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Effectiveness of cascade testing strategies in relatives for familial hypercholesterolemia: a systematic review and meta-analysis

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Abstract

Background and aims: Cascade testing in relatives of index cases is the most cost-effective approach to identifying people with familial hypercholesterolemia (FH); however, it is currently unclear which strategy to contact relatives would be the most effective. A systematic review was performed to quantify the effectiveness of different strategies in cascade testing of FH.

Methods: Comprehensive searches of three electronic databases and grey literature sources were searched (from inception to May 2020). Screening, data extraction and assessments of methodological quality were made independently by two reviewers. Meta-analyses of proportions were performed using random effects models. Effect measures are reported as percentages with 95% confidence intervals.

Results: 24 non-comparative studies were included, of which 11 used a direct, 8 used indirect, and 5 used a combination of both direct and indirect cascade strategies. The median number of new relatives with FH per known index case was approximately 1. The combination strategy resulted in the largest yields of relatives tested for FH out of those contacted (40%, 95% CI 37% to 42%, 1 study) and relatives responding to testing out of those contacted (54%, 1 study); however, the direct strategy had the largest yield of index cases participating in cascade testing out of those with FH confirmed (94%, 8 studies) compared to other strategies ($p \leq 0.01$ for all comparisons).

Conclusions: Evidence is limited; however, a combination strategy, which allows the index case to decide on method of contacting relatives, appears to lead to better yields compared to using the direct or indirect strategy.

Introduction

Familial Hypercholesterolemia (FH) is the commonest autosomal dominant (monogenic) disorder with between 1 in 270 and 1 in 500 individuals affected by the more common heterozygote form of the condition.(1, 2) Left untreated, around 50% of men with FH develop coronary heart disease (CHD) by the age of 50 and women approximately 10 years later. (3) CHD can be effectively prevented by high intensity lipid lowering treatment, with recent research suggesting initiation of statin therapy during childhood in children with FH significantly reduces CHD events and mortality. (4) Further, around 50% of first-degree relatives of individuals with confirmed diagnosis of heterozygote FH will also have the condition. Despite clear recommendations in the national and international guidelines (1, 5, 6), the majority of individuals with heterozygote FH are still not identified. For example, over 90% of people with FH in the general population have not yet been diagnosed (around 234,000 people in the UK). (1, 7)

The typical pathway for FH identification involves physicians, often the primary care provider, referring individuals with suspected FH to a specialist who confirms diagnosis, often using genetic testing. The specialist will then arrange testing of relatives of confirmed FH cases, usually by the patient contacting the relatives themselves (indirect cascade testing). The exception could be testing children of affected parents, which may be done directly. In fact, the family are often traced to two or three generations. (5, 8) Initially, this usually starts with the affected individuals' children (9). Internationally, most cascade testing starts with adult index patients and cascading testing to other relatives including children ("forward cascade testing"). Also "reverse" cascade testing is under consideration, starting identification from affected children. (6, 10) However, despite cascade testing being a recommended approach by NICE (5) and international guidelines,

(11, 12) and recognised as a cost-effective strategy (13), there are still many patients not being diagnosed, with one of the reasons being the relatively low yield, which could be related, partly, to using the indirect approach. The alternative strategy to indirect, is direct cascade testing where testing is initiated by the clinicians or other healthcare professionals contacting the relatives directly.

Recently a systematic review found that the proportion of cascade tested relatives was higher with direct contact (14); however, as this review did not synthesise the studies quantitatively, the magnitude of the differences between the strategies remains unclear. Therefore, we have performed a systematic review and meta-analysis to quantify the yield of different strategies (direct, indirect, combination) for cascade testing for familial hypercholesterolemia.

Materials and Methods

The protocol for the systematic review was registered in PROSPERO (CRD42019125775). Additionally, PRISMA guidelines (15) were adhered to throughout the conduct and reporting of the systematic review.

The systematic review encompasses relevant study designs, including controlled trials and epidemiological studies, which assessed the effectiveness of cascade testing in relatives for FH. Eligible participants were first and second degree relatives of index cases with confirmed FH, determined using either a clinical diagnosis (e.g. Simone Broome (16), Dutch Lipid Clinical Network (DCLN) (1), MEDPED (17) or another criterion appropriate to the population which is being studied); low density lipoprotein cholesterol levels using age specific cut offs; or genetic

