

Shailendra B. Patel

Introduction

Sterols are an integral part of the lipoprotein, as well as all cell membranes. Cholesterol is the single most abundant mammalian species representing >99% of body sterols. Cholesterol is synthesized *de novo* in the body, and it is also absorbed from the diet. In nonmammalian organisms, other sterols can be used to fulfill similar functions; ergosterol is the primary sterol in yeast and fungi, sitosterol and campesterol (as well as a host of many other phytosterols) fulfill these functions in plants and there are organisms, such as shellfish, crustaceans, etc., which utilize a mixture of different sterols species, including cholesterol. Humans, being omnivorous, are exposed to dietary cholesterol, as well as these xenosterols (sterols that are not made by the mammalian body). Thus, an understanding of how these sterols are handled physiologically is of importance. Disruption of pathways regulating sterol metabolism (synthesis, transport, and breakdown) can lead to dyslipidemia, but in many cases, the astute clinician is led astray as the standard lipid test (which does not discriminate between these sterols) may not offer clues to allow these conditions to

be identified. This chapter focuses on disorders that can affect sterol trafficking, sterol synthesis, and sterol breakdown, using one disease entity in each group to highlight the key points that allows for better diagnosis and management.

Sterol Trafficking Disorders

Clearly, any disorder of lipoprotein trafficking *per se* will also affect sterol trafficking, since sterols are a necessary constituent of these particles. Unfortunately, while sterol trafficking disorders lead to human disease, many such disorders do not result in dyslipidemia, as judged by blood lipid analyses. Thus, Niemann–Pick C (NPC) disease is a progressive neurological disorder caused by defects in one of the two genes, *NPC1* or rarely *NPC2*, and involve a failure of release of the lysosomal sterols into the cells for further metabolism and transport [1]. Or the loss of cholesterol transport from the outer to the inner mitochondrial membrane, mediated by steroidogenic acute regulatory (StAR) protein, results in congenital lipid adrenal hyperplasia, an endocrine disorder, but does not result in dyslipidemia [2].

This section focuses on sitosterolemia that specifically results in disruption of whole body sterol trafficking and may be more relevant to the practicing lipidologist. Sitosterolemia, also known as phytosterolemia, results in failure to traffic sterols, xenosterols as well as cholesterol.

S. B. Patel (✉)
Department of Medicine and Division of Endocrinology,
HRC4850, Division of Endocrinology, Clement J
Zablocki Veterans Affairs Medical Center, and Medical
College of Wisconsin, 8701 Watertown Plank Road,
Milwaukee, WI 53226, USA
e-mai: sbpatel@mcw.edu

Sitosterolemia/Phytosterolemia

History

In a review in 2007, we wrote, “The history of how mammals can distinguish between dietary non-cholesterol sterols and cholesterol is intertwined with the history of cholesterol itself; whether cholesterol could be synthesized by the body or was wholly absorbed from the diet, whether the body degraded cholesterol, what determines its absorption and biliary secretion, and whether cholesterol was involved in the process of atherosclerosis are all questions that have involved or continue to involve non-cholesterol sterols. Investigations of such observations led to the discovery that plant sterols were excluded by the body, but could compete with bulk cholesterol for entry into the micelles formed during digestion, thus preventing dietary absorption of cholesterol.” [3]. Historically, these concepts were considered and investigated from the early parts of the last century [4, 5]. The average human diet consists of about 250 mg of cholesterol and 300 mg of plant sterols a day, yet plant sterols are barely detectable in human blood or tissue. For almost a century, the molecular and physiological pathways responsible for these observations were never fully explored. Conventional wisdom held that plant sterols were essentially unabsorbable, and, thus, this was the main reason for their exclusion. How then cholesterol from the diet absorbed remained essentially ignored? In a classical paper, Bhattacharyya and Connor [6] reported two sisters who had signs and symptoms suggestive of familial hypercholesterolemia, FH, with arthralgia and tendon xanthomas, yet did not have elevated plasma cholesterol and their parents were not hypercholesterolemic either [7]. Examination of the blood sterols by gas chromatography led to the discovery that these two sisters had massive elevations in plant sterols. They named the disease β -sitosterolemia, after the most abundant plant sterol detected (this disease is more accurately phytosterolemia, as all xenosterols accumulate and some argue the term xenosterolemia is a better term) [7, 8]. This report prompted the investigations of two

subsequent families with sudden atherosclerotic cardiac death of teenagers, where familial hypercholesterolemia was suspected, but did not have elevated cholesterol [6]. One of these families was Amish, and an extended pedigree analyses confirmed that sitosterolemia behaved as an autosomal recessive condition, and thus a single locus was involved [9]. I posited that a single gene product was responsible for regulating dietary cholesterol, with a working hypothesis that this protein worked as a pump, to pump cholesterol in and noncholesterol sterols out. With the help of colleagues from across the globe, we assembled pedigrees with sitosterolemia, mapped the disease locus, *STSL*, to chromosome 2p21 and positionally cloned and identified the genetic defect responsible for this condition [10–13]. Helen Hobbs and coworkers also independently cloned the sitosterolemia genes [14]. To our surprise, not one, but two genes, *ABCG5* and *ABCG8*, comprised the *STSL* locus, and complete mutations in either gene resulted in the disease. These genes belong to the ATP-binding cassette transporters, family G. Current work suggests that *ABCG5* and *ABCG8* work as obligate heterodimers; they are expressed on the apical surfaces of the hepatocyte and enterocyte, and are responsible for pumping cholesterol and plant sterols out into the biliary lumen or intestinal lumen, respectively [14–16]. These pumps have a preference for non-cholesterol sterols, but in the absence of the latter, are bona fide cholesterol exporters. Although these transporters have been described as “defenses against cholesterol” [17] as well as defenses against xenosterols [18], the former may be relevant to the majority of people with variant forms of *ABCG5/ABCG8*, as opposed to the rare individuals with severe mutant forms that lead to xenosterolemia and disease.

Epidemiology

The true prevalence of sitosterolemia is unknown, but based on all families described and reported in the literature, this disease is not more common than 1 in 1,000,000. As for any rare disease, the true prevalence is always likely

to be higher because of under-detection; since plant sterol accumulation leads to some clinical manifestation, chances of underreporting are minimized (see Clinical Findings).

