Inherited Disorders of Cholesterol Biosynthesis

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Defects of cholesterol biosynthesis comprise a heterogeneous group of disorders, most of which have only recently been described and more are likely to follow in the near future.

Mevalonic adduria (MVA) and hyperimmunoglobulinemia D syndrome (HIDS) are due to allelic defects in mevalonate kinase, an enzyme located proximally in the pathway of cholesterol and nonsterol isoprene biosynthesis. Clinically, patients affected with these disorders present with recurrent febrile attacks. This is the only manifestation in most patients with HIDS, and, in the case of classical mevalonic adduria, is part of a severe multisystemic disease, including malformations, severe failure to thrive and neurological abnormalities. The other recognized defects of cholesterol biosynthesis are due to enzyme defects located distally in the pathway beyond the branching points of nonsterol isoprene biosynthesis and solely affecting cholesterol biosynthesis. Patients with these disorders all present with complex malformation syndromes involving different organ systems. The main characteristics of CHILD syndrome and Conradi-Huenermann syndrome are skeletal defects and ichthyosiform skin involvement. Smith-Lemli-Opitz syndrome and desmosterolosis are generalized malformation syndromes involving many different organs including the central nervous system.

The diagnosis of MVA and HIDS is based on determination of mevalonic acid in urine followed by determination of enzyme activity, whereas the search for the distally located defects of cholesterol biosynthesis requires sterol analysis in blood or tissues by GCMS.

Rational therapeutic approaches have been described for HIDS, WA and Smith-Lemli-Opitz syndrome.

• Key words: Cholesterol biosynthesis - Mevalonic adduria - Hyperimmunoglobulinemia D syndrome - Smith-Lemli-Opitz syndrome - Conradi-Huenermann syndrome - CHILD syndrome

Introduction

Although cholesterol metabolism has been the focus of intense research for decades and about 80 % of cholesterol is derived by endogeneous synthesis, mevalonic aciduria due to mevalonate kinase deficiency was only described in 1986 [21] as the first recognized inherited defect in cholesterol biosynthesis. It was known before that inhibitors of HMG-CoA reductase, an enzyme located proximally to mevalonate kinase, can lead to elevations of creatine kinase and liver enzymes and teratogenic sequelae [40], comparable to mevalonic aciduria. As early as 1966 there were hints that Smith-Lemli-Opitz syndrome (SLOS), a common autosomal recessive inherited malformation syndrome first described in 1964 [49], was associated with an abnormal cholesterol biosynthesis. Feeding inhibitors of 7-dehydrocholesterol reductase (DHCR7) to pregnant rats resulted in fetal malformations similar to those seen in SLOS [45]. It was not until 1993, however, that Tint and colleagues [26,50] recognized an accumulation of 7-dehydrocholesterol (7-DHC) in combination with a decrease of cholesterol in SLOS patients. As already suspected from the inhibitor studies, the underlying cause was indeed proven to be a deficiency of microsomal 7-dehydrocholesterol reductase (DHCR7), the last enzyme in cholesterol biosynthesis (Fig. 1). With elucidation of the biochemical abnormalities underlying Smith-Lemli-Opitz syndrome, the underlying pathophysiology of the severe disturbance of embryogenesis could be linked to a derangement of sterols affecting the sonic hedgehog embryonic signalling pathway [43]. In Smith-Lemli-Opitz syndrome, this results in a characteristic malformation pattern with dysmorphic fa-
cial features, midline defects, limb, and genital abnormalities, as well as malformations of the central nervous System and internal organs. The observation that one of the abnormalities of Smith-Lemli-Opitz syndrome can be rhizomesomelic shortening of the limbs led to the investigation and detection of abnormal sterols in Conradi-Hunermann syndrome, an X-linked dominant form of chondrodysplasia punctata (CDPX2). A deficiency of 3ß-hydroxysteroid-A8,A7-isomerase was confirmed by the discovery of mutations in the gene for this enzyme [3,8]. In patients with CHILD syndrome, a rare, again X-linked dominantly inherited disorder with unilateral ichthyosiform skin lesions and limb defects, two different enzyme defects seem to be responsible for the same clinical phenotype. Bio-chemical and mutational analysis gave evidence for a deficiency of the 4-sterol-demethylase in most of the patients, whereas a few patients have a defect of 3ß-hydroxysteroid-A8,A7-isomerase, as in CDPX2 [31,32].

