X-linked Dominant Chondrodysplasia Punctata (CDPX2): Multisystemic Impact of the Defect in Cholesterol Biosynthesis

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Abstract: Chondrodysplasia punctata represents clinically and genetically a heterogeneous group of disorders characterized by the presence of multiple congenital anomalies and stippled epiphyses. We present clinical course of the disease and the results of metabolic, X-ray and molecular analyses in 19-months old girl with X-linked dominant chondrodysplasia punctata with intrauterine growth retardation, craniofacial dysmorphy, cataracts, cutaneous anomalies including ichthyosis, asymmetric rhizomesomelic shortness of the limbs, deformity of the spine, club foot, polydactyly, syndactyly, epiphyseal stippling and low cholesterol (2.29 mmol/l). Spectrophotometric analysis revealed the presence of abnormal pattern of cholesterol precursors in blood. The increased level of 8-dehydrocholesterol (42.2 μmol/l, controls < 1) and 7-dehydrocholesterol (25.5 μmol/l, controls < 1) recognised with GC/MS suggested an endogenous defect of cholesterol biosynthesis. The diagnosis of X-linked dominant chondrodysplasia punctata (CDPX2) was confirmed by the molecular analysis. Sequencing of the EBP gene encoding for 3β-hydroxysteroid-Δ^8,Δ^7-isomerase revealed the presence of "de novo" heterozygous mutation c.327C>T (p.Arg110Stop). High cholesterol diet normalized cholesterol level (3.28 mmol/l) but it had no influence on the unfavourable prognosis of the disease. Low level of cholesterol with abnormal sterol profile in a child with congenital development anomalies represent an important laboratory marker suggesting an inherited defect of cholesterol biosynthesis.

Introduction

"Chondrodysplasia punctata" represents a heterogeneous group of genetic and acquired disorders characterized by the presence of multiple congenital anomalies with skeletal dysplasia and stippled epiphyses. To discriminate among individual types of chondrodysplasia punctata in affected children, a combination of clinical, radiological, biochemical and molecular approach is possible. One type of chondrodysplasia punctata is X-linked dominant chondrodysplasia punctata (CDPX2), earlier known as Conradi-Hunermann-Happle syndrome caused by 3β-hydroxysteroid-Δ^8,Δ^7-isomerase deficiency, the inherited metabolic defect of cholesterol biosynthesis [1–7].

We report here the clinical course of the disease and the results of X-ray, metabolic and molecular analyses in the girl with intrauterine growth retardation and multiple development defects including craniofacial dysmorphy, depressed nasal bridge, asymmetric rhizomesomelic shortness of the limbs, deformity of the spine, bilateral club food, cataracts, ichthyosis, polydactyly, syndactyly and hypocholesterolemia caused by X-linked dominant chondrodysplasia punctata (CDPX2).

Methods

The endogenous precursors of cholesterol in serum were studied spectrophotometrically. Quantitative analyses of sterol concentrations in plasma

were performed by high-resolution gas chromatography – mass spectrometry (GC-MS) [1,2]. Genomic DNA was isolated from cultivated amniocytes. Postnatally, after the clinical suspicion for X-linked dominant chondrodysplasia punctata, the PCR-amplification and subsequent direct sequencing of individual exons in EBP gene [1, 2, 8] encoding for the enzyme 3β-hydroxysteroid-Δ^8,Δ^7-isomerase were performed.

Case report

The girl was born from the first pregnancy at 35th week of gestation with birth weight 1870 g, length 34 cm and head circumference 30 cm. Both parents were healthy. The first sonographic investigation in the 20th week of gestation revealed, that foetal head circumference corresponds to 19th week of gestation and the length of legs correspond only to 18th week of gestation, but this findings may be still interpreted as normal. The second sonography in the 30th week of gestation already showed significant asymmetric shortening of upper and lower limbs with atypical curvature of the spine and hypoplasia of nasal bridge. The amniocentesis was recommended for chromosomal analyses, but the obtained foetal karyotype was normal 46 XX. The early postnatal adaptation was complicated with asphyxia and the low Apgar score 3-6-7 in the first, fifth and tenth minute, but after oxygen administration the next respiratory and circulatory adaptation was uneventful.

Craniofacial dysmorphy with prominent forehead, depressed nasal bridge and low set ears were observed since the birth. The girl had cataract on the right eye and cortical clouding of the left lens. Both upper and lower limbs were short, more pronounced on the right side till the picture of hemimicromelia. The girl has polydactyly on the right hand, partial syndactyly on both legs and bilateral club foot. The mobility of several joints is limited due to contractures of elbow and knee on the right side and fingers on the left side. Neurological examination revealed asymmetric reactions and general decrease in spontaneous movements.