Etiology and Pathogenesis

Mutations in one of the two *ATP binding cassette* transporters belonging to the *G* family, ABCG5 or ABCG8, cause the disease [11, 13, 14]. Both copies of ABCG5 or both copies of ABCG8 have to be mutant, and this was the first indication suggesting that they likely worked together, or in tight tandem for regulation of xenosterols. The Hobbs group has shown definitively that these proteins act as obligate heterodimers and that they need to be expressed at the apical surface for normal function [15, 16, 19, 20]. Figure 13.1 shows a depiction of the gene structure of the *STSL* locus and Fig. 13.2 shows mutations in ABCG5 and ABCG8 that lead to disease, as well as natural variants found in “normal” humans. The normal ABCG5/ABCG8 heterodimer needs to fold correctly in the endoplasmic reticulum (ER) to allow its progression through to the Golgi and then to the apical surface. There, in conjunction with proteins that export bile acids (ABCB11/BSEP) and phospholipids (MDR3), ABCG5/

ABCG8 facilitate the extrusion of sterols from the outer membrane into the lumen, though the exact mechanism(s) has not been defined. The mutational spectrum encompasses missense mutations, null mutations as well as microdeletions, and ones that affect transcript stability. As a rule, failure to express one of the two proteins results in mis-folding of the other subunit and its degradation in the ER of the hepatocytes and the enterocytes.

Clinical Findings

The most important step in making a diagnosis of sitosterolemia (and this principle applies to any rare disease) is considering the possibility of this diagnosis. The “classical” presentation would be a person who presents in a fashion similar to familial hypercholesterolemia (arthralgia, tendon xanthomas), but where the standard lipid tests show that the LDL-C is less than 200 mg/dL. While this was the case with the presentations of the earliest cases, a compilation of the presenting features of other subjects with sitosterolemia shows that these features (xanthomas) are not as frequent [8]. Table 13.1 lists all of the possible presenting features. One could consider the presence of premature atherosclerotic disease

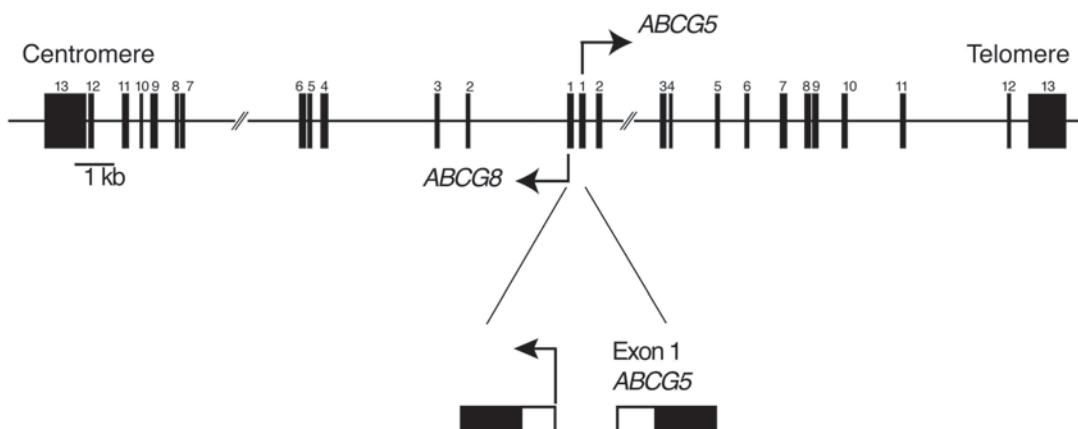


Fig. 13.1 Genetic organization of the sitosterolemia locus. The intron–exon structure of the *STSL* locus, containing the genes ABCG5 and ABCG8, is as shown. The genes likely arose as a result of gene duplication and re-

side on opposite strands of the DNA, being transcribed in opposite direction. Note that the promoter region separating the two genes is exceedingly small, suggesting non-conventional regulation

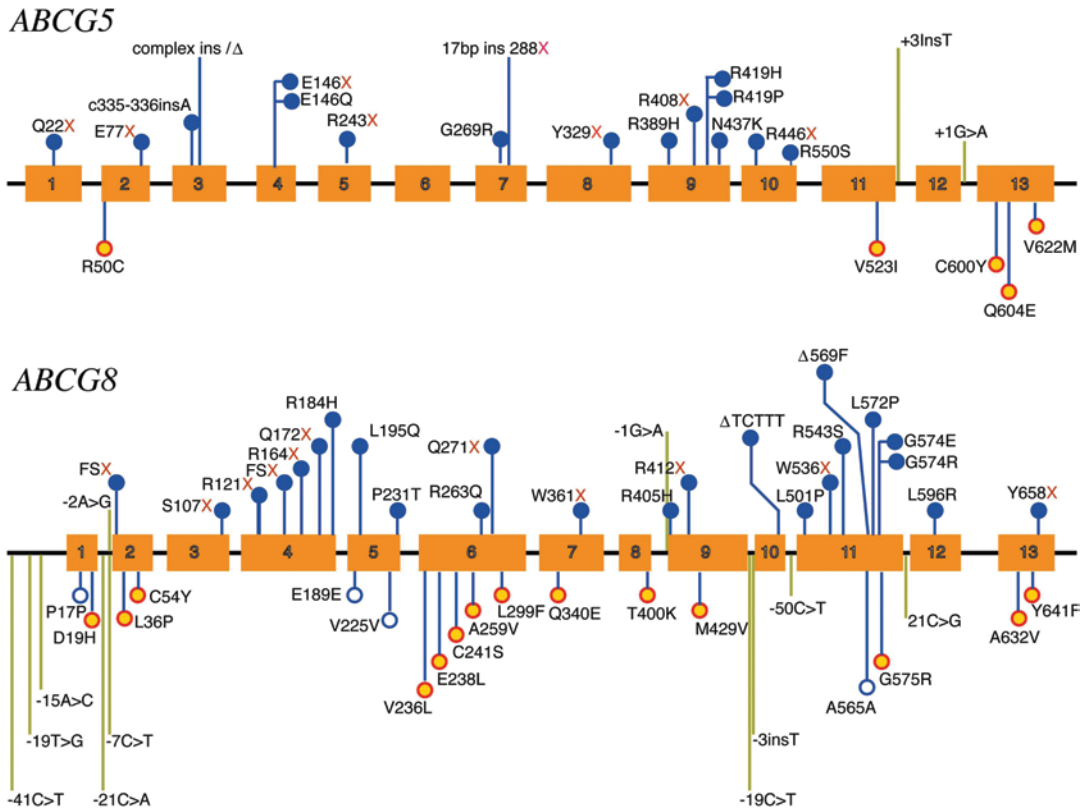


Fig. 13.2 Known mutations and common variations in ABCG5 and ABCG8 genes. Mutations in ABCG5 and ABCG8 that cause sitosterolemia are depicted *above* the gene structure, whereas polymorphic variants identified in normal humans are depicted *below* the gene. Some of the normal variants are very rare and their relevance is not established. Others have been shown to be associated with increased propensity to form gallstones [66]