A defect at the level of 3ß-hydroxycholesterol-A-24-reductase has been documented in two patients with elevated levels of desmosterol as the fifth defect of cholesterol biosynthesis discovered so far [2,13].

Mevalonic Aciduria and Hyperimmunoglobulinemia D Syndrome

**Clinical phenotype**

Mevalonic aciduria (MVA; MIM 251170) shows considerable clinical heterogeneity ranging from intrauterine or neonatal death and progressive fatal disease to relatively mild neurological Symptoms and a stable clinical course beyond childhood. Metabolic acidosis, lactic acidemia and hyperammonemia, the usual concomitants of organic acid disorders, are conspicuously absent and patients are cared for in a variety of different subspecialty clinics, such as dysmorphology, gastroenterology, hematology, immunology, and neurology.

Severely affected patients present from birth with minor anomalies such as microcephaly, dolichocephaly, and wide irregular fontanels as well as low set and posteriorly rotated ears, downslanted palpebral fissures, blue sclerae, and central cataracts [21] (Fig. 2). Stillbirths with skeletal malformations have been observed in affected families, possibly a result of the same genetic defect. From infancy, profound hypotonia, failure-to-thrive, short stature and psychomotor retardation are obvious. A progressive ataxia due to cerebellar atrophy.
Patient with mevalonic aciduria at the age of 21 months displaying the characteristic facial dysmorphism.

Cerebral magnetic resonance imaging of a patient with MVA at 5.8 years of age. Coronal section displaying marked atrophy of the hemispheres and the upper vermis of the cerebellum. The lower vermis is totally missing. Spin echo technique 1.0 T: time of repetition 2460 milliseconds, time of echo 30 milliseconds, time of inversion 600 milliseconds, slice thickness 9 mm.

Patient with MVA at 6 years of age during intercurrent crisis with high fever, diarrhea, vomiting, hepatosplenomegaly and rashes. In this girl these conditions occurred fortnightly.

(Fig. 3: MRI of a patient with MVA) develops at preschool age and can be the predominant neurological manifestation in milder cases.

Sometimes hematological abnormalities predominate with normocytic hypoplastic anemia, leukocytosis, thrombocytopenia, and abnormal blood cell forms [19], leading to misdiagnoses of congenital infection or myelodysplastic syndromes.

All patients have recurrent febrile crises with hepatosplenomegaly, lymphadenopathy, vomiting and diarrhea. Morbilliform rashes, edema, and arthralgia may also be present (Fig. 4) [21]. Most of these attacks appear to be non-infectious. However, they can also be triggered by intercurrent infections, making the differentiation between infectious and non-infectious crises difficult. In addition to high fever and general malaise, white blood cells, especially neutrophils, are highly increased as are acute phase proteins and the Sedimentation rate.

The first episode usually occurs in early childhood with up to 25 attacks per year. In some patients, they occur at regular monthly or even weekly intervals. Over years the severity and the frequency of these attacks usually decline.

In hyperimmunoglobulinemia D syndrome (HIDS; MIM 260920), which was recently found in some cases to be allelic to MVA [10,24], recurrent febrile attacks associated with abdominal pain, arthralgia, and rashes dominate the clinical course [51]. Neither malformations nor neurological abnormalities occur in this entity, and life expectancy is not reduced.

Genetics

Mevalonic aciduria and hyperimmunoglobulinemia D syndrome are autosomal recessive disorders. The gene encoding mevalonate kinase (MVK) is located on chromosome 12q24 [14]. In classical MVA, six mutations were identified in 10 of 20 known patients [20], all of which cluster in the C-terminal region of the protein.

In HIDS, three missense mutations affecting mevalonate kinase activity and stability have been found [10,24]. Although they are located in the same coding region as mutations in
classical MVA, up to now, no mutations common to both diseases have been identified.

