-time PCR
Wu *et al.* (63)	2006	USA	Mixed	Bladder cancer	604-595	Hospital	Taqman real-time PCR
Zhang *et al.* (46)	2005	China	Chinese	Breast cancer	220-310	Hospital	PCR-RFLP
Zheng[a] *et al.* (28)	2011	China	Chinese	Nasopharyngeal carcinoma	700-758	Hospital	PCR-RFLP
Zheng[b] *et al.*	2011	China	Chinese	Nasopharyngeal carcinoma	352-400	Hospital	PCR-RFLP
Zienolddiny *et al.* (7)	2006	Norway	Caucasian	Lung cancer	310-376	Hospital	Taqman real-time PCR
Ziólkowska *et al.* (42)	2007	Poland	Caucasian	Laryngeal cancer	260-193	Hospital	PCR-RFLP and direct sequencing
I171V							
Bogdanova[a] *et al.* (59)	2008	Germany	Caucasian	Breast cancer	1048-1017	Population	PCR-RFLP and direct sequencing
Bogdanova[b] *et al.*	2008	Belarus	Caucasian	Breast cancer	1636-1014	Population	PCR-RFLP and direct sequencing
Ciara *et al.* (34)	2010	Poland	Caucasian	Medulloblastoma	104-4227	Population	PCR-SSCP and direct sequencing
Desjardins *et al.* (37)	2009	Canada	Caucasian	Breast cancer	97-73	Population	PCR-SSCP and direct sequencing
Kanka *et al.* (71)	2007	Poland	Caucasian	Breast cancer	250-1300	Population	PCR-RFLP and direct sequencing
Mosor *et al.* (67)	2006	Poland	Caucasian	Lymphoid malignancies	135-500	Population	PCR-RFLP and direct sequencing
Nowak *et al.* (72)	2008	Poland	Caucasian	Mixed	658-600	Population	PCR-RFLP and direct sequencing
Roznowski *et al.* (41)	2008	Poland	Caucasian	Breast cancer	270-500	Population	PCR-SSCP and direct sequencing
Varon *et al.* (69)	2001	Germany	Caucasian	Lymphoid malignancies	47-220	Population	PCR-SSCP and direct sequencing
Ziólkowska *et al.* (42)	2007	Poland	Caucasian	Laryngeal cancer	268-500	Hospital	PCR-RFLP and direct sequencing
657del5							
Piekutowska-Abramczuk *et al.* (73)	2010	Poland	Caucasian	Astrocytic tumours	127-12484	Population	PCR-SSCP
Bogdanova[a] *et al.* (52)	2008	Germany	Caucasian	Breast cancer	1232-1017	Population	Allele-specific PCR assay and direct sequencing
Bogdanova[b] *et al.*	2008	Belarus	Caucasian	Breast cancer	1882-1014	Population	Allele-specific PCR assay and direct sequencing

Table I. *Continued*

Author (year)	Publication year	Country	Ethnicity	Cancer type	Case control	Source of control	Genotype method
Buslov *et al.* (57)	2005	Russia	Caucasian	Breast cancer	873-692	Population	Allele-specific PCR assay and direct sequencing
Carlomagno *et al.* (58)	1999	Germany	Caucasian	Breast cancer	477-866	Population	Allele-specific oligonucleotide hybridization assay
Chrzanowska *et al.* (64)	2006	Poland	Caucasian	Lymphoid malignancies	545-6984	Population	PCR-SSCP and direct sequencing
Ciara *et al.* (34)	2010	Poland	Caucasian	Medulloblastoma	104-12484	Population	PCR-SSCP and direct sequencing
Cybulski *et al.* (70)	2004	Poland	Caucasian	Prostate cancer	396-1500	Mixed	Allele-specific PCR assay and direct sequencing
Debniak *et al.* (76)	2003	Poland	Caucasian	Malignant melanoma	80-530	Population	Allele-specific PCR assay and direct sequencing
Górski *et al.* (55)	2003	Poland	Caucasian	Breast cancer	230-530	Population	Allele-specific PCR assay and direct sequencing
Górski *et al.* (56)	2005	Poland	Caucasian	Breast cancer	2012-4000	Population	Allele-specific PCR assay and direct sequencing
Hebbring *et al.* (45)	2006	USA	Mixed	Prostate cancer	3044-990	Mixed	Direct sequencing
Kanka *et al.* (71)	2007	Poland	Caucasian	Breast cancer	250-4000	Population	Allele-specific PCR assay and direct sequencing
Mateju *et al.* (79)	2012	Czech	Caucasian	Breast cancer	1303-915	Population	High-resolution melting analysis and direct sequencing
Mosor *et al.* (67)	2006	Poland	Caucasian	Lymphoid malignancies	135-195	Population	PCR-SSCP and direct sequencing
Resnick *et al.* (66)	2003	Russia	Caucasian	Lymphoid malignancies	68-548	Population	PCR-SSCP and direct sequencing
Roznowski *et al.* (41)	2008	Poland	Caucasian	Breast cancer	270-500	Population	PCR-SSCP and direct sequencing
Soucek *et al.* (65)	2003	Czech	Caucasian	Lymphoid malignancies	119-177	Population	Multiplex PCR reaction and capillary electrophoresis
Steffen *et al.* (54)	2004	Poland	Caucasian	Mixed	1289-1620	Population	PCR-SSCP and direct sequencing
Steffen *et al.* (53)	2006	Poland	Caucasian	Breast cancer	562-1620	Hospital	DHPLC
Steffen *et al.* (77)	2006	Poland	Caucasian	Lymphoid malignancies	186-1620	Population	PCR-RFLP and direct sequencing
R215W							
Piekutowska-Abramczuk *et al.* (73)	2010	Poland	Caucasian	Astrocytic tumours	127-2815	Population	PCR-SSCP
Bogdanova[a] *et al.* (52)	2008	Germany	Caucasian	Breast cancer	1232-1017	Population	Allele-specific PCR assay and direct sequencing
Bogdanova[b] *et al.*	2008	Belarus	Caucasian	Breast cancer	1882-1014	Population	Allele-specific PCR assay and direct sequencing
Desjardins *et al.* (37)	2009	Canada	Caucasian	Breast cancer	97-73	Population	PCR-SSCP and direct sequencing
Hebbring *et al.* (45)	2006	USA	Caucasian	Prostate cancer	477-319	Mixed	Direct sequencing
Mateju *et al.* (79)	2012	Czech	Caucasian	Breast cancer	1303-915	Population	High-resolution melting analysis and direct sequencing
Mosor *et al.* (67)	2006	Poland	Caucasian	Lymphoid malignancies	135-195	Population	PCR-SSCP and direct sequencing
Steffen *et al.* (54)	2004	Poland	Caucasian	Mixed	1289-1620	Population	PCR-SSCP and direct sequencing
Steffen *et al.* (77)	2006	Poland	Caucasian	Lymphoid malignancies	186-1620	Population	PCR-RFLP and direct sequencing
P266L							
Choudhury *et al.* (40)	2008	UK	Mixed	Bladder cancer	758-784	Mixed	Taqman real-time PCR
Desjardins *et al.* (37)	2009	Canada	Caucasian	Breast cancer	97-73	Population	PCR-SSCP and direct sequencing

