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EPIDEMIOLOGY

Association of two CASP8 polymorphisms with breast cancer risk: a meta-analysis

Theodoros N. Sergentanis · Konstantinos P. Economopoulos

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Abstract Caspase-8 (CASP8) is an initiator caspase implicated in the process of apoptosis in breast cancer cells. Attention has been drawn upon two polymorphisms: CASP8 D302H (rs1045485) and, more recently, CASP8 −652 6N del (rs3834129). The CASP8 −652 6N del polymorphism remains an open field, as studies are controversial. This meta-analysis aims to examine: (i) the association between CASP8 −652 6N del and breast cancer risk, separately in Chinese and Caucasian populations, and (ii) the association between CASP8 D302H and breast cancer risk. Eligible articles were identified by a search of MEDLINE, Cochrane, and EMBASE bibliographical databases for the period from June 1996 to July 2009. Regarding −652 6N del, five case–control studies were eligible (12,439 breast cancer cases, 13,253 controls) and four case–control studies were eligible for D302H (18,791 breast cancer cases, 20,318 controls). In case significant heterogeneity was detected, the random effects model was chosen; nevertheless, the fixed effects estimates are also secondarily reported as an alternative approach. Where appropriate, power calculations were performed. CASP8 −652 6N del was associated with reduced breast cancer risk at a borderline level (for *del* carriers: pooled OR = 0.884, 95% CI: 0.761–1.028); the power calculation pointed to lack of power in the individual studies. In the Caucasian populations, the same results seem valid (for *del* carriers: pooled OR = 0.944, 95% CI: 0.884–1.008). The random effects model in Chinese subjects has not reached statistical significance (for *del* carriers: pooled OR = 0.811, 95% CI: 0.492–1.338). CASP8 D302H was associated with reduced breast cancer risk (for H carriers: pooled OR = 0.874, 95% CI: 0.834–0.917). In conclusion, both CASP8 −652 6N del and D302H polymorphisms are associated with reduced cancer risk. Further studies are needed to gain the optimal power on −652 6N del, especially in Chinese subjects, as well as to gain insight into D302H in Chinese populations.

Keywords CASP8 D302H · CASP8 −652 6N del · Caspase-8 · Polymorphism · Breast cancer

T. N. Sergentanis · K. P. Economopoulos
School of Medicine, National University of Athens, Athens, Greece

T. N. Sergentanis (✉) · K. P. Economopoulos
Society of Junior Doctors, Athens, Greece
e-mail: tsergentanis@sni.gr
URL: www.sni.gr

Introduction

Caspase-8 (CASP8) is an initiator caspase implicated in the process of apoptosis in breast cancer cells [1, 2]. Given its involvement in apoptosis, a variety of studies have examined polymorphisms in CASP8 gene with respect to breast cancer risk. Attention has been mainly drawn upon two polymorphisms: CASP8 D302H (*rs1045485*) [3–6] and, more recently, CASP8 −652 6N del (*rs3834129*) [7–11]; secondarily one report on another polymorphism has appeared in the literature (*rs12693932* [12]).

The CASP8 −652 6N del polymorphism remains an open field. Studies are controversial; some studies have demonstrated reduced susceptibility [7], whereas other studies did not detect any association [8–11]. The controversy persists also at the level of race; two contradictory studies have appeared on Chinese populations [7, 8], whereas null results have appeared in Caucasian populations [8–11]. Importantly, no meta-analyses have appeared on the association between CASP8 −652 6N del and breast cancer risk.

On the other hand, there is relative unanimity regarding CASP8 D302H polymorphism. Two studies have reported a protective effect of the polymorphism [3, 5], which has been confirmed by a recent critical meta-analysis [13]; nevertheless, the majority of data come from the Breast Cancer Association Consortium [5], which seems to have established the protective action. Since then two studies have appeared [6, 14]; Palanca Suela et al. were in accordance with the protective effect, whereas Sigurdson et al. limited the effect of CASP8 D302H on homozygous carriers.

This meta-analysis aims to examine: (i) the association between CASP8 −652 6N del polymorphism and breast cancer risk, separately in Chinese and Caucasian populations, (ii) the association between CASP8 D302H polymorphism and breast cancer risk. Where appropriate, power calculations are presented so as to gain deeper understanding on the underlying associations.