diagnosis of mutation positive cases. The strategy for cascade testing was the intervention of interest, which could be conducted via either i) a direct method of contact (where the relatives of the index case are contacted directly by the clinic usually using personalized letters or phone calls, once consent has been sought from the index case; ii) an indirect method of contact (where the index case acts as an intermediary by passing on personalized letters or information to their relatives), or iii) where there is a choice of indirect or direct (combination of direct and indirect). The primary outcome measure was the proportion of relatives of the index cases tested out of those contacted, henceforth referred to as yield. Secondary outcome measures included the proportion of relatives contacted for FH testing out of those eligible; the proportion of relatives who responded out of those contacted for FH testing; the proportion of relatives with confirmed FH out of those tested; and the proportion of index cases who participated in cascade testing out of those confirmed with FH. We excluded studies which focused solely on index cases with homozygous FH; single case reports; studies that did not report on at least one outcome of interest; where the contact cascade testing method was unclear; or where the paper was a duplicate of another paper using the same cohort of index cases or relatives.

Comprehensive literature searches of three databases (Medline, from 1946 to May 2020; EMBASE, from 1980 to May 2020; and Cochrane CENTRAL, from 1966 to May 2020) were performed using a highly sensitive search strategy based on keywords and MeSH terms relating to the population (e.g. proband, index patient, relative, family, patient) and intervention of interest (e.g. cascade, mass screening, contact tracing) (Search terms for Medline are presented in Supplementary Table S1) and through contact with topic experts. Additionally, grey literature were identified from the following conferences: British Cardiovascular Society, Heart UK Annual

Scientific, European Human Genetics, European Atherosclerosis Society, from dates of inception to March 2020; through hand searching HEART UK and the Atherosclerosis journals; and the HEART UK (<http://www.heartuk.org.uk/>) and US Familial Hypercholesterolemia Foundation (<https://thefhfoundation.org/>) websites. No language restrictions were applied, and translations were sought where necessary.

Screening and study selection

Following the removal of duplicates, titles and abstracts, and full texts of potentially eligible studies were screened independently by two authors. Disagreements regarding eligibility of a study was resolved through discussion with a third author. Reasons for exclusion at the full text stage were documented.

Data extraction and quality assessment

A standardized form, developed by the authors and tailored to this review, was used for data extraction. Data relating to the study characteristics, methods used, and primary and secondary outcomes, were extracted independently by two authors. Where possible, the authors of any studies with missing data were contacted. Two authors independently assessed the methodological quality of the included studies using the JBI Critical Appraisal tool (18); studies which scored 'no' for more than two of the questions were rated as having low methodological quality, high methodological quality was assigned where all the domains were rated as 'yes', and the remaining studies were rated as moderate. Discrepancies were discussed between authors, as needed.

Data synthesis and investigations of heterogeneity

For each study, we calculated raw proportions with 95% score-based confidence intervals based on the appropriate numerator and denominator for each outcome measure. Variances of the raw proportions were stabilised before pooling using the Freeman-Tukey double arcsine transformation (19) to ensure studies which estimated proportions as 100% (standard error, SE=0) were not excluded from the analysis. The included studies only presented outcome data for one cascade strategy (direct, indirect, or combination); therefore, no relative effect measures could be estimated. Thus, pooled proportions for the outcome measures overall and for each cascade strategy were estimated using a random effects models where sufficient studies were included in the meta-analyses, to allow for anticipated heterogeneity resulting from inherent biases within the studies. Inconsistency (heterogeneity) was quantified using I^2 (20). We also planned to conduct subgroup analysis based on area of residence (inside or outside of the screening area), cascade testing programme characteristics (forward or reverse cascading, cascade testing location), and participants characteristics. However, subgroup analyses for the primary outcome could not be explored due to either insufficient information being reported in the studies or insufficient number of studies being included in some subgroups. Analyses were conducted in Stata version 16.0 (Stata-Corp, College Station, Texas, USA).

Results

The searches identified a total of 3742 studies. Following title and abstract screening, 217 studies were assessed for full text screening (Figure 1). At the full text screening, 193 studies were excluded, predominately related to ineligible study design (77 studies), ineligible or duplicate population (35 studies), ineligible or unclear intervention (62 studies) or ineligible outcome

reporting (19 studies); therefore, 24 studies were included in the systematic review and meta-analysis (21-44) (Table 1).

Of the 24 included studies, 16 were conducted in Europe (England (24, 25, 30, 34, 35, 41), Wales (28), Belgium (45), Denmark (22, 42), Latvia (32), the Netherlands (40), Norway (33), Spain (29), Malta (39), Estonia (21)), one from Australia (23), three in the Americas (North America (27, 37), Brazil (31)), one in New Zealand (36), two in Asia (India (38), Hong Kong (26)), and one in South Africa (44). All studies used an observational design to assess the outcome measures. Sixteen studies were published more recently, from 2010 onwards, and two studies were published before 2000 (22, 42). The average number of confirmed index cases enrolled in the studies was 242, with sample sizes ranging from 2 to approx. 1300 participants.