Table I. *Continued*

Author (year)	Publication year	Country	Ethnicity	Cancer type	Case control	Source of control	Genotype method
D95N							
Desjardins *et al.* (37)	2009	Canada	Caucasian	Breast cancer	97-73	Population	PCR-SSCP and direct sequencing
Hebbring *et al.* (45)	2006	USA	Caucasian	Prostate cancer	3044-990	Population	Direct sequencing
Mosor *et al.* (67)	2006	Poland	Caucasian	Lymphoid malignancies	135-195	Population	PCR-SSCP and direct sequencing
Varon *et al.* (69)	2001	Germany	Caucasian	Lymphoid malignancies	47-110	Population	PCR-SSCP and direct sequencing
Ziólkowska *et al.* (42)	2007	Poland	Caucasian	Laryngeal cancer	268-195	Hospital	PCR-RFLP and direct sequencing

[a,b; a–c; a–d; a–h], Studies included more than one case–control study or involved different caner types, when performed the meta-analysis they were divided as independent studies.
ALL, acute lymphoblastic leukemia; DHPLC, denaturing high-performance liquid chromatography; MALDI-TOF MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; PCR, polymerase chain reaction; PCR-SSCP, PCR-single-strand conformation polymorphism; RFLP, restriction fragment length polymorphism; UADT cancer, upper aerodigestive tract cancer.

$P = 0.343$ for heterogeneity. No significant association was found between this variant and bladder cancer (OR = 1.01, 95% CI: 0.79–1.30, $P = 0.920$, $P_{het.} = 0.419$), leukemia (OR = 0.93, 95% CI: 0.71–1.23, $P = 0.622$, $P_{het.} = 0.080$), nasopharyngeal cancer (OR = 0.94, 95% CI: 0.75–1.16, $P = 0.550$, $P_{het.} = 0.675$) and 'other cancers' (OR = 1.11, 95% CI: 0.91–1.35, $P = 0.317$, $P_{het.} = 0.786$) (Figure 2).

The frequency distributions of genotypes in control groups from all studies were in accordance with Hardy–Weinberg equilibrium ($P > 0.05$), so the sensitivity analysis was not performed. To evaluate publication bias, the rs2735383 genotypes were plotted against the precision ones in a funnel plot, which is approximately symmetrical. Egger's test suggested that there was no publication bias in the current meta-analysis ($t = -1.49$, $P = 0.165$) (Figure 3).

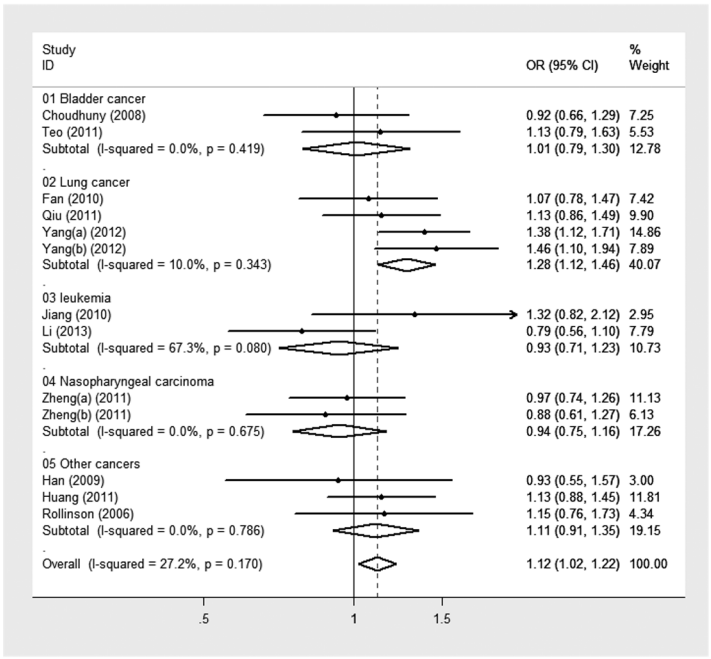
NBS1 *rs1063054*. Nine studies included 2757 cases and 5796 control subjects were analysed. The C allele frequency was 32.4% with 95% CI between 31.0 and 33.7%. Heterogeneity tests were negative for OR_1 (CC vs. AA, $\chi^2 = 12.6$, $P = 0.126$) and OR_2 (CA vs. AA, $\chi^2 = 4.59$, $P = 0.800$) but significant for OR_3 (CC vs. CA, $\chi^2 = 18.15$, $P = 0.020$). A number of factors was explored, including race and source of control groups, but the source of heterogeneity was not identified. We then pooled these studies by logistic regression with the random-effects model. The estimated OR_1, OR_2 and OR_3 were 1.15 (95% CI: 1.01–1.36), 1.11 (95% CI: 1.01–1.23) and 1.00 (95% CI: 0.77–1.29), respectively. The estimated λ by the genetic model-free approach was 0.83 (95% CI: −0.72–2.38). This seems to indicate a dominant mode of effect, and therefore AC and CC were combined and compared with AA (AC+CC vs. AA). The pooled OR was 1.12 (95% CI: 1.01–1.23, $P = 0.024$), $P = 0.941$ for heterogeneity (Figure 2). All studies were confirmed to Hardy–Weinberg equilibrium ($P > 0.05$). The shape of the funnel plot did not reveal any evidence of an obvious asymmetry. Moreover, the result of Egger's test did not show any evidence of publication bias ($t = 0.08$, $P = 0.940$) (Figure 3).