**Diagnostic methods**

In most cases of MVA, serum Fevels of creatine kinase and transaminases as well as leukotrienes and prostanooids in blood and urine are increased [38]. The levels fluctuate and in-crease further during intercurrent crises and seem to correlate with the severity of the disease. Levels of serum cholesterol are mostly low normal. An elevation of IgD is seen in HIDS patients and in MVA as well. The elevation of IgD levels is, however, not obligatory [9]. This implies that all these markers represent secondary phenomena with as yet unknown patho-physiological links to the primary defect. In fact, it appears likely that patients with periodic fever syndrome and normal levels of IgD will also be identified by analysis of mevalonic acid or determination of enzyme activity as suffering from the same genetic defect.

The only definitive biochemical abnormality is the demonstration of elevated concentrations of mevalonic acid.

In classical MVA this is easily accomplished by regular urinary organic acid analysis, as mevalonic acid is found to be highly elevated in all body fluids. However, in milder affected patients and especially in HIDS patients elevations of mevalonic acid may only be found during febrile attacks if measured by routine gas chromatography. The sensitivity of organic acid analysis is inadequate to recognize the very low concentrations of mevalonic acid present in control urine. Even moderate but definitely pathological elevations remain under the detection limit [22], as demonstrated by isotope dilution mass spectrometry [31]. As the absolute level of mevalonic acid is always increased in patients with mevalonate kinase deficiency, a specific suspicion should be followed by a specific and sensitive measurement of mevalonic acid by stable isotope dilution analysis or radioenzymatic assay to detect even slight abnormalities. The diagnosis of MVA should be confirmed by mutational analysis or by assay of mevalonate kinase in white blood cells or cultured fibroblasts [23]. Carrier detection is also possible by enzymatic assay [22], although the results are sometimes equivocal, or by DNA analysis, if mutations have been found in the index patient.

Prenatal diagnosis can be performed by enzyme assay in chorionic villus cells and amniocytes, by direct determination of mevalonic acid in amniotic fluid by GCMS, and by molecular analysis in informative families [22].

**Treatment**

There is no established therapeutic regime. Dietary supplementation of cholesterol may reduce frequency and severity of febrile attacks in some mildly affected patients, but has further compromised more severely affected patients ([23]; R. Kelley, unpublished observations). The severity of attacks can also be reduced in HIDS with leukotriene receptor inhibitors (R. Kelley, unpublished observations). Trials of corticosteroid therapy during clinical crises (2 mg prednisone/kg/d) resulted in positive responses. Additional long-term administration of ubiquinone-50 together with vitamin C and E appeared to further stabilize the clinical course and improve somatic and psy-

**Smith-Lemli-Opitz Syndrome**

**Clinical phenotype**

Similar to mevalonic aciduria, Smith-Lemli-Opitz syndrome (SLO; MIM 270400) shows a wide clinical variability. In affected families many pregnancies result in stillbirths with prominent skeletal and organ malformations. Severely affected SLO fetuses typically have nuchal edema, polydactyly, cleft palate, or structural abnormalities of brain, heart or kidneys. Those cases with major malformations were formerly classified as "type II", and most died shortly after birth [7], as opposed to the milder "type I", who at the other end of the spectrum may uniquely present with borderline mental retardation or abnormal behaviour [6]. In fact, children have been identified who successfully attend regular school with very mild clinical and biochemical manifestations [34]. Table 1 summarizes frequently occurring features as well as typical internal malformations, the latter being found in severe cases. After the biochemical and molecular diagnosis of SLO became available, the original differentiation between types I and II no longer appears justified. Biochemical and molecular studies have confirmed a continuum of severity in Smith-Lemli-Opitz syndrome with different mutations of a single gene responsible for part of the phenotypic variability. Affected siblings usually show similar clinical severity. However, the occurrence of malformations is not predictable and occasionally can cause individually very different consequences within the same family, which complicates family counselling.

Patients with Smith-Lemli-Opitz syndrome have characteristic facial features that consist of microcephaly, ptosis, anteverted nares, retrognathia and low-set, posteriorly rotated ears (Fig. 5). Newborns often have striking glabellar and other mid-

<table>
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<th>Table 1 Major clinical characteristics of Smith-Lemli-Opitz syndrome</th>
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<td><strong>Frequent Anomalies (&gt; 50% of patients)</strong></td>
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line capillary hemangiomas that tend to fade with age. The mouth is large and broad with a highly arched palate. Midline clefts ranging from a bifid uvula to lip and palatal clefts are common. A variety of ocular abnormalities, including cataracts, can occur.

