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Review Article

Homocysteine and risk of age-related macular degeneration: a systematic review and meta-analysis

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ABSTRACT.

There is still no agreement on total plasma homocysteine (tHcy) role in age-related macular degeneration (AMD), the leading cause of new blindness in industrialized countries. We performed a systematic review and meta-analysis of the published data on the correlation between tHcy and AMD. MEDLINE/PubMed and ISI Web of Sciences searches were performed according to MOOSE guidelines. Case–control studies were eligible for inclusion. Participants and controls were AMD patients and subjects without AMD. The main outcome measure was wet AMD. Homocysteine level was the main exposure variable. Data were pooled using a random-effects model. Twelve case–control studies were identified: 10 assessed wet AMD, four dry AMD, one early AMD, one late AMD, and one any AMD. As for wet AMD, there was a total of 453 cases and 514 controls. Mean tHcy was on average 1.1 μmol/l (95% confidence interval [CI] = 0.96–1.25) greater in wet AMD cases, but there was evidence of extreme between-study heterogeneity ($p < 0.001$, $I^2 = 91.8\%$). In a model homogenous for age, including six wet AMD studies (214 cases, 274 controls), mean tHcy was on average 0.58 μmol/l (95% CI = 0.35–0.73) greater in the case group, a not statistically significant result ($p = 0.144$) associated with moderate heterogeneity ($I^2 = 39.2\%$). Our meta-analysis indicates that there is some weak evidence that increased tHcy might be associated with wet AMD; however, this result should be interpreted cautiously, because of a marked between-study heterogeneity and the possible effect of publication bias. Future studies, preferably of cohort design, are necessary before any firm conclusions on the putative role of increased tHcy on AMD can be drawn.

Key words: age-related macular degeneration – meta-analysis – random-effects model – systematic review – total plasma homocysteine

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All the Authors contributed equally to this work.

Introduction

Age-related macular degeneration (AMD) is the main cause of new cases of blindness in the Western countries (Friedman et al. 2004). Early AMD, a clinical condition without overt functional loss, is said to be present when drusen and/or retinal pigment epithelium alterations are seen in the macular area (Age-Related Eye Disease Study Research Group 2001). The late-stage manifestations of AMD include neovascular (wet AMD) and geographic atrophy (dry AMD). The hallmark of wet AMD is the presence of choroidal neovascularization (CNV). Any disturbance of the Bruch's membrane (e.g. drusen and thickening of the inner aspect) can increase the likelihood that a break will occur, allowing buds of neovascular tissue from the choriocapillaris to perforate the outer aspect of the Bruch's membrane.

The exact pathophysiological mechanisms behind AMD remain to be determined, but genetic predisposition and environmental factors, such as oxidative stress and tobacco smoking, are believed to play an important role (Beatty et al. 2000; Despriet et al. 2006; Khan et al. 2006; Yang et al. 2006; Yates et al. 2007; Schmidl et al. 2015; Hong et al. 2016). It has been postulated that increased total plasma homocysteine (tHcy) is a risk factor for AMD (Axer-Siegel et al. 2004; Vine et al. 2005; Coral et al. 2006; Kamburoglu et al. 2006; Seddon et al. 2006a; Rochtchina et al. 2007; Javadzadeh et al. 2010; Mulero et al. 2014); however, several studies have failed to demonstrate such a relationship (Heuberger et al. 2002; Wang et al. 2008; Obeid et al. 2013; Pinna et al. 2016).

Hcy is a potentially cytotoxic sulphur-containing amino acid produced during methionine metabolism. Methionine, an essential amino acid from dietary protein, donates methyl groups to vital transmethylation reactions generating important molecules, such as phosphatidylcholine and creatine, and allows methylation of DNA, RNA and neurotransmitters (Finkelstein 1990). In the remethylation pathway, Hcy is an essential intermediate in the transfer of activated methyl groups from tetrahydrofolate to *S*-adenosylmethionine (SAM). The enzymes involved in DNA methylation depend on the availability of vitamin B12 and folate. If they are abundant, DNA methyl transferases (DNMTs) readily transfer methyl groups to cytosine residues. On the other hand, if they are scarce, methionine is converted into *S*-adenosyl homocysteine (SAH) and Hcy. Excess SAH inhibits DNMT activity, thus reducing/preventing DNA methylation and compromising gene silencing.

There are a significant number of studies with contrasting results on the role of plasma tHcy as a risk factor for AMD (Axer-Siegel et al. 2004; Vine et al. 2005; Coral et al. 2006; Kamburoglu et al. 2006; Seddon et al. 2006a; Rochtchina et al. 2007; Javadzadeh et al. 2010; Mulero et al. 2014; Heuberger et al. 2002; Wang et al. 2008; Obeid et al. 2013; Pinna et al. 2016). A recent meta-analysis reported that AMD is associated with elevated tHcy, thus suggesting that this plasma thiol may be a modulator of the risk for AMD (Huang et al. 2015). However, this meta-analysis has several controversial aspects, concerning the study inclusion process and the lack of analysis for appropriate age matching between cases and controls, a crucial confounding factor when assessing plasma tHcy role in vascular disorders (Nygard et al. 1998). Taking into account these considerations, we decided to perform a new, updated, systematic review and meta-analysis of the published data on the correlation between tHcy and AMD. The discovery of a conclusive link between tHcy and AMD risk could lead to preventative measures, because folate and vitamin B12 supplementation, decreasing tHcy levels (Woodside et al. 1998), might also reduce the risk of AMD development.

Patients and Methods

Eligibility criteria for considering studies for this review

Studies were considered eligible for this systematic review if they met the following criteria: (1) included laboratory assessment of plasma tHcy concentrations, (2) compared human subjects with or without AMD (case–control studies), and (3) had been published as articles or letters in peer-reviewed journals.

Search methods for identifying studies

Literature review was performed according to MOOSE guidelines for Meta-Analyses and Systematic Reviews of Observational Studies. Eligible studies were identified by searching MEDLINE/PubMed using this strategy: ('explode "homocysteine"[All Fields] AND "age related macular degeneration"[All Fields]') and ('explode "homocysteine"[All Fields] AND "age related maculopathy"[All Fields]'). A similar strategy was used in searches on ISI Web of Science: search (homocysteine) AND (age related macular degeneration OR maculopathy). On each database, the search was limited to studies on humans published up to and including January 2016.