Methods

Trial identification

Eligible articles were identified by a search of MEDLINE, Cochrane, and EMBASE bibliographical databases for the period from June 1996 to July 2009 (last search: July 3, 2009) using combinations of the following keywords: "breast cancer," "CASP8," "caspase-8," "polymorphism," "deletion," "D302H," "−652 6N del," "rs3834129," and "rs1045485". In addition, we checked all the references of relevant reviews and eligible articles that our search retrieved. Language restrictions were not used, and two investigators (TNS and KPE), working independently, searched the literature and extracted data from each eligible case–control study.

Eligible studies and data abstraction

All case–control studies with any sample size examining the association of CASP8 −652 6N del or CASP8 D302H polymorphisms with breast cancer (i.e., reporting the frequencies of *ins/ins*, *ins/del* and *del/del* and DD, DH, and HH genotypes in cases and controls, respectively) were considered eligible for this analysis. For each of the eligible case–control studies, the following data were collected: journal name, year of publication, inclusion and exclusion criteria, demographic characteristics of the population being studied, and frequencies of genotypes in cases and controls.

Statistics

Based on the genotype frequencies in cases and controls, crude odds ratios (ORs) as well as their standard errors (SEs) were calculated. Concerning the −652 6N del polymorphism, the ORs pertained to genotype *ins/del* (heterozygous versus *ins/ins*), genotype *del/del* (homozygous versus *ins/ins*), and the *del* allele (*del* carriers, i.e., *ins/del* merged with *del/del* versus *ins/ins*). With respect to the CASP8 D302H polymorphism, the ORs pertained to genotype DH (heterozygous versus HH), genotype DD (homozygous versus HH), and the D allele (D allele carriers, i.e., DH merged with DD versus HH). Where possible, subanalyses on Chinese and Caucasian populations were performed.

The fixed-effects model (Mantel–Haenszel method) as well as the random effects (DerSimonian Laird) model were used to calculate the pooled OR. Between-study heterogeneity and between-study inconsistency were assessed by using Cochran Q statistic and by estimating I^2, respectively [15]. In case significant heterogeneity was detected, the random effects model was chosen; nevertheless, the fixed effects estimates are also secondarily reported as an alternative approach. In case of borderline findings, power calculations were also performed to examine whether the lack of adequate power accounts for the blurring of associations.

Evidence of publication bias was determined using Begg's [16] and Egger's [17] formal statistical test and by visual inspection of the funnel plot. For the interpretation of Begg's test, statistical significance was defined as $P < 0.1$. Analyses were conducted using STATA 10.0 (STATA Corp. College Station, TX, USA) and meta-analysis was performed using the "metan" command.

Results

Figure 1 graphically illustrates the trial flow chart. Out of the 30 abstracts retrieved through the search criteria, 17 studies were irrelevant, two studies were excluded due to the fact that they did not report the allele frequencies [14, 18], and two articles were meta-analyses [4, 13]. As a result, nine case–control studies were included in this meta-analysis; five of them pertained to the −652 6N del polymorphism (12,439 breast cancer cases, 13,253 controls), and four of them concerned D302H polymorphism (18,791 breast cancer cases, 20,318 controls).

CASP8 −652 6N del polymorphism

In the overall analysis, CASP8 −652 6N del polymorphism was associated with reduced breast cancer risk at a borderline level (for *del* carriers: pooled OR = 0.884, 95% CI: 0.761–1.028, Fig. 2a). Interestingly, the power calculation on the pooled frequencies showed that the required sample size for the achievement of power equal to 0.8 (assuming

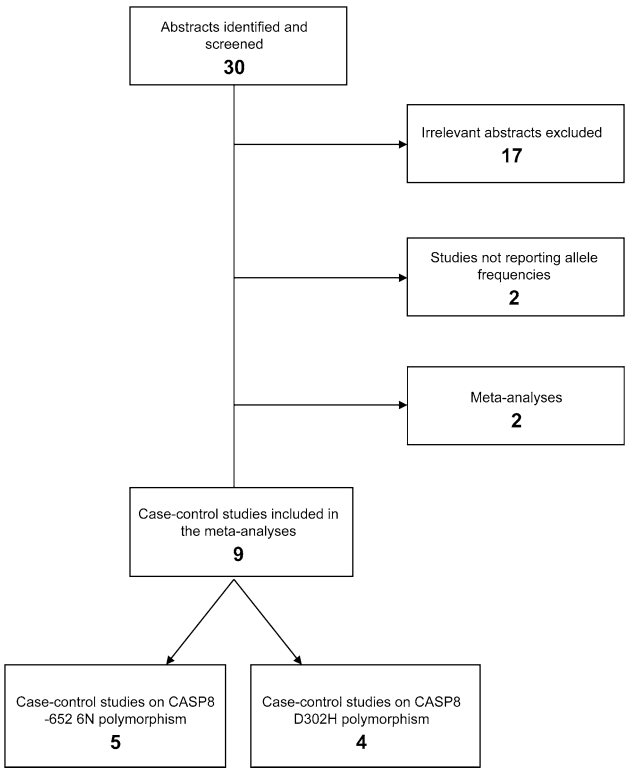


Fig. 1 Study flow chart explaining the selection of the nine eligible case–control studies

