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Chapter

Human Cholesterol Biosynthesis Defects

Erin Anderson and David Coman

Abstract

Cholesterol plays an essential role in normal embryogenesis and perturbations in its *de novo* synthesis are responsible for organ malformations in the cholesterol biosynthesis defects. Ten distinct inherited disorders have been linked to different enzyme defects in the isoprenoid/cholesterol biosynthetic pathway: mevalonic aciduria, hyperimmunoglobulinemia syndrome, squalene synthase deficiency, lanosterol synthase deficiency, hydrops-ectopic calcification-moth-eaten (Greenberg) skeletal dysplasia, X-linked dominant chondrodysplasia punctata, congenital hemidysplasia with ichthyosiform erythroderma and limb defects syndrome, lathosterolosis, Smith-Lemli-Opitz syndrome and desmosterolosis. These Mendelian disorders are clinically heterogeneous with protean manifestations reflecting the important role of cholesterol, and its intermediary metabolites, in embryogenesis and development. Key clinical features commonly represented by the cholesterol biosynthesis defects include structural brain malformations, axial skeletal developmental anomalies and genital and cardiac malformations. The aetiology of the underlying pathophysiology is unclear and multifactorial but may be due to lowered cholesterol and/or the elevated, teratogenic levels of the intermediate sterol precursors. Herein, we will review clinical, biochemical and molecular aspects of the known human cholesterol biosynthesis defects.

Keywords: cholesterol biosynthesis defects, mevalonate, squalene, skeletal dysplasia, chondrodysplasia, Smith-Lemli-Opitz

1. Introduction

Cholesterol is essential for normal cellular function. All nucleated cells can synthesise cholesterol from acetyl-CoA in the isoprenoid biosynthesis pathway via enzymatic reactions that are localised to the endoplasmic reticulum. Isoprenoids function in a variety of important cellular processes, including cell growth and differentiation, protein glycosylation, as precursors of oxysterols, steroid hormones and bile, in mitochondrial electron transport and signal transduction pathways, especially that of the hedgehog pathway [1–3]. Cholesterol biosynthesis is divided into two major pathways: pre-squalene cholesterol synthesis and post-squalene cholesterol synthesis. Pre-squalene cholesterol synthesis contributes to both sterol and isoprenoid synthesis, whereas post-squalene cholesterol synthesis is a committed pathway to sterol and vitamin D synthesis [3].

Isoprenoid biosynthesis (**Figure 1**) begins with the C2 compound acetyl-CoA, which, via six subsequent enzyme reactions, is converted into isopentenyl-pyrophosphate, the basic C5 isoprene unit used for synthesis of all subsequent

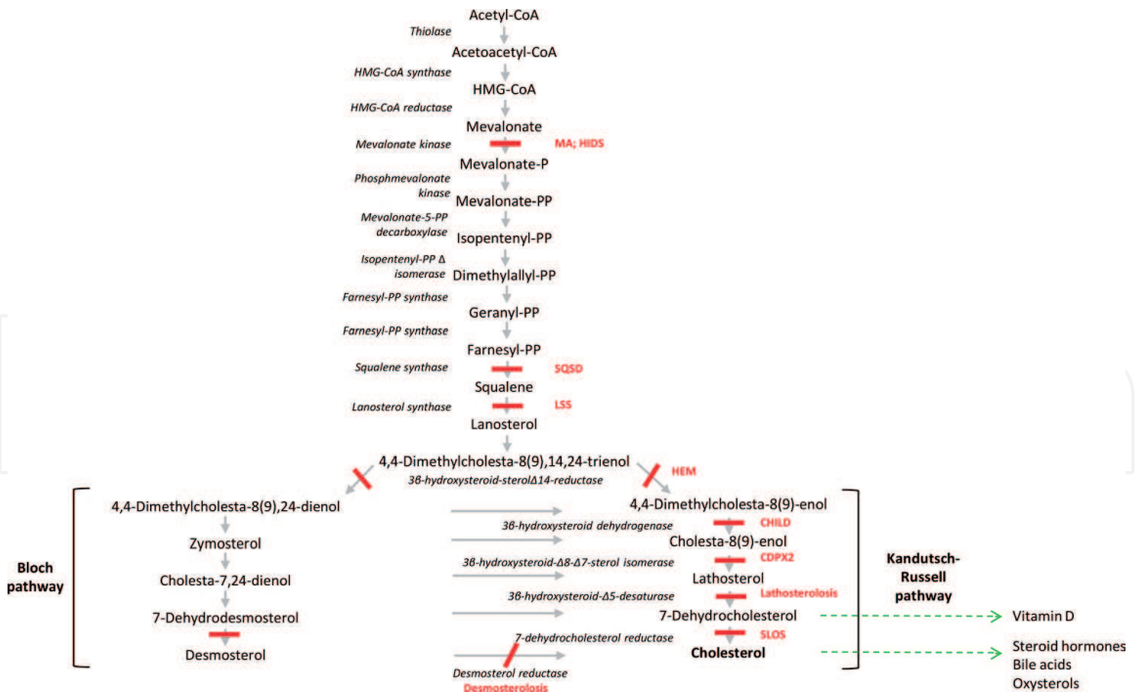

Figure 1.
Schematic representation of the human cholesterol biosynthesis pathway. HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; P, phosphate; PP, pyrophosphate; MA, mevalonic aciduria, HIDS, hyper IgD syndrome; SQSD, squalene synthase deficiency; LSS, lanosterol synthase deficiency; HEM, hydrops-ectopic calcification-moth-eaten; CHILD, congenital hemidysplasia with ichthyosiform erythroderma and limb defects; CDPX2, X-linked chondrodysplasia punctate 2; SLOS, Smith-Lemli-Opitz syndrome.

isoprenoids [3]. The first committed step to the production of sterol isoprenoids is C30 squalene (composed of 6 isoprene units) which, after cyclisation, is converted into C30 lanosterol (4,4,14-α-trimethyl-cholesta-8(9),24-dien-3β-ol) [4]. Following this transformation, cholesterol can be synthesised via one of two independent routes; the Bloch pathway [5] or the Kandutsch-Russell pathway [6]. Both pathways utilise the same enzymes, but in different orders in a tissue-dependent manner, leading to the formation of different intermediates [7]. C27 cholesterol is subsequently produced from lanosterol via a series of at least eight different enzyme reactions, including one demethylation at C14, two demethylations at C4, one isomerisation of the D8 [9] double bond to D7, three reductions of the D24, D14 and D7 double bonds and one desaturation between C-5 and C-6 [3].