Study selection

Abstracts were screened independently by three investigators (AP, FZ and FB) to establish whether studies were likely to provide relevant data based on the above-mentioned eligibility criteria. If the abstracts were considered to be relevant, full articles were obtained and examined. Any disagreement between the reviewers in the abstract review or following article selection for full-text review was resolved by discussion. Furthermore, the reference lists of all relevant articles were screened for additional articles.

Data collection and risk of bias assessment

Eligible studies were assessed independently by three reviewers (AP, FZ and FB) using a structured form to extract information about the study (country and year of publication), study subjects (number of cases and controls, selection of cases and controls, age) and tHcy data. We used the Newcastle–Ottawa Scale (NOS; available at: http://www.ohri.ca/programs/clinical_epidemiology/nosgen.pdf) to assess the quality of each study. A number of possible quality indicators were also extracted, including closeness of age matching, use of fasting samples and prestatement of exclusion criteria. The extracted data sets were cross-checked before meta-analysis was performed.

Data synthesis and analysis

The difference in mean tHcy between the AMD and control groups and 95% confidence intervals (CIs) were calculated for each study. In two studies (Seddon et al. 2006a; Obeid et al. 2013), the mean and standard deviation were estimated from formulas using the median and range (Hozo et al. 2005). Meta-analysis was then performed on the combined standard differences. Individual and combined standard differences were plotted on a graph with 95% CIs. Standard differences with 95% CIs that included a value of zero were not significant. Random-effects models were used to calculate pooled estimates. Chi-square tests were used to formally test for heterogeneity. Statistical heterogeneity between studies was evaluated using I^2 statistic (I^2 = 0–25%, no heterogeneity; I^2 = 25–50%, moderate heterogeneity; I^2 = 50–75%, large heterogeneity; and I^2 = 75–100%, extreme heterogeneity). Statistical analysis was performed with commercial software (STATA S/E 14.0 for Windows; StataCorp, College Station, TX, USA).

According to the Italian law, no institutional board review was required for this systematic review.

Results

The PRISMA flow chart of the selection process is shown in Fig. 1 (Moher et al. 2009). Using the above-mentioned criteria, we retrieved 35 records for tHcy and AMD. On individual examination of each of these, four were duplicate publications of the same data set (Nowak et al. 2004; Seddon et al. 2006b; Javadzadeh et al. 2012; Manresa et al. 2015b). After removal of duplicates, 31 records were screened; of these, nine were excluded, because four were cohort studies (Robman et al. 2004; Christen et al. 2009, 2015; Gopinath et al. 2013), two were review

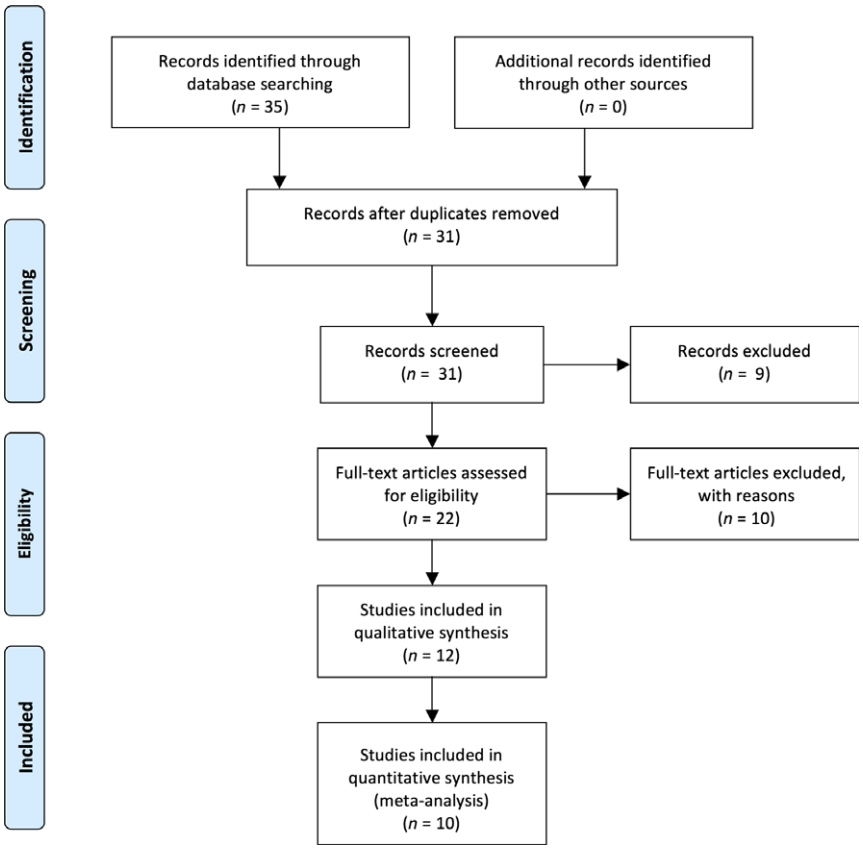

Fig. 1. PRISMA flow chart outlining the selection process for the inclusion of the studies in the systematic review and meta-analysis.

articles (McCarty 2000; Wong et al. 2015), one was a meta-analysis (Huang et al. 2015), one was a cell culture study (Roybal et al. 2005) and one was about Eales' disease (Bharathselvi et al. 2013). Twenty-two full-text articles were assessed for eligibility; of these, 10 were excluded, because two were commentaries (Evans 2013; Kawada 2013), two provided no data on plasma tHcy concentrations (Parmeggiani et al. 2007; Saá et al. 2014) and six did not report suitable or sufficient data for calculation (Heuberger et al. 2002; Vine et al. 2005; Rochtchina et al. 2007; Wu et al. 2007; Keles et al. 2014; Manresa et al. 2015a). In total, 12 of 35 (34.3%) initially identified studies met selection criteria and were finally selected for qualitative synthesis (Table 1): 10 assessed wet AMD, four dry AMD, one early AMD, one late AMD and one any AMD (224 early, 54 late).

