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ABSTRACT

Background: Familial hypercholesterolemia (FH) is the most common genetic disorder associated with a high risk for premature atherosclerotic cardiovascular disease attributable to increased levels of LDL-cholesterol (LDL-C) from birth. FH is both underdiagnosed and undertreated.

Objective: We describe the clinical, biological, and genetic characteristics of 147 patients in France with clinical FH (including a group of 26 subjects aged < 20 years); we explore how best to detect patients with monogenic FH.

Methods: We retrospectively reviewed all available data on patients undergoing genetic tests for FH from 2009 to 2019. FH diagnoses were based on the Dutch Lipid Clinics Network (DLCN) scores of adults, and elevated LDL-C levels in subjects < 20 years of age. We evaluated *LDLR*, *APOB*, and *PCSK9* status.

Results: The mutations of adults (in 25.6% of all adults) were associated with DLCN scores indicating "possible FH," probable FH," and "definitive FH" at rates of 4%, 16%, and 53%, respectively. The areas under the ROC curves of the DLCN score and the maximum LDL-C level did not differ ($p = 0.32$). We found that the pediatric group evidenced more monogenic etiologies (77%, increasing to 91% when an elevated LDL-C level was combined with a family history of hypercholesterolemia and/or premature coronary artery disease).

Conclusion: Diagnosis of monogenic FH may be optimized by screening children in terms of their LDL-C levels, associated with reverse-cascade screening of relatives when the children serve as index cases.

KEYWORDS: familial hypercholesterolemia, adult population, pediatric population, monogenic disease, DLCN score, LDL cholesterol

INTRODUCTION

Familial hypercholesterolemia (FH) is a common genetic disorder. It is an autosomal codominant disease usually attributable to loss-of-function mutations in genes encoding the low-density lipoprotein receptor (*LDLR*) or apolipoprotein B (*APOB*), or to gain-of-function mutations in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene.[1] The overall prevalence of clinical FH in general populations is about 1:310,[2] but it is 1:120 in French subjects.[3] Biallelic *LDLR*-related FH is rare (estimated prevalence ~1/160,000–1/300,000).[4] FH is characterized by elevated low-density lipoprotein-cholesterol (LDL-C) levels and a high risk for premature atherosclerotic cardiovascular disease (ACD), particularly in those not or inadequately treated.[5,6] FH diagnosis is based on clinical and biological factors that contribute to the Dutch Lipid Clinic Network (DLCN) score[7,8] in adults but on the LDL-C level in children. Genetic testing (of at least the *LDLR*, *APOB*, and *PCSK9* genes) is strongly recommended for patients with clinically confirmed or suspected monogenic FH; this formalizes the diagnosis and facilitates molecular screening of relatives.[9] Here, we present the clinical, biological, and genetic data on 147 French patients diagnosed with FH based on the DLCN score (for adults) and on severe elevations in LDL-C levels in 26 subjects < 20 years of age.

MATERIALS AND METHODS

Study design and patients

We describe unrelated patients with clinical FH who underwent genetic analyses. None had been identified via cascade testing following a diagnosis of FH in a relative. All had been referred to specialist physicians (principally endocrinologists, cardiologists, and pediatricians) of the Bordeaux University Hospital between October 2009 and December 2019. Patients who underwent monogenic FH genetic testing were retrospectively selected (regardless of outcome). Written informed consent was obtained from all patients or their legal representatives. We adhered to the requirements for protection of personal health data and privacy set out in Article 65–2 of the (amended) Data Protection Act and the General Regulation on the Protection of Personal Data. The study was approved by our institutional ethics committee (CHU Bordeaux, France).

Adult patients were stratified by the DLCN criteria prior to genetic testing as recommended by the guidelines of the Consensus Statement of the European Atherosclerosis Society (EAS).[8] The highest known LDL-C level was used for scoring. A diagnosis of FH was considered “possible” (3–5 points), “probable” (6–8 points), or “definitive” (>8 points). Patients who could not be scored due to missing data were excluded. The pediatric population consisted of children or adolescents < 20 years of age in whom FH was suspected on the

basis an elevated LDL-C level (>4 mmol/L [155 mg/dL]) and a family history of hypercholesterolemia and/or premature coronary artery disease (CAD).