Currently, 10 Mendelian disorders of cholesterol biosynthesis have been characterised, all with complex multisystem clinical phenotypes, supporting the importance of cholesterol in embryogenesis and development (see **Figure 1** and **Table 1**). Currently, the only reported defects in the pre-squalene pathway are the mevalonate kinase deficiency allelic conditions of mevalonic aciduria (MA, OMIM 610377) and hyper IgD syndrome (HIDS, OMIM 260960), squalene synthase deficiency (SQSD, OMIM 618156) and lanosterol synthase deficiency (LSS, OMIM 600909). Six Mendelian diseases in the post-squalene pathway have been reported: hydrops-ectopic calcification-moth-eaten skeletal dysplasia (HEM, OMIM 215140), congenital hemidysplasia with ichthyosiform erythroderma and limb defects syndrome (CHILD, OMIM 308050), chondrodysplasia punctate 2 (CDPX2, OMIM 302960), lathosterolosis (OMIM 607330), Smith-Lemli-Opitz syndrome (SLOS, OMIM 270440) and desmosterolosis (OMIM 602398). Improved understanding of molecular mechanisms associated with intracellular trafficking of cholesterol and regulation of key rate limiting steps in cholesterol synthesis (e.g. via the ubiquitin proteasome system) has generated opportunities for identification of other novel Mendelian defects associated with cholesterol homeostasis [8, 9].

Human Cholesterol Biosynthesis Defects
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Syndrome	OMIM	Chromosome location	Gene	Enzyme	Key features	Inheritance
MA	610377	12q24.11	*MVK*	Mevalonate kinase	Autoinflammatory flares, dysmorphia, DD, psychomotor retardation and hepatosplenomegaly	AR
HIDS	260960	12q24.11	*MVK*	Mevalonate kinase	Recurrent cyclical fevers and abdominal pain	AR
SQSD	618156	8p23.1	*FDFT1*	Squalene synthase	Dysmorphia, DD, male genital malformations, brain malformations, seizures and abnormal urine organic acids	AR
LSS	600909	21q22.3	*LSS*	Lanosterol synthase	Congenital cataracts and hypotrichosis	AR
HEM skeletal dysplasia	215140	1q42.12	*LBR*	3β-hydroxysteroid-sterol Δ^{14}-reductase	Non-immune hydrops fetalis, stippling and erroneous calcification and dwarfism	AR
CHILD	308050	Xq28	*NSDHL*	Sterol C4-demethylase aka 3β-hydroxysteroid dehydrogenase	Unilateral ichthyosis, male-lethal, ipsilateral limb reduction	XLD
CDPX2	302960	Xp11.22–23	*EBP*	3β-hydroxysteroid-Δ^8-Δ^7-sterol isomerase	Rhizomelia, calcific stippling cataracts	XLD
Lathosterolosis	607330	11q23.3-q24.1	*SC5DL*	3β-hydroxysteroid-Δ^5-desaturase	Microcephaly, cataracts, poly and syndactyly, DD, II	AR
SLOS	270400	11q13.4	*DHCR7*	7-dehydrocholesterol reductase	2–3 syndactyly, cleft palate, II, typical craniofacial stigmata	AR
Desmosterolosis	602398	1p32.3	*DHCR24*	24-dehydrocholesterol reductase	SLOS-like dysmorphia, CHD, microcephaly, DD, II	AR

Table 1.
Known human defects of cholesterol biosynthesis.

Modulating flux through the cholesterol biosynthesis pathway has been of interest for many years as a pharmacological treatment option for hypercholesterolemia. The statin family of drugs inhibit HMG-CoA reductase, the rate limiting step in the pre-squalene pathway, and similar efforts have focused on inhibitors of squalene synthase as this enzyme is the first committed step in cholesterol biosynthesis. Animal and human models of squalene synthase inhibitors generated a complex array of farnesol-derived metabolites [10–12], the recognition of which was instrumental in describing SQSD, a newly described pre-squalene cholesterol biosynthesis defect [4]. That pathogenesis of the cholesterol biosynthesis defects is complex, reflective of the multisystem nature of the clinical phenotypes.

2. Disorders of the pre-squalene cholesterol pathway

2.1 Mevalonate kinase deficiency

Mevalonate kinase phosphorylates mevalonate, the product of the reduction of 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA), which is important in cholesterol biosynthesis and for farnesylation and isoprenylation of proteins [13]. Mevalonate kinase deficiency (MKD) is a recessively inherited autoinflammatory disorder in the isoprenoid biosynthetic pathway with a spectrum of manifestations, including the well-defined allelic clinical phenotypes of HIDS and MA [14], both of which were identified in the mid-1980s [15, 16].

Mevalonate kinase is essential for the biosynthesis of non-sterol isoprenoids, which mediate protein prenylation. MKD is caused by mutations in the *MKD* gene which encodes mevalonate kinase, with the degree of residual enzyme activity largely determining disease severity. MKD leads to perturbations in the mevalonate pathway of cholesterol synthesis with episodes of hyperinflammation [17]. MKD is now viewed as a phenotypic continuum based on the degree of enzyme deficiency, with MA the most severe phenotype and HIDS the mild end of the spectrum [18].

