SUPPLEMENTARY APPENDIX

Estimating the prevalence of heterozygous familial hypercholesterolemia: a systematic review and meta-analysis

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eTable 1 | Search strategy for Medline

Database: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>
Search Strategy:
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1 exp Hyperlipoproteinemia Type II/ (5647)

2 ("familial hypercholesterolemia" or "familial hypercholesterolaemia").mp. (5157)

3 exp Coronary Disease/ or exp Atherosclerosis/ (224905)

4 exp Mortality/ or exp Mortality, Premature/ (314243)

5 exp Myocardial Infarction/ (156095)

6 exp Stroke/ (102093)

7 exp Heart Failure/ (98464)

8 exp Peripheral Vascular Diseases/ (48037)

9 exp Myocardial Ischemia/ (383424)

10 exp Cardiovascular Diseases/ (2068438)

11 exp Risk/ or exp Risk Factors/ or exp Prevalence/ or exp Incidence/ or exp Prognosis/ (2274061)

12 (prevalence or "risk factors" or incidence or prevalence or prognosis).mp. (2177998)

13 ('familial hypercholesterolemia'.mp. or exp Hyperlipoproteinemia Type II/) and ('systematic review' or 'meta-analysis').mp. (51)

14 1 or 2 (7403)

15 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 (2314576)

16 11 or 12 (3062771)

17 14 and 15 and 16 (942)

18 13 or 17 (985)

19 limit 18 to (human and english language and yr="1990 -Current") (724)

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eTable 2 | Detailed inclusion/exclusion criteria for systematic review of FH prevalence

1. **FULL-TEXT peer-reviewed publication?**
   - Yes (**include**)
   - No - e.g., conference abstract/proceeding (exclude)
   - Can't decide (**include**)

2. **Live HUMAN subjects or study participants?**
   - Yes (**include**)
   - No (exclude)
   - Can't decide (**include**)

3. **Is the study in HETEROZYGOUS familial hypercholesterolemia?**
   - Yes (**include**)
   - No (exclude)
   - Can't decide (**include**)

4. **AGEs of subjects or study participants:**
   - Adults 18 years and over (**include**)
   - Children / Adolescents (**include** – separate)
   - Can't decide (**include**)

5. **TYPE of study reported in this article:**
   - Report of a cohort/registry (**include**)
   - Other observational studies (e.g. Case Control, Cross-Sectional, Case Report/Series, Survey) (**include**)
   - Meta-analyses/systematic reviews/health technology assessments (exclude – separate)
   - Findings from a controlled clinical trial (exclude – separate)
   - Protocol of methods for a controlled clinical trial (exclude)
   - Practice/treatment guideline (exclude)
   - Academic/Narrative Review, Comment, Editorial, Letter, Note, Patient Handout, Study Design Description (exclude)
   - Can't decide (**include**)

6. **Is this study in ENGLISH?**
   - Yes (**include**)
   - No (exclude)
   - Can't decide (**include**)

7. **Does the study report disease PREVALENCE in the subjects or study participants?**
   - Yes (**include**)
   - No (exclude)
   - Can't decide (**include**)

   If PREVALENCE is reported, how is it determined?
   - A) DNA-based evidence of an LDL-receptor mutation, familial defective apo B-100, or a PCSK9 mutation
   - B) Dutch Lipid Clinic Network Criteria
   - C) Simon Broome Registry Criteria
   - D) Making Early Diagnosis to Prevent Early Death (MEDPED) Criteria
   - E) ADULT: Total cholesterol levels > 290 mg/dL (7.5 mmol/L) or LDL-C > 190 mg/dL (4.9 mmol/L)
   - F) CHILD: (< 16 years of age): Total cholesterol levels > 260 mg/dL (6.7 mmol/L) or LDL-C > 155 mg/dL (4.0 mmol/L)
   - G) Hardy-Weinberg equilibrium (exclude)