Healthy subjects were used as controls in five studies (Coral et al. 2006; Kamburoglu et al. 2006; Ates et al. 2009; Javadzadeh et al. 2010; Mulero et al. 2014), whereas three studies used cataract patients without AMD (Axer-Siegel et al. 2004; Obeid et al. 2013; Pinna et al. 2016). Other control groups included healthy outpatients with refractive errors (Nowak et al. 2005), atherosclerotic cardiovascular disease-matched patients without AMD (Ghosh et al. 2013) and patients without AMD (Seddon et al. 2006a; Wang et al. 2008). In eight studies, blood samples were obtained in a fasting state (Axer-Siegel et al. 2004; Nowak et al. 2005; Coral et al. 2006; Kamburoglu et al. 2006; Javadzadeh et al. 2010; Ghosh et al. 2013; Mulero et al. 2014; Pinna et al. 2016); in nine, subjects with decreased renal function were excluded (Axer-Siegel et al. 2004; Coral et al. 2006; Kamburoglu et al. 2006; Ates et al. 2009; Javadzadeh et al. 2010; Ghosh et al. 2013; Obeid et al. 2013; Mulero et al. 2014; Pinna et al. 2016). In seven studies, plasma tHcy was determined by high-performance liquid chromatography (HPLC) (Axer-Siegel et al. 2004; Nowak et al. 2005; Coral et al. 2006; Seddon et al. 2006a; Kamburoglu et al. 2006; Ates et al. 2009; Ghosh et al. 2013), whereas in five surveys, other laboratory methods were used, including fluorescence polarization immunoassay with IMX analyzer (Wang et al. 2008), ELISA (Javadzadeh et al. 2010), gas chromatography–mass spectrometry (Obeid et al. 2013), intensifying immunonephelometric particle test with BN ProSpec® analyzer (Mulero et al. 2014) and capillary electrophoresis (Pinna et al. 2016).

Overall, there were a very small number of studies assessing dry AMD, early AMD, late AMD and any (both early and late) AMD. As a result, meta-analysis was performed only on the 10 studies investigating wet AMD (Table 2), which represents 28.6% of the initially identified articles. A total of 453 cases and 514 controls were included.

The forest plot for tHcy and wet AMD is shown in Fig. 2. There was evidence of a greater mean tHcy in the cases compared with the controls in the majority of studies. Overall, the mean tHcy was on average 1.1 μmol/l (95% CI = 0.96–1.25) greater in wet AMD cases, but there was extreme between-study heterogeneity ($p < 0.001$, $I^2 = 91.8\%$), thus suggesting the need for cautious interpretation. As most studies were homogenous for fasting state and renal function, we hypothesized that age could be a potential moderator variable accounting for the extreme heterogeneity. Therefore, we tried to identify subsets of participants homogeneous for age. After exclusion of the surveys with higher age heterogeneity, meta-analysis was performed on the remaining six studies (Fig. 3). In this model, including 214 cases and 274 controls, the mean tHcy was on average 0.58 μmol/l (95% CI = 0.35–0.73) greater in wet AMD patients, a not statistically significant result ($p = 0.144$) associated with moderate between-study heterogeneity ($I^2 = 39.2\%$).

Marked heterogeneity persisted despite reanalysis in the following subgroups: (1) European studies ($n = 4$, $p < 0.001$, $I^2 = 95.9\%$; Nowak et al. 2005; Obeid et al. 2013; Mulero et al. 2014; Pinna et al. 2016), (2) non-European studies ($n = 6$, $p < 0.001$, $I^2 = 81.1\%$; Axer-Siegel et al. 2004; Coral et al. 2006; Kamburoglu et al. 2006; Ates et al. 2009; Javadzadeh et al. 2010; Ghosh et al. 2013), (3) studies awarded six or seven stars in

Table 1. Characteristics of studies examining plasma homocysteine (tHcy) and age-related macular degeneration (AMD) included in qualitative synthesis.

						Cases			Controls		
Study	Year	Origin	AMD type	Fasting/ non-fasting state	Renal function	NOS[†]	No. of patients	tHcy, μmol/l mean ± SD	Age, years mean ± SD	No. of patients	tHcy, μmol/l mean ± SD	Age, years mean ± SD
Axer-Siegel	2004	Israel	Wet	Fasting	Checked	Six stars	59	16.4 ± 11.9	78.4 ± 8.4	56	12.5 ± 3.5	77.3 ± 8.2
Axer-Siegel	2004	Israel	Dry	Fasting	Checked	Six stars	58	11.9 ± 4.1	76.3 ± 8.4	56	12.5 ± 3.5	77.3 ± 8.2
Nowak	2005	Poland	Wet	Fasting	Not reported	Five stars	30	14.88 ± 6.23	66.2 ± 3.6	20	8.72 ± 3.34	65.8 ± 5.2
Coral	2006	India	Wet	Fasting	Checked	Five stars	16	18.39 ± 5.29	66.9 ± 7.5*	20	6.7 ± 1.81	62 ± 5*
Kamburoglu	2006	Turkey	Wet	Fasting	Checked	Five stars	30	14.19 ± 3.11	69.7 ± 7.2	30	10.79 ± 2.56	69.9 ± 6.8
Kamburoglu	2006	Turkey	Dry	Fasting	Checked	Five stars	30	13.07 ± 2.9	69.9 ± 7	30	10.79 ± 2.56	69.9 ± 6.8
Seddon	2006	USA	Late	Not reported	Not reported	Seven stars	222	9.51 ± 2.97*	71 ± 5.1	184	8.81 ± 2.74*	67 ± 4.2
Wang	2008	Australia	Any	Not reported	Not reported	Seven stars	278	14 ± 5.2	75.6 ± 8.5	557	13.5 ± 6	74.9 ± 7.9
Ates	2009	Turkey	Wet	Not reported	Checked	Five stars	40	11.6 ± 2.9	63.3 ± 5	40	9.8 ± 1.5	61 ± 4
Javadzadeh	2010	Iran	Wet	Fasting	Checked	Six stars	45	15.4 ± 7.2	71 ± 7	45	10.7 ± 3.7	69 ± 5
Obeid	2013	Germany	Wet	Not reported	Checked	Six stars	31	16.3 ± 4*	78 ± 4.8*	47	15.4 ± 0.9*	74 ± 5.3*
Obeid	2013	Germany	Dry	Not reported	Checked	Six stars	38	14.3 ± 5*	77 ± 4.5*	47	15.4 ± 0.9*	74 ± 5.3*
Ghosh	2013	India	Wet	Fasting	Checked	Six stars	12	18.3 ± 3.39	67.4 ± 6.5	32	14.53 ± 4.08	66.5 ± 5.9
Ghosh	2013	India	Dry	Fasting	Checked	Six stars	20	15.99 ± 3.37	—	32	14.53 ± 4.08	66.5 ± 5.9
Mulero	2014	Spain	Wet	Fasting	Checked	Seven stars	163	13.66 ± 1.47	71 ± 7.3	170	10.35 ± 1.72	71 ± 6.68
Pinna	2016	Italy	Early	Fasting	Checked	Six stars	39	14 ± 4.56	77.8 ± 6	78	14.4 ± 4.55	77 ± 4.7
Pinna	2016	Italy	Wet	Fasting	Checked	Six stars	27	14.5 ± 5.24	79.1 ± 6.9	54	13.8 ± 5.39	77.2 ± 5.4