MKD is characterised by autoinflammatory flares with fever, abdominal pain, mucoid and cutaneous lesions and arthralgias [14]. The more severely affected patients with MA classically have developmental delay, dysmorphism, psychomotor retardation, hepatosplenomegaly and ocular abnormalities [14]. During attacks, patients with MKD have increased levels of acute-phase proteins including C-reactive protein and cytokines such as TNF-α, IL-6 and interferon-γ [19, 20]. The MA phenotype characteristically presents in the first few months of life, with antenatal presentations linked with a high rate of stillbirth [21]. Commonly reported dysmorphic features include frontal bossing, hypertelorism, long eyelashes and triangular-shaped facies, as well as failure to thrive, developmental delay, ataxia, seizures, myopathies and autoinflammatory attacks [21, 22]. MA is a multisystem phenotype with gastrointestinal manifestations including cholestasis and liver dysfunction [23], and ocular findings including recurrent conjunctivitis, cataracts and uveitis [24].

The HIDS phenotype typically presents with recurrent (four-to-six weekly) self-limited bouts of multisystem inflammation characterised by fever, abdominal pain, adenopathy, rash and arthralgia [14]. As these are common symptoms of many childhood infectious illnesses, the diagnosis of HIDS is often delayed for many years. HIDS episodes usually last 3–7 days, occurring in a cyclical fashion or induced by a provocative physiological stress such as illness, injury or vaccination. Acute abdominal pain may be the most marked and debilitating feature of systemic inflammation and can mimic a ‘surgical acute abdomen’ [24]. A long-term follow-up study of 103 HIDS patients revealed that the frequency of the attacks decreases

over time, but 50% of patients greater than 20 years of age still experience six or more attacks per year, impacting on the quality of life [24].

The epidemiology of MKD is largely unknown. At least 300 people are documented worldwide, the majority with HIDS, although this is likely to be underdiagnosed as recurrent fevers in childhood are a common occurrence. The highest documented prevalence is in the Netherlands, with an estimated 1:200,000 affected nationwide, consequent to a high carrier rate which is estimated at 1:65 [24, 25].

Elevations in IgD in MKD are inconsistent and can be normal in up to 20% of cases [24]. Serum amyloidosis is a long-term sequela of prolonged inflammatory activation, with elevations in serum amyloid A noted in approximately 3% of HIDS patients [24]. Urinary excretion of mevalonic acid can persist in MA and maybe present in some HIDS patients during febrile attacks [21]. The diagnosis is confirmed by identifying pathogenic mutations in the *MVK* gene; currently more than 120 sequence variants in this gene have been reported in association with MKD [26], most of which are missense mutations that impair mevalonate kinase stability [27]. Some genotype-phenotype correlations exist: *MVK* variants located in the core of the protein (affecting folding and stability) are highly associated with the more severe MA phenotype [25, 28, 29]. In contrast, other variants such as the C-terminal V377I substitution typically manifest as the HIDS phenotype and are rarely associated with MA [25].

Although the precise pathogenesis of MKD remains unclear, increasing evidence suggests that deficiency in protein prenylation leads to innate immune activation and systemic hyperinflammation, which has assisted in the development of cytokine-directed biologic therapy. Corticosteroids induce a complete response in 24% of HIDS patients [30]. Biologics targeting IL-1, including anakinra and canakinumab, and TNF-α blocking agents such as etanercept and adalimumab, have been used with varying success [30]. Some cases that have failed to respond to anakinra have demonstrated a successful reduction in symptoms with tocilizumab, a monoclonal antibody targeted against the IL-6 receptor [31]. One patient with MKD, treated with alendronate for steroid-induced osteoporosis, subsequently achieved complete remission [32]. Alendronate inhibits farnesol-pyrophosphate synthase. For refractory cases of MA phenotype, the last consideration for therapy includes liver transplantation or haematopoietic stem cell transplantation [14].

Blockade of the mevalonate pathway with the HMG-CoA reductase inhibitors reduces both mevalonic acid levels and residual isoprenoid production and but can trigger disease flares [22]. The inflammatory hyper-responsiveness in MKD appears to be due to lack of isoprenoid products and not accumulation of mevalonic acid. This appears to be due to the need for geranylgeranylation rather than other mevalonate pathway products, such as cholesterol biosynthesis, in mediating the hypersecretion of IL-1β [27]. The use of statins in this disease process has therefore largely been abandoned [30]. *Mvk*$^{+/-}$ mice do have some features of immune dysfunction, including increased serum IgD and TNF-α levels, as well as increased expression of activation markers on T-lymphocytes and macrophages [33].

2.2 Squalene synthase deficiency

Squalene synthase deficiency (SQSD) is a recently identified pre-squalene defect to have been characterised. In 2018, three patients were reported with this novel cholesterol biosynthesis defect [4]. Salient clinical features include facial dysmorphism, dry skin with photosensitivity, generalised tonic-clonic seizures, structural brain malformations, cortical visual impairment, profound global developmental delay and genital malformations in the two males [4].

Gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy profiles yielded a consistent and complex pattern of abnormal metabolites including accumulation of methylsuccinic acid, mevalonate lactone, mesaconic acid, 3-methyladipic acid, saturated and unsaturated branched-chain dicarboxylic acids and glucuronides derived from farnesol [4]. A similar metabolite profile has previously been observed in the urine of animal models and humans treated with pharmacological inhibitors of squalene synthase, as well as in animals loaded with farnesol [10–12]. This urine metabolic profile is specific to and thus diagnostic of SQSD. Plasma total farnesol levels (the sum of free farnesol and farnesyl-pyrophosphate) in affected individuals were, however, significantly increased (1.5–3.9 mmol/L; reference <0.12) while plasma squalene levels were reduced or normal (0.17–0.93 mmol/L, reference 0.36–1.04).

A range of pathogenic *FDFT1* molecular variants have been described in the three SQSD patients identified thus far (a sibship and an unrelated patient) [4]. The sibship was compound heterozygous for a maternally-inherited 120 kb deletion, resulting in loss of exons 6–10 of *FDFT1* and the entire coding sequence of the neighbouring *CTSB* gene (encoding cathepsin B (OMIM 116810)); and a paternally inherited variant c.88024_88023delinsAG, which created a novel splice acceptor site. The unrelated patient was homozygous for a novel 16-bp intronic deletion. Functional characterisation of the variants demonstrated a partial splicing defect and altered promoter and/or enhancer activity, reflecting essential mechanisms for regulating cholesterol biosynthesis and/or uptake in steady state [4].