Very typical are skeletal anomalies. In some series, 99% of the biochemically proven cases have unilateral or bilateral syndactyly of the 2nd and 3rd toe [6]. The thumb is often short and proximally placed. Many of the more severely affected patients have postaxial polydactyly, and in some children a rhizomeso-melic shortening of the limbs has been observed [7,46]. Varus or valgus foot deformities as well as clubfoot can also occur [7,46].

Genital anomalies are commonly found even in mildly affected boys, and range from cryptorchidism to severe hypospadias and complete apparent sex reversal [7,47]. In females, hypo-plastic labia majora and minora have been found, but usually the external genitalia are normal.

In addition to microcephaly, structural abnormalities of the central nervous System are frequent, such as frontal lobe hypoplasia, enlarged ventricles, agenesis of the corpus callosum, and cerebellar hypoplasia. Various forms of holoprosencephaly can occur [29]. Most patients are severely retarded. Even in the absence of structural defects, mild to moderate mental retardation is one of the principal characteristics of Smith-Lemli-Opitz syndrome. Nevertheless, some patients with borderline or even normal intelligence have been described [34,37].

Behavioural problems are another common feature. They range from aggressive, self-injurious behaviour to autistic features.

In early childhood sleeping problems as well as excessive screaming are often reported [46]. Feeding is typically very difficult from birth and becomes often almost impossible later in infancy, resulting in tube feeding of most patients (see below). These behavioural and somatic abnormalities tend to slowly diminish in later childhood. Marked photosensitivity is seen in many patients and requires effective sun protection [46]. As malformations can be found in nearly every organ, patients often have heart defects, renal and gastrointestinal anomalies, and in severe cases tracheal and pulmonary malformations. In the cardiovascular System, endocardial cushion defects such as AV canal, secundum atrial septal defect, patent ductus arteriosus, and membranous ventricular septal defect are typical [35]. Severe cases often have pulmonary hypertension in the newborn period. Defects of the urinary tract include renal hypoplasia or aplasia, renal cortical cysts, hydronephrosis, and ureteral duplication [46]. In most patients, the intestinal tract is impaired due to moderate to severe disturbance of motility. Severely affected children often have intestinal aganglionosis [55] but even without histological evidence of an abnormal myenteric system, refractory dysmotility is frequently present. Pyloric Stenosis is a prominent problem [46], and feeding difficulties and vomiting often persist after surgical correction. Due to this, many patients require a feeding tube during the first years if not indefinitely.

Pulmonary hypoplasia or abnormal lobation can occur in severe cases [7] and limit life expectancy.

**Pathogenesis**

The underlying defect of Smith-Lemli-Opitz syndrome is a deficiency of microsomal 7-dehydrocholesterol reductase (DHCR7), the enzyme catalyzing the last step of cholesterol biosynthesis. As a result, the cholesterol precursor 7-DHC and its isomer, 8-dehydrocholesterol (8-DHC), are elevated in plasma and all tissues, whereas, in most patients, cholesterol is markedly decreased.

Unlike many other Substrates, only small amounts of cholesterol are transported through the placenta [4], so that the fetus is mostly dependent on its own biosynthesis of cholesterol. In tissues of aborted fetuses who were found prenatally to suffer from SLO, sterol analysis demonstrated 7-DHC and its isomers as the predominant sterols with low concentrations of cholesterol (G. F. Hoffmann, unpublished data). Interestingly, high maternal cholesterol levels may have a protective effect in the early embryonic period resulting in a less severe phenotype than predicted by the genotype (R. Kelley, unpublished data). In humans cholesterol is synthesized locally and does not cross the blood-brain barrier [41]. Protection from cerebral malformations by sufficient amniotic cholesterol concentrations may result from the influence of cholesterol on embryonic signalling proteins.