* Mean and standard deviation (SD) were estimated from formulas using the median and range (Hozo et al. 2005).
† NOS: Newcastle–Ottawa quality assessment scale for case–control studies.

the NOS ($n = 6$, $p < 0.001$, $I^2 = 94.6\%$; Nowak et al. 2005; Javadzadeh et al. 2010; Ghosh et al. 2013; Obeid et al. 2013; Mulero et al. 2014; Pinna et al. 2016), (4) studies awarded five stars in the NOS ($n = 4$, $p < 0.001$, $I^2 = 88.6\%$; Axer-Siegel et al. 2004; Coral et al. 2006; Kamburoglu et al. 2006; Ates et al. 2009), (5) studies using HPLC for tHcy determination ($n = 6$, $p < 0.001$, $I^2 = 81.6\%$; Axer-Siegel et al. 2004; Nowak et al. 2005; Coral et al. 2006; Kamburoglu et al. 2006; Ates et al. 2009; Ghosh et al. 2013), and (6) studies using an assay different from HPLC ($n = 4$, $p < 0.001$, $I^2 = 96.1\%$; Javadzadeh et al. 2010; Obeid et al. 2013; Mulero et al. 2014; Pinna et al. 2016).

Discussion

We performed a meta-analysis to determine the association between tHcy and AMD risk. In contrast to a similar, recent study merging all AMD forms (Huang et al. 2015), our investigation was focused only on wet AMD, due to the small number of reports addressing dry AMD, early AMD and late AMD. We found a statistically significant correlation between increased tHcy levels and wet AMD risk; however, this result must be interpreted cautiously, because of extreme between-study heterogeneity.

In their recent meta-analysis, Huang et al. (2015) reported that AMD is associated with elevated tHcy. In spite of the evidence of extreme between-study heterogeneity ($I^2 = 92\%$), they concluded that tHcy may be a modulator of the risk for AMD. In their study, patients with early and late AMD or with wet and dry AMD were pooled together. This strategy is rather questionable, as wet and dry AMD are different clinical entities with different natural histories, and therefore, their analysis should ideally carried out independently. Indeed, although wet and dry AMD share some common underlying pathological features and causes, there are unique drivers for each form (van Lookeren Campagne et al. 2014). To this regard, after subgroup analysis, Huang et al. (2015) found that wet AMD patients showed higher levels of tHcy than 'all AMD' patients, whereas there was no difference between dry AMD and 'all AMD' patients. Overall, these results confirm

Table 2. Characteristics of studies examining plasma homocysteine (tHcy) and wet age-related macular degeneration (AMD) included in quantitative synthesis.

						Cases			Controls		
Study	Year	Origin	Fasting/ non-fasting state	Renal function	NOS†	No. of patients	tHcy, μmol/l mean ± SD	Age, years mean ± SD	No. of patients	tHcy, μmol/l mean ± SD	Age, years mean ± SD
Axer-Siegel	2004	Israel	Fasting	Checked	Six stars	59	16.4 ± 11.9	78.4 ± 8.4	56	12.5 ± 3.5	77.3 ± 8.2
Nowak	2005	Poland	Fasting	Not reported	Five stars	30	14.88 ± 6.23	66.2 ± 3.6	20	8.72 ± 3.34	65.8 ± 5.2
Coral	2006	India	Fasting	Checked	Five stars	16	18.39 ± 5.29	66.9 ± 7.5*	20	6.7 ± 1.81	62 ± 5*
Kamburoglu	2006	Turkey	Fasting	Checked	Five stars	30	14.19 ± 3.11	69.7 ± 7.2	30	10.79 ± 2.56	69.9 ± 6.8
Ates	2009	Turkey	Not reported	Checked	Five stars	40	11.6 ± 2.9	63.3 ± 5	40	9.8 ± 1.5	61 ± 4
Javadzadeh	2010	Iran	Fasting	Checked	Six stars	45	15.4 ± 7.2	71 ± 7	45	10.7 ± 3.7	69 ± 5
Obeid	2013	Germany	Not reported	Checked	Six stars	31	16.3 ± 4*	78 ± 4.8*	47	15.4 ± 0.9*	74 ± 5.3*
Ghosh	2013	India	Fasting	Checked	Six stars	12	18.3 ± 3.39	67.4 ± 6.5	32	14.53 ± 4.08	66.5 ± 5.9
Mulero	2014	Spain	Fasting	Checked	Seven stars	163	13.66 ± 1.47	71 ± 7.3	170	10.35 ± 1.72	71 ± 6.68
Pinna	2016	Italy	Fasting	Checked	Six stars	27	14.5 ± 5.24	79.1 ± 6.9	54	13.8 ± 5.39	77.2 ± 5.4

* Mean and standard deviation (SD) were estimated from formulas using the median and range (Hozo et al. 2005).
† NOS: Newcastle–Ottawa quality assessment scale for case–control studies.