Fdft1-null mice demonstrate embryonic lethality at day 12.5 in conjunction with growth restriction and neurodevelopmental disorders [34]. The fact that the *FDFT1* variants in the human SQSD cases are compatible with life may be explained by the fact that all individuals have some form of residual FDFT1 activity, either resulting from the diminished levels of correctly-spliced enzyme or by functional compensation for disrupted regulation [4].

2.3 Lanosterol synthase deficiency

In the cholesterol biosynthesis pathway, lanosterol synthase leads to the cyclisation of (S)-2,3-oxidosqualene into lanosterol. Pathogenic mutations in the *LSS* gene have recently been reported in a spectrum of clinical phenotypes including congenital cataracts in three families [35], hypotrichosis simplex (HS) in three families [36] and a more severe neuroectodermal syndrome formerly named alopecia with mental retardation (APMR) syndrome in six unrelated families [37]. HS (OMIM 618275) is a rare form of hereditary alopecia characterised by childhood onset of diffuse and progressive scalp and body hair loss [36]. APMR syndrome (OMIM 203650) is a rare disorder with autosomal recessive transmission. A recent report identified 11 individuals from seven unrelated families affected with alopecia, male genital abnormalities, variable MRI abnormalities and neurological symptoms [37]. In this cohort, total alopecia was universal with other common dermatological manifestations including ichthyosis and erythroderma. Neurological manifestations included significant developmental delay, microcephaly, epilepsy and hypomyelination [37].

Sterol profiling in lanosterol synthase deficiency cases has not identified any specific abnormalities, thus supporting the previously proposed hypothesis of an alternative cholesterol pathway [36]. *LSS* variants identified to date include truncating, missense and splicing variants. *LSS* has also been associated with congenital cataracts in rat [38]. Mice homozygous for the $Lss^{tm1b(KOMP)Wtsi}$ allele demonstrate variable lethality, from embryonic day 9.5 to postnatal prior to weaning [39].

3. Disorders of the post-squalene cholesterol pathway

3.1 Hydrops-ectopic calcification-moth-eaten skeletal dysplasia

Most proximal in the post-squalene pathway is hydrops-ectopic calcification-moth-eaten (HEM) skeletal dysplasia, or Greenberg dysplasia. This very rare and severe autosomal recessive disorder was first described in 1988 [40] with only 11 examples identified in the literature to date. All but one of these have been lethal *in utero*, with the remaining case dying at 2 days of age [41]. HEM skeletal dysplasia is characterised by significant non-immune hydrops fetalis, erroneous chondro-osseous calcification of vertebrae, ribs, pelvis, larynx and trachea as well as a diagnostic mottled 'moth-eaten' appearance of long bones on radiography [42–44]. Further skeletal abnormalities can include rhizomelic and mesomelic shortening of the limbs, platyspondyly, decreased skull ossification and distal dysmorphisms such as absent phalanges or postaxial polydactyly [42–45]. Non-skeletal congenital malformations include pulmonary hypoplasia, intestinal malrotation, cystic hygroma and excessive extramedullary haematopoiesis [45, 46]. Histology shows significant bone and cartilage disorganisation [43, 45].

HEM skeletal dysplasia was first suggested as an inborn error of cholesterol biosynthesis by Kelley et al. [47] with identification of increased levels of 4,4-dimethylcholesta-8 [9],14-dien-3β-ol and 4,4-dimethylcholesta-8(9),14,24-trien-3β-ol in cultured fibroblasts, indicating a deficiency of sterol Δ^{14}-reductase. This enzyme converts these sterols to 4,4-dimethylcholesta-8(9)-en-3β-ol and 4,4-dimethylcholesta-8(9),24-dien-3β-ol, respectively. This point on the cholesterol biosynthesis pathway is unique with sterol Δ^{14}-reductase activity by both the lamin B receptor (LBR) and a second enzyme DHCR14 (TM7FS2), although functional redundancy is disputed [48, 49]. It was originally thought that the more prominent role in sterol biosynthesis was that of DHCR14 compared to the lamin B receptor. However, it has more recently been demonstrated that it is a deficiency in the lamin B receptor due to mutations in *LBR* at 1q42.12 that is causative for HEM skeletal dysplasia [50, 51] and that it is the LBR, not DHCR14, that is required for cholesterol biosynthesis [48, 52].

The involvement of *LBR* has raised contention as to whether HEM skeletal dysplasia should be classified as a laminopathy rather than as an error of cholesterol synthesis [49]; however, it is appropriate to recognise that mutations in *LBR* can cause different disorders in different contexts. The type of mutation (missense, nonsense or splice-site), the functional location of each mutation in the *LBR* gene and the residual protein activity affect the clinical outcome of this disorder [53, 54]. The LBR protein has both a nuclear domain involved in anchoring chromatin to the nuclear membrane, and a transmembrane domain with sterol Δ^{14}-reductase activity critical for cholesterol synthesis [48], the latter primarily where mutations causing HEM dysplasia are located [50]. Some mutations found in *LBR* in HEM dysplasia patients have been identified in the heterozygous state in the relatively benign autosomal dominant condition of Pelger-Huët anomaly in which granulocytes have bilobed nuclei but patients are otherwise clinically normal. These two conditions may represent different allele patterns of the same disorder for some mutations [53, 55]. The less common homozygous Pelger-Huët is clinically more severe with round or ovoid granulocyte nuclei and some cases with mild skeletal abnormalities [56, 57]. This highlights the role of the lamin B receptor sterol reductase function as essential in prenatal development but also the phenotypic continuum that can occur for various allele combinations of the *LBR* gene.

Species variation with respect to the role of the LBR can make mouse model outcomes difficult to elucidate. Studies of mutations in both *LBR* and *DHCR14/TM7FS2* have been investigated in ichthyosis (*ic*) mice with contrasting conclusions, including those highlighted above and as reviewed by Herman and Kratz [58].