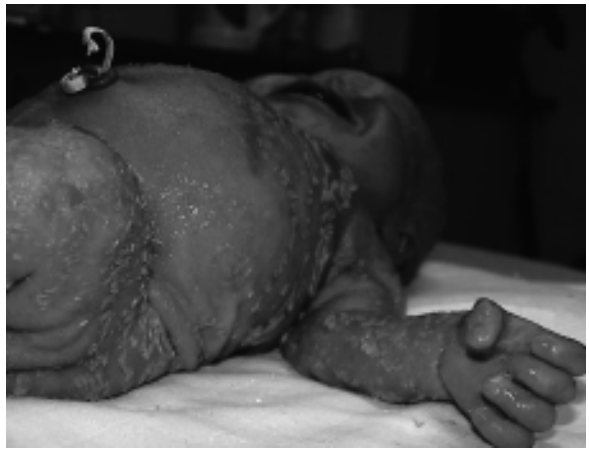

Figure 1 – Cutaneous anomalies including severe ichthyosis in the infant girl with X-linked dominant chondrodysplasia punctata.

The cutaneous involvement was striking with fragile and easily bleeding skin and generalised ichthyosis with dry and desquamate skin especially around lips, ears, elbows and knees (Figure 1). Hairs were very sparse without eyebrow or eyelashes. The incipient hyperkeratosis with sebum cutaneum was later exchanged with more dry and desquamating skin, erythematous and infectious complications were frequent. The skin care was very difficult, frequent skin lubrication and repeated baths with Linola fett Olbad were of importance.

In neonatal period, the girl got ill with acute pyelonephritis, urosepsis, renal sonography revealed hyperechogenic pyramides and frequent calcification. X-ray showed typical picture of chondrodysplasia punctata with asymmetric rhizomesomelic shortness of the limbs, deformity of the spine, multiple stippled foci of calcification in hyaline cartilage of the long bones, pelvis and around the vertebral column (Figure 2). Heart shadow was enlarged, echocardiography revealed mild pulmonar valvular stenosis and foramen ovale persistent without any significant shunt. Sonography of the brain was uneventful; brain stem evoked potentials were absent.

The level of total cholesterol was decreased (2.29 mmol/l) with lower concentration of both HDL-cholesterol (0.8 mmol/l) and LDL-cholesterol (0.85 mmol/l). Metabolic analyses revealed hyperlactaciduria (175–420 mmol/mol creatinine, controls < 60) with normal profiles of amino acids, organic acids oligosacharides and glycosaminoglycanes.

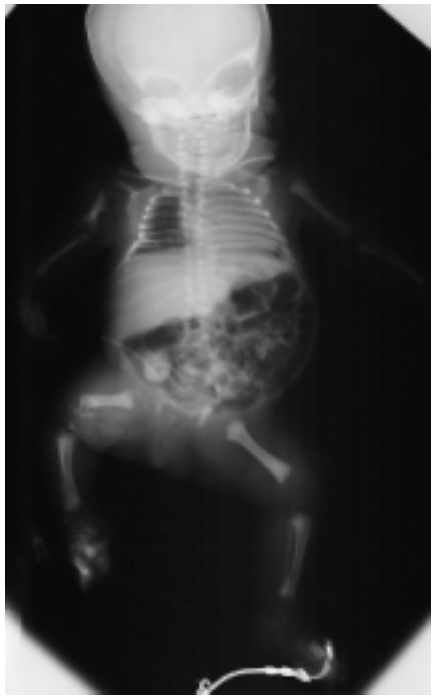

Figure 2 – X-ray of the newborn girl with X-linked dominant chondrodysplasia punctata.

Spectrophotometric screening of 7-dehydrocholesterol in serum showed abnormal profile of cholesterol precursors, quantitative analysis using GC-MS revealed increased level of 8-dehydrocholesterol (42,2 μmol/l, controls < 1) and 7-dehydrocholesterol (25,5 μmol/l, controls < 1). Former hypocalcemia in neonatal period (Ca^{++} 0,83 mmol/l) normalised after calcium supplementation, the levels of parathormone, alkaline phosphatase and 25-OH-cholecalciferol were normal. The level of 1,25-dihydroxycholecalciferol was mildly increased (95,8 ng/l). The diagnosis of X-linked dominant chondrodysplasia punctata (CDPX2) was confirmed by molecular analysis. In the proband, the "de novo" mutation c.327C>T (p.Arg110Stop) was found in *EBP* gene for 3β-hydroxysteroid-Δ^8, Δ^7-isomerase.