Collection of data

Clinical and biological data were collected from medical records, as were the DLCN scores calculated by clinicians. Demographics (age and sex), family or personal histories of hypercholesterolemia and cardiovascular events, evidence for the presence of lipid deposits (tendon xanthomas, xanthelasmas, and corneal arci), lipid profiles, any lipid-lowering therapy at the time of genetic analysis, and genetic test results were recorded. The maximum LDL-C was the highest recorded LDL-C level. Blood samples for lipid profiling were obtained after a 12 h fast. Serum levels of total cholesterol (TC), triglycerides (TGs), and HDL cholesterol (HDL-C) were quantified enzymatically on an autoanalyzer (AU5800, Beckman). LDL-C levels were obtained using either the Friedewald equation[10] (when TG < 3.5 mmol/L) or quantified enzymatically (AU5800, Beckman). Acquired causes of hypercholesterolemia[11] including hypothyroidism, chronic kidney disease, nephrotic syndrome, and cholestasis, and the use of medications that may increase LDL-C levels, were recorded. Smoking status, high blood pressure (HBP) readings, diabetes mellitus status, and the body mass index (BMI) were also collected.

Genetic analysis

All EDTA-containing blood samples were sent to the laboratory of Saint-Antoine Hospital (Paris, France). Genomic DNA was extracted from peripheral leukocytes using a QIAsymphony DSP DNA Midi Kit (Qiagen, Hilden, Germany). Sanger sequencing included the promoter region, all 18 exons, the flanking intronic sequences of *LDLR*, and exon 26 of *APOB*. Of patients with no identified variant in *LDLR* or *APOB*, 59 underwent multiplex ligation-dependent probe amplification (Salsa MLPA Kit P062, MRC-Holland, Amsterdam, the Netherlands) to search for large *LDLR* rearrangements and 49 underwent *PCSK9* sequencing. Sequencing was performed using a 3500xL Dx Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, USA) and the chromatograms were analyzed using SeqScape software (Applied Biosystems, Thermo Fisher Scientific).

All identified variants were sought in the Leiden Open Variation Database (LOVD v.3.0, www.lovd.nl/) and ClinVar (www.ncbi.nlm.nih.gov/clinvar/). Functional prediction was performed using Sorting Intolerant From Tolerant software (SIFT 4.0.3, sift.bii.a-star.edu.sg/)[12] and Polymorphism Phenotyping software (Polyphen2, genetics.bwh.harvard.edu/pph2/).[13] Mutation Taster (www.mutationtaster.org/)[14] and Combined Annotation Dependent Depletion (CADD, cadd.gs.washington.edu/)[15] were used to evaluate missense variants. Human Splicing Finder (HSF, www.umd.be/HSF3/)[16] was employed to predict the effects of splice variants. All variants were evaluated in terms of

pathogenicity following the recommendations of the American College of Medical Genetics (ACMG).[17] Only pathogenic variants, likely pathogenic variants, and variants of uncertain significance (VUS) are reported here.

Statistical analyses

Categorical variables are presented as numbers (n) with percentages (%). Differences between groups were compared using the Pearson chi-square test (or the Fisher exact test when values < 5 were expected). The distributions of continuous data were tested employing the Shapiro-Wilk test. Normally distributed continuous variables are reported as means with standard deviations (SDs) and differences among groups were analyzed via one-way ANOVA. Non-normally distributed parameters were compared using the Kruskal-Wallis test and are described as medians with interquartile ranges (IQRs). The significance level (alpha) was set to 0.05. All statistical analyses were performed using Rcmdr ver. 2.7–1. The Proc package was used to draw receiver operating characteristic (ROC) curves and the DeLong test was employed to compare areas under the ROC curves (AUROCs).

RESULTS

Populations

A total of 166 subjects genetically tested in terms of monogenic FH were eligible (according to their DLCN scores); 19 were excluded because of missing data. The final study population thus consisted of 147 individuals including 26 children. Acquired causes of hypercholesterolemia were investigated. One instance of uncontrolled hypothyroidism, one of cholestasis, and 12 cases taking medications that could impact the LDL-C level were noted among the adults.