NBS1 *rs1805794*. Forty-two studies included 18 901 cases and 21 430 control subjects were analysed. The pooled frequency of C allele was 36.6% with 95% CI between 34.0 and 39.2%. All studies were in accordance with Hardy–Weinberg equilibrium except two (38,80). Significant heterogeneity was observed for OR_1 (CC vs. GG, $\chi^2 = 273.95$, $P < 0.01$), OR_2 (CG vs. GG, $\chi^2 = 79.77$, $P < 0.01$) and OR_3 (CC vs. CG, $\chi^2 = 164.96$, $P < 0.01$). Race and cancer type were explored as a potential cause. However, heterogeneity was still present in Chinese (for OR_1: $\chi^2 = 142.30$, $P < 0.01$; for OR_2: $\chi^2 = 28.37$, $P < 0.01$; for OR_3: $\chi^2 = 75.74$, $P < 0.01$) and Caucasians (for OR_1: $\chi^2 = 40.89$, $P = 0.024$; for OR_2: $\chi^2 = 17.58$, $P = 0.860$; for OR_3: $\chi^2 = 33.74$, $P = 0.089$). The estimated λ was 2.99 (95% CI: −16.18 to 22.16). It was not suitable to calculate the ORs by race. In the subgroup analysis of cancer type, the variant genotypes had a non-significantly increased risk of cancer (OR = 1.10, 95% CI: 0.96–1.28, $P = 0.053$, $P_{het.} = 0.135$) as estimated in a dominant effect model in four lung cancer studies. In the three colorectal cancer studies, the variant genotypes had also a non-significantly increased risk (OR = 1.12, 95% CI: 0.92–1.36, $P = 0.275$, $P_{het.} = 0.784$) as estimated in an overdominant effect model. In the 12 breast cancer studies, 5 bladder cancer studies and 4 ovarian cancer studies, all of the estimated OR_1, OR_2 and OR_3 were equal to 1, which suggest that there are no correlation between rs1805794 and cancer risks in these subgroups (Table II). No publication bias was detected by either the funnel plot or the Egger's test ($t = -1.06$, $P = 0.334$) (Figure 3).

NBS1 *I171V*. Ten eligible studies included 4516 cases and 9951 controls were analysed, all of whom were Caucasians. Heterogeneity was detected among the 10 studies ($\chi^2 = 31.89$, $P < 0.01$). Consequently, the random-effects model was applied. In the overall analysis, I171V mutation was significantly associated with cancer risk (carriers vs. non-carriers: OR = 3.93, 95% CI: 1.68–9.20, $P = 0.002$) (Figure 4).

In the subgroup analysis of tumour site, I171V mutation was associated with lymphoma risk (carriers vs. non-carriers: OR = 25.98, 95% CI: 4.57–147.77, $P < 0.001$, $P_{het.} = 0.641$) and 'other cancers' (carriers vs. non-carriers: OR = 7.70, 95% CI: 1.82–32.64, $P < 0.001$, $P_{het.} = 0.106$). No significant association was found between this variant and breast cancer (carriers vs. non-carriers: OR = 1.40, 95% CI: 0.69–2.38, $P = 0.458$, $P_{het.} = 0.120$) (Figure 4).

I171V mutation is causally associated with NBS and found particularly in individuals with Slavic populations (Polish, Czech and Ukrainian), so there is not an even distribution within different Caucasian populations. Of these 10 studies, 6 studies were conducted in Polish, 1 in French, 2 in German and 1 in Byelorussian. The results were analysed for Slavic populations



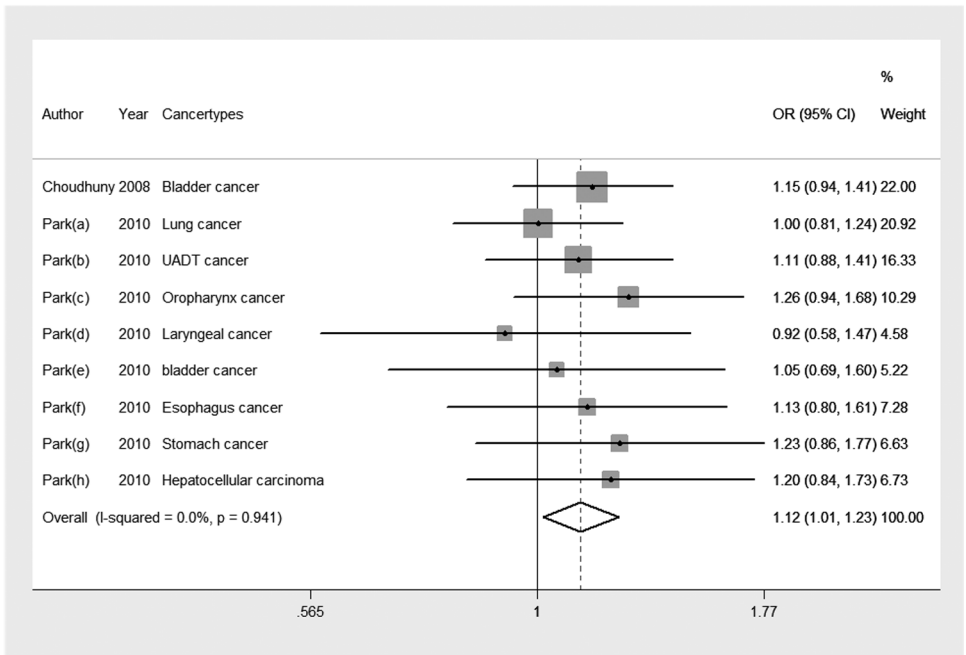


Fig. 2. Forest plots for the overall cancer risks associated with two *NBS1* variants. (**A**) rs2735383, the odds ratio was estimated under the recessive genetic model. (**B**) rs1063054, the odds ratio was estimated under the dominant genetic model.

and other Caucasian populations, respectively. The ORs were 5.93 (95% CI: 2.32–15.18, $P < 0.001$, $P_{het.} = 0.058$) and 1.70 (95% CI: 0.50–5.74, $P = 0.394$, $P_{het.} = 0.032$) for Slavic populations and other Caucasian populations, respectively. Publication bias was detected by the Egger's test ($t = 5.13$, $P = 0.001$) (Figure 3).

