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Review

Universal Paediatric and Newborn Screening for Familial Hypercholesterolaemia—Challenges and Opportunities: An Australian Perspective

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Abstract: Heterozygous familial hypercholesterolaemia is one of the most common genetic conditions leading to premature atherosclerotic cardiovascular disease. It can be diagnosed using a combination of clinical, biochemical, and genetic tools. Most guidelines recommend screening during childhood and treatment from the age of 8–10 years. However, screening remains sporadic in most countries and the majority of individuals remain undiagnosed. Registry studies have highlighted the ongoing delayed and low percentage of detection of FH in children. Universal early childhood screening models utilising a combination of biomarker-based and genetic testing have been trialled and are in practice in some countries. Newborn screening is a public health success story and one of the most effective public health measures. It offers universal screening for conditions that can result in significant morbidity or even death if left untreated. There has been renewed interest in including familial hypercholesterolaemia in newborn screening programmes. Using cord blood to identify familial hypercholesterolaemia has not yielded convincing results. However, novel screening approaches on dried blood spots that include biomarker-based lipid profile testing alone, in combination with confirmatory genetic testing, or first-line genetic testing have shown promising results. This provides the opportunity of early diagnosis and treatment of infants and their extended families. However, challenges are associated with the inclusion of familial hypercholesterolaemia in newborn screening programmes with significant impacts on the newborn, family members, and public health.

Keywords: genetic screening; screening; newborn; familial hypercholesterolaemia

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1. Background

Familial hypercholesterolaemia (FH) is one of the most common inheritable disorders and a leading cause of premature atherosclerotic cardiovascular disease (ASCVD). It results in the accumulation of low-density lipoproteins (LDL) that are rich in atherogenic cholesterol [1]. Apart from rare exceptions, FH is inherited in an autosomal (co-)-dominant

manner. Autosomal dominant variants of the LDL receptor gene (*LDLR*), apolipoprotein B gene (*APOB*), and proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*) account for most FH cases: >90%, ~5–8%, and ~1%, respectively. Most FH individuals are heterozygous for one of these variants [2]. A very rare form of autosomal recessive hypercholesterolaemia affects the LDL receptor adaptor protein (*LDLRAP1*). Some variants of *APOE* have also been described to cause FH. Furthermore, some conditions can result in phenotypes similar to FH, including: polygenic hypercholesterolaemia, variants of *LIPA* (Wolman disease), *ABCG5*/*ABCG8* (sitosterolaemia), *STAP1*, and *CYP27A1* [3].

Heterozygous FH is common and estimated to be present in 1 per 250 people. There is regional variation and in certain ethnic groups the prevalence has been reported to be higher [4,5]. Rarely, individuals with FH can have a homozygous genotype. This severe form of FH, named homozygous FH, is much rarer, with a reported prevalence of 1 in 250,000–360,000 [6]. Unless otherwise noted, FH in this article refers to the heterozygous form.

The diagnosis of FH in adults is based on a combination of clinical, biochemical, and genetic features. Clinical risk scores, such as the Dutch Lipid Clinic Network criteria, can help with diagnosis in adults. Genetic testing is considered the gold standard for diagnosis [7–9]. Diagnosis in children is more challenging as peripheral stigmata often do not develop until early adulthood or may not develop at all. However, significant differences in carotid intima media thickness (cIMT) of FH individuals compared to their unaffected siblings occur as early as age 8 [10].

Early diagnosis and treatment of FH is paramount to reduce the individual and economic burden of premature ASCVD. The cost-effectiveness of cascade screening children followed by statin treatment of affected FH individuals has been demonstrated in an Australian study [11]. A recent meta-analysis of 18 economic evaluations also supported the cost-effectiveness of various screening approaches [12].

Guidelines recommend treatment for FH from the age of 8–10 [13]. Homozygous FH requires immediate treatment [6]. Pravastatin treatment in children aged 8–18 years has been shown to reduce atherosclerotic burden in children with FH using cIMT as a marker over 20 years ago [14]. In the CHARON study, the effect of rosuvastatin treatment of children with FH aged $\geq$6 years was compared to their non-affected, untreated siblings. Rosuvastatin treatment mitigated the progression of cIMT, which had been significantly greater in FH individuals at baseline, and reduced it to the level of their unaffected siblings after two years of treatment [15]. Additionally, a 20-year follow-up study of children with FH treated with statins showed that treatment was efficacious and safe over a 20-year-period. It confirmed that statins slowed the progression of cIMT significantly and demonstrated that the cumulative incidence of cardiovascular events at the age of 39 years was lower among statin-treated patients compared to their affected parents (1% versus 26%) [16]. A meta-analysis of ten randomised controlled trials of statin use in children confirmed the efficacy of statins in lowering LDL-C, reducing cIMT, and confirmed their safety. No significant differences were seen in hepatic or renal function, creatine kinase concentrations, or growth or sexual development when compared to children who received a placebo [17]. More recently, the use of PCSK9 inhibitors in statin-treated children aged 10–17 years with FH showed significant regression of cIMT compared to placebo over 24 months [18].

Early diagnosis and treatment can also increase important early treatment years in female FH patients, who are recommended to discontinue lipid-lowering therapy during pre-conception, pregnancy, and breastfeeding [19].