The direct method of cascade testing contact in relatives was used in 11 studies (22, 24, 27, 29, 31, 35, 36, 38, 40, 42, 44), and a further eight studies used an indirect method (21, 25, 26, 32-34, 37, 43). The remaining five studies used a combination of direct and indirect methods (23, 28, 30, 39, 41), where the decision regarding which method was used was decided by the index case. Contact could be made through a range of approaches, including postal invitation, telephone, in person or a combination of approaches. In the studies using either a direct or combination strategy which reported the type of person that reached out to the relatives, a nurse was used in seven studies (23, 24, 29-31, 36, 40) compared to only single studies reporting using either a genetic counsellor (28) or an outpatient clinician.(22) Forward cascade testing was used in the majority of included studies (23 studies) with the remaining study using reverse cascade testing (42). Fourteen of the included studies reported the extent of cascade; the majority cascaded to

second degree relatives (eight studies, (21, 22, 26, 31, 35, 38, 40, 43)), with only five studies cascading to first degree (24, 27, 32, 39, 44) and one study cascading to third degree (23).

The majority of included studies confirmed FH diagnosis in the index cases using genetic testing (14 studies, (21, 23, 25-29, 31, 33, 36-38, 40, 41), nine studies confirmed FH diagnosis in the index cases using clinical assessment based either on SB (four studies, (24, 30, 34, 35), DCLN (three studies, (32, 39, 43), or study-specific criteria (serum cholesterol $\geq 8\text{mmol/L}$ and LDL cholesterol $\geq 6\text{mmol/L}$ and family history of hypercholesterolemia (22); apolipoprotein B: apolipoprotein A-1 ratio $>97^{\text{th}}$ centile or apolipoprotein B $>99^{\text{th}}$ centile, and LDL cholesterol $>95^{\text{th}}$ centile and no secondary causes for raised cholesterol (42)); and one study stated diagnosis was based on either genetic or clinical criteria but did not provide additional details. (44) For the relatives, genetic confirmation of FH was used in the majority of studies (15 studies, (21, 23, 25-29, 31, 33, 36-38, 40, 41, 46). A further eight studies used clinical assessment based on either SB (24, 30), DCLN (32, 39), MEDPED (34), combination of DCLN and MEDPED (43), or study-specific criteria (serum cholesterol $\geq 7\text{mmol/L}$ (22); LDL cholesterol $>95^{\text{th}}$ centile (42)). The final study used genetic testing or clinical assessment based on SB criteria depending on which arm of the trial the proband had been randomized to (35).

For the 16 studies using genetic testing for confirmation of FH, testing of only the LDLR gene was performed in three studies (36, 38, 40), testing of LDLR and APOB genes was performed in two studies (33, 35), testing of LDLR, APOB and PCSK9 genes was performed in eight studies (21, 23, 26, 27, 29, 31, 37, 41), and testing of LDLR, APoB, PCSK9, and LDLRAP1 genes was performed in one study (44); the genes considered in the remaining two studies were unclear (25, 28).

The median number of new relatives with FH per known index case was 0.98 (range 0.15 to 8.6), with the largest medians seen in the studies using the direct testing strategy (median 1.71, range 0.15 to 3.86) compared to the indirect (median 0.95, range 0.22 to 8.60) or combination (median 0.72, range 0.26 to 1.88) strategies; however, this is a crude analysis which does not consider the relative contribution of each study in terms of its sample size.

Quality assessment

The majority of studies were rated as having a moderate risk of bias; only three studies had a high methodological quality score (23, 32, 43) and two studies were rated with low methodological quality (36, 37) (Supplementary Table S2). The reasons for lower methodological quality were primarily related to less clarity regarding consecutive inclusion of participants (Question 4) and incomplete inclusion of participants (Question 5). Furthermore, high numbers of studies scored as 'no' on clear reporting of the demographics and clinical information of the participants (Question 6: 10 studies; Question 7: 10 studies, respectively).