Table 13.1 Presenting features of sitosterolemia

Signs and symptoms	Relative frequency ^a
Arthralgia	Common
Tendon or tuberos xanthomas	Common
Mildly elevated total cholesterol	Common
Mild anemia	Common
Thrombocytopenia	Common
Elevated liver enzymes (<3 ULN)	Common
Valvular thickening	Infrequent
Carotid bruits	Infrequent
Premature coronary artery disease	Infrequent
Severe hypercholesterolemia	In childhood only
Sudden death <40 years of age	Rare
Endocrine insufficiency	Very rare
Progressive liver disease	Very rare

^aBased upon clinical observations reported only, no formal study available. Based upon the ezetimibe studies, we define common as ~30%, infrequent as <10%, rare as ~1%, and very rare as <1%. (Adapted from reference [8])

in the face of seemingly normal lipid profiles (e.g., total cholesterol <200 mg/dL or LDL-C <160 mg/dL), yet these two phenotypes are still much more frequent than sitosterolemia, and will lead to many more futile tests than uncover this disease. Some notable features should lead to increased suspicion. With the permission of Dr. Goldstein and Dr. Brown, we were able to re-contact some children they had investigated for FH, presenting with massively elevated LDL-C levels, but did not have any defects involving the LDL receptor. These children were deemed to have “pseudohomozygous” FH. We were able to redraw their blood, and showed that they had diagnostically elevated plant sterol levels (see Fig. 13.3). Why children go through a phase where plasma cholesterol levels are so high is not well understood. Prospective study of any child who may present with such high levels of cholesterol and does not have homozygous FH may shed light on the mechanism(s). Another feature that was also known early on was hemolysis, with some teenagers developing splenomegaly and therefore coming to the attention of hematologists. The hematological aspects have now

gained more attention since the discovery that the only presenting feature may be macrothrombocytopenia (see Fig. 13.4), a condition initially reported in association with stomatocytosis [21]. Mutations affecting ABCG5 or ABCG8 have now been shown to cause this disease and the reported subjects did not seem to manifest any tendon xanthomas or arthralgias. Interestingly, mouse models of sitosterolemia have been reported to show these hematological features [22, 23], though the “pseudohomozygous FH” phase has not been reported. As with any rare disease, cases are now reported where for several years the correct diagnosis has not been made or delayed until this possibility is considered. A case of liver failure [24], a case of adrenal and ovarian failure [25], and the presence of valvular heart disease [26, 27] have all been described. Since the manifestation of any of these clinical features is depend upon exposure to xenosterols, it is important to consider the dietary components; an Iranian girl remained asymptomatic in her native country, where intake of plant-based foods was relatively minimal, but when she moved to Europe and started a “healthier” diet, she developed

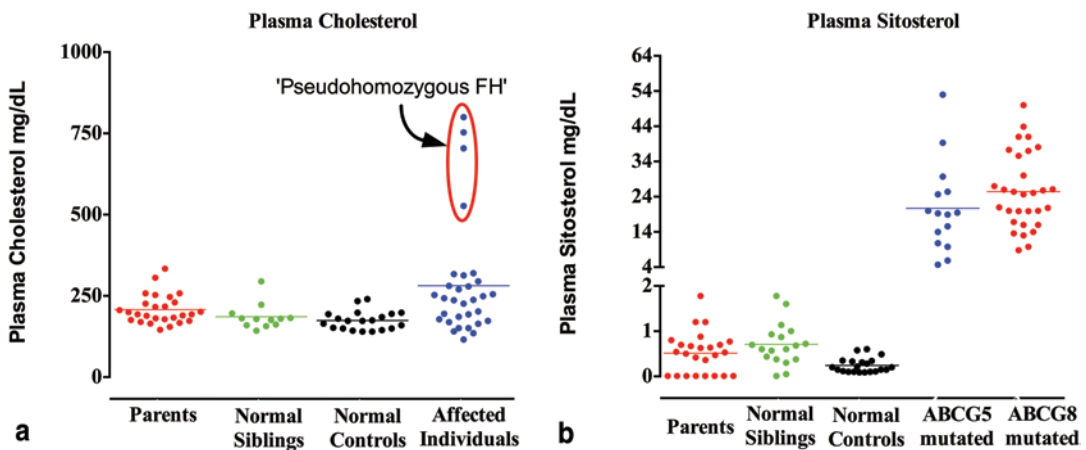


Fig. 13.3 Sterol profiles in families with sitosterolemia. The cholesterol profiles, determined by GC-MS, in parents (obligate carriers), normal siblings, sitosterolemic subjects, or random controls are shown. As can be seen, in general the cholesterol values are indistinguishable, except in four subjects who had massively elevated cholesterol. All four were previously labeled as “pseudohomozygous FH” and are only noted when subjects are

children; adults with this pattern have not been reported. The panel on the *right* shows the plasma sitosterol levels, this time the affected subjects are segregated by whether they have mutations in ABCG5 or ABCG8. As can be seen, this does not affect the level of phytosterolemia. Additionally, the pseudohomozygous FH pattern has been seen in subjects with mutant ABCG5 and ABCG8