In embryogenesis covalent binding of cholesterol to the sonic hedgehog (SHH) signalling protein plays an important role in controlling embryonic development of the forebrain and the limbs [43]. Mutations in the SHH gene result in holoprosencephaly [44], a pattern also found in some SLOS patients.
Although cholesterol was at first thought to be essential for the "autoprocessing" of SHH, a Splitting of the protein in an inactive and a signalling part, recently 7-DHC was found to have the same effect on SHH autoprocessing as cholesterol [5]. Thus the malformations in SLOS patients are now thought to result from an as yet unknown effect of the sterol abnormalities on tissues that respond to SHH signalling [5]. A genetic mouse model for Smith-Lemli-Opitz syndrome has been produced by disrupting the DHCR7 gene in mouse embryonic stem cells, but homozygotes die within 24 hours because of feeding problems [42]. The knock-out mouse is therefore not yet available for study of therapeutic interventions.

Genetics

The human DHCR7 gene is located on chromosome 11 q13 [12,52,53]. It contains nine exons and eight introns and spans approximately 14 kb. More than 40 different mutations have been identified in SLOS patients, 90% of which are missense mutations [54]. More than half of all mutations are in exon 9. Of the non-missense mutations the most interesting is a splice-site mutation (IVS8-1 G > C) resulting in a 134 basepair insertion, which represents 30% of all SLOS alleles. Homozygotes for this mutation are predicted to have no DHCR7 activity, and indeed most of them have a severely affected phenotype. However, all IVS8 -1 C > G homozygotes still have measurable cholesterol levels even without administration of exogenous cholesterol, and one IVS8 -1 G > C homozygote only had moderate malformations and a cholesterol level of 50 mg/dl, suggesting the existence of either an alternative pathway in cholesterol biosynthesis not requiring DHCR7 or an alternate enzyme with DHCR7-ac-tivity.

Diagnostic methods

Elevated levels of 7-DHC and 8-DHC (normal < 0.1 mg/dl or< 2.6pmol/l) in serum or plasma detected by GCMS confirm the diagnosis biochemically [28]. Measuring blood cholesterol levels as a screening method is not reliable because approximately 10% of affected individuals have normal cholesterol levels. In addition, the cholesterol oxidase assay method used in routine chemistry laboratories overestimates cholesterol levels because it reacts equally well with cholesterol and the dehydrosterols [28]. If serum or plasma is not available, sterol analysis can be performed in any tissue or cell culture.

In very mildly affected patients, the elevation of 7-DHC in plasma can be within the upper normal range but 8-DHC usually is distinctly if mildly elevated. Sterol analysis in lymphoblasts or fibroblasts grown in delipidated culture medium should be performed if results of plasma analysis are ambiguous or normal, yet the clinical phenotype is very suggestive of SLOS.

Prenatal diagnosis is possible by sterol analysis of chorionic villi from 10 weeks on [39]. The 7-DHC/cholesterol ratio usually shows a more than 30-fold increase in SLOS fetuses. In amniotic fluid the 7-DHC/cholesterol ratio is increased even more, often 500-fold [33]. Because biochemical analysis is fast and reliable there is no need for enzymatic or molecular test-

Table 2 Characteristic malformations of internal organs in severely affected Smith-Lemli-Opitz patients

<table>
<thead>
<tr>
<th>CNS</th>
<th>Endocrine</th>
<th>Gastrointestinal</th>
<th>Pulmonary</th>
<th>Cardiovascular</th>
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<td>frontal lobe hypoplasia</td>
<td>adrenal insufficiency</td>
<td>pyloric Stenosis</td>
<td>pulmonary hypoplasia</td>
<td>AV canal</td>
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<td>enlarged ventricies</td>
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<td>refractory dysmotility</td>
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<td>secundum atrial septal defect</td>
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<td>agenesis of corpus callosum</td>
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<td>cholestatic and non-cholestatic progress</td>
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<td>patent ductus arteriosus</td>
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<td>cerebellar hypoplasia</td>
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<td>renal cortical cysts</td>
<td>membranous ventricular septal defect</td>
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<td>holoprosencephaly</td>
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<td>hydromecephrosis</td>
<td>renal hypo- or apiasia</td>
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<td>ureteral duplication</td>
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<td>Hirschsprung disease</td>
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Table 2 summarizes the characteristic malformations of internal organs in severely affected Smith-Lemli-Opitz patients. A low maternal estriol level (< 0.5 multiples of the median) can be an early sign of a fetus affected with SLOS [1,33] and should lead to further investigations, especially when associated with abnormal ultrasonographic findings (Table 2).