Despite the knowledge of the benefits of early treatment, FH is underdiagnosed. It is estimated that worldwide less than 10% of FH affected individuals have been detected [20].

Global registry data from the European Atherosclerosis Society Familial Hypercholesterolaemia Studies Collaboration showed that the mean age of FH diagnosis was 43 years in men and 46 years in women, with only 2% of participants diagnosed before the age of 18 [21]. Children and adolescents were not the focus of most primary detection strategies. The same registry showed that most of the 11,848 individuals with FH younger than 18 years were not index cases and were likely detected via cascade screening [22]. Australian registry data shows similar results; the mean age at enrolment into the registry was 53.4 +/− 15.1 years and assessment for FH occurred 1.7 +/− 4.1 years prior [23].

2. Paediatric Screening for FH

2.1. National and International Guideline Recommendations

Screening for FH meets the classic criteria as per Wilson and Jungner [24,25] and both patients and clinicians have highlighted the importance of and need for early screening for FH in recent years [26,27]. However, screening children and adolescents for FH remains challenging and varies between and even within countries [28]. Major cardiovascular and lipid societies recommend screening at different ages. A brief schematized overview that aims to simplify and divide the paediatric screening indications found within major consensus documents by categorising them into selective and universal categories alone can be found in Table 1.

Table 1. Paediatric screening recommendations from major cardiovascular and lipid societies.

Year	Issuing Body	Approach of Screening Age at First Screening	Initial Test	Reference
2011	National Heart, Lung, and Blood Institute's Expert Panel (NHLBI)	Selective: age 2–8 Universal: age 9–11 and 17–21	Lipid profile	[29]
2011	American Academy of Pediatrics (*adopted NHLBI*)	Selective: age 2–8 Universal: age 9–11 and 17–21	Lipid profile	[29]
2011 and 2015	National Lipid Association	Selective: age 2 Universal: age 9–11, repeat at age 20 or earlier	Lipid profile	[30,31]
2018	American Heart Association	Selective: age 2 Universal: age 9–11 and 17–21	Lipid profile	[32]
2015 and 2019	European Society of Cardiology/European Atherosclerosis Society	Selective: age of 5, or as early as possible if homozygous FH suspected Universal: may be considered	Lipid profile	[13,33]
2021	FH Australasia Network Consensus Working Group (endorsed by the Australian Atherosclerosis Society)	Selective: - Suspected or at risk of homozygous FH: newborns or by age 2 - At risk of FH: age 5–10 Universal: before puberty, preferably age 1–2 at time of immunisations	Lipid profile	[8]
2023	US Preventative Services Task Force	Insufficient data to screen asymptomatic children aged 20 years or younger	Not applicable	[34]
2023	International Atherosclerosis Society	Selective: - Suspected or at risk of homozygous FH: newborns or by age 2 - At risk of FH: age 2 or by age 5 Universal: should be considered	Lipid profile	[35]

2.2. Possible Paediatric FH Screening Strategies

Screening can take place in various settings and be initiated by various healthcare professionals, including (but not limited to) General Practitioner (GP) specialists, non-GP specialists, nurse practitioners, and genetic counsellors: see Table 2.

Table 2. Paediatric screening strategies for FH.

Strategy	Situation	Test	Reference
Selective			
Opportunistic	- After cardiac event in a parent or grandparent	Lipid profile	[36,37]
	- Routine visit or wellness check	Lipid profile	[35]
	- Prior to commencing isotretinoin/steroids, as part of diabetes mellitus routine care	Lipid profile	
	- Interpretative comments suggesting possibility of FH if LDL-cholesterol (LDL-C)/total cholesterol (TC) elevated		[38,39]
	- Reporting eligibility criteria of FH genetic testing if LDL-C/TC meeting criteria		
Systematic Cascade testing	- Test children (and other first and second-degree relatives) of parents diagnosed with FH	Lipid profile/genetic testing	[7,40]
Universal			
Opportunistic	- Newborns - At immunisations - Early childhood at school	Lipid profile/genetic testing	See Sections 3 and 4
Systematic Reverse cascade Child parent	- Test siblings (and other first and second-degree relatives) after FH diagnosis in infant/child	Lipid profile/genetic testing	See Sections 3 and 4

3. Large Population Paediatric FH Screening (Non-Newborns)

Slovenia is one of the few countries that has an established universal screening programme. Pre-school children aged 5 are tested with non-fasting lipid profiles. If TC levels are >6 mmol/L or >5 mmol/L with a positive family history, a referral to a lipid clinic is made. A fasted lipid profile is then measured and, if it meets the criteria, is reflexed to the sequencing of the coding and promoter regions of *LDLR*, *PCSK9*, *APOE*, and *APOB* exon 26. If children are found to have FH, cascade screening is initiated. Data over a 4-year period showed that 44.7% of 170 children who were fully genotyped had FH-causing variants. The sensitivity and specificity were 55.2% and 74.7% for the 5mmol/L plus family history cut-off, and 78.9% and 62.6% for the 6 mmol/L cut-off. In total, 94.4% of children with genetically confirmed FH had at least one parent with elevated TC levels. The study demonstrated the feasibility of a universal screening programme in real-life with a strategy of sequential biochemical and genetic testing. The authors published an implementation algorithm that could be adapted for use in other countries [41].