type I error 0.05) is 28,228 subjects (14,114 cases and 14,114 controls); this indicates that all the published studies are underpowered. Given that this meta-analysis has included a slightly less number of subjects than the above, the borderline character of the association may be due to relatively inadequate overall power. Noticeably, the less strict fixed effects procedure has yielded a formally statistically significant result.

In the Caucasian populations, the same results appear to be valid (for *del* carriers: pooled OR = 0.944, 95% CI: 0.884–1.008, Fig. 2b). For the distinction between heterozygous and homozygous carriers, see Table 1. Only two studies have appeared on Chinese subjects [7, 8]; the random effects model has not yet reached statistical significance (for *del* carriers: pooled OR = 0.811, 95% CI: 0.492–1.338).

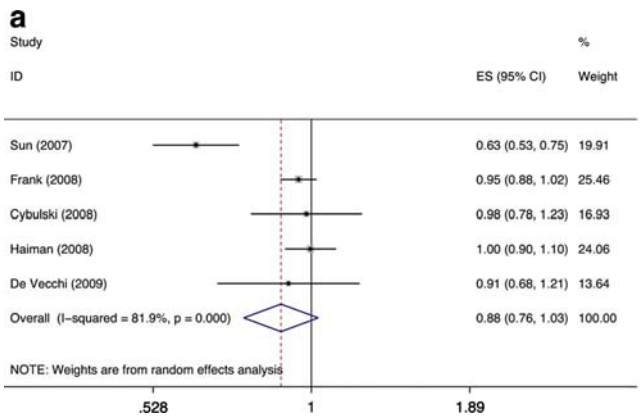
CASP8 D302H polymorphism was associated with reduced breast cancer risk (for H carriers: pooled OR = 0.874, 95% CI: 0.834–0.917, Fig. 3). For further details, see Table 1.

The Begg's and Egger's test did not demonstrate any statistically significant publication bias in either polymorphisms.

Discussion

This is the first meta-analysis on CASP8 −652 6N del polymorphism; a clear trend indicating a protective effect of the polymorphism became evident. The protective association seems to pertain both to Caucasian and Chinese subjects; for the latter, however, the inadequate number of studies did not allow the random effects procedure to reach significant findings. The protective association demonstrated by this meta-analysis can be inscribed into a wider context, as CASP8 −652 6N del polymorphism has been associated with reduced risk for bladder [19], melanoma [20], pancreatic [21], lung, gastrointestinal, and cervical [7] cancer.

Interestingly, given the results of the power calculations, all individual published studies seem underpowered to detect the slight association between CASP8 −652 6N del



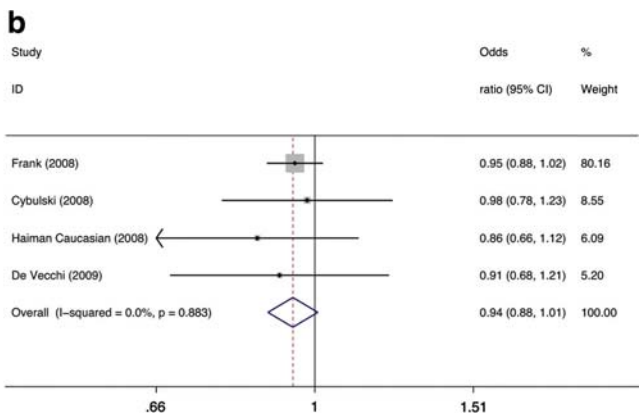


Fig. 2 Forest plot for the **a** overall association between CASP8 −652 6N del status and breast cancer risk (for *del* allele carriers versus *ins/ins*), random effects, **b** association between CASP8 −652 6N del status and breast cancer risk in Caucasian subjects (for *del* allele carriers versus *ins/ins*), fixed effects. Each study is shown by the point estimate of the Odds Ratio (OR) (the size of the square is proportional to the weight of each study) and 95% confidence interval for the OR (*extending lines*); the pooled OR and 95% confidence interval are shown by *diamonds*