eTable 3 | The Dutch Lipid Clinic Network (DLCN) criteria	
Criteria	**Score**
Family History	
First-degree relative with premature coronary and/or vascular disease (men < 55 years, women < 60 years) **OR** First-degree relative with known LDL-cholesterol (LDL-C) > 95th percentile for age and sex	**1**
First-degree relative with tendon xanthomata and/or arcus cornealis **OR** Children aged < 18 years with known LDL-C > 95th percentile for age and sex	**2**
Clinical History	
Patient with premature coronary artery disease (age as above)	**2**
Patient with premature cerebral or peripheral vascular disease (age as above)	**1**
Physical Examination	
Tendon xanthomas	**6**
Arcus cornealis at age < 45 years	**4**
LDL-C mmol/L (mg/dL)	
LDL-C > 8.5 (330)	**8**
LDL-C 6.5-8.4 (250-329)	**5**
LDL-C 5.0-6.4 (190-249)	**3**
LDL-C 4.0-4.9 (155-189)	**1**
DNA Analysis	
Functional mutation in LDLR, APOB or PCSK9	**8**
Stratification	**Total Score**
Definite FH	**8**
Probable FH	**6-8**
Possible FH	**3-5**
Unlikely FH	**<3**

eTable 4 | Simon Broome Register diagnostic criteria	
A diagnosis of <u>DEFINITE</u> FH requires either (1), (2) or (3)	
(1)	Total cholesterol > 290 mg/dL (7.5 mmol/L) or LDL-C > 189 mg/dL (4.9 mmol/L) in adults
	Tendon xanthomas in patient or a first- or second-degree relative
(2)	Total cholesterol > 259 mg/dL (6.7 mmol/L) or LDL-C > 155 mg/dL (4.0 mmol/L) in a child under 16 years of age
	Tendon xanthomas in patient or a first- or second-degree relative
(3)	DNA-based evidence of a function LDLR, PCSK9 or ApoB mutation
A diagnosis of <u>PROBABLE</u> FH requires either (1), (2) or (3)	
(1)	Total cholesterol > 290 mg/dL (7.5 mmol/L) or LDL-C > 189 mg/dL (4.9 mmol/L)
	Family history of myocardial infarction
(2)	Total cholesterol > 259 mg/dL (6.7 mmol/L) or LDL-C > 4.0 mmol/L in a child under 16 years of age
	Family history of myocardial infarction before 50 years of age in a second-degree relative or below age 60 in a first-degree relative
(3)	Family history of elevated total cholesterol in a first or second-degree relative (> 7.5 mmol/L in an adult; > 6.7mmol/L in child or sibling aged under 16 years)

eTable 5 | MEDPED Program diagnostic criteria for FH				
	Total cholesterol threshold (mmol/L)			
	First-degree relative with FH	Second-degree relative with FH	Third-degree relative with FH	General population
Age (years)				
<20	5.7	5.9	6.2	7.0
20-29	6.2	6.5	6.7	7.5
30-39	7.0	7.2	7.5	8.8
> 40	7.5	7.8	8.0	9.3
FH is diagnosed if the total cholesterol levels exceed the specified threshold.				

eTable 6 | Considerations of the Effect Public Health Practice Project Quality Assessment Tool	
Component Ratings	**Domains Assessed**
A) Selection Bias	1. Are the individuals selected to participate in the study likely to be representative of the target population? 2. What percentage of selected individuals agreed to participate?
B) Study Design	1. Indicate the study design. 2. Was the study described as randomized? If NO, go to component C. 3. If YES, was the method of randomization described? 4. If YES, was the method of randomization appropriate?
C) Confounders	1. Were there important differences between groups prior to the intervention? 2. If yes, indicate the percentage of relevant confounders that were controlled (either in the design (e.g., stratification, matching) or analysis)?
D) Blinding	1. Was (were) the outcome assessor(s) aware of the intervention or exposure status of participants? 2. Were the study participants aware of the research question?
E) Data Collection Methods	1. Were data collection tools shown to be valid? 2. Were data collection tools shown to be reliable?
F) Withdrawals & Dropouts	1. Were withdrawals and drop-outs reported in terms of numbers and/or reasons per group? 2. Indicate the percentage of participants completing the study.
G) Intervention Integrity	1. What percentage of participants received the allocated intervention or exposure of interest? 2. Was the consistency of the intervention measured? 3. Is it likely that the subjects received an unintended intervention (contamination or co-intervention) that may influence the results?
H) Analyses	1. Indicate the unit of allocation. 2. Indicate the unit of analysis. 3. Are the statistical methods appropriate for the study design? 4. Is the analysis performed by intervention allocation status (i.e. intention to treat) rather than the actual intervention received?