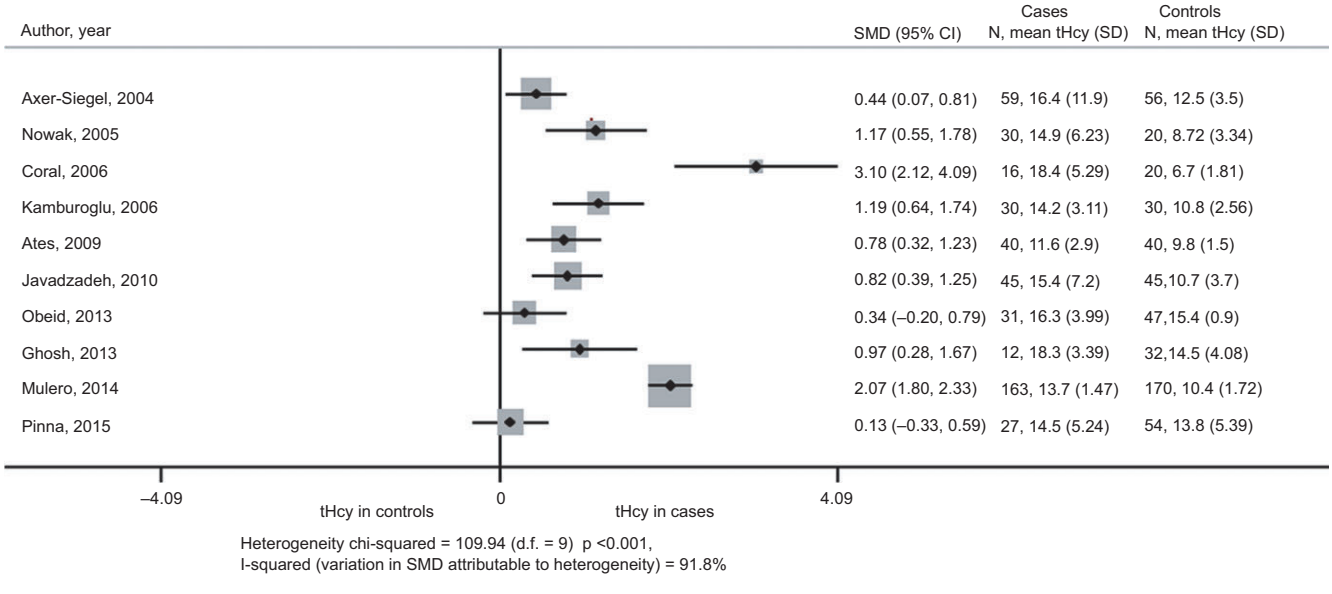

Fig. 2. Forest plot of studies examining plasma homocysteine (tHcy, μmol/l) and wet age-related macular degeneration (AMD), ordered by date of publication. SMD = Standard mean difference; CI = confidence interval; SD = standard deviation.

the need for separate analysis of the different AMD types, while assessing plasma tHcy. Another controversial aspect regarding the study by Huang et al. (2015) is the meta-analysis performed on dry AMD, including only four studies for a total of 147 patients and 165 controls, too small a sample to draw any conclusion. Furthermore, these authors did not perform subgroup analysis for age, on the assumption that all the eligible studies were age-matched and the mean age of the participants was >60 years.

Age is an important confounder when analysing the potential role of plasma tHcy in vascular disorders. Indeed, ageing is associated with increasing tHcy (Nygard et al. 1998; Pinna et al. 2016). In a recent survey on AMD, Pinna et al. (2016) found a ~2 mmol/l increase in tHcy values for every 5 years of increasing age. Although many of the studies identifying tHcy as a risk factor for AMD state that patients and controls were age-matched, the precision of matching and a statistical test of the outcome of matching are rarely presented. It is noteworthy that in the survey by Seddon et al. (2006a), the controls were on average 4 years younger. Similarly, Coral et al. (2006) reported that their controls were significantly younger ($p < 0.001$). In these studies, the use of controls younger than patients could account for the finding of

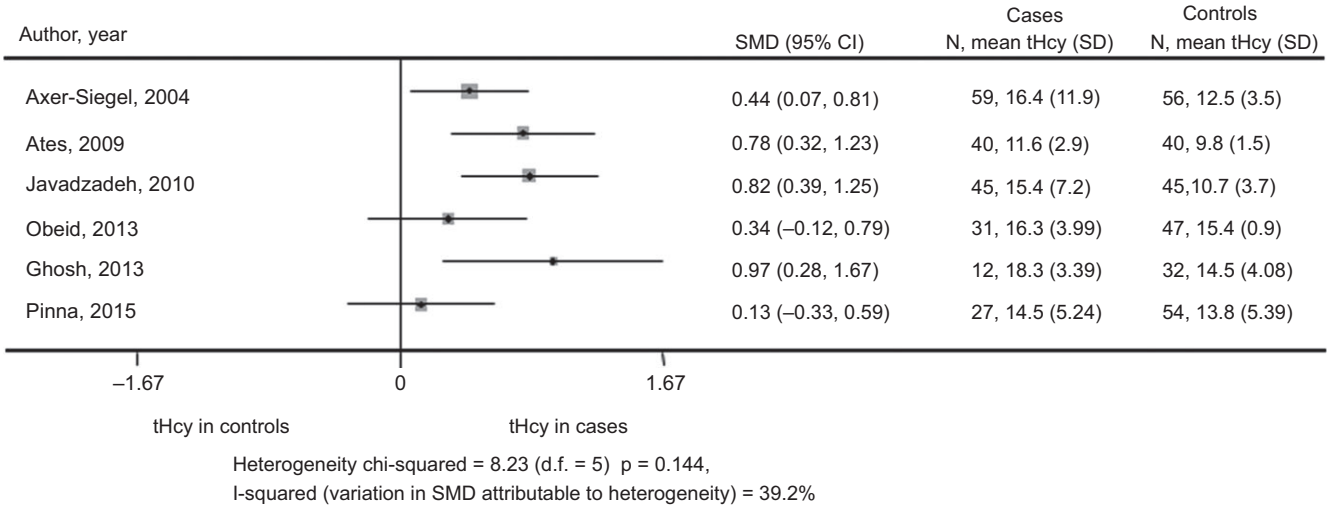

Fig. 3. Forest plot of a subgroup of studies homogeneous for age, examining plasma homocysteine (tHcy, μmol/l) and wet age-related macular degeneration (AMD), ordered by date of publication. SMD = Standard mean difference; CI = confidence interval; SD = standard deviation.

significant differences in tHcy concentrations. These results re-emphasize the need for precise age matching when planning studies on the effects of plasma tHcy (Nygard et al. 1998; Pinna et al. 2016).

Quantifying heterogeneity is one of the most troublesome aspects of meta-analysis. Some possible sources of heterogeneity may be the clinical differences between studies, such as inclusion criteria, patients' and controls' characteristics, baseline disease severity or others.