Many other countries offer paediatric universal screening programmes; Greece offers universal screening of children at the age of 3 years combined with cascade testing. Regional German programmes screen children between the ages of 2 and 6 or 5 and 14. Slovakia has

universal screening programmes at the age of 11. Most screening programmes, however, are not universal nor nation-wide [28,42]. As a result of differing screening approaches, the age of FH diagnosis varies significantly; a European registry study demonstrated that the age of diagnosis ranged from 3 years in Greece to 11 years in the Netherlands and Belgium [43]. A recent comparison of the implemented Slovenian model and the German Fr1dolin pilot project concluded that opt-out screening programmes, such as in Slovenia, might have a higher yield and reliability [44]. Some guidelines in the United States of America recommend universal screening at the age of 9–11 and 17–21 years with lipid profiles: see Table 1.

Multiple recent studies have attempted to develop optimal universal screening programmes. A meta-analysis assessed TC and LDL-C in individuals with and without FH to determine at which age and concentration results are most discriminatory using multiples of the median (MoMs). The detection rate (DR) appeared to peak at 1–2 years. The newborn period returned much lower DRs but was based on two small case series using umbilical blood. A concentration of 1.53 MoM for TC was reported to identify 88% of affected children, with a false positive rate (FPR) of 0.1%. LDL-C was also assessed and performed similarly (85% detected) at 1.84 MoM with a FPR of 0.1% [45]. This model was applied to a prospective study in the United Kingdom (UK); data from 10,095 children with a median age of 12.7 months with heel prick capillary blood collection for lipid profiles and targeted genetic analysis of FH causing variants was analysed. The overall DR using this approach was 62% with a FPR of 0.6%. They defined FH as either carrying a FH variant or as a total cholesterol >1.53 MoM at baseline and 3 months—this harbours a risk of mixing polygenic hypercholesterolaemia with FH. DRs fell to 47% with a FPR of 0.7% if only genetically confirmed FH cases were included [46].

A similar model was studied locally in Western Australia. Children were screened at the age of 1–2 years with a point of care TC at the time of routine immunisations with reflex genetic testing if the TC was above the 95th percentile ($\geq$5.3 mmol/L). This was followed by reverse cascade screening of parents. In total, 448 children were screened, with 7.1% having a TC above the threshold. FH was confirmed in three children and five family members. This model of early childhood screening with reverse cascade screening was cost-effective and feasible [47].

Another major study investigated the application of the aforementioned biochemical screening thresholds to the cross-sectional Avon Longitudinal Study of Parents and Children (ALSPAC); venous lipid profiles of 5083 ALSPAC children aged 9 years were available. The previously reported heel prick thresholds of TC > 1.53 MoM and LDL-C > 1.84 MoM were applied, and the results were compared to available low-read depth whole genome sequencing (WGS) and high-read depth sequencing of FH causative genes. When data from high-read depth sequenced participants alone was used, the DR for LDL-C > 1.84 MoM was 83% with a FPR of 0.8%, with a positive predictive value (PPV) of 29.4% and a negative predictive value (NPV) of 99.9%. For TC > 1.53 MoM, the DR was only 33% with a FPR of 0.9%, PPV 12.5%, and NPV of 99.7%. However, when this model was adjusted for verification bias, performance decreased significantly; the DR for LDL-C fell to 62.5% and even further to 25% for TC. It is noteworthy that TC and LDL-C concentrations diverge around this study's age group and an overlap of FH and non-FH dyslipidaemias may be contributory to low DR [48].

The DECOPIN project in Spain highlighted a successful combination of child–parent and parent–child screening; children aged 9–11 years were screened via lipid profiles added to routine blood tests. If a child's LDL-C was $\geq$4.9 mmol/L (or $\geq$3.5 mmol/L plus cardiac family history), the parents were instead assessed and underwent genetic testing if appropriate. Children only underwent genetic testing in the case of a positive

parental genetic test result. The alternative pathway consisted of performing targeted cascade screening on all offspring of parents with genetically confirmed FH. This combined strategy of opportunistic childhood screening and systematic cascade and reverse cascade screening was effective. Of the referred children, 87% were found to have a FH variant. In addition, this approach yielded a new diagnosis of FH in many parents [49]. Another comparable approach of universal lipid profile screening in Japan was recently published: Almost 8000 Japanese school children aged 9–10 were annually screened with lipid profiles. Individuals identified as being at risk of FH were referred to tertiary centres with reflex genetic testing and the reverse cascade screening of relatives was initiated. The approach was deemed successful and effective over the 5-year trial period [50].

It remains important to emphasise that while universal screening methods are developed, the combination of various established screening approaches including various age groups can be highly successful. For instance, the Netherlands, where one of the first nation-wide screening programmes for FH was established in 1994, implemented a systematic cascade screening approach. Individuals suspected to have FH underwent genetic testing for variants in *LDLR*, *APOB*, and *PCSK9*. If a variant was found, extensive cascade testing of relatives ensued with the help of genetic field workers [51].