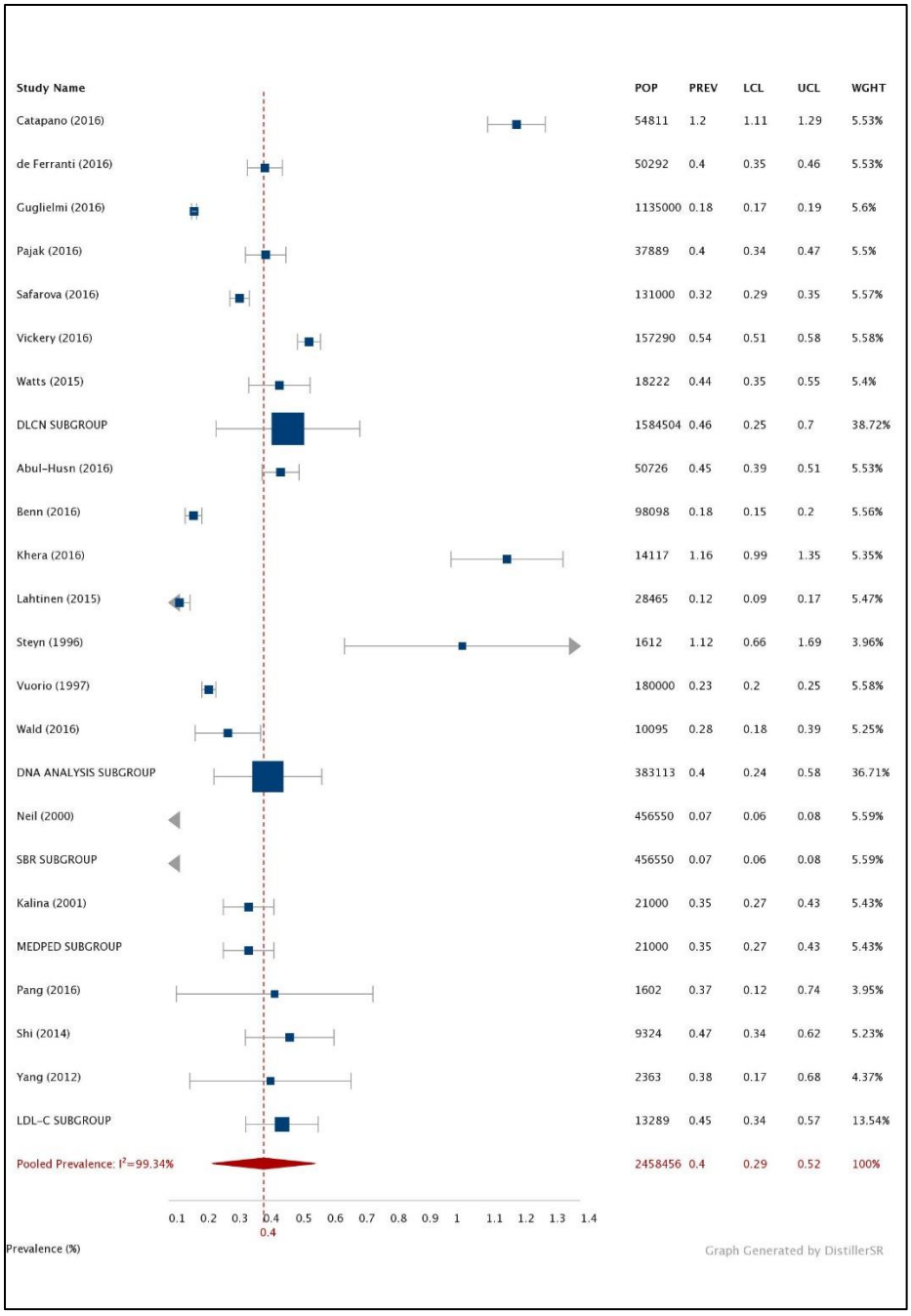
Source: http://www.ephpp.ca/tools.html
Note: Only sections A-F are used in generating the global assessment of study quality.