Primary outcome measure

Proportion of relatives of index cases tested for FH out of those contacted

Four studies (21, 30, 34, 35) provided data to estimate the primary outcome. On average 39% of relatives were tested for FH out of those contacted (95% CI 31% to 47%, 4 studies); however, the estimates varied significantly by the cascade strategy used (p value for subgroup differences, $p=0.01$; Figure 2). The largest yield was seen in the study conducted in England which used a combination strategy (40%, 95% CI 37% to 42%, 1 study); however, similar but slightly lower yields were seen for the direct and indirect strategies (direct 33%, 95% CI 28% to 39% [1 study

conducted in England]; indirect 34%, 95% CI 30% to 37% [2 studies, conducted in England and Estonia]); although the results from the latter two studies varied considerably (57% (21) and 20% (34)).

Secondary outcome measures

The proportion of relatives contacted for FH testing out of those eligible

Only three studies reported data to estimate the proportion of relatives contacted for FH testing out of those eligible (30, 34, 35). For the studies that reported this outcome, on average, 95% relatives were contacted out of those eligible (95% CI 59% to 100%, 3 studies). Using either a direct or an indirect strategy resulted in all of the relatives who were eligible for testing being contacted (Direct: 100%, 95% CI 99% to 100%, 1 study; Indirect: 100%, 95% CI 99% to 100%; 1 study; Supplementary Figure S1). However, in the single study that use a combination of direct and indirect methods a significantly lower proportion of relatives were contacted out of those eligible (65%, 95% CI 63% to 67%; p value for subgroup differences, $p < 0.001$). However, in this latter study, only 26% of the index cases had a diagnosis of definite FH, with the remaining having a possible diagnosis of FH (30).

The proportion of relatives who responded out of those contacted for FH testing

Three studies reported data on the proportion of relatives who responded to cascade screening out of those contacted (30, 34, 35), which found on average 43% of relatives responded out of those contacted (95% CI 28% to 58%; 3 studies) (Figure 3). Using a combination of direct or indirect strategy yielded a significantly greater proportion of relatives responding out of those contacted (54%, 95% CI 51% to 56%, 1 study) compared to using either an indirect strategy (31%,

95% CI 27% to 35%, 1 study) or using a direct strategy (45%, 95% CI 39% to 51%, 1 study) (p value for subgroup difference, $p<0.001$).

The proportion of relatives with confirmed FH of those tested

Twenty-one of the included studies reported data on the proportion of relatives confirmed as FH out of the number of relatives tested (Supplementary Figure S2). On average 47% of relatives were confirmed to have FH out of those tested (95% CI 42% to 52%; 21 studies). Contact strategies were found to produce similar pooled results (Direct: 51%, 95% CI 41% to 60%, $I^2=97\%$, 9 studies; Indirect: 44%, 95% CI 38% to 50%, $I^2=81\%$, 8 studies; Combination: 43%, 95% CI 29% to 58%, $I^2=98\%$, 4 studies; p value for subgroup differences, $p=0.48$).

The proportion of index cases who participated in cascade testing out of those confirmed with FH

Seventeen studies reported data on the proportion of index cases who participated in FH cascade testing out of those confirmed with FH (Supplementary Figure S3). On average, 89% of index cases participated in cascade testing out of those confirmed with a diagnosis of FH (95% CI 73% to 99%, 17 studies); however, the estimates varied significantly by the cascade strategy used (p value for subgroup differences, $p<0.001$). The yield was highest using a direct strategy (94%, 95% CI 79% to 100%, $I^2=98\%$, 8 studies), a slightly lower yield was seen using an indirect strategy (84%, 95% CI 52% to 100%, $I^2=99\%$, 7 studies), and the lowest yield was seen using a combination of direct and indirect strategies (60%, 95% CI 56% to 63%, 2 studies).

Discussion

Principal Findings

Overall, the combination strategy, which allows the index case to decide how the relatives are contacted, appeared to lead to a higher proportion of relatives being tested, compared to direct or indirect approach, which had similar yields. Further, a higher proportion of relatives contacted responded to the offer of testing was seen in the combination strategy; however, the evidence was limited for most outcome measures. Three studies presented data for both of these outcome measures (30, 34, 35). The UK study using a direct approach was a randomized controlled trial that compared routine clinical diagnosis plus genetic testing to routine clinical diagnosis alone in index cases and their relatives (35). Therefore, the study design may have had an impact due to it being recruitment into a trial; whereas the participants recruited into the UK study using indirect approach were part of a cascade testing programme (34) and therefore, probably more generalisable. Also, for the study assessing a combined approach to cascading, it was noted that only 26% of index cases had a definite diagnosis of FH, with the remaining having a probable diagnosis (30); therefore this was likely to result in an reduction in the efficiency of the cascade programme compared to restricting cascading to relatives in whom index cases had a definite diagnosis of FH, thus the true yield using the combination approach could be substantially greater.

