Genetic associations for keratoconus: a systematic review and meta-analysis

Shi Song Rong[1,5], Sarah Tsz Ue Ma[1], Xinting Yu[1,3], Li Ma[1], Wai Kit Chu[1], Tommy Chung Yan Chan[4], Yu Meng Wang[1], Alvin L. Young[1,2], Chi Pui Pang[1], Vishal Jhanji[1,2], Li Jia Chen[1,2]

[1] Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong, China;

[2] Department of Ophthalmology and Visual Sciences, Prince of Wales Hospital, Hong Kong, China;

[3] Faculty of Medicine, the Chinese University of Hong Kong, Hong Kong, China;

[4] Hong Kong Eye Hospital, Kowloon, Hong Kong, China;

[5] Current affiliation: Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear, Boston, Massachusetts, USA.

[6] Current affiliation: Department of Gastroenterology, Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA.

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Supplementary Table 1. Summary of reported and meta-analysed genes/loci and polymorphisms for keratoconus

	Offical Symbol	No. of SNPs reported	No. of SNPs meta-analysed
1	*VSX1*	19	5
2	*HGF*	15	3
3	*CAST*	14	0
4	*COL4A3*	11	9
5	*COL4A4*	7	6
6	*IL1B*	5	2
7	*IL1RN*	5	1
8	*LOX*	5	4
9	*COL5A1*	4	2
10	*IL1A*	3	1
11	*TF*	3	0
12	*TGFBI*	3	0
13	*13q33.3*	2	2
14	*CHSY1*	2	0
15	*FEN1*	2	0
16	*FGF9-SGCG*	2	0
17	*HS3ST3B1-PMP22*	2	0
18	*IMMP2L*	2	2
19	*MPDZ-NFIB*	2	1
20	*RAD51*	2	0
21	*SMAD3*	2	0
22	*XRCC1*	2	0
23	*12p13.3*	1	1
24	*19p12*	1	1
25	*3q26.2*	1	1
26	*ADAMTS6*	1	0
27	*ARHGAP20-POU2AF1*	1	0
28	*ARID5B*	1	0
29	*BANP-ZNF469*	1	1
30	*BHLHE40*	1	1
31	*BIRC8*	1	1
32	*C7orf42*	1	0
33	*COL8A2*	1	0
34	*DOCK9*	1	0
35	*FAM46A-IBTK*	1	0
36	*FAS*	1	0
37	*FASLG*	1	0
38	*FNDC3B*	1	1
39	*FOXO1*	1	1
40	*GLT8D2*	1	0
41	*hsa-mir-568*	1	0
42	*KCND3*	1	1
43	*KIF26B*	1	1
44	*LCN12-PTGDS*	1	0
45	*LPAR1*	1	0
46	*LRRK1*	1	0
47	*LRRN1*	1	1
48	*MIR184*	1	0
49	*MUTYH*	1	0
50	*NEIL1*	1	0
51	*NR3C2*	1	0
52	*OGG1*	1	0
53	*PARP1*	1	0
54	*POLG*	1	0
55	*PPP3CA*	1	1
56	*PTGDS*	1	0
57	*RAB3GAP1*	1	1
58	*RXRA-COL5A1*	1	1
59	*SOD1*	1	0
60	*TBL1XR1-KCNMB2*	1	0
61	*TJP1*	1	0
62	*UBXN4*	1	1
63	*VKORC1L1*	1	0
64	*WNT10A*	1	0

No. = number; SNPs = single nucleotide polymorphism.

Supplementary Table 2. Allelic associations of gene variations with keratoconus in different populations