Finding the optimal timepoint for biochemistry screening is controversial. As treatment is recommended from the age of 8–10 years, diagnosis before this age is ideal [13]. The age in childhood seems optimal around 1–2 years [45]. A recent study examined lipid parameters during the first 14–16 months of life using data from the Copenhagen Baby Heart Study. It demonstrated that LDL-C, non-high-density lipoprotein cholesterol (nonHDL-C), apolipoprotein B (apoB), and lipoprotein(a) Lp(a) increased stepwise when measured at birth, 2 months, and 14-16 months when adult concentrations are reached. From birth to 14–16 months, the median of LDL-C, non-HDL-C, apoB, and Lp(a) rose by 1.35 mmol/L, 1.6 mmol/L, 46 mg/dL, and 5 mg/dL, respectively. TC initially rose from a median of 2.0 mmol/L to 3.8 mmol/L and then declined slightly to 3.7 mmol/L. Importantly, lipid concentrations differed depending on sex, gestational age, birth weight, breastfeeding, and parental lipid concentrations [52].

Lastly, if sequential LDL-C based paediatric screening programmes with reflex genetic testing are introduced, it is important to establish LDL-C cut-offs in the paediatric population, preferably sex-, age-, and country-specific. LDL-C cutoffs used are often derived from the adult population and risk missing children with FH. Paediatric reference intervals for non-fasted LDL-C were recently published for the CALIPER cohort but may not be applicable to other countries [53]. The Dutch lifelines cohort study also provided contemporary reference values for LDL-C in children aged 8–18 years established via the analysis of lipid profiles from 8071 children. It demonstrated age- and gender-related changes in the 95th percentile of LDL-C [54]. Global registry data from the European Atherosclerosis Society Familial Hypercholesterolaemia Studies Collaboration showed that if only a single cut-off for LDL-C for all children had been applied to prompt suspicion of FH, many children may have been missed that were subsequently diagnosed via genetic testing [22]. Further research is required to inform LDL-C thresholds in the paediatric population reflecting each country's local paediatric population.

4. Universal Paediatric FH Screening (Newborns)

There have been efforts to implement FH diagnostics in newborns, with studies dating back to the 1960s and 1970s. Interest waned for a while, but there has been a renewed interest in newborn screening for lipid disorders more recently [55]. Screening methods include cord blood or heel prick biochemical testing and/or genetic testing.

4.1. Biochemical Testing

Biochemical testing for FH includes measuring lipid parameters such as TC, LDL-C, high density lipoprotein cholesterol (HDL-C), apoB, apolipoprotein A1 (apoA1), and nonHDL-C on cord blood or dried blood spots (DBS).

4.1.1. Cord Blood Testing

The first larger studies investigating cord blood screening for FH emerged as early as the 1960s [56,57]. In 1971, umbilical cord blood TC was measured in 1800 newborns. In total, 65 neonates had cord blood TC concentrations greater than the mean plus 2 standard deviations. At 6 weeks post-partum, parents' lipid profiles were assessed and a parent with hypercholesterolaemia was detected in 14 out of the 65 neonates' parents (but many parents were not tested). The findings were promising but the overall sensitivity of this screening method was low, there was a lack of clinical information regarding the neonates, and datasets were incomplete [58]. A follow-up study from the same cohort at age 1 showed promise for TC and LDL-C, but results were found to be significantly affected by the neonates' diet [59]. A study shortly after examined the cord blood of 2937 neonates and reviewed multiple other publications since 1960. Their data yielded similar results, but comparison to other cord blood studies showed a dispersion of results. Prematurity, maternal diabetes, prolonged labour, gender, ethnicity, maternal lipid status and cord blood contamination with maternal blood were contributors to the wide range of results [60]. Another study of cord blood lipid concentrations of 2815 newborns also demonstrated variability of results due to similar factors, albeit to a lesser degree than the previous study [61]. The validity of using cord blood TC or LDL-C was then criticised by studies thereafter [62–64]. While FH-affected neonates tended to have higher cord blood cholesterol levels, there was a significant overlap of results and the effectiveness of cord blood testing for FH remains questionable [65–67]. The Copenhagen Baby Heart study found good correlation of various lipid biomarkers in umbilical cord blood and venous bloods taken 2–3 h after birth. However, it also demonstrated a significant effect of gender, gestational age, birth weight, breastfeeding, and parental lipid concentrations [52]. While most of these findings were in keeping with previous studies, lower mean and median concentrations of TC, LDL-C, HDL-C, and triglycerides (TG) were found compared to other studies [68,69]. A pilot study was launched in the Czech Republic that tests TC and LDL-C concentrations in the cord blood of 10,000 newborns with reflex genetic testing in newborns with the highest LDL-C. The outcome of the study has not been published yet [70].

4.1.2. Heel Prick DBS Testing

FH newborn screening can be performed by measuring lipid parameters on heel prick DBS. Analysis of cholesterol DBS has been reported [71] but was criticised due to its analytical complexities and unreliability [72]. Multiple publications subsequently reported on the use of apolipoproteins on DBS. One group examined apoA1 and apoB and its ratio via immunonephelometry [73], another via immunoturbidimetry [74]. Others measured apoB via enzyme-linked immunosorbent assays [75,76], turbidimetric methods [77], and radial immunodiffusion assays [78–80]. However, apoB levels in DBS were also affected by gender, gestational age, and birthweight, with the selection of threshold cut-offs proving difficult [81].

Recently, FH screening via DBS has gained attention again. Efforts have been made to develop assays for TC, TG, and apoB that can be automated and are more efficient compared to the previously published time-intensive methods [82–84].