The acceptability of different contact strategies may partly explain their effectiveness. Qualitative interviews with FH patients have demonstrated mixed views about the approach to cascade testing, a Scottish study favoured indirect cascade testing (47), whilst in a more recent Australian study index patients supported health professionals directly contacting relatives, perceiving health professionals to have greater credibility and authority. (48) Further, it has been suggested indirect contact by index patients may lead to inadequate counselling and sense of social pressure

to be tested in solidarity with other family members. (49) With the autosomal dominant mode of inheritance, 50% of the first-degree relatives of index cases will be affected. This finding was confirmed in this review, with similar proportions seen for each cascade strategy.

Our results differ to those from a recent systematic review of studies which found cascade testing strategies for FH tended to be higher with direct contact of relatives; (14) however, our systematic review has several strengths compared to the previous review. While both systematic reviews had searched similar electronic databases, the previous review included only 10 studies (14) compared to our review which included 24 studies due to a comprehensive search of grey literature. Additionally, the previous review only conducted a narrative synthesis of the studies, (14) whereas we pooled the magnitudes of yield from the studies to provide new estimates of the yield for a range of relevant outcome measures and compare the yields between cascade strategies using subgroup analysis; thereby highlighting the apparent effectiveness of the combination strategy.

However, there are some limitations for this and the previous review, (14) predominately related to the nature of the studies available. The most robust evidence for comparing the effectiveness of the strategies for cascade testing would have been from studies which made within study comparisons, such as randomized controlled trials; however, no studies using such designs were identified; therefore, we had to rely on comparing strategies across studies. Therefore, we have assumed that the differences in yield between the cascade strategies can be wholly ascribed to the contact method used; however, it is likely that differences in the setting, approaches, and time may all have influenced the yield. Additionally, we pooled studies together irrespective of the method used to diagnose FH in index cases and relatives, using genetic testing or clinical

criteria, which could have affected the estimates of yield in relatives since clinical criteria alone are less likely to confirm FH than genetic testing.(50) Also, only four of the 24 included studies provided information to estimate the proportion of relatives tested out of those contacted for cascade testing. Of the other 20 studies, 18 did not report the number of relatives contacted. Only approximately half of the included studies reported the extent of cascading to other relatives; therefore, we were unable to explore whether there were differences in yields by cascade strategy related to extent of cascading to other relatives. Additionally, inadequate reporting in the included studies meant that we were unable to assess whether there was a difference in the ages of the relatives being identified. Family size is an important factor to consider when estimating the effectiveness of cascade testing, with indications that cascading to smaller families may be less effective.(9) Unfortunately, the included studies did not report details on family size thereby limiting our ability to investigate this further. The majority of the included studies were regional based and did not report on yield of relatives outside of catchment area (including those residing in different countries); therefore, we were unable to explore whether the effective of cascade strategies varied by within/out of catchment area. A further limitation is that three included studies did not report the number of confirmed index cases enrolled into the study, therefore we used the number that were included in the cascade strategy when estimating one of the secondary outcomes; however, it is unlikely that this would have overtly influenced the findings since this accounted for only a small number of studies reporting this outcome. Only one study using reverse cascade testing was included in the review (42); however, other reverse cascade testing studies were identified from the searches, but no information was available on the modality and/or outcomes of approach and therefore they were

excluded from the review. Whilst the testing to parents would be through direct cascade, indirect or direct cascade could be used to more distant relatives. Finally, the overall methodological quality of the included studies was rated as moderate, with only three studies scoring high quality (23, 32, 43), however, lower scores tended to be related to poorer reporting therefore, quality is unlikely to have biased the estimates of yield from the studies.

Clinical Implications

Although there is limited evidence of moderate quality, this systematic review provides evidence to support a combination approach to cascade testing improves the identification of further FH cases. Historically, traditional genetic cascade testing to relatives has used the indirect approach. This aligns with national laws and standards on privacy and confidentiality (51, 52). However, the recent ABC vs St Georges court case has highlighted that clinicians have a legal duty to inform a patient's relatives, but the court decision did not specify the approach to contact relatives. (53) In certain circumstances, where there is identification of an index case with an incurable condition, such as Duchene muscular dystrophy, it may be more appropriate to indirectly approach the relatives. However, the direct approach may be more justified in FH, as this is a treatable condition with readily available medication. Further, irrespective if using the direct or indirect approach, the genetic testing of relatives has been demonstrated to be more cost-effective than using clinical phenotype (5) but not all health services have the facilities to offer cascade genetic screening. In these circumstances clinical data based on age and gender-specific LDL levels in relatives, may still need to be utilized. (51)