Treatment

Up to now, orally administered cholesterol has been the only causal treatment [11]. Increasing cholesterol levels from exogenous cholesterol inhibits HMG-CoA reductase and therefore decreases the synthesis of abnormal sterols. In most treatment protocols purified cholesterol at a daily dosage of 100 mg/kg is provided. In one study, the addition of bile acids to the therapy did not show any additional beneficial effects [11].

When enteral administration of cholesterol is not possible (e.g., after surgical procedures) fresh frozen plasma (FFP) given intravenously increases serum cholesterol levels (R. Kelley, un-published observations). This is of special importance as many patients suffer from compensated adrenal insufficiency that might decompensate due to the stress of surgery or prolonged illness.

Feeding problems often are a prominent feature of SLOS so that supplemental gastrostomy feeding is required in a majority of patients, at least for the first years of life. It is important to provide an adequate energy intake but not to overfeed the children in order to achieve a better growth. SLOS patients have a genetically determined short stature, and additionally, as a result of their muscle hypoplasia, their normal well-nourished weight typically is 1-2 Standard deviations less than their length. Increasing adipose tissue by trying to reach a weight above the 3rd percentile limits the availability of cholesterol to the organs. In addition, overfeeding often leads to increased vomiting.

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The benefit of cholesterol substitution can be seen shortly after onset of the treatment by increased weight, improved growth, and behavioural changes. However, the sterol levels in plasma often improve very slowly despite obvious immediate clinical benefits. After initiation of treatment cholesterol levels sometimes paradoxically decrease further and 7-DHC levels increase. These temporary changes can be explained by the distribution of cholesterol from the blood to the tissues, and after several months, cholesterol levels increase in most patients.

HMG-CoA reductase inhibitors have not been routinely used in SLO, because a postnatal "toxic" effect of the abnormal sterols has never been proved, and because the potential inhibition of the biosynthesis of non-sterol isoprenoids could lead to additional pathology. Actually 7-DHC might replace cholesterol in some of its functions so that depletion of 7-DHC without a rising cholesterol level could theoretically be harmful. However, a preliminary clinical trial of the HMG-CoA reductase inhibitor simvastatin in five mildly affected SLO patients resulted in a rapid fall of 7-DHC levels and an unexpected and as yet unexplained rise of serum cholesterol. Even more importantly, preliminary clinical evaluation after 12 to 24 months indicated improved somatic development without signs of unwarranted side effects [27]. This therapeutic approach is currently being tested in a larger European multicenter study.

**Conradi-Huenermann Syndrome**

Chondrodysplasia punctata is a term used for abnormal punctate calcification of cartilaginous structures, most prominent in the epiphyses of the long bones. the larynx, and the pelvis, resulting in bilateral disproportionate shortening of the limbs (Fig. 6). It has been associated with a variety of genetic disorders, the most common being classical rhizomelic chondrodysplasia punctata (RCDP), caused by several individual defects of peroxisomal plasmalogen biosynthesis.

The X-linked dominant form (CDPX2 or Conradi-Huenermann syndrome, MIM 302960) is thought to be lethal in males. Be-sides the typical calcifications, asymmetric rhizomelic limb shortness (Fig. 7), and ichthyosis, cataracts and mental retardation have been found in a few affected females [16].

Biochemically, CDPX2 patients have normal or, occasionally, decreased cholesterol levels and elevated concentrations of 8-DHC and 8(9)-cholesterol in plasma and tissues due to a defect of 3β-hydroxy steroid-A8,A7-isomerase [30]. The EBP (emopamil binding protein) gene encoding for the sterol-AS-isomerase is located on Xpn.22-pll.23, and mutations in this gene have been found in all CDPX2 patients with this abnormality in sterol metabolism [3]. EBP was first identified as a high-affinity binding target for the drug emopamil. It was later demonstrated to possess sterol isomerase activity in a high-affinity binding target for the drug emopamil. It was later demonstrated to possess sterol isomerase activity in mammalian cells and to complement sterol isomerase deficient mutants of *S. cerevisiae* [48]. The same biochemical abnormalities associated with mutations in EBP were detected in tattered mouse, an X-linked semi-dominant mouse mutation with a clinical phenotype resembling CDPX2 [8].