NBS1 *657del5*. A total of 21 studies met the inclusion criteria, in which 15 184 cases and 54 081 controls were included. For the overall analysis, significant association between the risk of cancer and 657del5 mutation was found (carriers vs. non-carriers: OR = 2.79, 95% CI: 2.12–3.68, $P < 0.001$, by fixed effects; $P = 0.609$ for heterogeneity) (Figure 4).

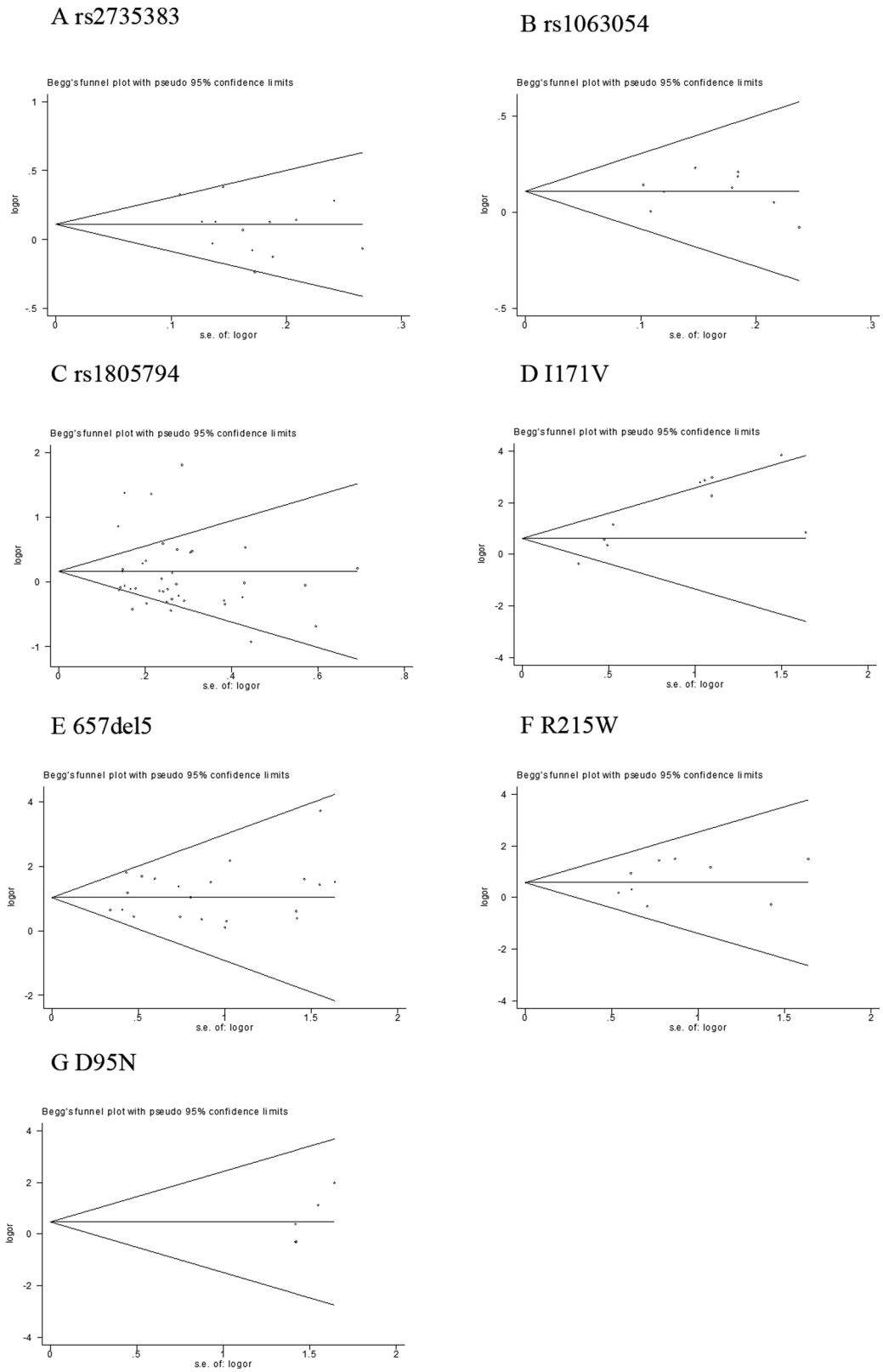


Fig. 3. Funnel plot analysis to detect publication bias for each of the *NBS1* variants. (**A**) rs2735383, (**B**) rs1063054, (**C**) rs1805794, (**D**) I171V, (**E**) 657del5, (**F**) R215W and (**G**) D95N. Each point represents an individual study for the indicated association.

In the stratified analysis of cancer type, significant risk was observed in breast cancer (carriers vs. non-carriers: OR = 2.51, 95% CI: 1.68–3.73, $P < 0.001$, $P_{het.} = 0.869$), lymphoma (carriers vs. non-carriers: OR = 2.93, 95% CI: 1.62–5.29, $P < 0.001$, $P_{het.} = 0.170$), prostate cancer (carriers vs. non-carriers: OR = 5.87, 95% CI: 2.51–13.75, $P < 0.001$, $P_{het.} = 0.883$) and 'other cancers' (carriers vs. non-carriers: OR = 2.29, 95% CI: 1.23–4.26, $P = 0.009$, $P_{het.} = 0.435$) (Figure 4).