On a related issue, despite a high proportion of eligible relatives contacted, there is a significant drop off in relatives tested and a wide variation in the number of relatives confirmed with FH per

index case. This may relate to limited resources available for cascade testing. The implementation of cascade testing can be improved by better integration of the cascade testing process with primary care engagement and community outreach specialists, and offer index cases the option of either them contacting their at-risk relatives themselves or the clinical service directly contacting the relatives. (51)

Research recommendations

All of the studies included in this review reported on a single cascade strategy; therefore, future studies should be conducted which compare different cascade strategies, using either quasi-experimental (such as controlled before-after studies) or preferably using randomized controlled designed studies. Also, to enable the results from future studies to be compared to determine whether combination approach to cascade testing has a greater yield compared to direct or indirect approaches, it is important that a core set of outcome measures for such studies is agreed and reported. Aligned with current guidelines, index cases should have FH genetically confirmed or with definitive clinical diagnosis. We recommend that as a minimum, studies report on the numbers of relatives eligible and contacted for, and responded to a request for, cascade testing, and the number of relatives tested and confirmed to have a diagnosis of FH. Another hypothesis worth exploring is whether the efficiency of cascade testing is related to family size and the extent of index' patients contact with other family members.

Conclusion

The review provides tentative support for the combination approach to cascade testing where the index case determines which method is used to contact relatives. However, further evidence

to support the combination approach requires experimental studies to compare the cascade approaches or interrogation of routine datasets and FH registers held on the cascade testing and the modality of contact with relatives.

Conflict of interest: The authors declare no competing financial interests in relation to the work described.

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Figures and Tables

Figure legends

Figure 1 PRSIMA Flow Chart

Figure 2 Proportion of relatives tested out of those contacted for FH cascade testing by cascade strategy

Black square indicates the pooled effect size for individual study, horizontal lines indicate the 95% confidence intervals for individual study, Diamond indicates the pooled estimate and 95% confidence intervals, ES Effect Size, CI Confidence Interval

Figure 3 Proportion of relatives responded out of those contacted for FH cascade testing by cascade strategy

Black square indicates the pooled effect size for individual study, horizontal lines indicate the 95% confidence intervals for individual study, Diamond indicates the pooled estimate and 95% confidence intervals, ES Effect Size, CI Confidence Interval

Table legends

Table 1 Characteristics of included studies

^a Number of confirmed index cases involved in cascade testing strategy used because number of confirmed index cases enrolled in study was not reported, ^b An additional 43 index cases were included in the cascade testing strategy who did not meet criteria for FH diagnosis

Table 1 **Characteristics of included studies**

Study, year	Country	Number of confirmed index cases	Contact method	Format of cascade	Extent of cascading in relatives	FH diagnosis method in index cases	FH diagnosis method in relatives	Quality score
Alver, 2019	Estonia	27	Indirect	Forward	Second degree	Genetic	Genetic	Moderate
Andersen, 1997	Denmark	62	Direct	Forward	Second degree	Clinical (study-specific)	Clinical (study-specific)	Moderate
Bell, 2015	Australia	100 ^a	Both	Forward	Third degree	Genetic	Genetic	High
Bhatnagar, 2000	England	262	Direct	Forward	First degree	Clinical (SB)	Clinical (SB)	Moderate
Breen, 2011	England	72	Indirect	Forward	Not reported	Genetic	Genetic	Moderate
Chan, 2018	Hong Kong	64	Indirect	Forward	Second degree	Genetic	Genetic	Moderate
Davis, 2016	US	5	Direct	Forward	First degree	Genetic	Genetic	Moderate
Descamps, 2020	Belgium	127	Indirect	Forward	Second degree	Clinical (DCLN)	Clinical (MEDPED/DCLN)	Moderate
Edwards, 2013	Wales	270	Both	Forward	Not reported	Genetic	Genetic	Moderate
Ellis, 2019	Spain	755	Direct	Forward	Not reported	Genetic	Genetic	Moderate
Hadfield, 2009	England	931	Both	Forward	Not reported	Clinical (SB)	Clinical (SB)	Moderate
Jannes, 2015	Brazil	125	Direct	Forward	Second degree	Genetic	Genetic	Moderate
Latkovskis, 2018	Latvia	140	Indirect	Forward	First degree	Clinical (DCLN)	Clinical (DCLN)	High