Table 1 lists the characteristics of the adult population (n = 121, age 20 to 77 years, 38.8% males). Family histories of hypercholesterolemia and/or premature CAD were found in only 82.1% of cases (no data for four patients). As expected, the lipid profiles obtained at the time of genetic testing revealed increases in serum concentrations of LDL-C and premature CHD in 24% of patients, but the groups did not differ significantly in terms of the DLCN score (p = 0.09; data not shown). By contrast, physical examination identified tendon xanthomas and other extravascular lipid deposits in 7.5% and 14.2% of the patients, respectively, almost all of whom were in the “definite FH” group. A total of 114 patients (95%) underwent lipid-lowering therapy using either statins (n = 113) or an alternative such as anti-PCSK9 (n = 16) combined with statins if the desired decrease in LDL-C was not achieved using statins, and in patients exhibiting statin intolerance.

Table 2 lists the data for the pediatric population (n = 26, age 3 to 18 years, 42.3% males). Of these, 92.3% had family histories of hypercholesterolemia and/or premature CAD

and 22 family histories of hypercholesterolemia. Only two patients had no known family history of either hypercholesterolemia or premature CAD. One underwent lipid profiling during follow-up of diabetes (this revealed an elevated LDL-C level) whereas the other achieved a normalized LDL-C level after a dietary change. The maximum LDL-C level was 4–10.1 mmol/L (155–394 mg/dL); 92% of patients lacked lipid deposits. Prior to genetic testing, 50% of young patients eligible for lipid-lowering therapy (≥8 years of age, n = 18) were prescribed statins.

Table 1. Clinical and biochemical characteristics of the adult population

	N=121
Demographics	
Age, years	53.2±12.2
Male, n (%)	47 (38.8)
DLCN score	
Possible (3-5 points)	n=24
Probable (6-8 points)	n=57
Definitive (> 8 points)	n=40
Family history of hypercholesterolemia and/or premature CAD, n (%)	96 (82,1)
Hypercholesterolemia, n (%)	
Yes	72 (62.6)
No	43 (37.4)
Premature CAD, n (%)	
Yes	58 (48.7)
No	61 (51.3)
Clinical history	
BMI, kg/m²	(n=102)
Median (IQR)	26.8 (24.0-31.5)
Smoker, n (%)	
Ever smoker	60 (50.8)
Non smoker	58 (49.2)
Hypertension, n (%)	
Yes	47 (39.8)
No	71 (60.2)
Diabetes mellitus, n (%)	
Yes	17 (14.3)
No	102 (85.7)
Premature CHD, n (%)	
Yes	29 (24.0)
No	92 (76.0)
Ischemic stroke, n (%)	
Yes	11 (9.1)
No	110 (90.9)
Physical examination	
Tendinous Xanthomata, n (%)	
Yes	9 (7.5)
No	111 (92.5)
Other lipid deposits*, n (%)	
Yes	17 (14.2)
No	103 (85.8)
Biochemical profile, mmol/L	
Total cholesterol	(n=108)
Median (IQR)	8.1 (6.5-9.4)
LDL cholesterol	(n=114)
Median (IQR)	5.8 (4.6-6.9)
HDL cholesterol	(n=110)
Median (IQR)	1.3 (1.1-1.6)
Triglycerides	(n=110)
Median (IQR)	1.7 (1.2-2.6)
LDL cholesterol maximum	(n=106)
Median (IQR)	7.1 (6.2-8.5)
LLT, n (%)	
Yes	114 (95.0)
No	6 (5.0)

◄ * including corneal arcus and xanthelasmas
Continuous variables are presented as mean ± SD for normally distributed data and as median with IQR for non-normal distributed data. Categorical variables are described as absolute values and frequency.
Patients with missing data were excluded from the statistical analysis.
Cholesterol : mmol/L x 38.7 = mg/dL. Triglycerides : mmol/L x 87.5 = mg/dL

Abbreviations: FH, familial hypercholesterolemia; DLCN, Dutch Lipid Clinic Network, SD, standard deviation; BMI, body mass index; IQR, interquartile range; CAD, coronary artery disease; CHD, coronary heart disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LLT, lipid-lowering therapy.