Table II. Determination of the genetic effects of rs1805794 on cancers

Genotype	Case number	Control number	Multivariate method		Model-free approach	
			OR	95% CI	λ	95% CI
Caucasian	11 471	12 738				
CC vs. GG			1.04	0.92, 1.17	2.99	−16.18, 22.16
CG vs. GG			1.05	0.99, 1.11		
CC vs. CG			0.97	0.87, 1.08		
Chinese	4333	4880				
CC vs. GG			1.71	1.51, 1.93	0.40	0.21, 0.59
CG vs. GG			1.24	1.12, 1.38		
CC vs. CG			1.37	1.23, 1.53		
Lung cancer	1784	1843				
CC vs. GG			1.34	1.11, 1.62	0.86	0.21, 1.50
CG vs. GG			1.26	1.06, 1.48		
CC vs. CG			1.03	0.88, 1.21		
Colorectal cancer	797	783				
CC vs. GG			0.75	0.54, 1.04		
CG vs. GG			1.05	0.85, 1.30	−0.18	−1.01, 0.65
CC vs. CG			0.72	0.52, 0.99		
Ovarian cancer	1586	2792				
CC vs. GG			0.85	0.69, 1.05	0.02	−0.78, 0.81
CG vs. GG			1.00	0.87, 1.14		
CC vs. CG			0.85	0.69, 1.05		
Breast cancer	6566	6435				
CC vs. GG			0.98	0.86, 1.11	−2.12	−24.97, 20.73
CG vs. GG			1.04	0.96, 1.13		
CC vs. CG			0.95	0.85, 1.08		
Bladder cancer	2798	9270				
CC vs. GG			1.15	0.96, 1.37	0.84	−0.57, 2.25
CG vs. GG			1.13	1.01, 1.26		
CC vs. CG			1.01	0.76, 1.35		

657del5 mutation is associated with NBS and also found particularly in Slavic populations. Of these 21 studies, 13 studies were conducted in Polish, 1 in Czech, 2 in German, 1 in Byelorussian, 2 in Russian and 2 in mixed Caucasian. The ORs were 2.51 (95% CI: 1.87–3.36, $P < 0.001$, $P_{het.} = 0.440$) and 5.21 (95% CI: 1.95–13.95, $P = 0.001$, $P_{het.} = 0.525$) for Slavic populations and other Caucasian populations, respectively. The results did not show any evidence of publication bias ($t = 0.77$, $P = 0.453$) (Figure 3).

NBS1 *R215W.* Nine studies included 6728 cases and 9588 controls were pooled to analyse. In total, the summary OR for all the studies was 1.77 (95% CI: 1.07–2.91, $P = 0.025$, $P_{het.} = 0.641$) (Figure 5). It was shown that the R215W mutation was related to susceptibility to all cancers.

In the stratified analysis of cancer type, no significant risk was observed in breast cancer (carriers vs. non-carriers: OR = 1.44, 95% CI: 0.73–2.85, $P = 0.294$, $P_{het.} = 0.357$). Interestingly, statistically significantly ascending cancer risk was observed in 'other cancers' (carriers vs. non-carriers: OR = 2.24, 95% CI: 1.09–4.60, $P = 0.029$, $P_{het.} = 0.809$) (Figure 5).

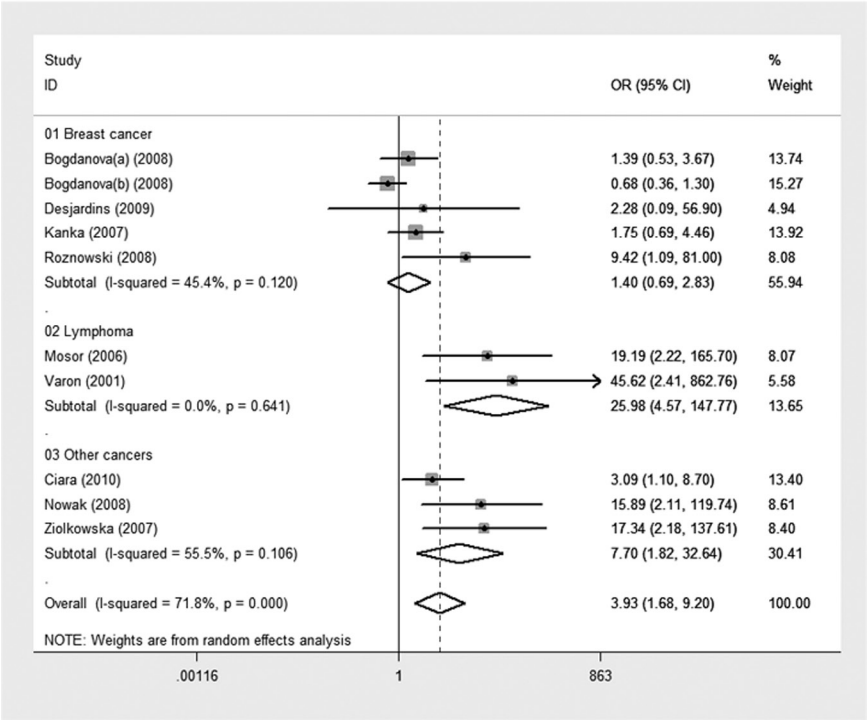
All of the studies were conducted in Caucasians. R215W mutation is also associated with NBS and found particularly in Slavic populations. Of these 9 studies, 4 studies were conducted in Polish, 1 in Czech, 1 in Byelorussian, 1 in French, 1 in German and 1 in mixed Caucasian. The ORs were 1.94 (95% CI: 0.93–4.06, $P = 0.080$, $P_{het.} = 0.465$) and 1.80 (95% CI: 0.80–4.02, $P = 0.154$, $P_{het.} = 0.344$) for Slavic populations and other Caucasian populations, respectively. Finally, Begger's funnel plot and Egger's test showed that publication bias was not significant ($t = 0.83$, $P = 0.431$) (Figure 3).