Leren, 2008	Norway	~1300	Indirect	Forward	Not reported	Genetic	Genetic	Moderate
Marks, 2006	England	354	Indirect	Forward	Not reported	Clinical (SB)	Clinical (MEDPED)	Moderate
Marteau, 2004	England	341	Direct	Forward	Second degree	Clinical (SB)	Genetic or clinical (SB)	Moderate
Muir, 2010	New Zealand	76	Direct	Forward	Not reported	Genetic	Genetic	Low
Neuner, 2019	US	2	Indirect	Forward	Not reported	Genetic	Genetic	Low
Raal, 2020	South Africa	252 ^{ab}	Direct	Forward	First degree	Genetic or clinical (not specified)	Genetic	Low
Setia, 2018	India	31	Direct	Forward	Second degree	Genetic	Genetic	Moderate
Skovby, 1991	Denmark	17	Direct	Reverse	Not reported	Clinical (study-specific)	Clinical (study-specific)	Moderate
Tilney, 2019	Malta	9	Both	Forward	First degree	Clinical (DCLN)	Clinical (DCLN)	Moderate
Umans-Eckenhansen, 2001	Netherlands	237 ^a	Direct	Forward	Second degree	Genetic	Genetic	Moderate
Webster, 2019	England	215	Both	Forward	Not reported	Genetic	Genetic	Moderate

^a Number of confirmed index cases involved in cascade testing strategy used because number of confirmed index cases enrolled in study was not reported, ^b An additional 43 index cases were included in the cascade testing strategy who did not meet criteria for FH diagnosis