Table 2. Clinical and biochemical characteristics of the pediatric population

	Children and adolescents N=26
Demographics	
Age, years	(n=26)
Mean ± SD	11.2 ± 4.8
Male, n (%)	11 (42.3)
Family history of hypercholesterolemia and/or premature CAD, n (%)	24 (92.3)
Clinical phenotype	
Lipid deposits*, n (%)	
Yes	2 (8.0)
No	24 (92.0)
Biochemical profile, mmol/L	
Total cholesterol	(n=22)
Median (IQR)	7.8 (7.1-8.3)
LDL cholesterol	(n=22)
Median (IQR)	6.0 (5.3-6.8)
HDL cholesterol	(n=22)
Median, (IQR)	1.5 (1.1-1.7)
Triglycerides	(n=22)
Median, (IQR)	0.8 (0.6-1.2)
LDL cholesterol max	(n=25)
Median, (IQR)	6.7 (5.7-8.0)
LLT, n (%)	
Yes	9 (36.0)
No	16 (64.0)

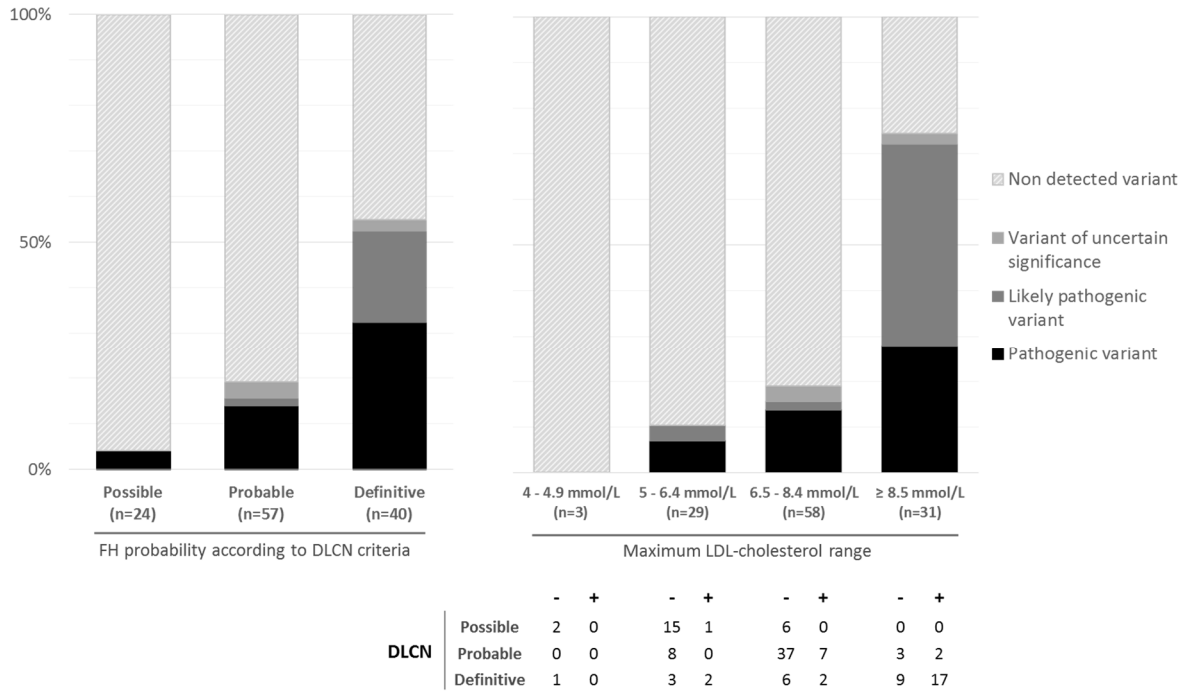
* including corneal arcus and xanthelasmas
Continuous variables are presented as mean ± SD for normally distributed data and as median with IQR for non-normal distributed data. Categorical variables are described as absolute value and frequency.
Cholesterol : mmol/L x 38.7 = mg/dL. Triglycerides : mmol/L x 87.5 = mg/dL

Abbreviations: SD, standard deviation; IQR, interquartile range; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LLT, lipid-lowering therapy.