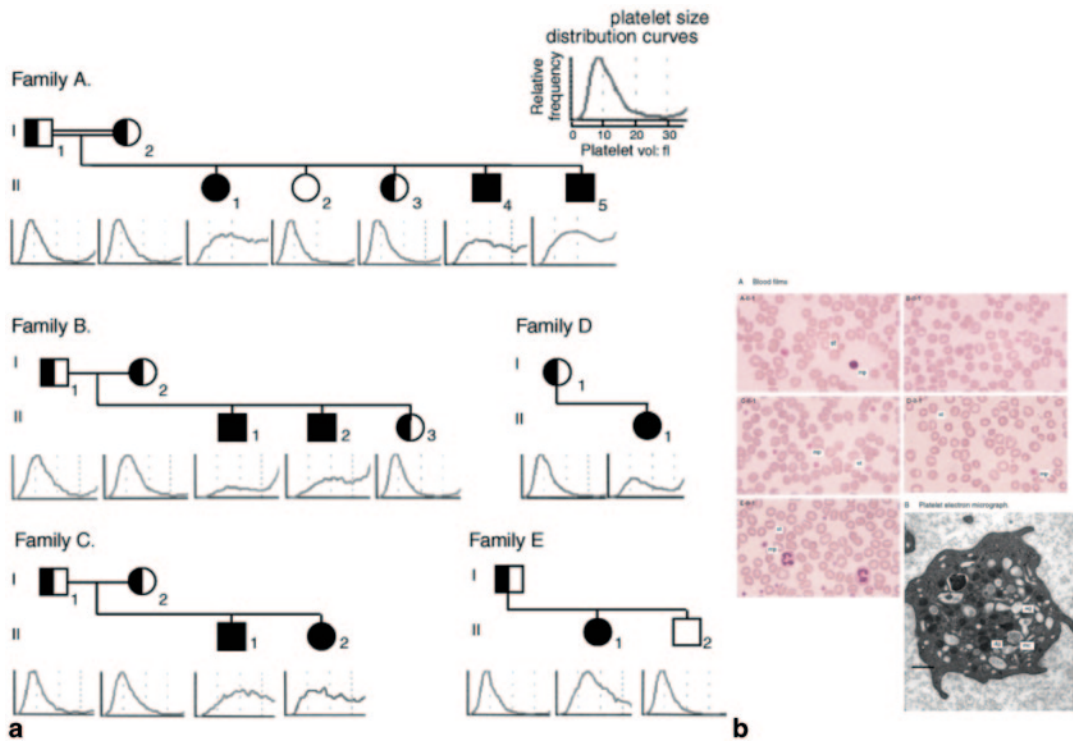


Fig. 13.4 Macrothrombocytopenia and stomatocytosis as presenting features of sitosterolemia. The panel on the *left* shows the platelet volume profile plotted versus the relative frequency (*y*-axis), in each of the family members. The filled symbols indicate sitosterolemic subjects; obligate carriers are in half-filled symbols and normal are unfilled symbols. As can be seen, sitosterolemia leads to

reduced and larger platelets in all of the affected subjects. The panel on the *right* shows the blood films from affected subjects and shows the presence of macroplatelets, as well as stomatocytes and an electron micrograph shows that the platelet is very large, but does not have any abnormal structures or granules. (Reproduced with reference [21], with permission from John Wiley and Sons)

many of the features that led to the diagnosis of sitosterolemia [28]. This also highlights another key feature, namely the key xenosterol(s) that is pathological has not been established. The various different xenosterols in any food component will vary significantly, not only between different types of plant sources (Brassica foods compared to starches, etc.). While sitosterol is clearly the most abundant plant sterol, *in vitro* other plant sterols, such as avenosterol, fucosterol, stigmasterol, etc., seem to be more potent at activation of the transcriptional factor LXR [29]. These latter sterols are also less abundant and no study has correlated levels of these relatively harder to measure sterols and whether they are causatively related to the clinical features.

Laboratory Tests

The diagnostic test is to measure the plant sterol in the plasma or serum (or tissue) [7]. Elevated plant sterols are diagnostic for sitosterolemia and no other disease condition has been shown to mimic this [30]. Conventional “cholesterol” tests are performed using enzymatic assays that measure all sterols, and thus do not distinguish between xenosterols and cholesterol. However, under normal circumstances, more than 99% of the sterols in normal humans is cholesterol, thus the utilization of this assay is valid. However, to detect plant sterols, one needs to utilize methods that can separate and distinguish between these different sterols. This is accomplished using

either gas chromatography or high-performance liquid chromatography, and can be aided by using mass spectroscopy in tandem. In the USA, a limited number of centers perform these analyses for clinical diagnostic use and this can be ordered via all local laboratories as a send-out test. Normal humans have plant sterols that are typically <0.5 mg/dL, although rare normal individuals with levels as high as 1–2 mg/dL have been reported. All sitosterolemia subjects typically have plants sterols that are >10 mg/dL, making this diagnosis definitive [18]. Molecular diagnostic testing for mutations in ABCG5 or ABCG8 can also be used, but are not necessary, as the elevation of plant sterols is diagnostic.

Differential Diagnosis

Tendon xanthomas, in the presence of normal to moderately elevated cholesterol levels, may also suggest a diagnosis of cerebrotendinous xanthomatosis (CTX; see below). However, the plasma/serum plant sterol levels are diagnostic for sitosterolemia. Many other conditions can also result in macrothrombocytopenia, though most of these are also relatively rare. In cases of thrombocytopenia where no cause has been identified definitively, we would recommend a single determination of blood plant sterol levels. As these conditions are so much more prevalent than sitosterolemia, this diagnosis should be considered where the definitive diagnosis is absent and where other associated factors have been identified (e.g., presence of large platelets, >12 fl, or thrombocytopenia and valvular heart disease, or liver disease, or premature atherosclerosis, etc.).

Complications

Untreated sitosterolemia has been shown to be fatal [6, 26] with sudden cardiac deaths, premature atherosclerotic disease, and hematological disease that does not improve until a correct diagnosis has been made. The commonest complications of this disease are premature atherosclerotic disease and macrothrombocytopenia. However,

rare cases of liver, adrenal, and ovarian failure have been reported as well as fatal and nonfatal valvular disease.

Clinical Course and Treatment

Limiting the intake of foods that contain xenosterols would seem to be a reasonable strategy, except this is exceptionally difficult to achieve, as a balanced diet for healthy living requires a varied diet. Additionally, ABCG5/ABCG8 are also important players for biliary cholesterol excretion, and a diet only containing animal products may result in increased propensity to atherosclerosis. Therapy is therefore aimed at preventing xenosterol absorption. Bile acid resin therapy formed the mainstay, until the discovery of the dietary sterol-blocking agent, ezetimibe (Zetia®). Prior to the approval of this drug as a cholesterol-lowering agent, the mechanism of action was not known and ABCG5/ABCG8 were considered a potential target. In order to evaluate this hypothesis, subjects with sitosterolemia were enrolled in a short-term study with daily ezetimibe, and to the surprise of the investigators, lowered plant sterols by 21–24% [31]. An extension study showed maximal sitosterol reductions of 44% at 52 weeks on 10 mg of ezetimibe [32]. These data led to the approval of ezetimibe as a specific therapy for sitosterolemia and is a unique instance where a billion dollar drug was codeveloped for the benefit of a very rare condition prior to approval. The inventors of ezetimibe went on to identify the true target of this drug, and showed that the molecule NPC1L1, which is now known to function as a key molecule that allows dietary sterol entry into the enterocyte [33], and that ABCG5/ABCG8 allow for sterol exit. As this is a rare condition, while case reports suggest benefit at individual levels who are treated with ezetimibe, it is not clear if this will be translated to blocking all features of this disease. Prior to this, bile acid sequestrants were the mainstay of therapy, although these reduced plant sterols by only 10–15% (G. Salen, *pers commun*), but intolerance to these agents is high (mainly constipation or diarrhea).