NBS1 *D95N and P266L.* The association between D95N mutation and cancer risk was investigated in five studies with a total of 1281 cases and 1011 controls. All of the studies were conducted in Caucasians. In the overall analysis, no significant association was observed (carriers vs. non-carriers: OR = 1.69, 95% CI: 0.49–5.81, $P = 0.404$, $P_{het.} = 0.805$) (Figure 5). Source of controls in all studies was population based except for one (42), the result was similar with that of the overall population (carriers vs. non-carriers: OR = 2.08, 95% CI: 0.51–8.44, $P = 0.306$, $P_{het.} = 0.747$). Publication bias was found by Begger's funnel plot and Egger's test ($t = 5.16$, $P = 0.014$) (Figure 3). Only two studies were included for the analysis between P266L mutation and cancer risk, the result suggested that there was no correlation (OR = 1.31, 95% CI: 0.25–6.77, $P = 0.745$, $P_{het.} = 0.679$). Further subgroup analyses were not performed because of limited data for this variant.

Discussion

In this meta-analysis, including a total of 39 731 cancer cases and 64 957 controls from 60 independent publications, the associations of eight well-characterised variants (rs2735383, rs1063054, rs1805794, I171V, 657del5, R215W, P266L and D95N) of the *NBS1* gene with cancer risk were examined. It was demonstrated that the carriers of rs2735383, rs1063054, I171V, 657del5 and R215W variant genotypes were associated with a significant increase in overall cancer risks, whereas the others did not appear to have an influence on cancer susceptibility. From stratification analyses, an effect modification of cancer risks was found in the subgroups of tumour site and ethnicity for rs2735383, whereas the I171V, 657del5 and R215W showed a deleterious effect of cancer susceptibility in the subgroups of tumour site.

A I171V



B 657del5

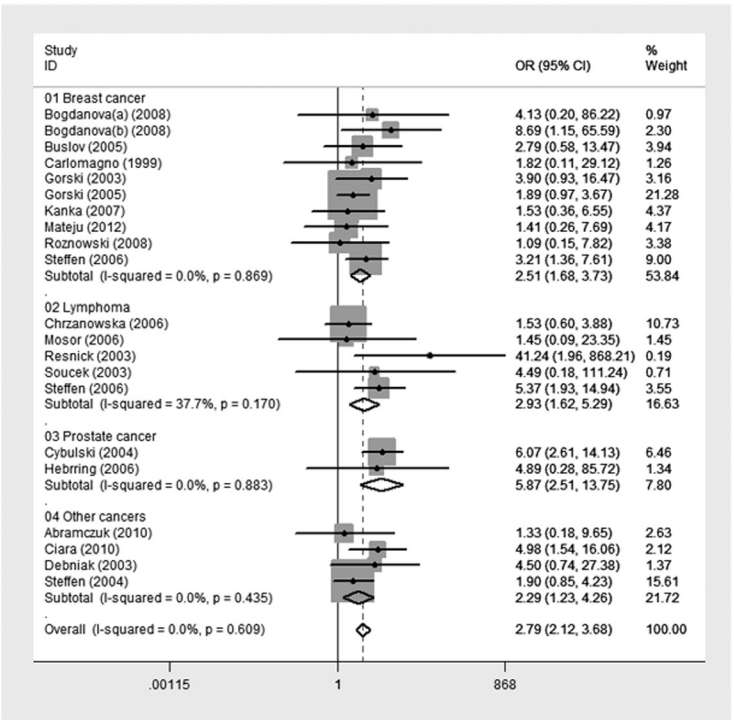


Fig. 4. Forest plots for the overall cancer risks associated with two *NBS1* variants. (**A**) I171V and (**B**) 657del5.