3.2 Congenital hemidysplasia with ichthyosiform erythroderma and limb defects syndrome

Congenital hemidysplasia with ichthyosiform erythroderma and limb defects (CHILD) syndrome is a rare X-linked dominant disorder of cholesterol biosynthesis, with fewer than 100 cases discussed in the literature [58]. The earliest identification of the condition is thought to be in 1903 [59], through the proposal of the syndromic acronym in 1980 [60]. CHILD syndrome is nearly always male-lethal although perhaps two males with this syndrome have been identified, one with a 46, XY karyotype, postulated to have survived due to a postzygotic mutation [61]. The distinguishing hallmark of the condition is that of unilateral skin lesions with ipsilateral limb defects [60, 62, 63]. The characteristic yellow, scaly plaques are usually present at birth or emerge in the first few months of life and while there may be some resolution over time, they often remain for life [60]. These markings may follow the lines of Blaschko, but more commonly, there is a striking delineation at the midline with the lesions showing a unique lateralisation pattern [62]. This has been proposed to be due to interactions between X-inactivation and the organisation of left-right axis symmetry in the developing embryo [60]. The lateralisation of these lesions and their persistence is a distinguishing feature of CHILD syndrome compared to differentials such as CDPX2, a similar but distinct inborn error of cholesterol biosynthesis [64].

CHILD syndrome demonstrates complete limb aplasia, severe phocomelia or severe hypoplasia on the same side of the body as ichthyosiform lesions [60]. Infant radiography may show epiphyseal stippling such as that seen in CDPX2, as well as milder skeletal malformations such as scoliosis, hypo or hemi-plastic vertebrae, distal digit shortening, syndactyly or polydactyly [65]. Non-skeletal manifestations include alopecia, verruciform xanthoma, dystrophic nails and congenital malformations on the affected side that can involve the heart, kidneys and CNS [60, 65]. Intelligence may be normal or slightly reduced. Despite the severity of these symptoms, mild cases of CHILD syndrome with no skeletal and/or cutaneous involvement have been identified through molecular analysis [66].

Molecular investigation has identified various mutations in the *NSDHL* (NADH steroid dehydrogenase-like) gene as causative for CHILD syndrome [67]. *NSHDL* is located at Xq28 and encodes 3β-hydroxysteroid dehydrogenase, part of a three-part enzyme complex. This C4 demethylation complex acts on the sterol ring in the post-squalene pathway, converting 4,4-dimethylcholesta-8(9),24-dien-3β-ol to zymosterol and 4,4-diemthylcholesta-8(9)-en-3β-ol to cholesta-8(9)-en-3β-ol. Mutations are most often loss-of-function [68]. Cholesterol and sterol levels are normal and so a diagnosis requires clinical and molecular assessment. Various *NSDHL* mutations have been studied in the allelic 'bare patches' (*Bpa*) and 'striated' (*Str*) murine models [69]. These have given insights into facets of CHILD syndrome and cholesterol synthesis disorders in general, for example, the roles of the maternal placenta [70] and Hedgehog signalling pathways [71] in disease presentation.

Treatment options for CHILD syndrome have generally focused on topical management of skin lesions with symptomatic remedies such as emollients or with pathogenesis-based therapies generally involving combinations of cholesterol and a cholesterol synthesis-inhibitor [72, 73], the latter with some efficacy.

3.3 X-linked dominant chondrodysplasia punctata 2

X-linked dominant chondrodysplasia punctata 2 (CDPX2), or Conradi-Hünermann-Happle syndrome, is estimated to have an incidence of 1/400,000 and, similarly to CHILD syndrome, is almost entirely male-lethal. The CDPX2 phenotype, like the other cholesterol biosynthesis disorders, is heavily based on the

skeletal and cutaneous domains, and there can be significant variability even within family lines [74–76] with generational anticipation [74]. Severe manifestations can result in neonatal or infant death with considerable skeletal and internal abnormalities, while mild cases may be nearly asymptomatic. This range of phenotypic variability is likely due to the combination of somatic and/or gonadal mosaicism and X-inactivation patterns [76]. Occasional male patients are identified with CDPX2, usually due to somatic mosaicism [77, 78] with one case of 46,XXY [79]. Gonadal mosaicism is possible which is relevant for recurrence risk [80].

Widespread epiphyseal stippling is seen on infant radiographs, often including not just the long bones but the trachea and vertebrae as well [81–83]. Additional skeletal stigmata include short stature and scoliosis (which can be congenital), clubfoot, joint contractures, and postaxial polydactyly [74, 77, 83]. Cutaneous manifestations include skin with patches of scaly hypo or hyper-pigmentation, which usually follows the lines of Blaschko. The initial skin scaling and erythroderma present at birth usually fades over the first few months of life, leaving follicular atrophoderma, pigmentation and alopecia, although ichthyosis can persist [84, 85]. The pattern and then resolution of skin scaling as well as its histological profile is a differentiating diagnostic feature for CDPX2 compared to CHILD syndrome. Diagnosis of CDPX2 in adulthood can be difficult due to the childhood resolution of the characteristic skin lesions and epiphyseal stippling [86]; however, a combination of cutaneous manifestations, asymmetric limb reduction and cataracts (found in 65% of patients) is a good suggestion of this condition for further investigation [86]. CDPX2 presents with characteristic facial features including frontal bossing, midface hypoplasia and flat nasal bridge [74, 81]. The condition is also associated with microphthalmia or microcornea, congenital heart disease, renal abnormalities including hypoplasia and hydronephrosis and sensorineural hearing loss [87]. Cognition is usually normal [87].

CDPX2 is caused by mutations in the *EBP* (emopamil binding protein) gene [88, 89] located at Xp11.23 and encoding a Δ^8-Δ^7-sterol isomerase. This enzyme functions downstream of the C4-demethylation complex affected in CHILD syndrome and converts zymosterol and cholesta-8(9)-en-3β-ol to cholesta-7,24-dien-3β-ol and lathosterol, respectively. There is a phenotypic correlation with enzyme function with lethality of homozygous females and clinically affected heterozygous females; however, there is no clear genotype-phenotype correlation, presumably due to X-inactivation patterns [74, 76]. Surviving males with CDPX2 are almost always due to mosaic postzygotic mutations as a hemizygous male genotype is lethal *in utero*. CDPX2 mutations (including deletions, insertions, nonsense, missense and splice-site) of *EBP* have been identified as both *de novo* and inherited mutations and are found throughout the entire length of the gene [74].