No.	Gene/locus	SNP	No. of cohorts	Ethnicity	Associated allele vs. Reference allele	Pooled sample size		Outcome*		Heterogeneity		Egger's test (P)
						Case	Control	P	OR (95% CI)	P (Q)	I^2 (%)	
Based on both GWAS and replication studies												
1	*FOXO1*	rs2721051	3	Whites	C vs. T	1,031	6,757	**1.5×10^{-9}**	0.63 (0.54 - 0.73)	0.40	11	0.56
2	*MPDZ-NFIB*	rs1324183	3	Whites	C vs. A	1,032	6,759	**1.8×10^{-4}**	0.72 (0.61 - 0.86)	0.14	49	0.083
3	*BANP-ZNF469*	rs9938149	3	Whites	C vs. A	1,030	6,757	**2.6×10^{-4}**	0.76 (0.66 - 0.88)	0.18	42	0.066
4	*COL5A1*	rs7044529	4	Whites	C vs. T	1335	7275	**9.9×10^{-4}**	0.81 (0.71 - 0.92)	0.41	12	0.12
5	*HGF*	rs3735520	5	Whites	T vs. C	1,101	4,356	**3.6×10^{-3}**	1.32 (1.10 - 1.60)	0.014	66	0.79
6	*LOX*	rs2956540	3	Whites	G vs. C	692	6,598	0.60	0.89 (0.57 - 1.38)	2.9×10^{-4}	91	6.0×10^{-5}
Based on candidate gene studies												
1	*COL4A4*	rs2229813	3	Whites	G vs. A	262	336	**1.3×10^{-12}**	2.38 (1.87 - 3.03)	0.68	0	0.39
		rs2229813	2	Chinese and Arabic	G vs. A	209	252	**0.047**	0.74 (0.56 - 1.00)	0.27	16	n.a.
		rs2228557	3	Whites	T vs. C	261	335	**4.5×10^{-7}**	0.54 (0.42 - 0.68)	0.59	0	0.31
		rs2229814	2	Whites	T vs. C	218	258	0.59	1.07 (0.83 - 1.39)	0.99	0	n.a.
		rs2228555	2	Whites	G vs. A	217	257	0.67	1.06 (0.82 - 1.38)	1.00	0	n.a.
2	*FNDC3B*	rs4894535	2	Chinese and Arabic	T vs. C	307	477	0.078	1.25 (0.98 - 1.60)	0.48	0	n.a.
3	*COL4A3*	rs55703767	3	Whites	T vs. G	262	334	0.11	0.19 (0.03 - 1.44)	3.0×10^{-14}	96	3.4×10^{-15}
		rs28381984	2	Whites	T vs. C	217	257	0.25	0.86 (0.66 - 1.11)	0.972	0	n.a.
		rs34019152	2	Whites	A vs. G	217	257	0.63	0.86 (0.47 - 1.58)	0.945	0	n.a.
		rs13424243	2	Whites	C vs. G	217	257	0.82	1.08 (0.57 - 2.04)	0.928	0	n.a.
		rs10178458	2	Whites	T vs. C	216	257	0.89	1.02 (0.73 - 1.44)	0.972	0	n.a.
		rs11677877	2	Whites	G vs. A	218	256	0.93	1.02 (0.62 - 1.69)	0.971	0	n.a.
		rs6436669	2	Whites	G vs. A	217	258	0.94	0.99 (0.70 - 1.40)	0.986	0	n.a.
4	*COL5A1*	rs7044529	3	Whites	C vs. T	684	4,514	0.11	0.88 (0.74 - 1.03)	0.678	0	0.52
		rs7044529	2	Chinese and Arabic	C vs. T	317	491	0.95	1.01 (0.73 - 1.40)	0.590	0	n.a.
5	*VSX1*	rs12480307	2	Chinese and Indian	G vs. A	214	209	0.29	2.06 (0.54 - 7.92)	0.163	49	n.a.
		rs6138482	2	Whites	A vs. G	155	149	0.59	1.10 (0.77 - 1.57)	0.411	0	n.a.
		rs6138482	3	Chinese, Korean, and Indian	A vs. G	459	406	0.98	1.01 (0.70 - 1.45)	0.068	64	0.026
6	*FOXO1*	rs2721051	2	Chinese and Arabic	C vs. T	314	489	0.31	0.79 (0.50 - 1.25)	0.377	0	n.a.
7	*BANP-ZNF469*	rs9938149	2	Chinese and Arabic	C vs. A	316	491	0.32	0.86 (0.65 - 1.15)	0.893	0	n.a.
8	*IL1B*	rs16944	3	Chinese, Korean, and Japanese	T vs. C	366	590	0.43	0.86 (0.60 - 1.25)	0.043	70	0.66
9	*MPDZ-NFIB*	rs1324183	2	Chinese and Arabic	C vs. A	317	491	0.63	0.87 (0.49 - 1.54)	0.020	82	n.a.

* A random-effects model was used.

† Asian ancestries included 2 or more ethnic groups from Arabic, Chinese, Korean, Japanese, or Indian populations.

CI=confidence interval; OR=odds ration; SNP = single nucleotide polymorphism; n.a. = not applicable; No. = number.