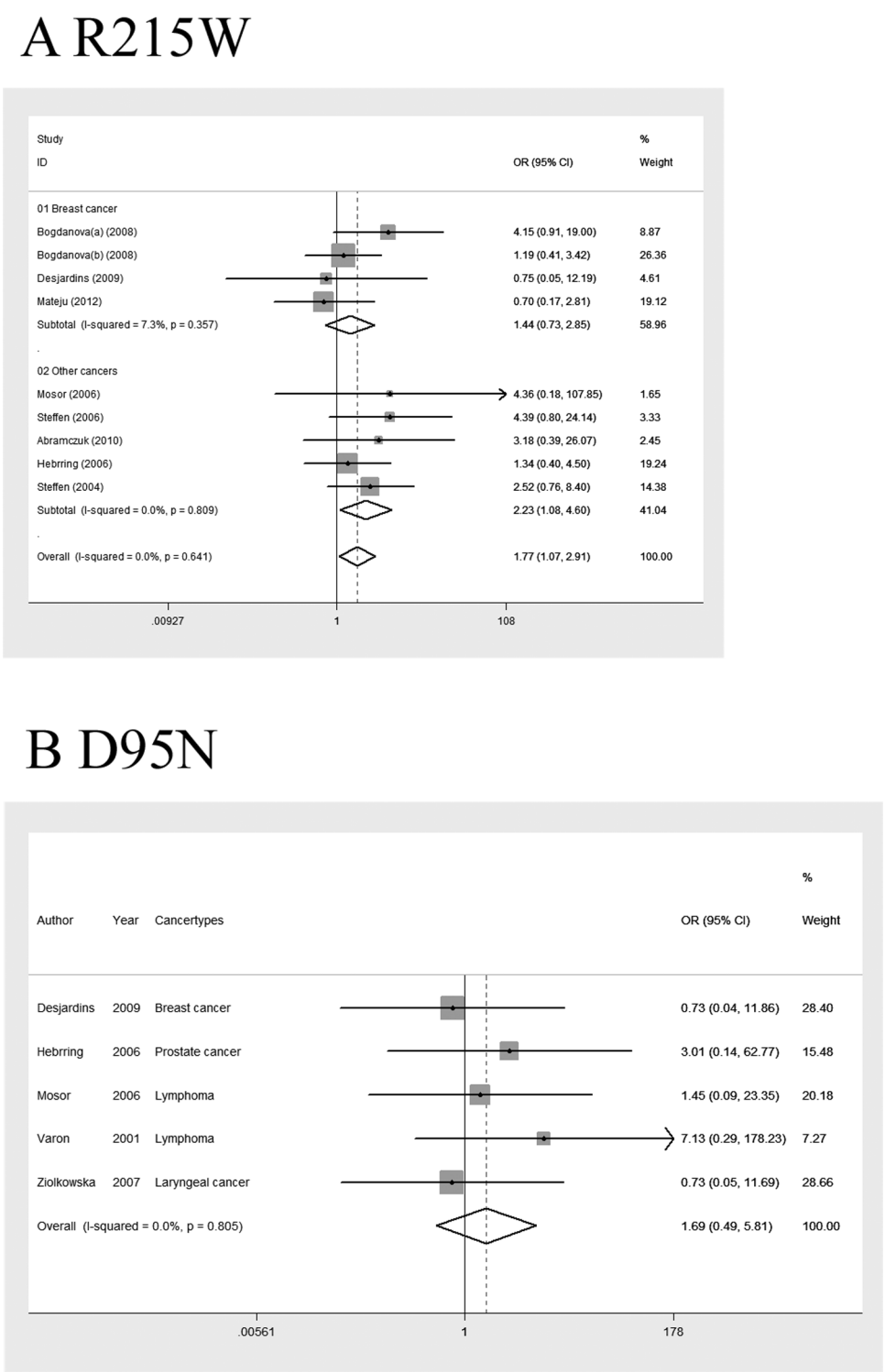


Fig. 5. Forest plots for the overall cancer risks associated with two *NBS1* variants. (**A**) R215W and (**B**) D95N.

Most meta-analyses of genetic association studies were done by assuming a specific genetic model. For example, a polymorphism has two alleles (G and g), the allele of G is thought to be associated with a disease, association studies will usually collect information on the numbers of diseased and non-diseased subjects with each of the three genotypes (gg, Gg and GG). Actually, if genotypes were compared by assuming a special genetic model (e.g. dominant model, recessive model and so on), the conclusion will be sensitive to the assumed genetic model (83). When unsure about the genetic model, some researchers fit multiple models and/or perform pairwise comparisons. However, adjustment for multiple testing is rarely made. The OR of GG vs. gg and the OR of Gg vs. gg are usually obtained by carrying out two separate meta-analyses, but ignoring the correlation between the two ORs induced by the same population (the carriers of gg genotype), thus increasing the probability of making type I error. Several studies indicated that if 5 independent significant tests in 4 genetic models were

made, the false-positive rate will be increased to 0.23 (using 0.05 as the critical significance level in each separate test) (84–86). Minelli's result suggested that adopting the wrong genetic model can lead to erroneous pooled estimates with deceptively high precision by given five meta-analysis examples (11).

Thus, Thakkinstian *et al.* (10) presented a method to determine the best genetic method by comparing OR_1 (GG vs. gg), OR_2 (Gg vs. gg) and OR_3 (GG vs. Gg). The detailed method can be found in the reference (10). Besides, Minelli *et al.* (11) suggested a genetic model-free approach to the meta-analysis, which does not assume the mode of inheritance. The underlying genetic model is estimated from the original data. The model is based on a simple reparameterization and use the parameter of λ ($\log OR_{Gg}/\log OR_{GG}$) to determine the mode of inheritance. Details of this model are given in the reference (11). In this study, the two methods mentioned above were combined to systematically review the association between functional *NBS1* variants and cancer risks. Hence, the association between rs2735383 and cancer risk was investigated under the recessive model of effect. The relationship between rs1063054 and cancer risk was investigated under the dominant model of effect.

As a component of the MRN complex, NBS1 plays an important role in cellular response to DNA damage and the maintenance of chromosomal integrity (30). Previous studies have proved that NBN is a protein with both caretaker and gatekeeper functions in the prevention of tumourigenesis (87–89). The present meta-analysis supports a significant impact of *NBS1* variants on overall cancer risks, particularly in rs2735383, rs1063054, I171V, 657del5 and R215W. The rs2735383 is located at the 3′-UTR of *NBS1*. Single-nucleotide polymorphisms (SNPs) in 3′-UTR region may disrupt or create a microRNA (miRNA) binding site so as to repress translation or destabilise mRNA. Bioinformatics analysis revealed that the rs2735383 variant locates at the binding site of three miRNAs (hsa-miR-629, hsa-miR-499-5p and hsa-miR-509-5p). Yang *et al.* (26) revealed that the rs2735383C allele had a lower transcription activity than G allele, and the hsa-miR-629 but not the other two miRNAs had effect on modulation of *NBS1* gene *in vitro* by luciferase assay in lung cancer. Zheng *et al.* (28) also revealed that the rs2735383C allele had a lower transcription activity than G allele in nasopharyngeal carcinoma, but its function was modulated by hsa-miR-509-5p rather than hsa-miR-629. As for rs1063054, its biological functions have not yet been clarified, but it is also located at the 3′-UTR of *NBS1*. It is predicted that rs1063054 may be combined with hsa-miR-1178, hsa-miR-513a-3p, hsa-miR-654-3p or hsa-miR-657. The exact binding miRNAs need to be validated by functional tests. I171V variant is a transition A>G in 511 position. The consequence of this missense mutation is an exchange of isoleucine for valine in position 171 of NBN. The effect of I171V mutation is a change in this protein's structure. One of its N-terminal domains BRCT is affected (4). The BRCT domain is responsible for a proper interaction with other proteins in DNA repair and cell cycle regulation. Our results indicated that carriers who had the I171V variant were about 293% more likely to have cancers than were non-carriers. 657del5 variant is the most common hypomorphic variant in the *NBS1* gene, which results in two alternative forms of the NBN protein with a lower molecular weight of ~26 and 70 kDa. In particular, the 5-bp deletion in position 657 splits the BRCT tandem domain exactly in the linker region that connects the two BRCT domains. Null mutation is embryonically lethal in the mouse (90). Murine B cells with a conditional null mutation had defective G2/M-phase and intra-S-phase checkpoints (91). Carriers who had the 657del5 variant were about 179% more likely to have cancers than were non-carriers. Significant results were also found in breast cancer and lymphoma subgroups. Zhang *et al.* (92) researched the relationship between 657del5 and breast cancer risk, the pooled OR was 2.63-fold of the referenced genotype, which was similar with the result of this meta-analysis. The R215W mutation in *NBS1* impairs histone γ-H_2AX binding after induction of DNA damage, leading to a delay in DNA-DSB rejoining. Molecular modelling (93) reveals that the 215 residue is located between the two BRCT domains, affecting their relative orientation that appears critical for γ-H_2AX binding. The result of this study indicated that the R215W mutation is a risk factor for cancers. Meanwhile, it is noteworthy that statistically increased risk was observed in 'other cancers', which was combined with a number of single studies with different cancer types, implying that significant association may really exist.