While there is no clear CDPX2 genotype-phenotype correlation, there is a distinct association between genotype and CDPX2 sterol profile [74], and plasma sterol assay is a highly specific indicator for an *EBP* mutation [83]. Plasma shows increased 8-dehydrocholesterol and 8(9)cholesterol levels, with the ratios compared to cholesterol increased 0.71–0.80% [74]. Plasma cholesterol is usually normal. Treatment and surveillance are symptomatic, and studies in these areas have been advanced by the 'tattered' (*Td*) mouse which shares both phenotypic and molecular similarities with human CDPX2 [89].

3.4 Lathosterolosis

Lathosterolosis (OMIM 607330) results from impaired 3-hydroxysteroid-5-desaturase (SC5D) activity [90]. In the Kandutsch-Russel synthetic pathway, SC5D catalyses the conversion of lathosterol to 7-dehydrocholesterol (7DHC)

in the enzymatic step immediately preceding the defect in SLOS, whereas in the Bloch pathway of cholesterol synthesis, SC5D catalyses the conversion of cholesta-7,24-dienol to 7-dehydrodesmosterol [90].

To date, deleterious missense mutations of *SC5D* have been reported in six patients from three families [91–95]. The clinical features include microcephaly, facial dysmorphism, bitemporal narrowing, ptosis, cataracts, anteverted nares, micrognathia, postaxial polydactyly, syndactyly, ambiguous genitalia, non-neuronal mucolipidosis, global developmental delay, intellectual impairment, hepatic cirrhosis, and early lethality [91–95]). One surviving patient who developed end-stage hepatic failure and received a liver transplantation had improvement of lathosterolosis symptoms [96]. Another patient had a milder clinical phenotype of microcephaly and learning defects with cataracts [91] highlighting the possible under-diagnosis of the syndrome without plasma sterol analysis.

Plasma cholesterol levels are normal with accumulation of lathosterol in plasma and in cultured fibroblasts, and lamellar inclusions within cellular lysosomes [95]. *Sc5d*−/− pups are stillborn and demonstrate craniofacial malformations including cleft palate and limb defects such as postaxial polydactyly [94].

3.5 Smith-Lemli-Opitz syndrome

Smith-Lemli-Opitz syndrome (SLOS) is the prototypical inborn error of cholesterol biosynthesis first described in 1964 [97]. It is by far the most common disorder in this group, with an incidence of approximately 1/40,000 although this can range from 1/70,000 to 1/10,000 depending on the population in question [98]. The carrier frequency can range from approximately 1:100 in North American Caucasians to 1:50–1:30 in various Central European populations [99]. While these carrier rates would imply a far greater incidence than is clinically observed, there is thought to be a level of misdiagnosis or non-diagnosis in mildly-affected patients, and *in utero* prenatal demise is estimated to affect 42–88% of conceptuses [100], mostly in the first trimester [98, 101].

SLOS has a broad range of phenotypic variabilities: mild cases can comprise minor physical abnormalities and behavioural or learning difficulties through a wide spectrum to a severe phenotype comprising major and life-limiting congenital abnormalities. Cognition can range from near-normal [102] to profound intellectual impairment, and on MRI, up to 96% of SLOS patients have a structural brain abnormality [103]. There is a correlation of atypical sterol profiles with both intellectual impairment and brain malformations, particularly abnormalities of the septum pellucidum and corpus callosum [103]. CNS myelination is normal despite its high proportion of cholesterol content and the mostly *in situ* synthesis of cholesterol in the CNS [104]. As well as intellectual impairment, patients are often diagnosed with language delays or impairment, autistic spectrum disorder and sleep disturbances, and can engage in self-harm. Global developmental delay, hypotonia and failure to thrive are common [105–107]. The most common physical manifestation reported with SLOS is that of 2,3 toe syndactyly, and a combination of this with other structural or cognitive symptoms should suggest a possible SLOS diagnosis for investigation [108]. Limb anomalies are common, including polydactyly, short proximal thumbs and a single palmar crease [105–107]. Other structural malformations that can occur include microcephaly, cleft palate, bifid uvula and characteristic facies with micrognathia, ptosis and broad nasal tip with anteverted nares [105–107]. This facial dysmorphia can be less recognisable in older patients [107]. Congenital abnormalities can also affect the heart and lungs, gastrointestinal tract and genitalia [105–107]. Patients with SLOS often have severe ultraviolet photosensitivity [109].

The final steps of the post-squalene cholesterol biosynthesis pathway are conversion of 7-dehydrodesmosterol to desmosterol and 7-dehydrocholesterol (7-DHC) to cholesterol. The latter is catalysed by the 3β-hydroxysteroid-Δ^7-reductase (or 7-dehydrocholesterol reductase, DHCR7) enzyme, encoded by the *DHCR7* gene at 11q13.4. Increased levels of 7-DHC and decreased levels of cholesterol led to SLOS being identified as a disorder of sterol biosynthesis in 1993 [110, 111]. This altered plasma profile is a useful diagnostic tool for SLOS, and there is evidence of a relationship between serum sterols and disease severity [112, 113].

Over 100 mutations in *DHCR7* have been identified in SLOS [114] with no clear genotype-phenotype correlations [115, 116], although some mutations are associated with more mild phenotypes due to some residual enzyme activity [117]. There is a significant correlation between SLOS patient phenotype and maternal genotype for *ApoE* and *ABCA1* [118, 119]. These correlations are positive for amelioration of SLOS symptomatology and pathogenesis and with the potential for therapeutic mediation [120].

As well as being the precursor to cholesterol, 7-DHC is also the precursor to vitamin D with exposure of cutaneous 7-DHC to ultraviolet B and subsequent synthesis to vitamin D by the liver and kidney. Increased levels of circulating vitamin D are seen in patients with SLOS [121], despite their increased photosensitivity and ensuing limited sun exposure. One of the primary theories for a possible heterozygous advantage of *DHCR7* mutations is that of protection against vitamin D deficiency [105], particularly given the greater carrier rate seen in populations of northern Europe [98, 99].