A crucial issue in case–control studies is the control-group selection. An ideal control group will be as similar as possible to the cases, apart from not having the disease under investigation. In our systematic review, we found quite a variety of control groups, which could partly explain the elevated heterogeneity of the results. The controls usually consisted of healthy volunteers or patients attending hospital (cataract patients without AMD or healthy outpatients with refractive errors), whereas in some studies, the source of controls was not clearly stated (simply 'patients without AMD'). Actually, tHcy tends to increase in cardiovascular disease, and the use of healthy controls (e.g. excluding those with cardiovascular disease) may enhance mild associations with a phenomenon known as the 'healthy participant effect' (McGimpsey et al. 2009). Even though the NOS recommends the use of community controls, the use of hospital-based controls is more appropriate in this context, because their characteristics may be closer to those of the case group.

Our combined estimates, as well as the meta-analysis by Huang et al. (2015), showed a positive correlation between increased tHcy and wet AMD risk, but there was evidence of extreme between-study heterogeneity. When heterogeneity is present, one should question whether and how to generalize the results. To overcome this problem, we tried to identify homogenous subgroups of participants (Haidich 2010). As most studies were homogenous for fasting state and renal function, but heterogeneous for age, we assumed that the latter could be a potential moderator variable accounting for heterogeneity. In a model assessing studies homogenous for age, heterogeneity changed from extreme (I^2 = 91.8%) to moderate (I^2 = 39.2%) and the mean tHcy, although on average 0.58 μmol/l greater in wet AMD patients, became not statistically significant. We also tried to reduce heterogeneity by considering the geographic origin of the studies, classifying them as European and non-European, but this approach was unsuccessful. A similar outcome was found after categorization of the studies on the basis of their quality according the NOS.

There are a number of methods for determining tHcy, including HPLC, capillary electrophoresis, ELISA, immunonephelometry, fluorescence polarization immunoassay and mass spectrometry. In our systematic review, we found that HPLC was used in seven studies, whereas five other studies used a different assay. This is an important aspect, because the use of different laboratory tests may result in different tHcy levels and contribute to heterogeneity. However, our attempt to reduce heterogeneity by considering the type of test for tHcy (HPLC or other assay) was unsuccessful.

Overall, the results from our meta-analysis do not support a sufficiently robust link between tHcy and wet AMD risk.

Folate and vitamin B12 play a pivotal role in Hcy metabolism, as they are essential for the conversion of Hcy into methionine in the remethylation pathway (Pinna et al. 2006). It has been shown that dietary levels of folate and vitamin B12 are inversely related to plasma tHcy levels (Selhub et al. 1993). Therefore, the discovery of a conclusive link between increased tHcy and AMD risk could lead to preventative measures, because folate and vitamin B supplementation can decrease serum tHcy levels (Woodside et al. 1998). As reported by Huang et al. (2015), there are only three studies assessing folate and vitamin B12 in AMD (Nowak et al. 2005; Kamburoglu et al. 2006; Obeid et al. 2013). Their meta-analysis revealed no difference in the serum folate and vitamin B12 levels between the 'all AMD' patients and the controls. When subgroup analyses were performed, they found that folate and vitamin B12 were significantly lower in the

wet AMD group, but there were only 91 cases and 97 controls, too small a sample to draw any conclusion about the role of these nutrients in AMD.

It is well established that plasma tHcy levels are lower in women than in men (Nygard et al. 1998). Women's tHcy concentrations increase after menopause, possibly due to decreased oestrogen production. In our systematic review, we found two studies showing tHcy concentrations higher in male than in female patients (Axer-Siegel et al. 2004; Pinna et al. 2016), whereas two other studies reported no gender differences (Kamburoglu et al. 2006; Obeid et al. 2013). Gender subgroup analysis was not possible because of the limited data.

Cigarette smoking and the use of drugs such as carbamazepine or phenytoin are other possible causes of elevated tHcy (Cahill et al. 2003). In our systematic review, we found only one study analysing the impact of tobacco smoking on tHcy (Ates et al. 2009), which failed to show any statistical difference between non-smoking and smoking patients. In four surveys, smoking was considered an exclusion criterion (Nowak et al. 2005; Coral et al. 2006; Javadzadeh et al. 2010; Mulero et al. 2014). Furthermore, four more studies stated that data about smoking habits had been collected, but they did not present any results (Axer-Siegel et al. 2004; Kamburoglu et al. 2006; Ghosh et al. 2013; Obeid et al. 2013). Regarding the use of drugs affecting Hcy metabolism, there was no information available.

An intrinsic limitation of our study is that we restricted our meta-analysis to reports using a case–control design. The case–control design, as well as the cross-sectional design, is often unable to determine the temporal relationship of exposure and disease, and therefore, it can be unclear whether elevated tHcy is a potential cause of AMD, a consequence of AMD and its underlying mechanisms, or simply a correlate of other factors associated with AMD. Prospective data can provide more reliable evidence to evaluate a possible causal relationship between tHcy and AMD, but they are limited to just two cohort studies from Australia and the USA (Gopinath et al. 2013; Christen et al. 2015). Gopinath et al. (2013) examined the association between serum tHcy and the 10-year incidence of AMD in an Australian cohort of 1760 men and women aged 55 years and older. In that study, each one standard deviation increase in tHcy was associated with a modest 33% increased risk of early and any AMD, but no significant association was found with late AMD. In a more recent study from the USA, Christen et al. (2015) evaluated the relationship between baseline levels of plasma tHcy and the 10-year incidence of AMD among 27 479 apparently healthy women aged 40 years or older. These authors found that elevated tHcy at baseline was associated with a modest, but statistically non-significant, increased risk of AMD after adjustment for AMD risk factors. Overall, the findings from both cohort studies do not support a major role for elevated tHcy as an independent risk factor for the development of AMD, a result consistent with our meta-analysis.

It is well known that studies yielding inconclusive or contrary results are more likely not be published, leading to publication bias. This is a potentially serious limitation of a meta-analysis. Publication bias can be estimated using a number of tests, such as the Egger regression asymmetry test and funnel plots (Egger et al. 1997; Sterne et al. 2000). However, in meta-analyses with <20 studies, such as ours, the sensitivity of these tests is low, and funnel plots and Egger tests performed on the small number of reports included in our study were inconclusive (data not shown).

Despite the inclusion of one additional case–control study (Pinna et al. 2016) and restriction of the meta-analysis to the most numerous subgroup (i.e. wet AMD), we were still unable to draw firm conclusions on the relationship between tHcy and the risk of AMD. The majority of studies published have tended to conclude that an association between tHcy and AMD risk exists, and our meta-analysis would seem to support this view through the finding of a significantly increased pooled estimate. However, the presence of marked heterogeneity and likely publication bias makes direct comparison and pooling of the results unreliable.