Now at the age of 19 months, the girl has failure to thrive. The anthropometric parameters are profoundly bellow the 3. percentile with the weight 6360 g, length 66 cm and head circumference 43 cm. Her psychomotor development is delayed, she is able to sit but not to sit up, she is able to crawl but not to climb or to walk. She started to use syllables but not words after she obtained the hearing aids. She has still problems with the joint contractures. Her body and head have the tendency to curl up to the left side. Therefore she has tailored orthopaedic cot partially correcting her spine deformity. Echocardiography normalised. Using several times per day a special skin care, the skin lesions and ichthyosis improved. Right side cataract was operated in infancy, since than is she able to watch. High cholesterol diet supplemented with cholesterol-module resulted in an increase of cholesterol level (3.28 mmol/l), but the diet had no clinical impact on the course of the disease.

Discussion

High blood level of cholesterol represents unfavourable prognostic "biochemical marker" increasing the risk of cardiovascular diseases, but neither low cholesterol level may be always beneficial. Cholesterol is relevant for number of metabolic pathways in man and especially it is important for development of central nervous system [9]. Inherited defects of endogenous cholesterol biosynthesis accompanied by hypocholesterolaemia result in several metabolic disorders. One of them is Smith-Lemli-Opitz syndrome [10, 11] with multiple developmental anomalies including microcephaly, hypospadia, and polydactyly or syndactyly. X-linked dominant chondrodysplasia punctata belongs to this group of diseases, too.

X-linked dominant chondrodysplasia punctata (CDPX2) is caused due to decreased activity of 3β-hydroxysteroid-Δ^8,Δ^7-isomerase (*EBP*, EC 5.3.3.5), the enzyme involved in the cholesterol biosynthesis. In patients with CDPX2, more than 55 mutations were described in the *EBP* gene localised on the X-chromosome. Clinical impact is heterogeneous with considerable intra- and interfamiliar variability of bone and skin impairment resulting from various inactivation of X-chromosome [12].

In our patient, the suspicion for X-linked dominant chondrodysplasia punctata resulted from the clinical course of the disease with ichthyosis and the X-ray with stippled epiphyses [2, 3, 13]. Metabolic analyses targeted on cholesterol biosynthesis disturbances were positive. Spectrophotometric analysis of 7-dehydrocholesterol in blood, which is usually performed for metabolic screening in children with suspicion on syndrome Smith-Lemli-Opitz showed increased concentration of several cholesterol precursors and quantitative analysis with GC/MS revealed first of all the increased level of 8-dehydrocholesterol. The diagnosis of X-linked dominant chondrodysplasia punctata (CDPX2) was confirmed by molecular-genetic analysis. In the proband, the nonsense mutation c.327C>T (p.Arg110Stop) was found in the *EBP* gene encoding enzyme 3β-hydroxysteroid-Δ^8,Δ^7-isomerase.

The mutation c.327C>T in the *EBP* gene in our child similarly to other nonsense mutations in *EBP* gene produces truncated protein resulting in severe phenotype of the disease in affected children, whereas the phenotypes resulting from missense mutations are not always typical for CDPX2 [14]. On the other hand, the mutation c.327C>T was also described in a child with CHILD syndrome – Congenital Hemidysplasia with Ichthyosiform erythrodermia and Limb Defects [15]. It was suggested, that at least a part of CHILD syndrome patients may represent an allelic variant to CDPX2.

The disease is usually lethal in affected males, but also in girls with X-linked dominant chondrodysplasia punctata is the prognosis unfavourable and the therapy is only symptomatic. We learned from our patients with Smith-Lemli-Opitz syndrome, which also represents metabolic disorder due to inherited disturbance of endogenous cholesterol biosynthesis, that high cholesterol diet may normalize cholesterol concentration in affected children. Unfortunately, the increase in cholesterol blood level is not accompanied by significant improvement of the clinical course of the disease in most of the patients. The same experience we now obtained in our patient with CDPX2.

The very unfavourable prognosis of children with CDPX2 is caused by decreased availability of cholesterol during the foetal development resulting among other things in congenital anomalies and the impairment of advance development of the central nervous system. The early diagnostics of inherited cholesterol biosynthesis defects is important, because the results of molecular analyses may be used in affected families to genetic counselling and eventually for prenatal diagnostics.

Conclusion

The experience with the diagnostic of CDPX2 in our patient documents, that the low blood level of cholesterol in infants with congenital developmental anomalies may represent an important "biochemical marker" in the diagnostics of metabolic disorders due to inherited disturbance of endogenous cholesterol biosynthesis, especially if hypocholesterolaemia is accompanied with increased concentrations of cholesterol precursors.

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