No attempt of metabolic therapy has yet been reported.

<table>
<thead>
<tr>
<th></th>
<th>Mevalonic aciduria</th>
<th>Hyper-IgD syndrome</th>
<th>CHILD syndrome</th>
<th>CDPX2</th>
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<tr>
<td>Symptoms</td>
<td>Developmental delay</td>
<td>Febrile crises with diarrhea and vomiting</td>
<td>Unilateral ichthyosiform skin lesions</td>
<td>Asymmetrical rhizomesomelic limb shortness</td>
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<td>Progressive ataxia</td>
<td>Skin rashes</td>
<td>Congenital hemidystrophia</td>
<td>Growth retardation</td>
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<tr>
<td></td>
<td>Febrile crises with diarrhea and vomiting</td>
<td>Lymphadenopathy</td>
<td>Unilateral limb defects</td>
<td>Punctate calcification of cartilaginous structures</td>
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<td></td>
<td>Fluctuating hepatosplenomegaly and lymphadenopathy</td>
<td>Skin rashes</td>
<td>Cataracts</td>
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<td>Skin rashes</td>
<td>Lymphadenopathy</td>
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<td>Failure to thrive</td>
<td>Anthritis and arthralgia</td>
<td>Ambiguous genitalia</td>
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<td>Short stature</td>
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<td>Dysmorphic facial features</td>
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<td>Malformation of internal organs</td>
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Fig. 6 Clinical signs and Symptoms in disorders of cholesterol biosynthesis.

**CHILD Syndrome**

CHILD (MIM 308050) is an acronym for congenital hemidysplasia, ichthyosiform erythroderma and limb defects. The disorder is characterised by unilateral ichthyotic skin lesions with a sharp demarcation at the midline of the trunk [17], typically with the facial area spared (Fig. 8). Punctate calcifications similar to those in CDPX2 are present in the epiphyses and other cartilaginous structures of the affected side, which is usually the right side [15,32]. The mode of inheritance is thought to be X-linked dominant and lethal in males, although two affected males have been described and explained by the possibility of somatic mosaicism [18].

Biochemically, patients with the CHILD phenotype show one of two distinct sterol patterns: most of the patients have elevated levels of 4-methylsterols, a pattern also found in the "bare-patches (BPA)" mouse mutant [36] with a deficiency of the NSDHL (NAD[P]H steroid dehydrogenase-like) subunit of the sterol-4-demethylase complex, the enzymatic step just prior to sterol-A8-isomerase. In these patients mutations in the NSDHL gene have been detected [31,32].

**Table 2 for clinical synopsis**

- Febrile crises with diarrhea and vomiting
- Skin rashes
- Lymphadenopathy
- Anthritis and arthralgia
- Unilateral ichthyosiform skin lesions
- Congenital hemidystrophia
- Unilateral limb defects
- Punctate calcification of cartilaginous structures
- Cataracts
- Mental retardation
- Asymmetrical rhizomesomelic limb shortness
- Growth retardation
- Punctate calcification of cartilaginous structures
- Cataracts
- Mental retardation
- - Rhizomesomelic limb shortening
- - Osteosclerosis
- - Ambiguous genitalia
- - Facial dysmorphic features with cleft palate
- - Malformation of internal organs

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<td><strong>Smith-Lemli-Opitz syndrome</strong></td>
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Fig. 7 Rx of the hip and both legs of a patient with CDPX2. The long bones are disproportionately short and punctate calcification is present in the epiphyses.

Fig. 8 Patient with CHILD syndrome.

However, in two patients with the CHILD phenotype the same sterol pattern as in CDPX2 patients has been found. In plasma and tissues 8-DHC and 8(9)-cholesterol are abnormally increased, and subsequently mutations were discovered in the sterol-A8-isomerase gene [15] indicating that the CHILD phenotype comprises two different genetic disorders and that CDPX2 and CHILD syndrome are allelic conditions. As in CDPX2, there are no reports about therapeutic trials yet.