In summary, there is some weak evidence suggesting an association between tHcy and wet AMD; yet, because of the presence of extreme heterogeneity and likely publication bias, this association remains tentative. High-quality epidemiologic studies, preferably of cohort design, are necessary before firm conclusions on the putative role of elevated tHcy on AMD can be drawn. At this stage, as well as in retinal vein occlusion (McGimpsey et al. 2009), no recommendation can be made with regard to routine investigation and treatment of elevated tHcy in wet AMD.

References

Age-Related Eye Disease Study Research Group (2001): A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report number 8. Arch Ophthalmol **119**: 1417–1436.

Ates O, Azizi S, Alp HH, Kiziltunc A, Beydemir S, Cinici E, Kocer I & Baykal O (2009): Decreased serum paraoxonase 1 activity and increased serum homocysteine and malondialdehyde levels in age-related macular degeneration. Tohoku J Exp Med **217**: 17–22.

Axer-Siegel R, Bourla D, Ehrlich R, Dotan G, Benjamini Y, Gavendo S, Weinberger D & Sela BA (2004): Association of neovascular age-related macular degeneration and hyperhomocysteinemia. Am J Ophthalmol **137**: 84–89.

Beatty S, Koh H, Phil M, Henson D & Boulton M (2000): The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol **45**: 115–134.

Bharathselvi M, Biswas J, Selvi R, Coral K, Narayanasamy A, Ramakrishnan S & Sulochana KN (2013): Increased homocysteine, homocysteine-thiolactone, protein homocysteinylation and oxidative stress in the circulation of patients with Eales' disease. Ann Clin Biochem **50**: 330–338.

Cahill MT, Stinnett SS & Fekrat S (2003): Meta-analysis of plasma homocysteine, serum folate, serum vitamin B12, and thermolabile MTHFR genotype as risk factors for retinal vascular occlusive disease. Am J Ophthalmol **136**: 1136–1150.

Christen WG, Glynn RJ, Chew EY, Albert CM & Manson JE (2009): Folic acid, pyridoxine, and cyanocobalamin combination treatment and age-related macular degeneration in women: the Women's Antioxidant and Folic Acid Cardiovascular Study. Arch Intern Med **169**: 335–341.

Christen WG, Cook NR, Ridker PM & Buring JE (2015): Prospective study of plasma homocysteine level and risk of age-related macular degeneration in women. Ophthalmic Epidemiol **22**: 85–93.

Coral K, Raman R, Rathi S, Rajesh M, Sulochana KN, Angayarkanni N, Paul PG & Ramakrishnan S (2006): Plasma homocysteine and total thiol content in patients with exudative age-related macular degeneration. Eye **20**: 203–207.

Despriet DD, Klaver CC, Witteman JC et al. (2006): Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration. JAMA **296**: 301–309.

Egger M, Davey Smith D, Schneider M & Minder C (1997): Bias in meta-analysis detected by a simple, graphical test. BMJ **315**: 629–634.

Evans J (2013): Should we be taking B vitamins to prevent age-related macular degeneration? Not yet, but worth doing more research [letter] Am J Clin Nutr **98**: 4–5.

Finkelstein JD (1990): Methionine metabolism in mammals. J Nutr Biochem **1**: 228–237.

Friedman DS, O'Colmain BJ, Muñoz B et al. (2004): Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol **122**: 564–572.

Ghosh S, Saha M & Das D (2013): A study on plasma homocysteine level in age-related macular degeneration. Nepal J Ophthalmol **5**: 195–200.

Gopinath B, Flood VM, Rochtchina E, Wang JJ & Mitchell P (2013): Homocysteine, folate, vitamin B-12, and 10-y incidence of age-related macular degeneration [letter]. Am J Clin Nutr **98**: 129–135.

Haidich AB (2010): Meta-analysis in medical research. Hippokratia **14**(Suppl. 1): 29–37.

Heuberger RA, Fisher AI, Jacques PF, Klein R, Klein BE, Palta M & Mares-Perlman JA (2002): Relation of blood homocysteine and its nutritional determinants to age-related maculopathy in the third National Health and Nutrition Examination Survey. Am J Clin Nutr **76**: 897–902.

Hong N, Shen Y, Yu CY, Wang SQ & Tong JP (2016): Association of the polymorphism Y402H in the CFH gene with response to anti-VEGF treatment in age-related macular degeneration: a systematic review and meta-analysis. Acta Ophthalmol **94**: 334–345.

Hozo SP, Djulbegovic B & Hozo I (2005): Estimating the mean and variance from the median, range, and the size of a sample. BMC Med Res Methodol **5**: 13.

Huang P, Wang F, Sah BK, Jiang J, Ni Z, Wang J & Sun X (2015): Homocysteine and the risk of age-related macular degeneration: a systematic review and meta-analysis. Sci Rep **5**: 10585.

Javadzadeh A, Ghorbanihaghjo A, Bahreini E, Rashtchizadeh N, Argani H & Alizadeh S (2010): Plasma oxidized LDL and thiol-containing molecules in patients with exudative age-related macular degeneration. Mol Vis **16**: 2578–2584.

Javadzadeh A, Ghorbanihaghjo A, Bahreini E, Rashtchizadeh N, Argani H & Alizadeh S (2012): Serum paraoxonase phenotype distribution in exudative age-related macular degeneration and its relationship to homocysteine and oxidized low-density lipoprotein. Retina **32**: 658–666.

Kamburoglu G, Gumus K, Kadayifcilar S & Eldem B (2006): Plasma homocysteine, vitamin B12 and folate levels in age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol **244**: 565–569.

Kawada T (2013): Nutrients related to the incidence of early and late age-related macular degeneration [letter]. Am J Clin Nutr **98**: 1144.

Keles S, Ates O, Kartal B et al. (2014): Evaluation of cardiovascular biomarkers in patients with age-related wet macular degeneration. Clin Ophthalmol **8**: 1573–1578.