A higher throughput method for TC, LDL-C, and apoB on DBS and subsequent analysis of results from 10,000 de-identified neonatal DBS were published [85,86]. Results were

reported as MoMs, and the 99th percentile of each analyte adjusted for birth weight, gestational age, gender, and race was determined. LDL-C and apoB were potential candidates for neonatal FH screening. However, future correlation studies with genetic testing need to be performed to assess the clinical robustness and sensitivity/specificity of this approach. Importantly, birth weight, gestational age, and gender significantly affected results, in addition to seasonal variation. TC and LDL-C were stable analytes but apoB concentrations degraded at room temperature. A recent publication investigated pre-analytical effects on various analytes measured on DBS, including TC: TC was affected by suboptimal collections (small spots), prolonged shipping time, excessive heat, and humidity, resulting in lower concentrations. It highlights an important issue in using biomarker-based testing for FH screening [87].

4.2. Genetic Testing

There are several countries currently undertaking larger scale observational or randomised genomic newborn screening studies. Most of these studies, including Australia's BabyScreen+ study, use WGS to evaluate genomic newborn screening [88]. Others such as Baby Detect and Screen4Care in Europe use genetic panels [89].

Panel sequencing involves analysing a specific set of genes that are known to be disease causative. WGS involves sequencing the entire genome (coding, non-coding, +/− mitochondrial DNA) with subsequent analysis of pre-defined (or all) areas of the genome. Sequencing the entire genome and the required bioinformatic processing can be associated with higher cost, lower speed, increased amount of data, and a higher number of secondary and incidental findings and variants of unknown significance (VUS) compared to panel testing. However, as both sequencing technologies and interpretative pipelines evolve, both cost and turnaround time are improving. WGS also provides the opportunity to add novel conditions prospectively, quickly, and at minimal cost. It allows the re-analysis of an individual's data retrospectively as knowledge regarding new disease-causing genes emerge. WGS may also have diagnostic superiority for newborn screening, including broader variant detection [90].

4.2.1. Second Tier/Confirmatory Genetic Testing

Due to the pre-analytical issues affecting newborn lipid testing on DBS, there has been interest in incorporating genetic testing for FH into newborn screening and a few publications have emerged since 2022. Most studies applied a process of screening via lipids on DBS followed by genetic testing. In a recent study, DBS from 5248 newborns screened for LDL-C, TC, and apoB were reviewed. The samples identified as having the most extreme elevations were referred for genetic testing, including *LDLR*, *APOB*, *PCSK9*, *LDLRAP1*, *APOE*, *STAP1*, *LIPA*, *ABCG5*, and *ABCG8* [91]. Of the first 2500 samples, 192 were selected for genetic testing. A pathogenic variant for FH was found in one sample, and risk factors for polygenic FH in another four samples. Shortly after, the same group analysed a total of 10,004 newborn DBS with results for LDL-C, TC, and apoB. Mahalanobis distance (determining MoM different from 1) was employed to select the top 8% of distances which then proceeded to genetic testing. A total of 768 specimens were selected for genetic testing and 11 pathogenic and 4 likely pathogenic variants were detected. Combining apoB and LDL-C Mahalanobis distances yielded the highest detection rate. FH testing via biochemical and then reflex genetic testing was concluded to be feasible. However, it was also noted that more extensive genetic testing would likely lead to additional FH diagnoses. Furthermore, clinical validation of this model, especially phenotypic correlation of genetic results in childhood, should be completed [92].

4.2.2. First Tier/Primary Genetic Testing

Another approach is upfront genetic testing for FH on DBS. The feasibility of utilising Targeted Gene Sequencing on newborn DBS for a panel of genes which also included FH was recently demonstrated in Australia. In a pilot project, 2552 DBS from newborns were analysed. The DBS were available after routine newborn screening had been completed. Fifteen pathogenic/likely pathogenic variants causative of FH were detected (1 in 170), including 12 in *LDLR*, 2 in *APOB*, and 1 in *PCSK9* [93]. Analytical sensitivity using this approach was ≥99%, specificity was 100%. However, this model requires clinical validation via a prospective study. Another group used WGS on DBS from 6820 newborns as part of a pilot in China and identified 35 heterozygous FH carrier variants (1 in 195), with 32 described as pathogenic (1 in 213) and 2 as VUS. In total, 15 variants were detected in *LDLR*, 19 in *APOB*, and 1 in *PCSK9*. Importantly, at the age of 1.3–1.4 years, biochemical follow-up testing with TC, LDL-C, and HDL-C was performed in 22 out of the 35 infants who had been identified to have FH via genetic testing. Additionally, their parents were screened. It confirmed genotype–phenotype correlation, with significantly higher TC and LDL-C concentrations in infants with FH and their affected parent. On average, FH infants' TC and LDL-C levels were 48.1% (6.2 +/− 0.95 mmol/L) and 42.9% (4.21 +/− 0.64 mmol/L) higher compared to non-FH infants. It was also confirmed that the FH infants' parent carriers had significantly higher TC and LDL-C levels compared to the non-FH infants' parents: 29.8% (5.71 +/− 1.27 mmol/L) and 55.5% (3.67 +/− 1.52 mmol/L). There was no significant difference between the TC or LDL-C levels between FH infants and their affected parent carrier [94]. This study is a first step in the important need of prospective genotype–phenotype correlation and clinical validation of first tier genetic testing for FH. Larger prospective studies will need to investigate this further.