Prevalence of FH-causing genetic variants

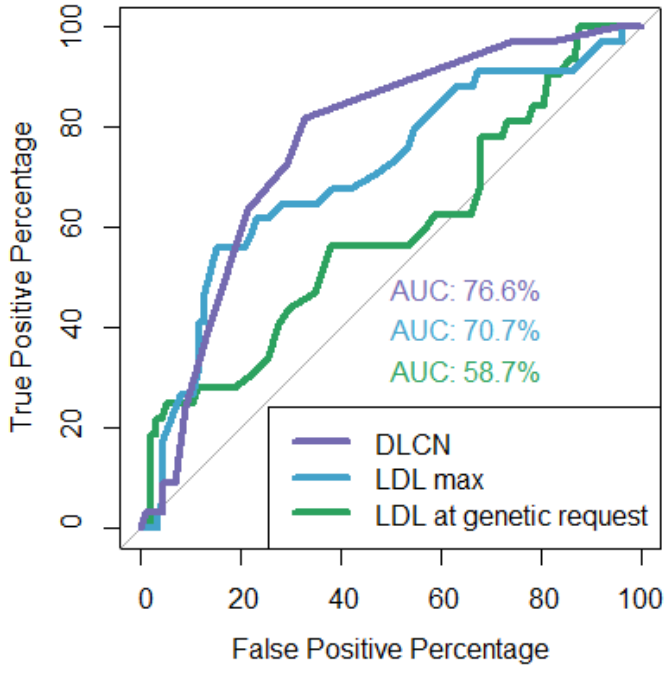
Thirty-one adults (25.6%) evidenced monogenic FH-causing genetic variants in *LDLR*, *APOB*, or *PCSK9* (87.1%, 9.7%, and 3.2%, respectively) and three VUS in *LDLR*. Twenty pediatric patients (77%) exhibited pathogenic (n = 15) or likely pathogenic (n = 5) variants (95% in *LDLR* and 5% in *APOB*). Overall, 40 variants were pathogenic or likely pathogenic, and 4 were VUS; one patient bore both a pathogenic variant (c.261G>A in *LDLR*) and a VUS (c.262A>G in *LDLR* Supplementary Data Table S1). The pathogenic or likely pathogenic variants affected *LDLR* (n = 38) more often than *APOB* (n = 1) and *PCSK9* (n = 1). The *LDLR* variants were distributed along the gene (Figure S1) and included missense (50%), nonsense (24%), frameshift (8%), and splicing (8%) mutations as well as large rearrangements (10%). To the best of our knowledge, *LDLR* (NM_000527) c.945del and c.2284del have not been previously described. Four individuals (three adults, one young patient) were heterozygous for the classic *APOB* missense variant p.R3527Q (rs5742904) associated with the monogenic FH phenotype. The *PCSK9* variant was detected in one adult and was classified as pathogenic.[18]

Figure 1 shows the proportions of adults among whom variants were reported by the DLCN scores and LDL-C subgroups. The variant detection rate was associated with the DLCN score (4%, 16%, and 53% in the "possible FH," "probable FH," and "definitive FH" groups, respectively), paralleling the LDL-C findings (0%, 10%, 16%, and 61% in the LDL-C ranges 4–4.9, 5–6.4, 6.5–8.4, and ≥ 8.5 mmol/L, respectively). The AUROCs significantly differed by the differences between the DLCN scores and the maximum LDL-C values (compared to the values at the time of genetic analysis) (76.6% vs. 58.7%, $p < 0.02$ and 70.7% vs. 58.7%, $p < 0.04$, respectively). No significant differences were noted when the DLCN score was compared to the maximum LDL-C level ($p = 0.32$) (Figure 2).



235

236 **Figure 1. Detection rate of FH-related variants according to the DLCN or LDL-C**
237 **subgroups.**
238 Frequency of detected variants in adults by DLCN score (left) or maximum LDL-cholesterol range (right).
239 The table shows the number of adult patients with (+) or without (-) likely pathogenic or pathogenic
240 variants according to both maximum LDL-C levels and DLCN score.
241
242 Abbreviations: FH, familial hypercholesterolemia; DLCN, Dutch Lipid Clinic Network
243



244

245 **Figure 2. Receiver Operating Characteristic (ROC) curves of three parameters in**
246 **predicting monogenic FH.**
247 AUC score for DLCN (purple line), LDL max (blue line) and LDL at the time of genetic analysis
248 request (green line) in adults.
249
250 Abbreviations: AUC, Area Under the Curve; DLCN, Dutch Lipid Clinic Network; LDL max,
251 maximum LDL-cholesterol

DISCUSSION

We describe the biochemical, clinical, and genetic characteristics of 147 unrelated patients referred to the Bordeaux University Hospital with suspected FH, including 26 patients <20 years of age. Our principal finding is the high level of monogenic FH in children with elevated LDL-C concentrations.