Supplementary Table 3. Quality assessment of case-control study using Newcastle - Ottawa scale (NOS)*

No.	First author (year)	Stars awarded	Selection				Comparability		Exposure		
			(1)	(2)	(3)	(4)	(1a)	(1b)	(1)	(2)	(3)
1	Abu-Amero, K. K., et al. (2015)	6	*	*	-	*	*	-	*	*	n.a.
2	Dudakova, L., et al. (2015)	5	*	*	-	*	*	-	*	-	n.a.
3	Hao, X. D., et al. (2015)	8	*	*	*	*	*	*	*	*	n.a.
4	Hasanian-Langroudi, F. (2015)	5	*	-	-	*	*	*	*	-	n.a.
5	Saravani, R. (2015)	6	*	-	-	*	*	*	*	*	n.a.
6	Kokolakis, N. S. (2014)	7	*	-	*	*	*	*	*	*	n.a.
7	Karolak, J. A., et al. (2014)	6	*	*	-	*	*	-	*	*	n.a.
8	Sahebjada, S. (2014)	6	*	-	*	*	*	-	*	*	n.a.
9	Palamar, M. (2014)	6	*	-	-	*	*	*	*	*	n.a.
10	Bae, H. A. (2013)	8	*	*	*	*	*	*	*	*	n.a.
11	Li, X. (2013)	6	*	*	*	-	*	-	*	*	n.a.
12	Sahebjada, S. (2013)	6	*	-	*	*	*	-	*	*	n.a.
13	Mikami, T. (2013)	6	*	-	-	*	*	*	*	*	n.a.
14	Verma, A. (2013)	5	*	-	-	*	*	-	*	*	n.a.
15	Lu, Y. (2013)†	7	*	*	*	*	*	-	*	*	n.a.
16	Wang, Y. (2013)	6	*	-	-	*	*	*	*	*	n.a.
17	Bykhovskaya, Y. (2012)†	6	*	*	*	*	*	-	*	-	n.a.
18	Li, X. (2012)†	6	*	*	*	*	*	-	*	*	n.a.
19	Burdon, K. P. (2011)†	6	*	*	*	*	*	-	*	*	n.a.
20	Stabuc-Silih, M. (2010)	5	*	-	-	*	*	-	*	*	n.a.
21	Stabuc-Silih, M. (2010)	5	*	-	-	*	*	-	*	*	n.a.
22	Stabuc-Silih, M. (2009)	5	*	-	-	*	*	-	*	*	n.a.
23	Kim, S. H. (2008)	8	*	*	*	*	*	*	*	*	n.a.
24	Mok, J. W. (2008)	7	*	*	*	*	*	-	*	*	n.a.

*Newcastle - Ottawa Quality assessment scale was detailed in Supplementary Appendix 1.

† Genome-wide association study

"-" means no star was awarded. n.a. = not applicable.

Supplementary Table 4. Sensitivity analysis of allelic associations by excluding cohorts deviated from Hardy-Weinberg Equilibrium

No.	**Gene/locus**	**SNP**	**No. of cohorts**	**Ethnicity**	**Associated allele vs. Reference allele**	**Pooled sample size**		**Outcome***		**Heterogeneity**		**Egger's test (P)**
						Case	**Control**	**P**	**OR (95% CI)**	**P (Q)**	**I^2 (%)**	
Based on both GWAS and replication studies												
1	*FOXO1*	rs2721051	4	Multiple ancestries†	C vs. T	1,139	7,058	**4.4×10^{-11}**	0.64 (0.56 - 0.73)	0.490	0	0.83
Based on candidate gene studies												
1	*FOXO1*	rs2721051	2	Multiple ancestries†	C vs. T	265	974	**7.5×10^{-3}**	0.68 (0.51 - 0.90)	0.523	0	n.a.
2	*COL4A3*	rs10178458	2	Multiple ancestries†	T vs. C	209	201	0.87	0.97 (0.65 - 1.44)	0.745	0	n.a.
		rs55703767	3	Multiple ancestries†	T vs. G	256	279	0.43	0.46 (0.07 - 3.08)	3.1×10^{-11}	96	0.45
		rs55703767	2	Whites	T vs. G	158	177	0.46	0.32 (0.016 - 6.54)	3.1×10^{-11}	98	n.a.
3	*COL4A4*	rs2228555	2	Multiple ancestries†	G vs. A	225	250	0.86	1.02 (0.79 - 1.33)	0.807	0	n.a.
		rs2229813	4	Multiple ancestries†	G vs. A	367	431	0.46	1.27 (0.68 - 2.40)	1.3×10^{-6}	89	0.42
		rs2229813	2	Whites	G vs. A	158	179	**3.5×10^{-7}**	2.30 (1.67 - 3.16)	0.42	0	n.a.
		rs2229814	2	Multiple ancestries†	T vs. C	211	201	0.95	1.01 (0.77 - 1.33)	0.634	0	n.a.
4	*VSX1*	rs12480307	2	Multiple ancestries†	G vs. A	159	158	0.23	1.27 (0.86 - 1.88)	0.625	0	n.a.