5. Opportunities, Challenges, and Ethical Considerations of Newborn Genetic Testing for FH

5.1. Opportunities

Screening for FH in the paediatric population is endorsed by major guidelines (Table 2), benefit and cost-effectiveness has been demonstrated for universal screening [41,46,48,95–98] and FH meets all classic screening criteria [24,25]. However, if universal screening were to be implemented, the question remains when the optimal timepoint for most effective screening would be and if newborn screening for this condition is ethical.

One advantage is that genetic newborn screening also identifies homozygous FH, a condition that needs to be treated immediately and has been incorporated into various newborn WGS pilot projects [6]. Despite being rare, modelling indicates that homozygous FH will rank approximately tenth out of the 65 conditions currently screened for in Queensland (internal, unpublished data). In isolation, this is a compelling rationale to include genetic screening for FH into routine newborn screening given the gravity of this disease and available treatment options to mitigate its consequences.

Genomic screening for FH at the newborn age has additional benefits. FH genetic testing on DBS can also help rule out conditions that may present similarly; sitosterolaemia, for instance, presents with elevated TC and LDL-C concentrations after the introduction of plant sterol-rich foods and genetic screenings allows the disease to be identified in the preclinical phase. Furthermore, genetic testing is not influenced by multiple pre-analytical variables that may result in false negative or false positive biomarker results [52,85,86]. Lipid parameters change over the first months in life and there is a reasonable chance that biomarker-based newborn screening will miss some individuals with FH [52].

Testing in the newborn period does not require any additional interventions or blood collections. A DBS can be sent for variant testing as part of the existing newborn screening

process. Furthermore, there is no need for additional infrastructure or collection procedures. Due to the high uptake (99%) of newborn screening in Queensland [99], almost the entire newborn population will be screened for FH and their relatives could be screened via reverse cascade testing. This would provide the opportunity to offer appropriate treatment, including early lifestyle changes and pharmacotherapy from the age of 8–10 years, for affected newborns [13] and immediate pharmacotherapy for affected relatives [1].

Newborn screening is the most successful universal screening programmes—an opportunity to reach such a broad number of individuals is difficult to achieve at later life stages. It has been demonstrated that even in countries like the Netherlands, with structured FH screening models, only a fraction of the expected FH individuals have been detected. The Netherlands had an extensive experience with a model of cascade screening using a team of highly skilled genetic field workers to facilitate cascade testing. Despite this government-funded model, only 38.6% of the expected 66,800 FH patients were diagnosed over a 20-year period [51].

After the success of the 100,000 genomes project in the UK, which involved WGS in 4660 participants with rare diseases [100], the Newborn Genomes Programme plans to perform WGS in 100,000 newborns with homozygous FH is one of the more than 200 targeted conditions that will be screened for [101]. Humphries et al. have recently made a strong argument that genomic newborn screening for FH should be considered in the UK. The genes causative of FH have been extensively studied, and technology is available that can readily diagnose these variants with high accuracy. Furthermore, there is clear evidence that both heterozygous and homozygous FH will lead to debilitating ASCVD with a significant impact on quality of life and life expectancy. This can be mitigated by early diagnosis and intervention in the pre-symptomatic stage with improved outcomes. As newborn screening is universal and FH treatment is cheap, equitable access for all is possible [25].

5.2. Ethical Considerations and Challenges

The distance between the time of diagnosis of FH in the newborn and recommended treatment at the age of 8–10 years is a considerable challenge and the ethics around consent for genetic testing at the newborn age is topical, with varying views [102]. The possible harm of diagnosing a genetic condition at an early age without the newborn's consent needs to be balanced carefully against the opportunities that an early diagnosis and treatment of FH can offer. This is particularly true for individuals with homozygous FH—a condition predicted to be detected with a frequency higher than most other conditions currently screened for in newborns in Queensland. This includes conditions with equally late onset forms, such as homocystinuria and some carnitine uptake disorders. Genetic privacy must be guaranteed for each individual but early screening and treatment of this important condition should be considered, as pharmacological treatment is recommended from age 8 for heterozygous FH [13] and immediately for homozygous FH—an age when informed consent by the affected child will also be difficult. Furthermore, early dietary and lifestyle changes implemented prior to pharmacological treatment have been moderately successful, highlighting further benefits of early screening [103]. In Slovenia, children are routinely screened for FH at age 5 [41] or at age 1–2 during a pilot project in the UK [46]. This raises the same issue as children might struggle to understand the concept and implications of genetic testing at this young age. Most guidelines recommend screening at age 9–11; however, the recent Australian guidelines advocate for universal screening before puberty, but preferably at age 1–2 at the time of immunisations. While this approach would be equally reasonable, the additional infrastructure and blood collections required and possibly lower opt-in rates, make screening in the newborn age more practical. Parents will be asked to provide

specific consent for genomic testing performed in addition to routine NBS and can decline participation. It is of utmost importance to highlight that the benefits of newborn screening lie with the opportunity to diagnose and treat children early rather than children being used as a tool to diagnose adults.