Sterol Synthesis Disorders

At the turn of the last century, it was assumed that cholesterol was a preformed molecule we absorbed from our diets, and it was not until the elegant work by Schoenheimer who showed that cholesterol not only was synthesized in the mammalian body but also could be destroyed by the mammalian body [5]. However, the identification of sterol synthesis disorders (as defined by affecting any sterol synthetic pathway beyond squalene, the first committed sterol synthesis intermediate) affecting humans is a very modern discovery. A number of human sterol synthesis disorders are now known; the Smith–Lemli–Opitz syndrome, demosterolosis, lathosterolosis, Conradi–Hunermann syndrome, CHILD congenital hemidysplasia with ichthyosiform erythroderma and limb defects) syndrome, CK syndrome, and sterol C4 methyloxidase deficiency [34]. These disorders are all congenital disorders and present as malformation syndromes, not dyslipidemia. However, all of these conditions do exhibit lower than normal cholesterol profiles, typically in the lower 5th centiles. Since these disorders do not typically present with dyslipidemia, their discussion is curtailed herein. The reader is directed to a recent review of this topic [35].

Smith–Lemli–Opitz Syndrome

History

A new dysmorphology syndrome, termed RSH syndrome, was described in 1964 by three astute clinical geneticists [36]. Their clinical definition led to more cases with similar dysmorphological findings identified (see Fig. 13.4), but the genetic basis for this syndrome remained elusive. The insight into this came when two other clinical geneticists, Drs. Mira Irons and Ellen Elias, collaborated with Dr. G. Stephen Tint's group and showed that a low serum cholesterol, but a very high precursor sterol, 7-DHC, was a strong marker for this condition [37, 38]. They proposed that the Smith–Lemli–Opitz syndrome (SLOS, now an accepted term after the discov-

ers) was actually a disease caused by a defect involving an enzyme, dehydrocholesterol $\Delta 7$ reductase (DHCR7), that catalyzes the conversion of 7-DHC to cholesterol. This was viewed very skeptically by the clinical geneticist community as no dysmorphology syndrome had been known previously to be caused by a defect in a metabolic enzyme. Over the ensuing years, the biochemical test of 7-DHC became a diagnostic test for this disease and garnered greater acceptance of this hypothesis. This was solidified when Drs. Glossmann, Utermann, and their colleagues cloned the gene for DHCR7 and demonstrated mutations on a cohort of subjects diagnosed with Smith–Lemli–Opitz syndrome [39, 40], a finding verified by several other groups [41–43].

Epidemiology

The incidence of SLOS varies by region with an estimated 1:40,000 live births in the USA, but increasing to 1:20,000 in Eastern Europe. SLOS is much less common in Asia and Africa [44].

The true incidence may be masked for this autosomal recessive condition, as loss of pregnancy early on may be caused by this condition, but the family would not be investigated further if a subsequent normal pregnancy results. If only when a live birth with dysmorphology, or an abortus with dysmorphology is detected would this diagnosis be entertained.

Etiology and Pathogenesis

Genetic defects of the enzyme dehydrocholesterol $\Delta 7$ reductase (DHCR7) are responsible for causing this condition. The reduction of the $\Delta 7$ bond in the sterol molecule is absolutely necessary for cholesterol synthesis, whether the synthesis follows the Bloch or the Kandutsch–Russell pathways. Failure to do so results in the accumulation of the immediate precursor 7DHC, which can spontaneously isomerize to 8DHC. Despite the knowledge of the enzymatic defect, the pathophysiological mechanisms that then lead to the highly specific dysmorphology syndrome

remains a challenge. Cholesterol fulfills a host of protean functions, ranging from its structural role in membranes, lipoproteins, in specialized membranes such as myelin, as part of the skin barrier, as a substrate for bile acid synthesis, or steroid hormones and even as esoteric as modifiers of protein structure, such as a covalent tail for the hedgehog proteins, etc. In addition, cholesterol metabolites have important regulatory roles. Each of these roles has been examined in the pathogenesis of SLOS, and while some evidence for each of these pathways being potentially disrupted has been accumulated, a unifying pathway linking these has not been easy to forge. A recent summary of potential mechanisms can be found in reference [45].

Clinical Findings

This condition is very strongly associated with dysmorphology, thus the presentation will be typically at the neonatal and pediatric stages [35]. It is highly unlikely to present to most general internists. However, as with all relatively rare diseases, knowledge about this disorder may alert consideration of this condition as a “missed” diagnosis. The presentation of SLOS ranges from a severe dysmorphology and intrauterine death and spontaneous abortion, to being born with a number of developmental defects (Fig. 13.4). These range from external characteristic facial dysmorphology (flat face, micrognathia or retrognathia, short palpebral fissures, low set ears, short nose with concave nasal ridge and anteverted nostrils, cleft palate), bifid uvula, cataracts, polydactyly, 2–3 syndactyly, hypospadias and ambiguous genitalia in males, together with internal organ developmental defects ranging from midline CNS malformations (holoprosencephaly, absent corpus callosum, cerebellar hypoplasia, etc.), hypotonia, congenital cardiac defects (almost all kinds), renal agenesis and cysts, pulmonary hypoplasia, to intestinal malformations and Hirschsprung disease [44]. Developmental cognitive defects are evident as these children age, with mental retardation and behavioral issues as very common sequelae. However, for the internist, rare cases of SLOS have been described

where there are almost no structural defects, may have the mildest of 2–3 syndactyly and no mental retardation [46]. In these cases, the diagnosis is suspected based upon the above clinical features and a consideration of the diagnosis, confirmed by biochemical testing (see below). In all of the cases, the “dyslipidemia” is a low plasma cholesterol level [38]. Thus for the internist facing a child, or a young adult in whom there are subtle signs of mild cognitive defects, with a remote history of some corrected midline organ defect, and an examination showing 2–3 syndactyly, one could suspect the diagnosis of SLOS.