As for rs1805794, it is a non-synonymous mutation, which causes the change of 185 Glu to Gln. The amino acid alteration caused by rs1805794 may possibly change the function of NBN, and then probably change the protein–protein interaction of NBS1 and other BRCA1. A recent research analysed for the association between rs1805794 and cancer risk (8). Their research included 16 studies and was done by assuming a genetic model. Their result indicated that the carriers of variant genotypes had a 1.06-fold elevated risk of cancer in a dominant model. However, the association was not found in an additive model nor in a recessive model. And they did not find associations in the subgroup of cancer type. In this meta-analysis, we adopted the multivariate method and the model-free method to determine the best genetic model. Because significant heterogeneity was observed for OR_1, OR_2, OR_3 and λ, it was not suitable to pool estimates in all of the studies. In the subgroup analysis of cancer type, the association was estimated under the dominant effect model in those lung cancer studies. In the colorectal cancer studies, the association was estimated under the overdominant effect model. This model is also called molecular heterosis. Molecular heterosis occurs when subjects heterozygous for a specific genetic polymorphism show a significantly greater effect (positive heterosis) or lesser effect (negative heterosis) than subjects homozygous for either allele. At a molecular level, heterosis appears counterintuitive, but a review indicates that this model is perhaps more common than we thought and cites many examples (94). Indeed, the SNP Leu432Val in *CYP1B1* gene has been associated with bladder cancer, and this also seems to observe a pattern of overdominant (95). The mechanism of molecular heterosis may include the following. First, the range of expression of gene products on different cells is greater in heterozygotes than in homozygotes. The second possibility is the inverted U effect, i.e. heterozygotes show optimal performance than homozygotes. Third, heterozygotes have advantages in having variation in a multimeric protein, such as better V_{max}. In the breast cancer studies, bladder cancer studies and ovarian cancer studies, all of the three ORs were equal to 1, which suggested there were no substantial associations between rs1805794 and cancer risks in these subgroups.

In the subgroup analysis, elevated risk was pronounced among breast cancer by I171V and 657del5. A common thread linking the main risks for developing breast cancer in women is cumulative, excessive exposure to oestrogen (96). The suggested mechanism is that oestrogen metabolites (i.e. CE-Qs)

bind to DNA, leading to the formation of depurinating adducts and resulting in DSB formation. Breast cells that have lost DSB-related checkpoint/repair due to the harbouring of at-risk genotypes have a growth advantage over DSB checkpoint/repair-proficient cells, resulting in an increased risk of developing breast cancer (97). Tobacco smoke is a well-characterised risk factor for lung cancer. In the subgroup analysis of lung cancer, elevated risk was only found in rs2735383, but not in rs1805794. In spite of this, some researchers (20) indicated that the rs1805794 variant could affect repair of DNA adducts and would allow the accumulation of G→T or C→A transversions in *p*53. Previous studies have reported that *p*53 mutations accumulate in lung tumours with high levels of chromosomal abnormalities (98,99), suggesting that *p*53 mutations may prevent the efficient repair of the chromosomal alterations caused by tobacco carcinogens. rs1805794 associates with *p*53 mutations in lung cancer, suggesting that it may contribute to human lung carcinogenesis in smokers. The non-significant result in our meta-analysis may be due to limited included studies, further investigations are required to validate the result of the present meta-analysis.

This is the first and the most comprehensive meta-analysis undertaken so far for quantitative analyses between *NBS1* variants and the risk of cancer. However, in interpreting the results of the current meta-analysis, some limitations need to be addressed. Firstly, most of the studies were conducted in Caucasian and Asian populations. Therefore, our results may be applicable only to these ethnic groups. Secondly, publication biases were found for I171V and D95N. For these variants, no published data were available for Asian populations, probably because these variants are rare in Asian populations. Thirdly, this meta-analysis was based on unadjusted evaluation, because not all published studies presented adjusted ORs or when they did, the ORs were not adjusted by the same potential confounders. Meanwhile, original data shortage limited our further evaluation of potential gene–environment interactions. Fourthly, our meta-analysis investigated the association between *NBS1* variants and all cancer risk. However, the HR repair pathway is not necessarily expected to be relevant in the carcinogenesis of certain cancers, such as basal cell carcinoma and melanoma, where the nucleotide excision repair would be expected to play a bigger role. The heterogeneity of the cancer types included thus reduces the paper's overall practical relevance to identify clinically useful genetic risk variants. We did calculate the summary ORs without studies of these cancer types. There were no substantial changes in the pooled ORs with or without these studies. In spite of these, our meta-analysis still has some advantages. On one hand, we adopted the multivariate method and the model-free method to determine the best genetic model, rather than assuming a specific genetic model, thereby decreasing the probability of making type I error. On the other hand, no substantial heterogeneities were found for rs2735383, rs1063054, 657del5, R215W, D95N and P266L. For rs1805794, instead of pooling the ORs in all studies directly, we explored the cause of heterogeneity and estimated ORs in subgroups when heterogeneity was eliminated. Merging studies with great heterogeneity can be very misleading (100). Besides, the subject number of >100 000 in the published studies is sufficient for a comprehensive analysis.

In summary, this meta-analysis supports an association between minor variants of rs2735383, rs1063054, I171V, 657del5 and R215W and elevated cancer risks. rs1805794, D95N and P266L were not associated with cancer susceptibility. Further studies with large sample sizes and tissue-specific biological characterization are required to confirm current findings.

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