eTable 7 | Quality assessment for studies included in systematic review of FH prevalence							
Study author	**Selection bias**	**Study design**	**Confounders**	**Blinding**	**Data collection methods**	**Withdrawal & dropouts**	**Globing rating**
Abul-Husn (2016)	★★★	★★	★★★	★★	★★★	★★★	★★★
Benn (2012)	★★★	★★	★★★	★★	★★★	★★★	★★★
Benn (2016)	★★★	★★	★★★	★★	★★★	★★★	★★★
Catapano (2016)	★	★★	★★	★★	★★★	★★★	★★
de Ferranti (2016)	★★★	★★	★★★	★★	★★★	★★★	★★★
Kalina (2001)	★★	★★	★★	★★	★★★	★★★	★★★
Guglielmi (2016)	★★★	★★	★★	★★	★★	★★★	★★★
Khera (2016)	★	★★	★★	★★	★★★	★★★	★★
Lahtinen (2015)	★★★	★★	★★★	★★	★★★	★★	★★★
Neil (2000)	★★★	★★	★	★★	★★★	★★★	★★
Pajak (2016)	★★★	★★	★★★	★★	★★	★★★	★★★
Pang (2016)	★★	★★	★	★★	★★	★	★
Perak (2016)	★	★★	★★★	★★	★★★	★★	★★
Safarova (2016)	★★★	★★	★★	★★	★★	★★★	★★★
Shi (2014)	★★★	★★	★★★	★★	★★★	★★★	★★★
Steyn (1996)	★	★★	★★★	★★	★★★	★★★	★★
Vickery (2016)	★★	★★	★★	★★	★★★	★★★	★★★
Vuorio (1997)	★★★	★★	★★★	★★	★★★	★★	★★★
Watts (2015)	★★	★★	★★	★★	★★★	★★★	★★
Wald (2016)	★★★	★★	★★★	★★	★★★	★★★	★★★
Yang (2012)	★★	★★	★★★	★★	★★★	★	★★
★ - weak; ★★ - moderate; ★★★ - strong							