The genetic basis of SLOS is an autosomal recessive genetic defect involving the enzyme dehydrocholesterol $\Delta 7$ reductase that is responsible for reducing 7-DHC to cholesterol [47]. This enzyme also reduces 7-dehydrodesmosterol to desmosterol (which is further reduced by 24-dehydrocholesterol reductase to form cholesterol). Failure to do so results in failure to synthesize cholesterol, with the diagnostic accumulation of the precursor sterol, 7DHC, in the blood and tissues.

Laboratory Tests

The diagnostic test is the determination of 7DHC in the blood, and requires the use of high-performance liquid chromatography (HPLC) or gas chromatography–mass spectrometry (GC-MS), but is available as a send-out laboratory test. Combined with clinical features, a low plasma cholesterol and an elevated 7DHC is diagnostic for SLOS. Very rarely, mild elevations in 7DHC can be seen in CTX, but the clinical features are very different (see below). Molecular diagnostic testing for mutations in *DHCR7* gene can also be used, especially as three mutations account for >70% of the mutations causing SLOS. Molecular testing may aid cases where the clinical features are very mild, the plasma 7DHC levels are minimally elevated, but SLOS is strongly suspected. Figure 13.5 shows depiction of some of the mutations of *DHCR7* that cause loss of enzyme activity. A newer compendium of more than 150 mutations has been assembled by Waterham and Hennekam [47].



Fig. 13.5 Dysmorphological features commonly seen in SLOS. Panels a–d shows typical features of Smith–Lemli–Optiz syndrome (SLOS) patients; microcephaly, ptosis, broad nasal bridge, upturned nose, and micrognathia. Panel e shows a short proximally placed thumb, clinodactyly, and postaxial polydactyly, with syndactyly (the commonest SLOS finding) of the second and third toes (f). (Reproduced from reference [45], with permission from Nature Publishing Group)

Differential Diagnosis

As stated above, a defect at almost any of the postsqualene sterol synthesis defect could mimic SLOS [35], although the sterol diagnostic determination allows for clear distinction. However, in the mildest of SLOS cases, where the elevation of 7-DHC may not be dramatic and the presence of any major structural defects absent, one may have to consider genetic analyses of the DHCR7 locus. Any condition that leads to partial inhibition of DHCR7 activity, as seen in untreated CTX, may lead to very mild elevations in 7DHC. However, these two conditions are sufficiently different to allow for distinction (Fig. 13.6).

Clinical Course and Treatment

The commonest clinical issues are the major structural defects involving almost any organ system that may require surgical repair or amelioration. Additionally, mental retardation, significant cognitive defects, as well as seizures, behavioral issues, hyperstimulation, and autism-like neurological issues are frequently described. The accumulation of 7-DHC in the skin is also thought to lead to skin photosensitivity in some cases. Cases of adrenal insufficiency have been reported.

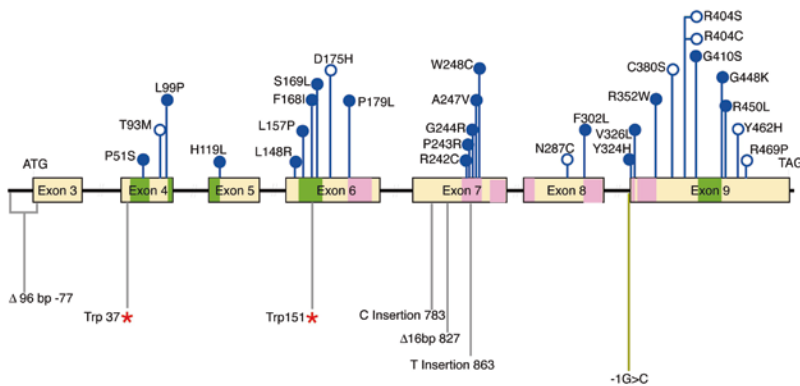


Fig. 13.6 Mutational spectrum of the DHCR7 gene in Smith–Lemli–Optiz syndrome (SLOS). The distribution and variety of mutations observed of the DHCR7 gene is shown. As can be seen, these affect almost any part of

the gene, although five gene mutations are highly prevalent, namely IVS8-1G>C (c.964-1G>C), R404C, T93M, W151X, and V326L. However, now more than 150 unique mutations have been reported (see ref. [47])

There are few well-designed randomized clinical studies to direct treatment [48]. Since the defect involves a failure to synthesize cholesterol, the standard approach is to increase dietary cholesterol with supplementation using purified products. Once the enterohepatic bile acid pools are restored, there is adequate absorption of cholesterol from the diet to reduce the plasma levels of 7-DHC. However, it is not clear if this affects any of the longer term consequences, such as behavioral issues or mental retardation, as controlled studies testing this have been difficult to conduct [49]. One approach, that to inhibit cholesterol synthesis by use of statin drugs (in conjunction with cholesterol supplementation), has not led to any meaningful conclusions to be drawn, despite improved biochemical changes [48]. Thus, long-term management is aimed at expectant management, or correction of any structural defect.

Sterol Breakdown Disorders

The major pathway by which the body can rid itself of cholesterol is to excrete cholesterol into the intestinal lumen (via biliary secretion, major, or directly via the intestine) or by breakdown of the cholesterol molecule via the bile acid synthesis pathway and excretion via the biliary system. There are a number of genetic or acquired defects involving the bile acid/biliary secretion pathway that can lead to dyslipidemia. For example, severe hyperlipidemia can be seen under conditions of cholestasis. This section focuses only on the genetic pathways that lead to bile acid synthesis defects using CTX as an example. The reader is directed to some excellent reviews on a wider aspect of genetic disorders that can affect bile acid secretion.

Cerebrotendinous Xanthomatosis

History

In 1937, Von Bogaert, Scherer, and Epstein described a case that manifested progressive motor and cognitive neurological deterioration (having

been very normal) and manifested juvenile cataracts, and tendon xanthomas [50]. In the subsequent years, many more similar cases were reported and a distinct clinical entity was formulated that consisted of the above, but with added observations that histological analyses showed increased cholesterol, but more importantly cholestanol deposits in brain samples [51–55]. Despite the presence of tendon xanthomas, the plasma cholesterol levels were not always elevated. The source of the cholestanol not well understood at that time, but it was felt that this was the primary reason for the neurological issues. With increasing cases, it became clear that this was a recessive trait, as the parents were normal, and so a genetic cause was suspected. The key observation by Salen that the bile acid amount was reduced by 50% allowed him and his colleagues to demonstrate that chenodeoxycholic acid (CDCA) was almost completely absent in CTX subjects and that there was an accumulation of bile alcohols, and an inability to detect C-26 hydroxylated intermediates (now renumbered to be C27-hydroxylated intermediates) [56, 57]. Ensuing work from a number of other groups also confirmed a putative defect in CYP27A1, establishing that CTX was caused by a defect in this key bile acid synthesis enzyme necessary for the synthesis of CDCA (but not cholic acid, which is relatively normal in CTX). The next breakthrough came when Russell and his colleagues cloned the gene and characterized the enzyme for CYP27A1 and showed that this was not only responsible for the biochemical defects observed by Salen and his colleagues on the bile acid pathway but mutations of this enzyme were responsible for causing CTX [58, 59].