In patients aged < 20 years, an elevated LDL-C level associated with a family history of hypercholesterolemia and/or premature CAD suggests FH.[19] Of our 26 children and adolescents, 2 had no relevant family histories and 2 attained normal LDL-C levels via dietary changes alone; all 4 lacked mutations. Thus, only 22 met the selection criteria prior to genetic testing. Twenty (91%) were genetically confirmed to have FH. All had family histories of hypercholesterolemia, consistent with the known semi-dominant pattern of inheritance. The two other cases remain uncharacterized; we lack MLPA and *PCSK9* sequence data.

It is more difficult to predict monogenic FH in adults than children, as evidenced by the lower yield (25.6%) of positive genetic tests in our adult population. The detection rate of FH-causing variants unsurprisingly increased with the DLCN score; the figures were 4%, 16%, and 53% for the "possible FH," "probable FH," and "definitive FH" groups, respectively. Other studies have reported comparable distributions[20] or higher frequencies[21,22] depending on patient ethnicity and/or the techniques used, but also the efforts made to eliminate acquired causes of hypercholesterolemia. Phenotypic FH in adult patients lacking pathogenic or likely pathogenic variants may reflect age-related hypercholesterolemia (particularly in patients with mild or moderate increases in LDL-C levels)[23] or an elevated lipoprotein a (Lpa) level (a known independent risk factor for ACD).[24] We lack Lpa data. However, note that a negative genetic analysis does not exclude the presence of an undetected FH-causing variant. Finally, when the AUROCs of the DLCN score, the maximum LDL-C level, and the LDL-C level at the time of genetic testing were analyzed, the maximum LDL-C level and the DLCN score were equally effective at predicting monogenic FH in adults.

Our results highlight the need to carefully explore family histories (elevated cholesterol levels in first-degree relatives, ages of onset of ACD events) and the maximum LDL-C levels, and to exclude all acquired factors that might trigger hypercholesterolemia. This should increase the yield of genetic testing. However, although family histories are helpful, they may be incomplete, inaccurate, or unavailable. Genetic confirmation is recommended from the perspectives of patient care and disease prevention. Thus, FH is recognized in France as a long-term illness that may require financial support. Genetic data may trigger LDL apheresis, which would aid the planning of patient management (including

a lower LDL-C target); such data would also facilitate the genetic counselling that must precede a "cascade" family analysis. Pathogenic/likely pathogenic variants are associated with an increased risk for ACD.[25,26] The cumulative LDL-C burden imposed since birth may play an important role in ACD development in monogenic FH individuals. Khera et al.[25] showed that a pathogenic variant increased the ACD risk independent of the LDL-C level, compared to that of patients lacking mutations. Adults with elevated LDL-C levels and monogenic variants exhibit earlier-onset ACD.[27] A diagnosis of monogenic FH should trigger the management suggested by the 2019 European guidelines,[6] commencing at a young age. Genetic diagnosis should be scheduled for children and adolescents with LDL-C levels > 4 mmol/L (155 mg/dL). The ESC/EAS guidelines[6] recommend FH testing from the age of 5 years, or earlier if biallelic FH is suspected. FH diagnosis is too often delayed; an appropriate diet and statin treatment commencing in childhood are essential to prevent ACD.[19,28] A recent review reported lower rates of ACD in FH patients placed on statins in childhood (compared to adulthood).[29] Several studies have advocated the screening of all children aged 5–10 years (at least via LDL-C testing).[30–32]

CONCLUSION

FH is underdiagnosed in general populations.[8] We recommend systematic evaluation of monogenic FH in children as young as 5 years based on LDL-C levels and genetic testing. Early FH diagnosis followed by lipid-lowering therapy is cost-effective and would successfully mitigate cardiovascular morbidity and mortality. Such a strategy should be complemented by reverse cascade screening of relatives; young FH patients should serve as index cases.

Declarations of interest: none

Contribution Statement:
MF: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft; **LL**: Formal analysis, Writing - Original Draft; **KB**: Resources, Writing - Review & Editing; **MDF**: Writing - Review & Editing; **VR**: Resources, Writing - Review & Editing; **TC:** Resources, Writing - Review & Editing; **YP**: Resources, Writing - Review & Editing; **PB**: Resources, Writing - Review & Editing; **CG**: Conceptualization, Writing - Review & Editing, Supervision; **AMB**: Conceptualization, Methodology, Formal analysis, Writing - Original Draft - Review & Editing, Supervision.
All authors have approved the final article.

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