Khan JC, Thurlby DA, Shahid H, Clayton DG, Yates JR, Bradley M, Moore AT & Bird AC (2006): Smoking and age related macular degeneration: the number of pack years of cigarette smoking is a major determinant of risk for both geographic atrophy and choroidal neovascularisation. Br J Ophthalmol **90**: 75–80.

van Lookeren Campagne M, LeCouter J, Yaspan BL & Ye W (2014): Mechanisms of age-related macular degeneration and therapeutic opportunities. J Pathol **232**: 151–164.

Manresa N, Mulero J, Losada M & Zafrilla P (2015a): Effect of Pegaptanib and Ranibizumab on plasma and vitreous homocysteine in patients with exudative age-related macular degeneration. Retina **35**: 1765–1771.

Manresa N, Mulero J, Losada M & Zafrilla P (2015b): Influence of anti-VEGF about cardiovascular biomarkers in age related macular degeneration. J Nutr Health Aging **19**: 228–231.

McCarty MF (2000): Up-regulation of endothelial nitric oxide activity as a central strategy for prevention of ischemic stroke - just say NO to stroke! Med Hypotheses **55**: 386–403.

McGimpsey SJ, Woodside JV, Cardwell C, Cahill M & Chakravarthy U (2009): Homocysteine, methylenetetrahydrofolate reductase C677T polymorphism, and risk of retinal vein occlusion: a meta-analysis. Ophthalmology **116**: 1778–1787.e1.

Moher D, Liberati A, Tetzlaff J, Altman DG & PRISMA Group (2009): Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med **6**: e1000097.

Mulero J, Manresa N, Zafrilla P & Losada M (2014): Markers of cardiovascular risk in elderly patients with age-related macular degeneration. Clin Hemorheol Microcirc **58**: 447–453.

Nowak M, Szapska B, Swietochowska E et al. (2004): Blood concentration of homocysteine, vitamin B (12), and folic acid in patients with exudative age related macular degeneration [in Polish]. Klin Oczna **106**: 429–430.

Nowak M, Swietochowska E, Wielkoszyński T et al. (2005): Homocysteine, vitamin B12, and folic acid in age-related macular degeneration. Eur J Ophthalmol **15**: 764–767.

Nygard O, Refsum H, Ueland PM & Vollset SE (1998): Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. Am J Clin Nutr **67**: 263–270.

Obeid R, Ninios K, Loew U, Gatzioufas Z, Hoffmann S, Seitz B, Geisel J & Herrmann W (2013): Aqueous humor glycation marker and plasma homocysteine in macular degeneration. Clin Chem Lab Med **51**: 657–663.

Parmeggiani F, Costagliola C, Gemmati D et al. (2007): Predictive role of coagulation-balance gene polymorphisms in the efficacy of photodynamic therapy with verteporfin for classic choroidal neovascularization secondary to age-related macular degeneration. Pharmacogenet Genomics **17**: 1039–1046.

Pinna A, Carru C, Zinellu A, Dore S, Deiana L & Carta F (2006): Plasma homocysteine and cysteine levels in retinal vein occlusion. Invest Ophthalmol Vis Sci **47**: 4067–4071.

Pinna A, Zinellu A, Tendas D, Blasetti F, Carru C & Castiglia P (2016): Plasma Homocysteine and Asymmetrical Dimethyl-l-Arginine (ADMA) and Whole Blood DNA Methylation in Early and Neovascular Age-Related Macular Degeneration: a Pilot Study. Curr Eye Res **41**: 88–96.

Robman L, McNeil J, Dimitrov P et al. (2004): Methodology of the Cardiovascular Health and Age-Related Maculopathy (CHARM) Study. Ophthalmic Epidemiol **11**: 161–179.

Rochtchina E, Wang JJ, Flood VM & Mitchell P (2007): Elevated serum homocysteine, low serum vitamin B12, folate, and age-related macular degeneration: the Blue Mountains Eye Study. Am J Ophthalmol **143**: 344–346.

Roybal CN, Hunsaker LA, Barbash O, Vander Jagt DL & Abcouwer SF (2005): The oxidative stressor arsenite activates vascular endothelial growth factor mRNA transcription by an ATF4-dependent mechanism. J Biol Chem **280**: 20331–20339.

Saá J, Fernández-Guinea O, García-Pravia P et al. (2014): Relationship between breast arterial calcifications seen on screening mammograms and age-related macular degeneration [letter]. Acta Ophthalmol **92**: e582–e584.

Schmidl D, Garhöfer G & Schmetterer L (2015): Nutritional supplements in age-related macular degeneration. Acta Ophthalmol **93**: 105–121.

Seddon JM, Gensler G, Klein ML & Milton RC (2006a): Evaluation of plasma homocysteine and risk of age-related macular degeneration. Am J Ophthalmol **141**: 201–203.

Seddon JM, Gensler G, Klein ML & Milton RC (2006b): C-reactive protein and homocysteine are associated with dietary and behavioural risk factors for age-related macular degeneration. Nutrition **22**: 441–443.

Selhub J, Jacques PF, Wilson PW, Rush D & Rosenberg IH (1993): Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA **270**: 2693–2698.

Sterne JA, Gavaghan D & Egger M (2000): Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. J Clin Epidemiol **53**: 1119–1129.

Vine AK, Stader J, Branham K, Musch DC & Swaroop A (2005): Biomarkers of cardiovascular disease as risk factors for age-related macular degeneration. Ophthalmology **112**: 2076–2080.

Wang JJ, Ross RJ, Tuo J et al. (2008): The LOC387715 polymorphism, inflammatory markers, smoking, and age-related macular degeneration. A population-based case-control study. Ophthalmology **115**: 693–699.

Wong CW, Wong TY & Cheung CM (2015): Polypoidal choroidal vasculopathy in Asians. J Clin Med **4**: 782–821.

Woodside JV, Yarnell JW, McMaster D et al. (1998): Effect of B-group vitamins and antioxidant vitamins on hyperhomocysteinemia: a double-blind, randomized, factorial-design, controlled trial. Am J Clin Nutr **67**: 858–866.

Wu KH, Tan AG, Rochtchina E, Favaloro EJ, Williams A, Mitchell P & Wang JJ (2007): Circulating inflammatory markers and hemostatic factors in age-related maculopathy: a population-based case-control study. Invest Ophthalmol Vis Sci **48**: 1983–1988.

Yang Z, Camp NJ, Sun H et al. (2006): A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. Science **314**: 992–993.

Yates JR, Sepp T, Matharu BK et al. (2007): Complement C3 variant and the risk of age-related macular degeneration. N Engl J Med **357**: 553–561.

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