A prenatal diagnosis can be obtained via molecular or biochemical analysis (e.g. of amniotic fluid sterols [122]); however, non-invasive techniques can also identify pregnancies requiring SLOS investigation. Measurement of a low maternal serum unconjugated estriol (uE3), particularly when combined with abnormal sonography results, can be utilised for prenatal screening although this can yield false positive results and uE3 levels can also be predictive for other disorders [101]. Baseline screening for a SLOS-affected pregnancy is also possible non-invasively via serial measurement of steroids such as pregnanetriol in maternal urine [123, 124]. Abnormal plasma sterol ratios in unaffected heterozygotes [125] mean that carrier status may be determined prior to pregnancy for increased reproductive options.

Current treatment protocols for SLOS usually involve endogenous cholesterol supplementation with or without adjunct therapies such as simvastatin [126]. There is broad anecdotal evidence throughout the literature as to the positive benefit of cholesterol supplementation for patient growth, overall health (including improved photosensitivity and response to infection) and behaviour, as well as measurable changes towards typical plasma sterols [127–129]. These improvements have been reported following initiation of cholesterol treatment in both children and adults [130], although with greater rate of improvement with earlier intervention [131]. Limitations to the efficacy of cholesterol treatment certainly exist, such as cholesterol's inability to cross the blood-brain barrier in any practical quantity (which makes the apparent behavioural improvements reported interesting). The real value of cholesterol supplementation is yet to be definitively determined as trials of increased dietary cholesterol both with and without placebo controls have yielded very mixed results [132–134]. Antioxidant [135, 136] and virus vector [137] therapies have also been explored as an avenue for improved patient outcomes for SLOS and other disorders of cholesterol synthesis. Both mouse and rat models of null and hypomorphic alleles in *DHCR7* have been useful homologues for characterisation and investigation of human SLOS [138, 139].

3.6 Desmosterolosis

Desmosterolosis (OMIM 602398) is currently the final inborn error of cholesterol biosynthesis and is caused by defective enzymatic function of 3-hydroxysterol-delta 24-reductase (DHCR24). This reaction causes the reduction of the C-24 bond in the aliphatic side chain of cholesterol [140]. Reduction of the C-24 bond catalysed by DHCR24 can occur at different times in the cholesterol synthetic pathway: this step occurs early in the Kandutsch-Russel cholesterol synthetic pathway [6] but is the penultimate step in the Bloch pathway of cholesterol synthesis [5].

While first described in 1998, the molecular mechanisms of desmosterolosis were not characterised until 2001 [140, 141]. To date, only nine cases have been reported and clinical features include SLOS-like dysmorphism, thick alveolar ridges, gingival nodules, cleft palate, short limbs, severe congenital heart defect, atherosclerosis, arthrogryposis, ambiguous genitalia, microcephaly, agenesis of the corpus callosum, global developmental delay and intellectual impairment [141–147]. The diagnosis of desmosterolosis is made by demonstrating elevated levels of desmosterol by GC-MS analysis, with serum cholesterol levels usually normal [141, 142]. Reported *DHCR24* pathogenic mutations thus far have all been missense mutations.

A targeted mouse model for desmosterolosis has been generated, and *Dhcr24*$^{-/-}$ mice are viable with some postnatal growth retardation and infertility [148]. Pharmacological inhibitors of DHCR24 have been developed for studies in rat models [135, 149, 150]. Treatment of pregnant rats with these inhibitors of sterol-D24-reductase is teratogenic and produces cataracts, CNS abnormalities, genitourinary and skeletal anomalies [149–151].

4. Cholesterol biosynthesis genes in other Mendelian diseases

Inherited defects in genes encoding cholesterol biosynthetic enzymes or regulators of cholesterol homeostasis create severe clinical phenotypes as discussed above and highlighted in **Table 1**. The central nervous system is highly susceptible to perturbations in cholesterol biosynthesis, with manifestations including structural brain malformations, defects in myelin structures and, in some cases, profound developmental delay. While the cholesterol biosynthesis defects are genetically distinct individual disorders, their characterisation has demonstrated interrelation between human disease processes. This underscores the importance of cholesterol in normal cellular function and opens the possibility of novel therapies for Mendelian disorders associated with cholesterol synthesis, transport and regulation. Lessons learnt from abrogation of the cholesterol biosynthesis pathway, either by deliberate pharmacological manipulation or via inherited Mendelian diseases, serve to provide vital information amongst a raft of seemingly unrelated human disease such as inflammatory bowel disease (IBD), the cholesterol trafficking disorders Niemann-Pick disease type C (NPC, OMIM 257220) and Tangier disease (TD, OMIM 205400) and neurodegenerative diseases such as Alzheimer's disease (OMIM 104300).

NPC is an autosomal recessive lysosomal storage disorder of cholesterol trafficking due to mutations in the *NPC1* and *NPC2* genes [152]. *NPC1* encodes a 13-transmembrane-spanning protein in late endosomes/lysosomes, while *NPC2* encodes a soluble lysosomal cholesterol-binding protein [153]. This is a devastating disease characterised by a relentless neurodegenerative disease course that is usually fatal in the second decade of life, although a subset of patients will die in infancy consequent to hepatic or pulmonary failure [152]. Free cholesterol is stored

in the late endosome/lysosome with minimal escape of cholesterol from the acidic compartment to the endoplasmic reticulum. NPC leads to a block in trafficking/fusion essential for the functioning of the endosomal/lysosomal system, causing the secondary storage of cholesterol, glycosphingolipids and sphingomyelin [154]. It is likely that cholesterol accumulation is a secondary storage metabolite in NPC [154].