Parental anxiety after a FH diagnosis is a concern that needs careful consideration. However, various studies have demonstrated that genetic testing for FH is acceptable to both parents and tested individuals. A Dutch study demonstrated that 87.1% of parents from FH families wanted their children to undergo a genetic test for FH [104]. Interviews conducted with 11 parents of children diagnosed with FH or cystic fibrosis demonstrated that all interviewed parents would be interested in having subsequent children screened via a biochemical test in the newborn period, and 10 would consent to genetic testing [105]. Parents indicated that early diagnosis aided with future planning and treatment expectations and there was a particular interest in reverse cascade screening. Parents also commented on screening during the newborn period being a preferred option due to the ease of sample collection. Research into how FH individuals respond to a genetic diagnosis is also promising, with evidence that they coped well with the diagnosis and there was even a trend towards believing more strongly in lipid-lowering medications [106,107]. Studies that examined the impact of a genetic diagnosis of an inherited cardiovascular disease (including FH) on children, demonstrated that children handled their diagnosis well [108,109]. With appropriate pre- and post-test counselling and emphasis on the highly treatable nature of FH and demonstrably improved ASCVD outcomes with early dietary changes and pharmacotherapy, parental anxiety is minimised. This should ideally involve highly skilled genetic counsellors when possible. However, all staff involved in the care of individuals with FH should be upskilled in genetic counselling.

The care of FH individuals will require the involvement of many stakeholders, especially primary care physicians. In a recent viewpoint published in the Royal Australasian College of General Practitioners' Journal, a call was made for universal screening for FH in newborns with demonstrated willingness of GP specialists to be involved in this process [110]. The role of GPs and paediatricians in establishing universal screening is of utmost importance and streamlined pathways of care must be established and agreed upon by all stakeholders. This will need to include referral pathways for newborns diagnosed with FH and clinics that can provide care and reverse cascade screening of family members, especially in complex cases that require multidisciplinary input. We estimate that 350 newborns will be diagnosed with FH per year in Queensland, resulting in many more family members being screened and receiving appropriate treatment. Providing the infrastructure to facilitate testing and treatment will require a multidisciplinary approach including nurses, genetic counsellors, GP specialists, and non-GP specialists including paediatricians with an interest in lipidology to guarantee success. This network and pathways of care must be established prior to commencement of routine universal newborn FH screening.

The impact of an early diagnosis of FH on access to insurance including private health, life, and income protection requires careful consideration. Predictive genetic testing is becoming more frequent, and governments need to take action to mitigate the risk of genetic discrimination. The *Private Health Insurance Act 2007* prevents private health insurers in Australia from using an individual's genetic test to determine access to or the price of a health insurance product. In 2019, Australia's life insurance industry agreed to a partial moratorium on the requirements to disclose genetic test results for policies that require underwriting. The moratorium meant that insurers could only ask for genetic test results if the insured amount of cover exceeds certain financial thresholds for life, total and permanent disability, trauma, or income protection [111]. The moratorium was reviewed in a recent consultation paper titled '*Use of genetic testing results in life insurance underwrit-*

ing', with an indefinite extension of the moratorium announced, as well as immunity for genetic tests taken before, or while the moratorium was in place, based on The Australian Government the Treasury data [112]. A risk remained due to the self-regulatory nature of the industry and the moratorium [111]. A recent study reported that health professionals were concerned with the industry's self-regulation and felt that government regulation is required [113]. The *A-GLIMMER* project from 2020–2023 demonstrated instances of non-compliance and shared the broad view of stakeholders that financial limits of insurance covered by the moratorium are too low and do not align with the cost of living [114]. This work resulted in the Australian government announcing a total ban on the use of genetic results in insurance underwriting in 2024 [115].

One issue is certainly the knowledge around interpreting and storing large amounts of data and the handling of VUS and incidental findings. A guideline regarding streamlined variant classification for *LDLR* variants has been published, and similar guidelines are in the pipeline for *APOB* and *PCSK9* [116]. Overall, it also appears that the frequency of VUS in FH is relatively rare; a UK study of tested index cases reported 2.8% of individuals with a VUS [117]. A similarly low rate was reported in a limited newborn screening project in Queensland [93]. However, with large, universal newborn screening, a higher VUS detection rate might be expected. Limiting analysis to panels will help reduce the number of incidental findings and the amount of data storage required. False negative or positive results are an important consideration and need to be addressed despite excellent analytical sensitivity and specificity [93]. The continuous review of assay performance and careful variant curation is paramount. Awareness will need to be raised that despite excellent analytical performance characteristics, genetic FH newborn screening remains a screening test at this stage. As with other screening in the newborn period (such as hypothyroidism), a positive result will need to be confirmed by further evaluation via a diagnostic test such as biochemical evaluation once lipid parameters have stabilised around the age of 1–2 years and by biochemical and/or genetic evaluation of relatives. The false positive rate of genetic screening has not yet been well defined but is expected to be lower compared with biomarker based screening [118]. Another consideration could be the introduction of functional variant profiling using cell-based assays, particularly in cases of rare missense variants [119,120]. It is furthermore important to emphasise that there will be an evolution of the interpretation of genetic test results. By the time the affected individual reaches the recommended treatment age of 8–10 years, the interpretation of the initial genetic results may have changed and may require review.

Lastly, if pilot projects for genomic newborn screening for FH are successful, government funding for genetic screening, which is currently still costly, will need to be obtained and equitable access to screening and treatment for all must be guaranteed. A summary of key opportunities and challenges is summarized in Table 3.

Table 3. Key opportunities and challenges with universal genomic newborn screening for FH.