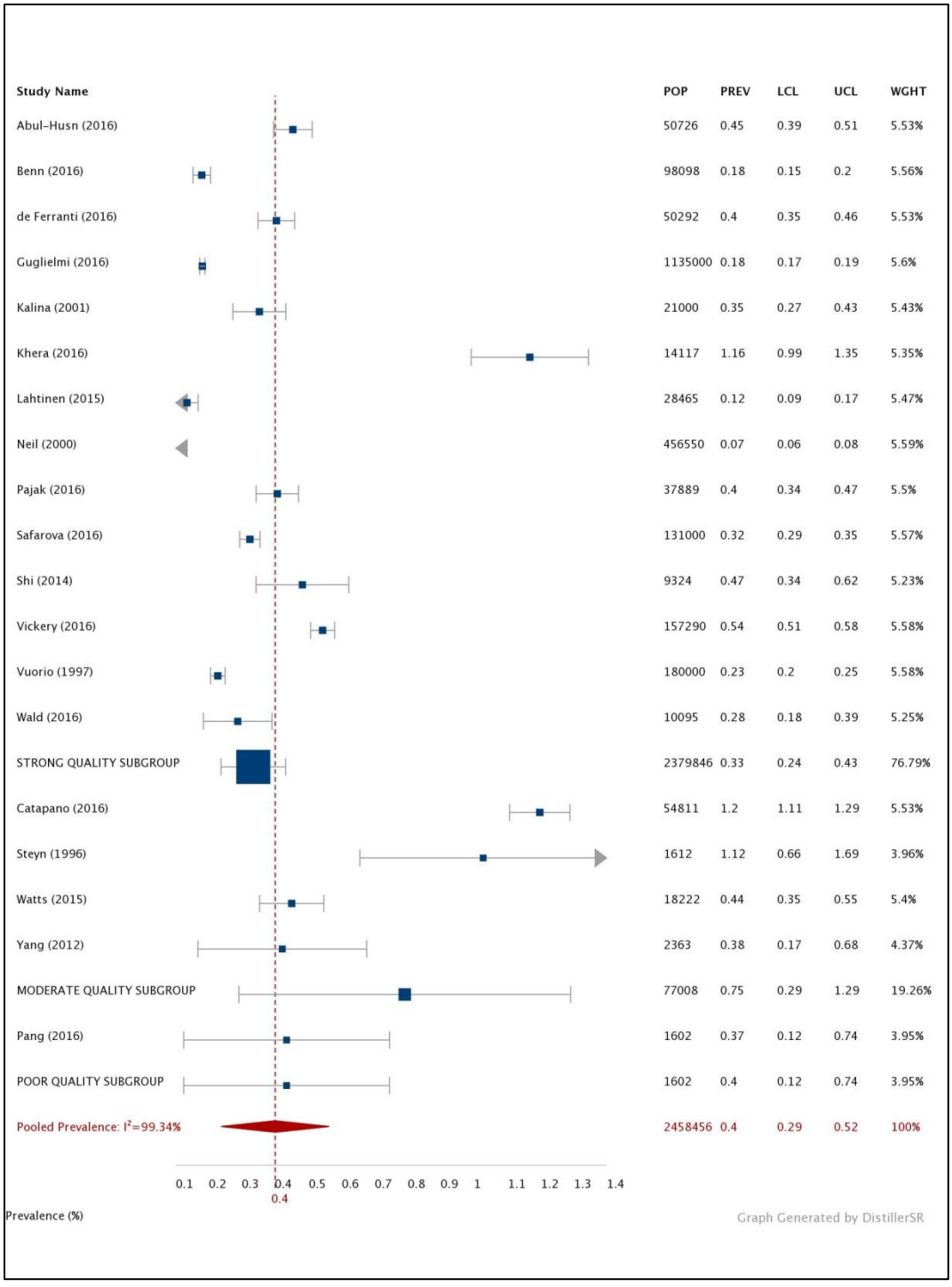
eTable 8 | Pooled prevalence of FH in children (ages 0 – 19)					
Study	**Prevalence (%)**	**LCL 95% (%)**	**UCL 95% (%)**	**Weight (%)**	**Population**
de Ferranti (2016)	0.42	0.32	0.50	45.60	13,343
Pang (2016)	0.37	0.12	0.74	7.16	1,602
Wald (2016)	0.28	0.18	0.39	36.90	10,095
Yang (2012)	0.38	0.17	0.68	10.35	2,363
Pooled	**0.36%**	**0.29**	**0.45**	**100**	**27,403**
Statistics					
I-squared	13.32%	0.00%	86.73%		
Cochran's Q	3.46				
Chi2, p	0.33				
tau2	0.00				

eTable 9 | Pooled prevalence of FH in adults (ages >20)					
Study	**Prevalence (%)**	**LCL 95% (%)**	**UCL 95% (%)**	**Weight (%)**	**Population**
Abul-Husn (2016)	0.45%	0.39%	0.51%	6.40	50,726
Benn (2016)	0.18%	0.15%	0.20%	6.44	98.098
Catapano (2016)	0.40%	0.33%	0.46%	6.37	54,811
de Ferranti (2016)	1.20%	1.11%	1.29%	6.41	50,292
Guglielmi (2016)	0.18%	0.17%	0.19%	6.48	1,135,000
Kalina (2001)	0.35%	0.27%	0.43%	6.28	21,000
Khera (2016)	1.16%	0.99%	1.35%	6.19	14,117
Lahtinen (2015)	0.40%	0.34%	0.47%	6.37	28,465
Neil (2000)	0.12%	0.09%	0.17%	6.33	456,550
Pajak (2016)	0.07%	0.06%	0.08%	6.47	37,889
Safarova (2016)	0.32%	0.29%	0.35%	6.45	131,000
Shi (2014)	0.28%	0.18%	0.40%	6.05	9,324
Steyn (1996)	1.12%	0.66%	1.69%	4.59	1,612
Vuorio (1997)	0.54%	0.51%	0.58%	6.46	157,290
Vickery (2016)	0.23%	0.20%	0.25%	6.46	180,000
Watts (2015)	0.44%	0.35%	0.55%	6.25	18,222
Pooled	**0.40%**	**0.29**	**0.54**	**100**	**2,431,053**
Statistics					
I-squared	99.44%	99.35%	99.52%		
Cochran's Q	2680.181				
Chi2, p	0.00				
tau2	0.00				