* A random-effects model was used.

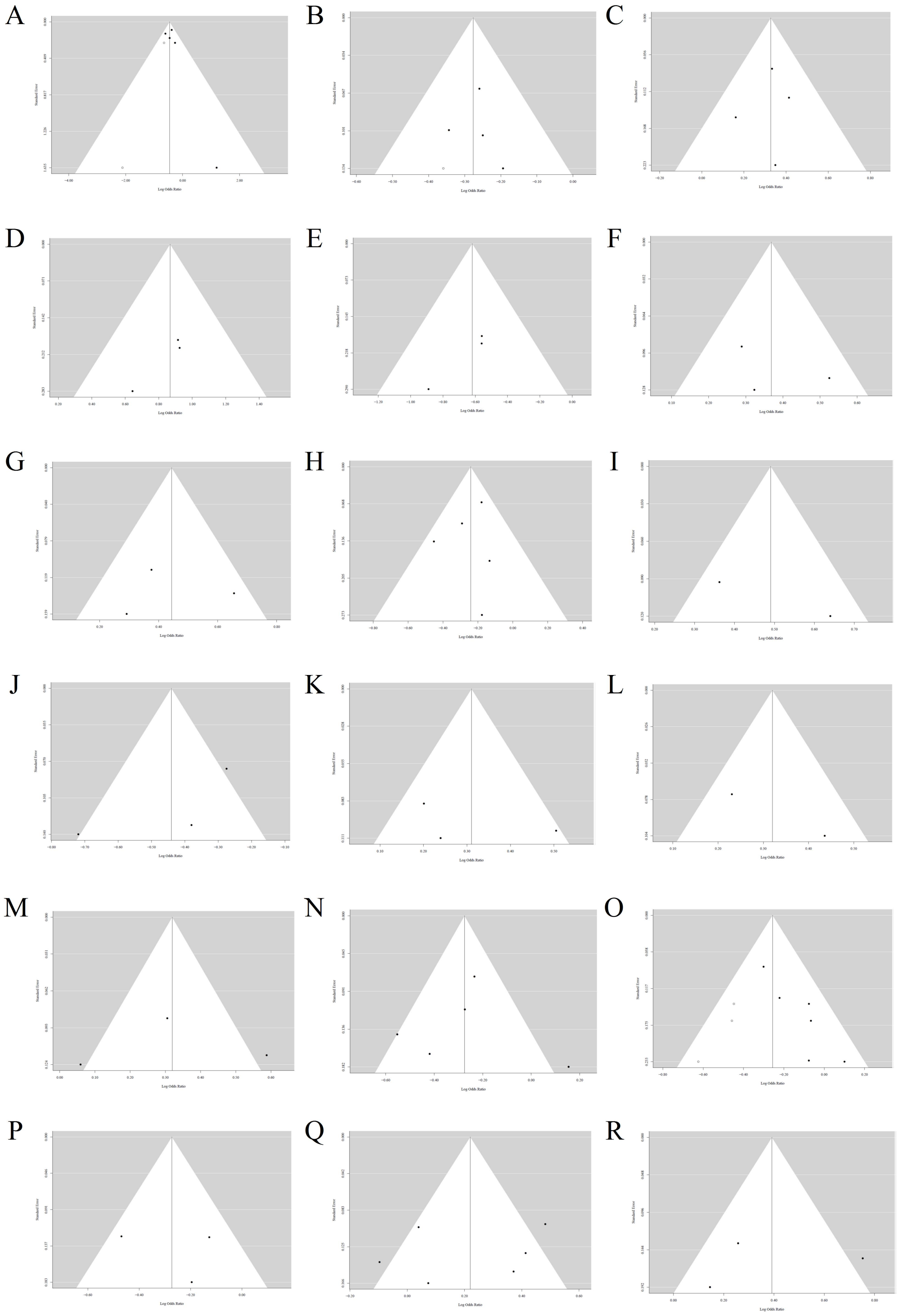
† Multiple ancestries included 2 or more ethnic groups from Whites and Asian (i.e., Arabic, Chinese, Korean, Japanese, or Indian).