Opportunities
Reduction in morbidity and mortality from ASCVD due to early treatment
Detection of homozygous FH
No requirement for additional blood collections
Unaffected by prematurity/gestational age, sex, maternal lipid concentrations, illness
Possibility of early reverse cascade screening
Ability of re-analysis of stored data as knowledge of FH variants expands
Equitable access to and high participation rate in newborn screening

Table 3. *Cont.*

Challenges
Time distance between diagnosis of heterozygous FH and start of treatment
Laboratory resource implications for large increase in genomic screening numbers
Cost of sequencing FH genes
Workforce and infrastructure requirements for follow-up of expected increase in diagnosed FH individuals
Informed consent without impact on standard newborn screening
Impact on insurance access
Data interpretation: variants of unknown significance, variable penetrance and/or expressivity
Maintaining genetic privacy and security
Concern regarding parental anxiety and impact on parent–child relationship

6. Local Models of Care

Comprehensive models of FH care incorporating several screening strategies have been established in Australia, including the recommendation to incorporate selective, opportunistic, and universal screening for FH [8]. Recent Australian guidelines suggest that testing of children with suspected FH should be considered between the ages of 5–10 years and universal screening between 1 and 2 years of age should be considered [121]. Despite these comprehensive and widely available models of care, gaps have been highlighted in the care of individuals with FH in an Australian registry study [23].

Universal, genetic newborn screening is currently under review in Queensland and models of care for the follow-up of neonates (and their family members) diagnosed with FH are currently being reviewed. This may include multidisciplinary lipid clinics with close collaboration of primary, secondary, and tertiary caregivers. A possible workflow for genetic newborn screening is summarised in Figure 1. However, the pathways of care require careful consideration, the definition of risk reduction pathways, and the involvement of all stakeholders prior to introduction.

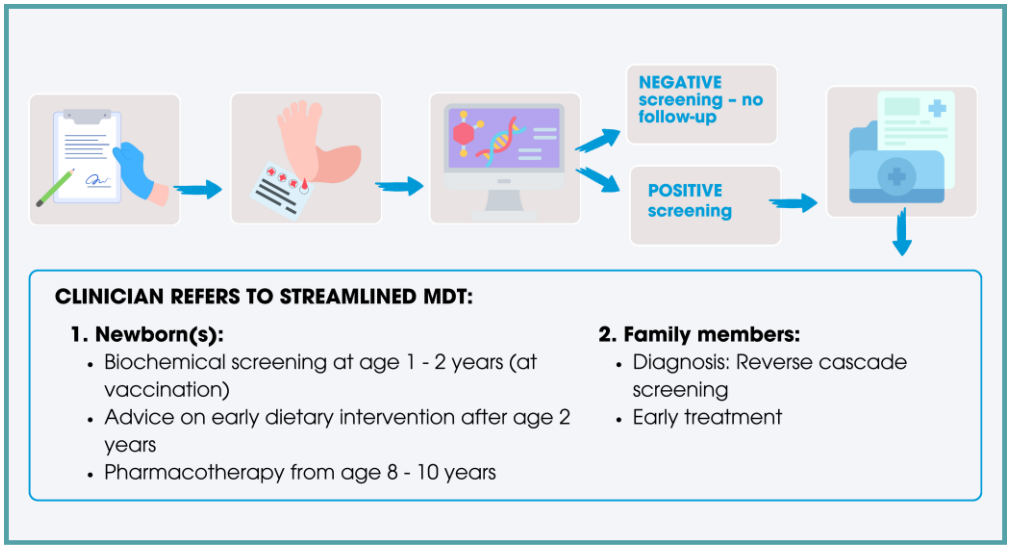

Figure 1. Possible workflow for genetic newborn screening for FH: After informed consent, a heel prick dried blood spot is sent to the laboratory for routine newborn screening tests, including targeted genetic screening for FH. Clinically significant variants are reported to the requesting clinician. The treating team offers referral of the newborn and parents to a multidisciplinary lipid clinic for follow-up.

7. Conclusions

FH is underdiagnosed and undertreated worldwide. Universal newborn genetic testing for FH presents an opportunity to reduce the burden of ASCVD and early death associated with this disease but needs to be carefully planned. Follow-up for families needs to be established via streamlined and multidisciplinary care pathways to ensure that individuals with FH and the health care system are adequately prepared. It is essential that each country establishes pathways suitable for its own background and resources. With careful implementation, newborn screening has the potential to revolutionise the future of families affected by FH.

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Abbreviations

The following abbreviations are used in this manuscript:

apoA1	Apolipoprotein A1
ASCVD	Atherosclerotic cardiovascular disease
ALSPAC	Avon Longitudinal Study of Parents and Children
cIMT	carotid intima media thickness
DR	Detection rate
DBS	Dried blood spots
FPR	False positive rate
FH	Familial hypercholesterolaemia
GP	General Practitioner
HDL-C	High density lipoprotein cholesterol
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
Lp(a)	Lipoprotein(a)
MOM	Multiples of the median
NHLBI	National Heart, Lung, and Blood Institute's Expert Panel
NPV	Negative predictive value
nonHDL-C	Non-high-density lipoprotein cholesterol
PPV	Positive predictive value
TC	Total cholesterol
TG	Triglycerides
UK	United Kingdom
VUS	Variants of unknown significance
WGS	Whole genome sequencing

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