TD has been reported in approximately 100 patients and is caused by mutations in the gene encoding ABCA1 [155, 156]. Patients have minimal circulating HDL and accumulate cholesterol, leading to the formation of foam cells and the development of cardiovascular disease, orange-coloured tonsils, enlarged spleen, liver and lymph nodes and peripheral neuropathy. The membrane-associated protein ABCA1 regulates cellular cholesterol and phospholipid homeostasis by functioning as a cholesterol efflux pump [157]. Tangier disease patients have structurally abnormal late endocytic vesicles, which are also observed in the cells of patients with NPC disease [158]. There exists a link between ABCA1 expression and function with the NPC pathway [158, 159]. NPC disease is characterised at the cellular level by storage of glycosphingolipids, fatty acids, cholesterol, sphingomyelin and sphingosine. NPC cells also have low levels of calcium in the late endosome/lysosome. These cellular hallmarks were also identified in TD patients, suggesting that the loss of function of ABCA1 inhibits the NPC pathway through an unknown mechanism. A recent serendipitous clinical observation has provided a further link between TD and the NPC pathway: an adult patient thought to have NPC1 was treated with miglustat and demonstrated measurable clinical improvements in neurological and haematological parameters. TD was ultimately diagnosed when the molecular investigations for NPC were negative [160].

SLOS cellular pathophysiology should theoretically be correctable with cholesterol replacement therapy, as this should bypass the enzymatic defect in the conversion of 7DHC to cholesterol. However, when SLOS patient fibroblasts are cultured in a lipid-depleted medium to induce *de novo* cholesterol synthesis, cells exhibit a significant cholesterol trafficking defect leading to the accumulation of unesterified cholesterol in the late endosome/lysosome, which mimics the fate of LDL-derived cholesterol in NPC cells [161]. This proposes a possible mechanistic convergence between these very different inborn errors of metabolism. 7DHC could be interfering with the function of NPC1 and NPC2 by inhibition, akin to the U18666A drug that induces NPC cellular phenotypes [162]. In SLOS patient fibroblasts, accumulation of 7DHC led to the accumulation of metabolic indicators of NPC, that is, the lysosomal storage of cholesterol, sphingomyelin and multiple glycosphingolipids [163]. Elevated sphingosine levels in SLOS patient cerebrospinal fluid have been described. This serendipitous discovery of a link between the NPC pathway, two cholesterol trafficking disorders and the prototypic cholesterol biosynthesis defect SLOS will prove important in delineating the pathogenesis of these diseases and the development of novel therapies. Miglustat is an iminosugar drug that inhibits glucosylceramide synthase, the enzyme that catalyses the first step in glycosphingolipid biosynthesis, and it is in use as a substrate reduction therapy for a number of lysosomal storage defects including NPC [164]. The finding that SLOS and TD involve secondary inhibition of the NPC pathway suggests that miglustat could be a novel therapy for SLOS and TD.

The recently described SQSD exhibits a characteristic sterol pattern dominated by farnesol-derived dicarboxylic acids secondary to accumulation of farnesol-PP proximal to the enzymatic block. The role of these metabolites in the pathogenesis of this rare disease remains to be determined but is of interest as farnesol and its products exhibit a wide variety of biological activities including cell growth inhibition, induction of apoptosis and regulation of bile acid secretion [165]. Evidence is emerging that dysregulation of the mevalonate pathway may be involved in the progression of neurodegeneration in disorders such as Alzheimer's disease [166].

Inflammatory bowel disease (IBD) comprises a spectrum of phenotypes from Crohn's disease to ulcerative colitis. IBD usually occurs in young adults; however, onset in infancy and childhood are described. IBD occurs both in isolation and in monogenic syndromes with early-onset autoinflammation including the *NOD2*, *ATG16L1*, *IL23R*, *IL10R*, *IL10* and *XIAP* genes which have previously been correlated with IBD both in multifactorial and in Mendelian models [167]. MVK mutations may perhaps then synergistically augment the risk of developing IBD, especially as severe neonatal onset colitis responsive to anakinra has been reported as a feature of MVK deficiency [14, 168, 169].

Recent studies have implicated the accumulation of pre-cholesterol sterols and the replacement of cholesterol with some of these sterols in lipid rafts as playing a key role in the underlying pathophysiology of cholesterol synthesis defects [170]. The meiosis-activating sterols were the first group of cholesterol biogenesis intermediates that were found to have important extrahepatic functions in mammals. Mutations in sterol-C4-methyl oxidase-like gene (*SC4MOL*) are causative for a rare autosomal recessive syndrome associated with psoriasiform dermatitis, arthralgias, congenital cataracts, microcephaly and developmental delay [171, 172]. This gene encodes a sterol-C4-methyl oxidase (SMO) which catalyses demethylation of C4-methylsterols in the cholesterol synthesis pathway [172]. C4-methylsterols are meiosis-activating sterols, and further work is required to understand the role of these novel biomolecules in the pathogenesis of the cholesterol biosynthesis defects.

5. Conclusion

Inborn errors of cholesterol metabolism have provided many fundamental insights into normal cholesterol homeostasis and cell biology over several decades. These disorders have been viewed as discrete diseases with their own unique genetic, biochemical and cellular consequences that in turn cause the clinical spectrum of symptoms associated with each disease. There remain specific pre-squalene enzymatic defects to be characterised and many unanswered questions regarding the pathogenesis of the cholesterol biosynthesis defects. What has been surprising is that at least three cholesterol-related disorders (SLOS, NPC and TD) all share a common pathological inhibition of the NPC pathway. The precise mechanism that inhibits this pathway in SLOS and TD remains to be fully elucidated, but these findings are suggestive of novel therapeutic approaches to treating SLOS and TD using drugs that modify the cell biology of NPC such as miglustat. Whether other human diseases also involve NPC pathway dysfunction remains to be determined. Current investigation of this question may pave the way for novel approaches to therapy for diseases that currently lack effective treatments.

MA, mevalonic aciduria; HIDS, hyper IgD syndrome; SQSD, squalene synthase deficiency; LSS, lanosterol synthase deficiency; HEM, hydrops-ectopic calcification-moth-eaten; CHILD, congenital hemidysplasia with ichthyosiform erythroderma and limb defects; CDPX2, X-linked chondrodysplasia punctate 2; SLOS, Smith-Lemli-Opitz syndrome; DD, developmental delay; II, intellectual impairment; CHD, congenital heart defect; AR, autosomal recessive; XLD, X-linked dominant.

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Conflict of interest

The authors have no COI to declare.

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