SUPPLEMENTAL MATERIAL

Search Strategy

A comprehensive search of several databases from each database's inception to March 12th, 2019 was conducted. The databases we searched included Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, and Daily, Ovid EMBASE, Ovid Cochrane Central Register of Controlled Trials, Ovid Cochrane Database of Systematic Reviews, and Scopus. The search strategy was designed and conducted by an experienced staff librarian (LP) with input from other investigators. Controlled vocabulary supplemented with keywords was used to search for cascade testing studies for FH. A complete list of terms used and how they were combined is included in the search strategies listed in the Data Supplement.

Study Inclusion

Inclusion criteria for studies in this systematic review were: 1) original published research; 2) utilized cascade testing for the identification of FH; 3) identified cases based on positive genetic testing for P/LP variants in *LDLR*, *APOB* or *PCSK9*, and/or utilized established diagnostic criteria such as the DLCN,[1] Simon Broome Diagnostic Criteria[2] or the MEDPED diagnostic criteria;[3] 4) provided data for both the total index cases and relatives tested; 5) stated the yield or provided sufficient data to allow reliable calculation of the yield; 6) stated the NCIC or provided sufficient data to allow reliable calculation; 7) methodology was not based on reverse cascade screening; 8) case finding did not utilize novel national registries that are not easily replicable; 9) not based exclusively on pediatric index cases; and 10) published in English language.

The initial search strategy returned 456 non-duplicate published studies (**Figure 1**). Abstract review identified 81 studies of interest. Further review facilitated by a third reviewer selected 23 papers which were examined in detail. Of these 23, 13 studies broadly meet inclusion criteria. We then excluded an additional three studies – one which comprised predominantly children;[4] a second which showed higher

LDL-C cutoff levels that do not conform to traditional diagnostic criteria (LDL-C >190 mg/dl in adults and >150 mg/dl in individuals under 18 years of age) and a third one because of a difficult to interpret methodology.[5,6]

Quality Assessment

To reduce bias, primary research was assessed for quality as part of this systematic review. This quality assessment was modeled on work by Hope et al[7] and utilized a previously designed quality assessment tool[8] originally based on the recommendations of the systematic review of quality assessment tools by Sanderson et al.[9] The quality assessment scored each study of interest based on participant selection, measurement of exposure and outcome variables, assessing for control of confounding factors and bias. Papers scoring 14 out of 17 or more were deemed of sufficient quality for inclusion.

Diagnostic Yield

Diagnostic yield was defined as the proportion of tested cases that were positive for FH, either by genetic or biochemical testing. Cascade testing by its nature has a greater positive predictive value and should typically be associated with much higher diagnostic yield than broader and less focused screening studies. Given the autosomal dominant inheritance pattern of FH the diagnostic yield is expected be close to 50%.

$$Diagnostic\ Yield = \frac{Positive\ (n)}{Total\ tested\ (n)}\%$$

Data from each individual study were extracted and a diagnostic yield was calculated as a percentage value.

New Cases per Index Case (NCIC)

NCIC is a ratio of the total number of newly diagnosed cases of FH to the total number of index cases. It is a useful measure of the efficiency of detecting new cases and is potentially influenced by study methodology and criteria used for identifying and confirming new cases of FH.

$$NCIC = \frac{Participants\ diagnosed\ with\ FH\ (n)}{Total\ number\ of\ index\ cases\ (n)}$$

Assessment of Study Methodology

For each study we assessed the following variables: 1) method for diagnosis of index cases, 2) total number of index cases, 3) total number of relatives tested, 4) geographical location of study, 5) the mode of contacting family members: direct (by study staff) or indirect (by proband), 6) degree of family relatedness (i.e. first – second – third-degree), 7) location and context of testing (in-home, primary care or testing at a study affiliated clinic), and 8) genetic vs. biochemical testing or combination thereof. The tentative search results for each of the eligible studies were independently reviewed by two of the authors (CJL & MRV). Any discrepancies between the results were reviewed by the senior author (IJK).

Data Analysis

We calculated the mean yield and NCIC in groups defined by method of contact (direct vs. indirect), relatedness of family members tested (FDR only vs. beyond FDR), sample collection (in-home vs clinic) and testing modality (genetic vs biochemical).

References

1. Fouchier SW, Defesche JC, Umans-Eckenhausen MW, Kastelein JP. The molecular basis of familial hypercholesterolemia in The Netherlands. *Hum Genet.* 2001;109:602-615.
2. Heath KE, Humphries SE, Middleton-Price H, Boxer M. A molecular genetic service for diagnosing individuals with familial hypercholesterolaemia (FH) in the United Kingdom. *Eur J Hum Genet.* 2001;9:244-252.
3. Williams RR, Hunt SC, Schumacher MC, Hegele RA, Leppert MF, Ludwig EH, et al. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. *Am J Cardiol.* 1993;72:171-176.
4. Setia N, Saxena R, Sawhney JPS, Verma IC. Familial hypercholesterolemia: cascade screening in children and relatives of the affected. *Indian J Pediatr.* 2018;85:339-343.
5. Rubio-Marin P, Michan-Dona A, Maraver-Delgado J, Arroyo-Olivares R, Barrado Varea R, Perez de Isla L, et al. Cascade screening program for familial hypercholesterolemia. *Endocrinol Diabetes Nutr.* 2018;65:280-286.
6. Leren TP, Finborud TH, Manshaus TE, Ose L, Berge KE. Diagnosis of familial hypercholesterolemia in general practice using clinical diagnostic criteria or genetic testing as part of cascade genetic screening. *Community Genet.* 2008;11:26-35.

7. Hope HF, Bluett J, Barton A, Hyrich KL, Cordingley L, Verstappen SM. Psychological factors predict adherence to methotrexate in rheumatoid arthritis; findings from a systematic review of rates, predictors and associations with patient-reported and clinical outcomes. *RMD Open.* 2016;2:e000171.
8. Hope HF, Binkley GM, Fenton S, Kitas GD, Verstappen SMM, Symmons DPM. Systematic review of the predictors of statin adherence for the primary prevention of cardiovascular disease. *PLoS One.* 2019;14:e0201196.
9. Sanderson S, Tatt ID, Higgins JP. Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: a systematic review and annotated bibliography. *Int J Epidemiol.* 2007;36:666-676.