Figure 1

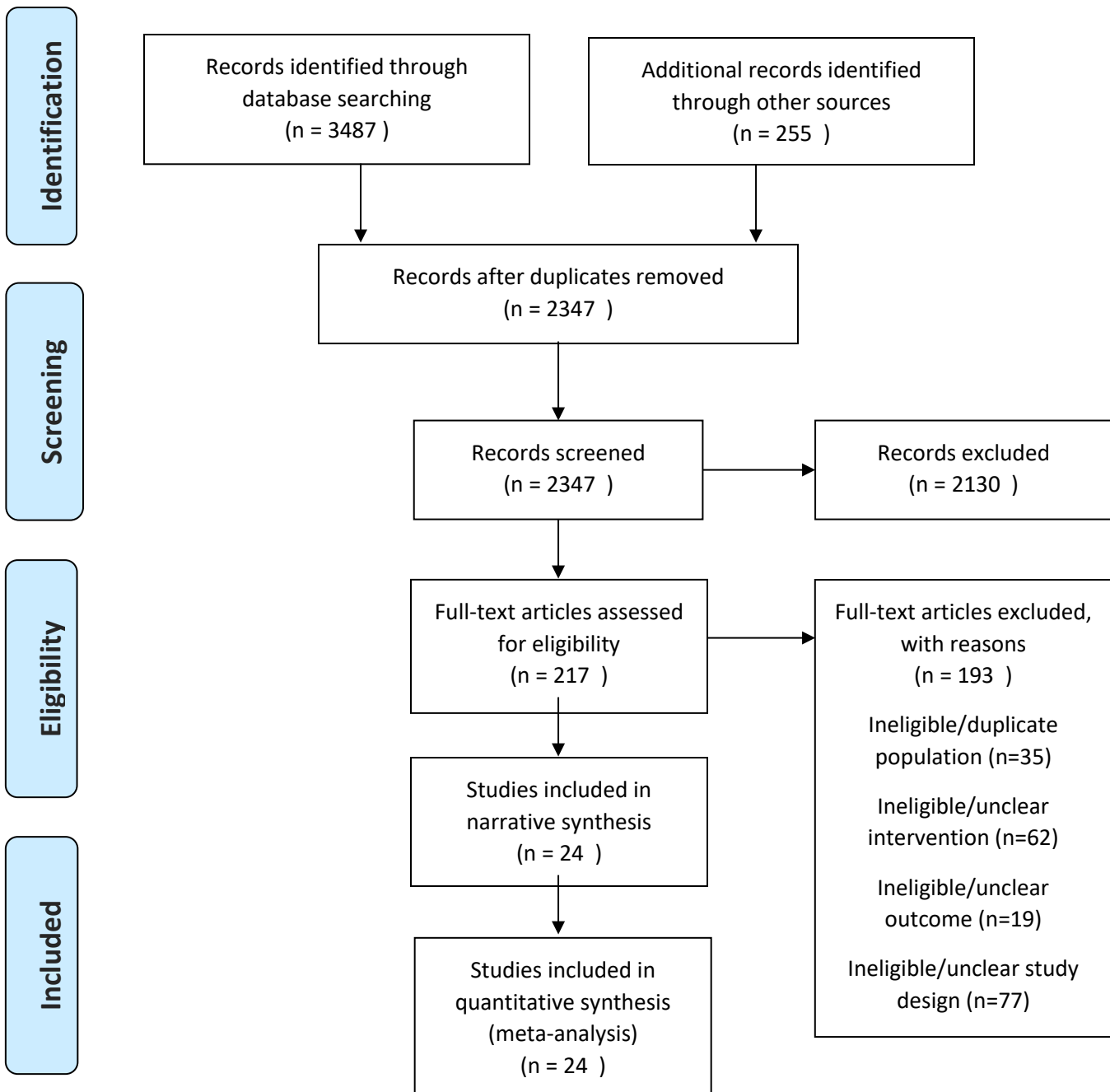


Figure 2

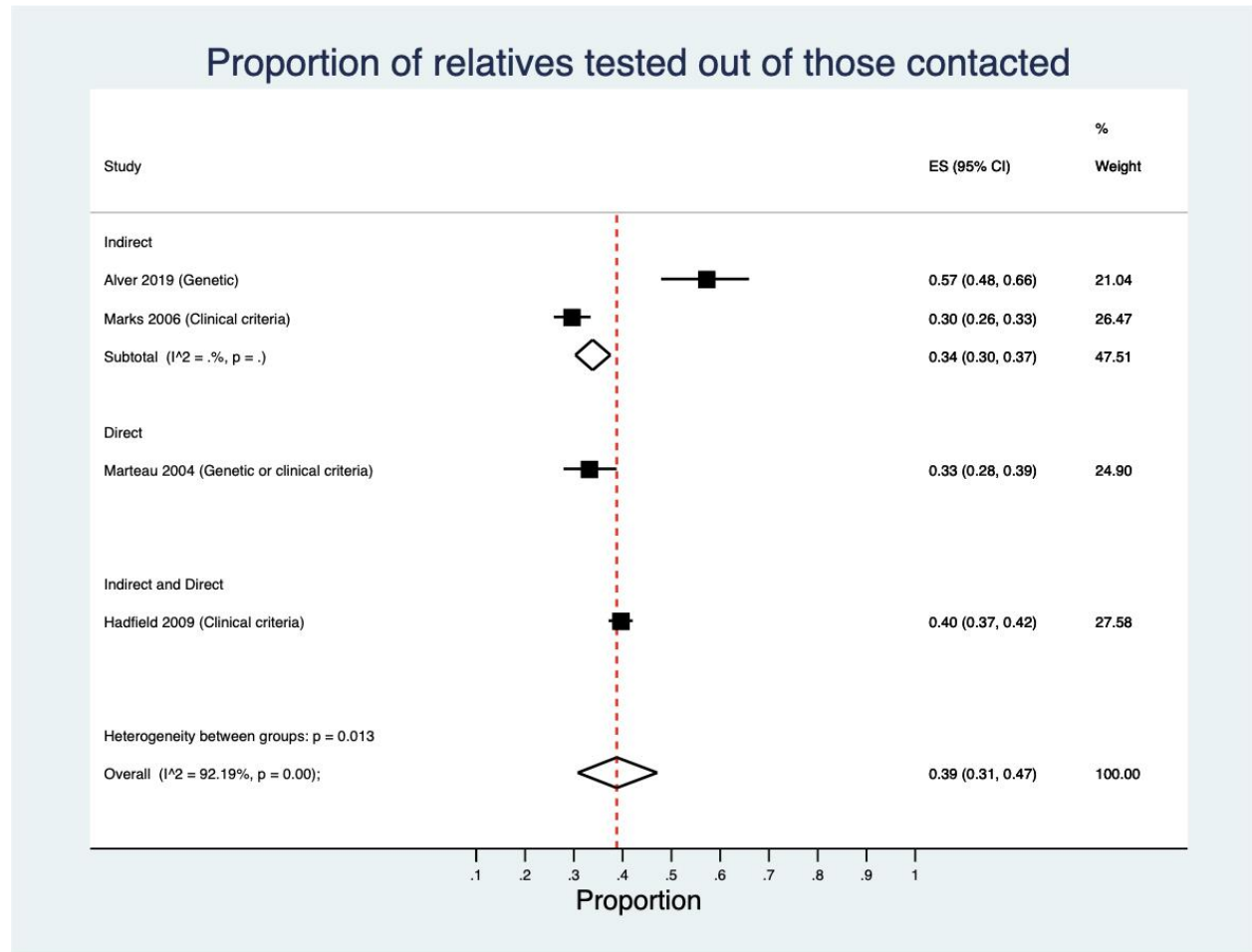


Figure 3