CI=confidence interval; OR=odds ration; SNP = single nucleotide polymorphism; n.a. = not applicable; No. = number.

Supplementary Table 5. Search strategies for MEDLINE and Embase databases on the Ovid platform

No.	**Search terms**
1	polymorphism*.mp.
2	nucleotide*.mp.
3	variant*.mp.
4	genom*.mp.
5	mutation*.mp.
6	(locus or loci).mp.
7	DNA.mp.
8	intron.mp.
9	(exon or exome).mp.
10	allel*.mp.
11	haplotype*.mp.
12	genotyp*.mp.
13	genetic*.mp.
14	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13
15	keratoconus.mp. or exp Keratoconus/
16	14 and 15

No.=number


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
Standard Error
Log Odds Ratio

Supplementary Figure 1. Funnel plots of significant genetic associations for keratoconus
A: *FOXO1* rs2721051 ($P_{meta}=\mathbf{5.6\times10^{-11}}$, $P_{Egger}=0.35$**);** B: *RXRA-COL5A1* rs1536482 ($P_{meta}=\mathbf{2.5\times10^{-9}}$, $P_{Egger}=0.89$); C: *FNDC3B* rs4894535 ($P_{meta}=\mathbf{1.4\times10^{-8}}$, $P_{Egger}=0.76$); D: *COL4A4* rs2229813 ($P_{meta}=\mathbf{1.3\times10^{-12}}$, $P_{Egger}=0.39$); E: *COL4A4* rs2228557 ($P_{meta}=\mathbf{4.5\times10^{-7}}$, $P_{Egger}=0.31$); F: *IMMP2L* rs757219 ($P_{meta}=\mathbf{6.1\times10^{-7}}$, $P_{Egger}=0.61$); G: *IMMP2L* rs214884 ($P_{meta}=\mathbf{2.3\times10^{-5}}$, $P_{Egger}=0.89$); H: *BANP-ZNF469* rs9938149 ($P_{meta}=\mathbf{1.3\times10^{-5}}$, $P_{Egger}=0.77$); I: *KCND3* rs4839200 ($P_{meta}=\mathbf{3.9\times10^{-4}}$, P_{Egger}=n.a.); J: *RAB3GAP1* rs4954218 ($P_{meta}=\mathbf{8.2\times10^{-4}}$, $P_{Egger}=0.19$); K: *UBXD2* rs6430585 ($P_{meta}=\mathbf{1.1\times10^{-3}}$, $P_{Egger}=0.62$); L: *13q33.3* rs1328089 ($P_{meta}=\mathbf{1.7\times10^{-3}}$, P_{Egger}=n.a.); M: *13q33.3* rs1328083 ($P_{meta}=\mathbf{3.0\times10^{-2}}$, $P_{Egger}=0.88$); N: *MPDZ-NFIB* rs1324183 ($P_{meta}=\mathbf{5.5\times10^{-3}}$, $P_{Egger}=0.75$); O: *COL5A1* rs7044529 ($P_{meta}=\mathbf{7.0\times10^{-3}}$, $P_{Egger}=0.051$); P: *LOX* rs10519694 ($P_{meta}=\mathbf{0.018}$, $P_{Egger}=0.74$); Q: *HGF* rs3735520 ($P_{meta}=\mathbf{0.027}$, $P_{Egger}=0.60$); R: *19p12* rs8111998 ($P_{meta}=\mathbf{0.035}$, $P_{Egger}=0.74$); n.a.=not applicable

Supplementary Appendix 1

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE

CASE CONTROL STUDIES

(accessed via http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   - a) yes, with independent validation
   - b) yes, e.g. record linkage or based on self reports
   - c) no description
2) Representativeness of the cases
   - a) consecutive or obviously representative series of cases
   - b) potential for selection biases or not stated
3) Selection of Controls
   - a) community controls
   - b) hospital controls
   - c) no description
4) Definition of Controls
   - a) no history of disease (endpoint)
   - b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   - a) study controls for ________________ (Select the most important factor: ethnicity)
   - b) study controls for any additional factor (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure
   - a) secure record (e.g. surgical records)
   - b) structured interview where blind to case/control status
   - c) interview not blinded to case/control status
   - d) written self report or medical record only
   - e) no description
2) Same method of ascertainment for cases and controls
   - a) yes
   - b) no

3) Non-Response rate (*This item was not applicable for this study.)

- a) same rate for both groups
- b) non respondents described
- c) rate different and no designation