Epidemiology

The prevalence of CTX is probably very close to that of sitosterolemia. In the USA, there are somewhere between 60 and 80 subjects known. Thus, this is a truly rare disease. There are pockets of this disease, based upon isolated populations where an increased incidence is noted (such as Jews of North African origin). The disease is worldwide and is present on all five continents.

Etiology and Pathogenesis

The condition is inherited as an autosomal recessive condition and is caused by mutations affecting CYP27A1, encoding cholesterol 27-hydroxylase. The biochemical defect prevents the synthesis of CDCA, but not cholic acid (Fig. 13.7). The liver is the only organ that has all of the necessary enzymes for the synthesis of bile acids (see Fig. 13.7). These enzymes are also located in different compartments within the hepatocyte, and CYP27A1 is a mitochondrial enzyme. Under normal circumstances, 5β -cholestane- $3\alpha,7\alpha,12\alpha$ -triol is transported to the mitochondrion for further oxidation of this by CYP27A1 [60]. In its absence, this bile acid intermediate is transported back to the microsomes, where it is acted upon by 25-hydroxylase, part of which results in the synthesis of

cholic acid but microsomal metabolism also leads to generation of tetrol, pentol, hexol bile alcohols, which are likely the major pathogenic molecules resulting in the pathogenesis of CTX. This microsomal pathway cannot generate CDCA, without 27-hydroxylation of the side chain. Bile acid alcohols are glucuronidated and can be found in significantly increased amounts in the blood, urine, and feces of untreated CTX subjects. Additionally, CTX subjects have increased generation of cholestanol. The bile alcohols are toxic, and lead to disruption of the blood–brain barrier, allowing increased accumulation of cholestanol, as well as disruption of CNS sterol metabolism, resulting in progressive neurological damage. This damage may also occur to peripheral nerves. It is this progressive damage and accumulation of sterols in the CNS that is responsible for the

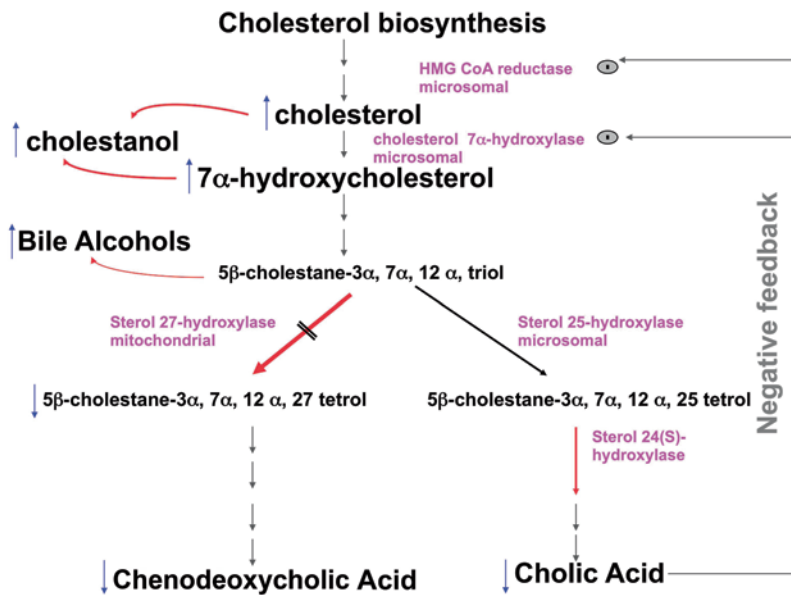


Fig. 13.7 Aberrant bile acid synthesis in cerebrotendinous xanthomatosis. Normal bile acid synthesis starts with 7α hydroxylation, which is quantitatively the most important pathway. Following the generation of 5β -cholestane- $3\alpha,7\alpha,12\alpha$ triol in the microsomal compartment, transfer of this sterol to the mitochondrion results in the production of 5β -cholestane- $3\alpha,7\alpha,12\alpha,27$ tetrol but the action of CYP27A1, which is necessary for the synthesis of chenodeoxycholic acid (CDCA) but also of cholic acid (not shown for simplification). In cerebrotendinous xanthomatosis (CTX), where CYP27A1 is

deficient, the block shown of the **bold red arrow**, leads to increased microsomal triols, where, through the action of CYP25, 5β -cholestane- $3\alpha,7\alpha,12\alpha,25$ tetrol can be synthesized and thus cholic acid, but this pathway cannot lead to synthesis of any CDCA. The production of cholic acid remained diminished though. The lack of enough CDCA leads to reduced feedback inhibition of both the synthesis of cholesterol, as well as CYP 7α , increasing flux via this pathway, thus further compounding the build-up of the intermediaries, resulting in accumulation of cholestanol, as well as bile alcohols

neurological defects. CYP27A1 is an enzyme also present in many other sites in the body, including macrophages. Accumulation of both cholesterol and cholestanol in macrophages is likely responsible for the pathogenesis of xanthomas, though the precise mechanisms have still to be defined. Additionally, this enzyme is also present in osteoclasts, and although osteoporosis is also a feature of CTX, the mechanism of this is also undefined at present.

Clinical Findings

The clinical presentation of CTX starts very early in life. There are two clinical presenting features that warrant highlighting; intractable diarrhea associated with failure to thrive in the first 3–4 years of life, and the development of juvenile cataracts, where there are no identifiable precipitating factors (such as steroid use, radiation, known genetic diagnosis, etc.). Many CTX patients report having had diarrhea that would not easily subside when they were very young, and cataracts by the age of 21 years seem to be almost universal in untreated cases. Other presenting features in childhood include psychomotor retardation and neurological damage early on may show signs of pyramidal as well as cerebellar damage. Rare cases of hepatitis have also been reported as presenting features in this age category. CTX, however, remains a misdiagnosis and the majority of cases continue to be diagnosed later in adult life, when they present with tendon or tuberous xanthomas (in almost all cases, the Achilles tendon is invariably involved), and neurological features that range from long tract signs, pyramidal paresis, bulbar palsies, cerebellar dysfunction, dystonia, and movement disorders (including signs of Parkinson's), peripheral neuropathies and progressive psychomotor and cognitive deficiencies. Thus, this diagnosis should be entertained in anyone who has juvenile cataracts removed, has any neurological signs and symptoms and especially if their Achilles tendons look bigger than normal. Other reported clinical manifestations include premature atherosclerotic disease, epilepsy, and osteoporosis.