eTable 10 | Sensitivity analyses						
Analysis	**Number of studies**	**Population**	**Prevalence (%)**	**LCL 95% (%)**	**UCL 95% (%)**	**I^2(%)**
2000s and later studies only	17	2,276,844	0.39	0.27	0.52	99.41
2010s and later studies only	15	1,799,294	0.42	0.29	0.57	99.23
General population studies only	10	444,581	0.45	0.26	0.68	98.97
Patient cohort studies only	9	2,013,875	0.33	0.21	0.47	99.37
LDL-C based studies excluded	15	2,248,379	0.39	0.27	0.52	99.41
Founder effects studies excluded	16	2,445,167	0.39	0.28	0.52	99.44
LDL-C + Founder studies excluded	13	2,152,048	0.40	0.27	0.56	99.55



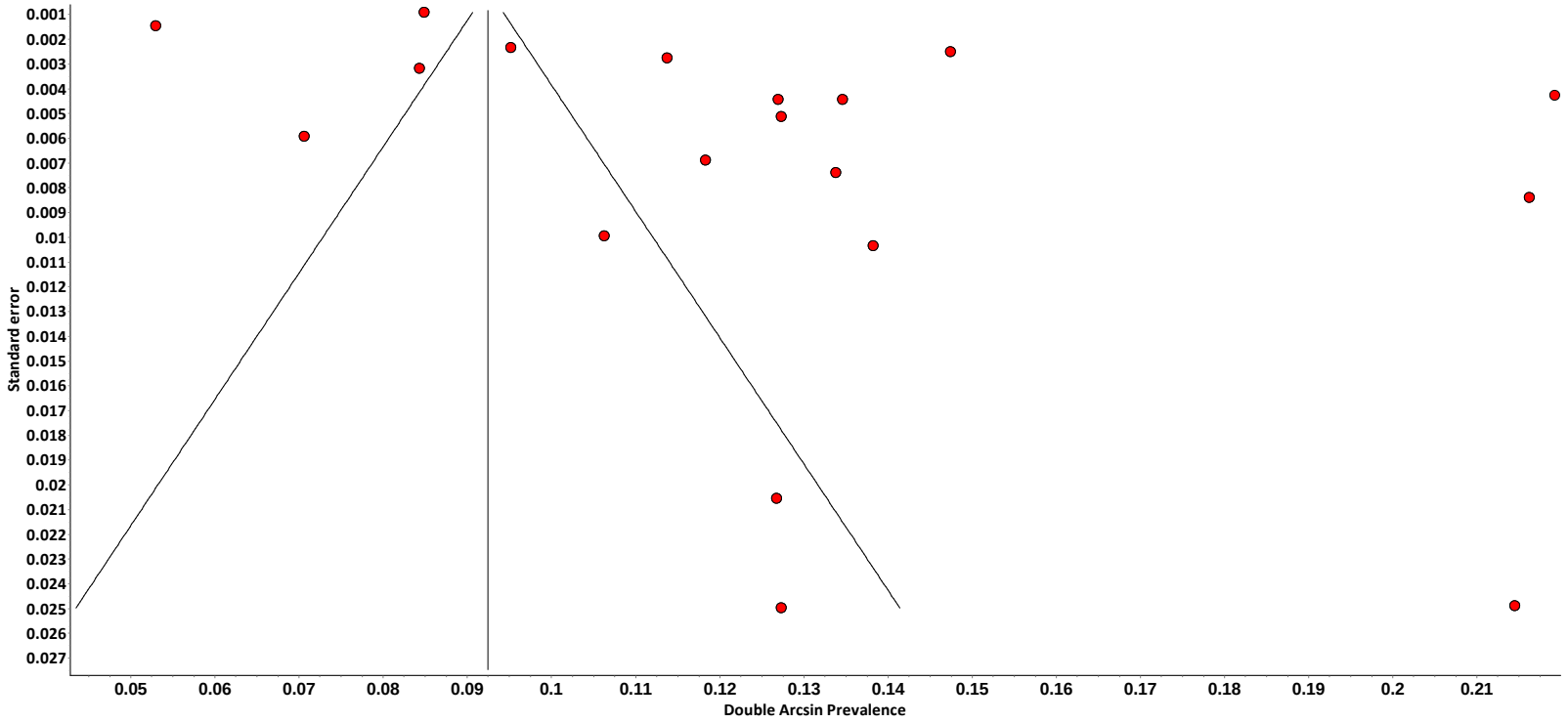
eFigure 1 | Forest plot of overall pooled prevalence (%) of heterozygous familial hypercholesterolemia stratified by diagnostic criteria employed. DLCN subgroup – Dutch Lipid Clinic Network Criteria; DNA subgroup – DNA-based evidence of an LDLR, ApoB, or PCSK9 mutation; LDL-C subgroup – low density lipoprotein-cholesterol > 189 mg/dL (4.9 mmol/L); MEDPED - Making Early Diagnosis to Prevent Early Death criteria; SBR –Simon Broome Registry criteria. I^2 – between-study heterogeneity; LCL – lower confidence limit; POP – population; PREV – prevalence; UCL – upper confidence limit; WGHT – weight under the random-effects model. Note: prevalence estimates were derived using the double-arcsine method, back-transformed and expressed as percentages for ease of interpretation.