**Desmosterolosis (MIM 602398)**

Up to now, reports of only two patients with an isolated increase of desmosterol and the clinical features of a lethal malformation syndrome have been published [2,13]. The patient described first was a newborn girl with bilaterally shortened limbs, generalized osteosclerosis, malformations of the brain and the internal organs as well as ambiguity of the external genitalia. Furthermore, a cleft palate and facial abnormalities, such as low set posteriorly rotated ears, a depressed nasal bridge and microstomia, were noted. The patient died shortly after birth.

Biochemically, desmosterol was found to be the major sterol compound in the brain of the patient with a desmosterol/cholesterol ratio of 1.06. In the other tissues examined, desmosterol was highly elevated as well.

The second patient had a less severe phenotype with dysmorphic facial features, microcephaly, limb anomalies, ambiguous genitalia and a profound developmental delay. Desmosterol accounted for 42% of total sterols in cultured cells but only about 5% of sterols in plasma.

The suspected enzyme deficiency is 3-hydroxysterol-A24-reductase, the enzyme that converts desmosterol to cholesterol. The gene for 3-hydroxysterol-A24-reductase has recently been cloned, and in both patients mutations have been found (Hans Waterham, personal communication).

**Discussion**

Disorders of cholesterol biosynthesis are evolving into a conceptually and numerically important group of metabolic diseases. In the last year, three more diseases have been found to be due to defects in this anabolic pathway, which result in quite varied clinical phenotypes with involvement of many different organ Systems. Therefore, patients may be found in different pediatric subspecialities depending on the predominant signs and symptoms of the disorder. HIDS was previously thought to be a primary immunological disorder, CHILD syndrome was known as a congenital malformation syndrome with skin involvement, and CDPX2 has clinical features of a peroxisomal disorder.

This broad range of abnormalities can be thought to reflect on the one hand the results of a lack of cholesterol combined with abnormal sterols accumulating before the enzymatic block, which are specific for each genetic defect. Both could impair embryonic signal transduction and result in congenital malformations in the defects distal from the branching of the initially combined pathway of cholesterol and nonsterol isoprene biosynthesis (Fig. 1). In the proximal defects a lack of nonsterol isoprenes appears to be critical for the pathophysiological consequences [25].

In some cases, disorders of cholesterol biosynthesis do not present as obviously as described in this article. The potential involvement of many different organ Systems makes it difficult to recognize variant forms of these disorders which present with atypical clinical features. In addition, biochemical tests can yield false negative results unless done by specially equipped laboratories. In HIDS, for example, the slightly elevated baseline excretion of MVA may not be detected by routine analysis of organic acids but can be detected by isotope-dilution methods.
In rare cases of Smith-Lemli-Opitz syndrome, 7-DHC serum levels in the upper normal range have been reported, so that culturing of lymphoblasts or fibroblasts in lipid-depleted medium may be required to establish an unequivocal diagnosis. In patients with Conradi-Huenerman and CHILD syndrome, plasma sterol analysis requires a very sensitive GCMS analytical System and experience as there often are only slight, but distinct elevations of the marker metabolites. Surprisingly, fibroblasts from affected skin areas can fail to show the typical sterol abnormalities because the dermis underlying the abnormal epidermis may express the normal X-linked allele [15]. Sterol analysis of lymphoblasts grown on delipidated medium may be the method of choice for cases with normal or equivocal abnormalities in plasma.

It is safe to assume that more defects in the biosynthetic pathway and probably in the intracellular transport of cholesterol are yet to be discovered. Because skeletal defects are common to all known disorders of cholesterol biosynthesis, it will be promising to investigate patients with different kinds of skeletal malformations for defects in the distal Steps of cholesterol biosynthesis and to analyze organic acids, HMG-CoA reductase activity, and sterols in patients with periodic fever syndrome, especially if signs of multisystem involvement or increased IgD or CK levels are recognized. Some defects might not be detectable by the current methods because analysis of urinary organic acids will only reveal elevations downstream of mevalonic-5-phosphate decarboxylase whereas GCMS sterol analysis will detect elevations downstream of squalene synthetase. For many of the intervening Steps in isoprenoid metabolism other screening techniques must be devised to evaluate candidate genetic deficiency syndromes.

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