Laboratory Tests

The laboratory tests for CTX include the determinations of plasma cholestanol levels, typically using a GC-MS, or HPLC techniques, looking for elevated cholestanol levels. The standard lipid test will not show major abnormalities, beyond perhaps some mild hyperlipidemia. Sterols need special testing. However, since cholestanol can be elevated under many other conditions (including sitosterolemia), the diagnostic tests include the determinations of bile alcohols in the plasma, urine, or feces of affected individuals. Molecular diagnostic testing for mutations in CYP27A1 can also be used. In isolated communities with a high rate of CTX, mutational screening is not only feasible, it may be cost-effective as therapy can be initiated as early as possible [61]. For the majority of CTX cases, this may not be feasible. Figure 13.8 shows a depiction of the mutations that have been reported by us, and how these map onto putative model of CYP27A1 [62]. However, the clinical presentation, together with the bile alcohol determinations, is usually all that is necessary to make an accurate diagnosis. In rare cases, where clinical signs suggest CTX, but no other features are helpful and diagnostic tests are equivocal, one can provoke accumulation of bile alcohols but depletion of body pools of bile acids using ingestion of bile acid resins for 48 h before the plasma and urine are analyzed. The loss of bile acids in the intestine leads to upregulation of the bile acid synthesis pathway and should exacerbate any enzymatic block in the pathway.

Differential Diagnosis

In any patient who presents at an early age with only tendon or tuberous xanthomas, the differential diagnosis includes familial hypercholesterolemia (plasma cholesterol is only moderately elevated CTX and is thus “diagnostic” excluder), and sitosterolemia. The test that measures cholestanol will also detect plant sterols, thus examination of the chromatogram allows for this distinction.

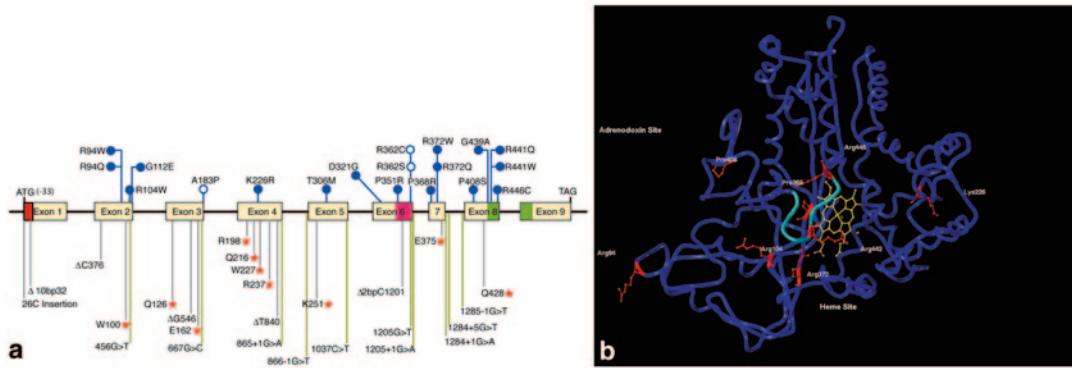


Fig. 13.8 Structural mapping and mutational spectrum affected in CYP27A1 in cerebrotendinous xanthomatosis (CTX). The positions of known mutations affecting CYP27A1 are shown on a depicted intron–exon structure of CYP27A1 in the panel on *right*. All of these are known to be pathogenic, except three (shown in *open circles*) which

may be normal variants. Despite the “scatter” of these mutations, mapping these onto a model of CYP27A1 (*left-hand panel*) shows that almost all of these affect the critical heme-adrenodoxin binding domain of CYP27A1 [62]. This domain is critical for enzymatic activity

Clinical Course and Treatment

With the institution of early and adequate replacement with oral CDCA, almost all of the CTX disease manifestations (except xanthomas) are greatly ameliorated and prevented [63–65]. CDCA therapy aims to suppress the bile acid synthesis pathway in the liver, greatly diminishing the generation of bile acid intermediaries and thus the bile alcohols. This allows for any damaged blood–brain barrier to heal and thus prevent any neurological damage from accumulation of cholestanol (and likely other toxic products) in the CNS. Restoration of CDCA into the enterohepatic circulation also improves digestion and absorption of fat and fat-soluble vitamins with an increase in weight. As this disease is rare, there are no large long-term prospective studies that have accumulated enough subjects treated very early on with CDCA to document the outcome. However, retrospective studies show that initiation of CDCA can result in significant reversal of signs and symptoms in many subjects, with consolidation of these gains with continued therapy. The addition of a statin, to suppress cholesterol synthesis, has also been shown to improve biochemistry and some clinical features in anecdotal case reports. The key to successful therapy

is not only to lower the cholestanol levels (frequently used as a marker of the disease) but also to ensure that all bile alcohols have been cleared from the blood (or urine), as the latter are more directly an indicator of disease activity. Cataract development may not be affected by early therapy, though this aspect has not been well studied. Finally, the development of xanthomas, especially at sites of repeated trauma may also continue to be an issue, despite good biochemical control. Presumably, this is because the pathogenesis of xanthoma formation may be related more closely to the absence of CYP27A1 in the macrophages, than to the direct effects of the bile acid intermediaries on macrophage biology. Removal of xanthomas may further aggravate local xanthoma formation and is not recommended. To improve bone health, supplementation of Vitamin D is recommended, with monitoring of bone density where clinically necessary. Atherosclerotic heart disease has also been reported in CTX and thus lowering of plasma cholesterol with statins is also recommended in middle-aged adults and in women unlikely to be childbearing. Women with treated CTX have successfully carried pregnancy to term with no complications, suggesting fertility is not affected and there are no major defects of the endocrine system. Regular medical

monitoring is mandatory to ensure that no ongoing damage to the CNS accrues, as neurological damage is the most important comorbid factor for a good quality of life.

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