Table 1 Pooled ORs for the examined CASP8 polymorphisms

Polymorphism	Overall associations			Associations by race				
				Caucasian		Chinese		
	Fixed effects OR (95% CI)	Random effects OR (95% CI)	Test for heterogeneity	Fixed effects OR (95% CI)	Test for heterogeneity	Fixed effects OR (95% CI)	Random effects OR (95% CI)	Test for heterogeneity
CASP8 −652 6N del								
CASP8 −652 6N del carriers	**0.925 (0.877–0.976)***	0.884 (0.761–1.028)	$P < 0.001$	**0.944 (0.884–1.008)**	$P = 0.883$	**0.826 (0.735–0.930)***	0.811 (0.492–1.338)	$P < 0.001$
Heterozygous	**0.934 (0.882–0.988)***	0.899 (0.779–1.037)	$P = 0.001$	0.949 (0.886–1.017)	$P = 0.751$	**0.854 (0.755–0.965)***	0.838 (0.510–1.379)	$P < 0.001$
Homozygous	**0.918 (0.854–0.988)***	0.871 (0.740–1.025)	$P = 0.024$	0.933 (0.860–1.013)	$P = 0.833$	**0.637 (0.484–0.838)***	0.641 (0.391–1.052)	$P = 0.071$
CASP8 D302H								
CASP8 D302H carriers	–	–	–	**0.874 (0.834–0.917)**	$P = 0.659$	–	–	–
Heterozygous	–	–	–	**0.889 (0.847–0.933)**	$P = 0.684$	–	–	–
Homozygous	–	–	–	**0.711 (0.606–0.833)**	$P = 0.297$	–	–	–

The values given in bold represent statistically significant results; the values marked with a star represent the results of the alternative approach (fixed effects despite heterogeneity)

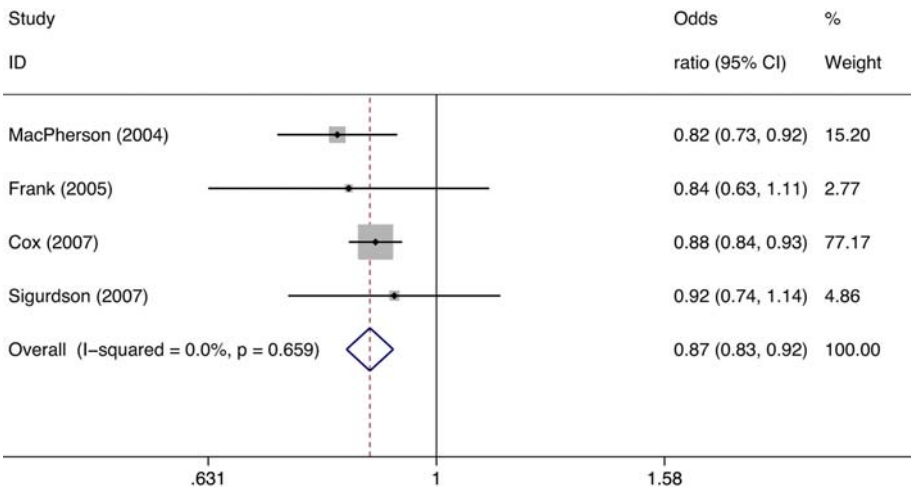


Fig. 3 Forest plot for the association between CASP8 D302H status and breast cancer risk in Caucasians (for H allele carriers versus DD), fixed effects

and breast cancer. Consequently, this meta-analysis gives the message that large studies are needed to provide a clear picture of the association. Indeed, the underpowered character of individual studies may have accounted for the fact that none of them was able per se to reach statistical significance, although all of them pointed to the same, protective direction; the protective effect became thus evident solely at the meta-analytical level.

Concerning CASP8 D302H, this meta-analysis is in accordance with previous ones [4, 13], which had been performed on a smaller number of studies. It is worth mentioning, however, that the lack of studies on Chinese women does not permit the safe extrapolation of findings onto the former race. Concerning the race-CASP8 D302H interplay, half the picture solely has become evident.

In conclusion, both CASP8 −652 6N del and D302H polymorphisms are associated with reduced cancer risk. This meta-analysis makes clear that further studies are needed to (i) gain the optimal power vis-à-vis −652 6N del, especially in Chinese subjects and (ii) gain insight into D302H in the above-mentioned race. It is tempting to anticipate studies simultaneously assessing both polymorphisms, so as to detect whether they mediate independent (additive) or synergistic (multiplicative) effects.

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