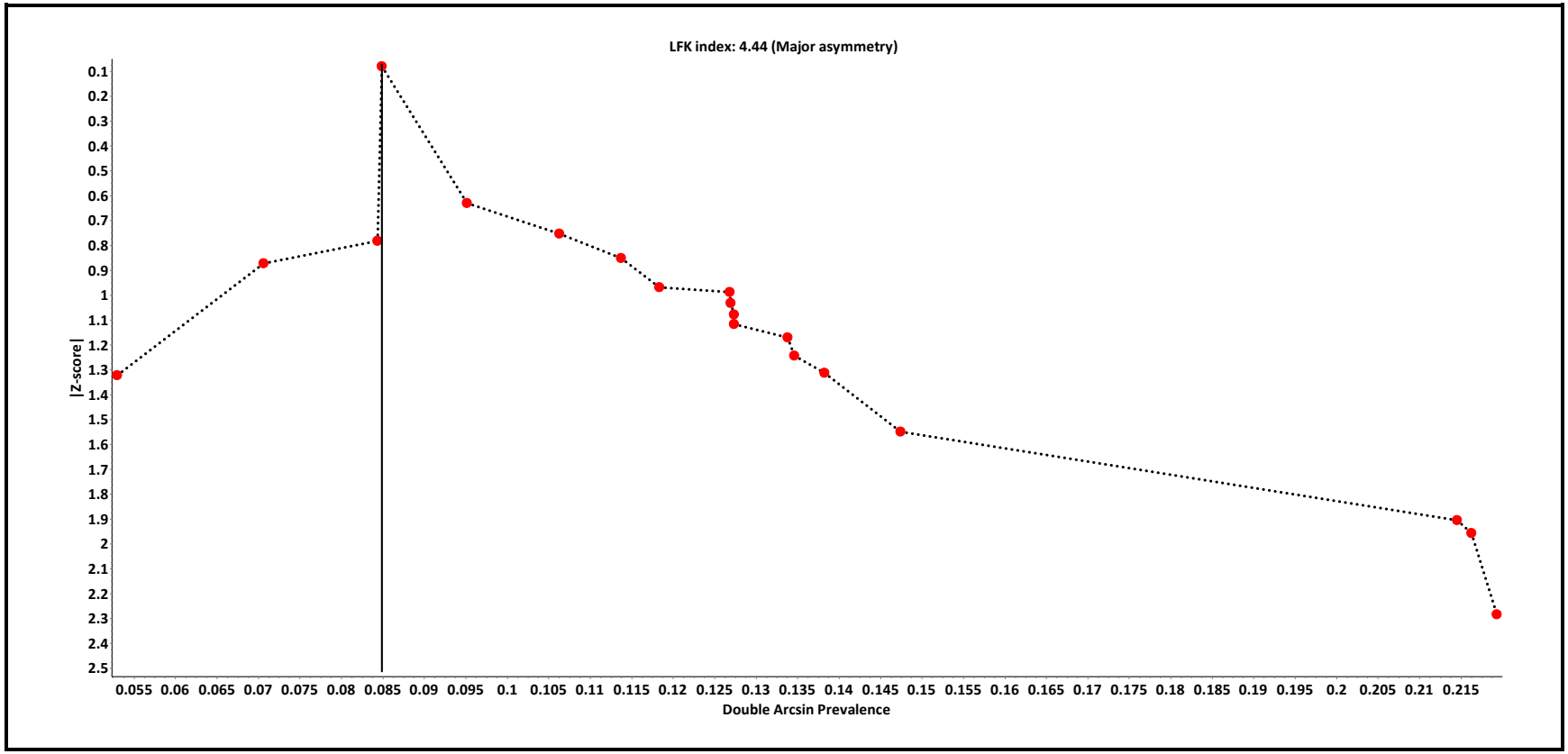
eFigure 2 | Forest plot of overall pooled prevalence (%) of heterozygous familial hypercholesterolemia stratified by study quality. I^2 – between-study heterogeneity; LCL – lower confidence limit; POP – population; PREV – prevalence; UCL – upper confidence limit; WGHT – weight under the random-effects model. Note: prevalence estimates were derived using the double-arcsine method, back-transformed and expressed as percentages for ease of interpretation.

eFigure 3 | Publication bias in studies reporting on adult FH prevalence

(A)



(B)


LFK index: 4.44 (Major asymmetry)
|Z-score|
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2
2.1
2.2
2.3
2.4
2.5
0.055 0.06 0.065 0.07 0.075 0.08 0.085 0.09 0.095 0.1 0.105 0.11 0.115 0.12 0.125 0.13 0.135 0.14 0.145 0.15 0.155 0.16 0.165 0.17 0.175 0.18 0.185 0.19 0.195 0.2 0.205 0.21 0.215
Double Arcsin Prevalence

Interpretation of eFigure 3

We present the Funnel plot in (A). Here, the vertical line indicates a fixed-effects summary estimate derived under inverse variance weighting. The sloping lines that straddle the horizontal demonstrate the expected 95% confidence intervals for the given standard error, assuming no heterogeneity between studies. We plot the standard error of individual study's effect sizes on the vertical axis and the effect sizes (i.e., prevalence estimates) on the vertical axis.

The Doi plot for publication bias is presented in (B). Here, double arcsine transformed prevalence estimates derived under random effects meta-analysis are plotted against an absolute value of a z-score attained by assigning each study a rank based on the standard error of its effect size. When studies included in an analysis are symmetrical, the most precise studies will approach zero on the z-score axis and define a midpoint around which other studies will scatter. By contrast, smaller, less precise studies should scatter widely as their absolute z-score increases and studies become more likely to report findings on either side of the midpoint. The result, in the absence of asymmetry should resemble a symmetrical triangle, with a z-score approaching zero as its peak. A dissimilar number of studies on either side of the triangle or a lack of equal spread or both are indicative of the existence of asymmetry.

Summary

Visually assessed, both the Forest plot (A) and the Doi plot(B) suggest asymmetry among estimates derived from included studies. This asymmetry was confirmed by Egger's weighted regression ($p = 0.03$).