Clinical Manifestation in Females with X-linked Metabolic Disorders: Genetic and Pathophysiological Considerations

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Abstract

Inborn errors of metabolism are predominantly autosomal-recessive disorders, but several follow an X-linked pattern of inheritance. They are called X-linked recessive, if the female carriers are asymptomatic, and are called X-linked dominant disorders, if almost all females are affected. Conditions, in which some females have symptoms while others are asymptomatic lifelong are simply referred to as X-linked. The aim of this review is to point out the variability in clinical manifestation of affected females in some X-linked metabolic disorders and to discuss on the basis of these examples possible mechanisms that may explain the broad phenotypic spectrum, such as the type of the underlying mutation, the issue of autonomous versus non-autonomous gene expression and the degree of skewing of X-inactivation. The use of the terms "X-linked dominant" and "X-linked recessive" will be discussed.

Keywords

X-linked disorders, metabolic disorders, X-inactivation, pyruvate dehydrogenase, creatine transporter, cholesterol biosynthesis, hypophosphatemic rickets, lysosomal storage disorder.

Introduction

In X-linked disorders an important, but not only, factor for disease expression in heterozygous patients is the degree of X-inactivation skewing. In females, one of the two X-chromosomes in every somatic cell becomes inactive in early embryonic life. The purpose of this mechanism, also called Lyonization, is to prevent female cells from expressing twice the amount of X-linked gene products compared with male cells. X-inactivation is controlled by the non-protein coding RNA gene *XIST*, which is located in the long arm of the X-chromosome. The choice of which of the two X-chromosomes is inactivated is generally random and does not depend on maternal or paternal origin. Skewed X-inactivation, that means the preferential expression (ratio more than 0.5) of either the paternal or the maternal chromosome, is not uncommon in the general female population.[1] Skewing may occur simply by chance[2] or may be influenced by selective pressure, whereby a variant on one of the X-chromosomes is associated with limited survival or lethality and will be subject to negative selection.[3] This phenomenon, which prevents carriers from becoming symptomatic, has been observed for example in incontinentia pigmenti.[4]. However, selection against cells containing the wild type allele on the active X-chromosome has also been reported, such as in cultured fibroblasts and blood cells from females heterozygous for X-linked adrenoleucodystrophy.[5] Since blood cells have a very great number of cell divisions, even a slight selection against the normal allele in blood cells may lead to a non-random X inactivation pattern in these cells.

X-inactivation, however, is not complete, in female cells about one-third of X-chromosome genes are expressed from both, the active and inactive chromosomes.[6]

In general, X-linked diseases are divided in recessive and dominant conditions This classification, however, cannot account for the phenotypic variability observed in female heterozygotes. Although, as expected, disease penetrance and severity are higher in males than in females, a relatively high proportion of X-linked disorders show an intermediate expression in heterozygotes. In order to explain this variable clinical manifestation, Dobyns *et al.* analysed in a pioneering article the pattern of inheritance and phenotypic expression of 32 X-linked diseases.[7] This study revealed several factors that can explain the variable

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clinical expression: These factors include the degree of skewed X-inactivation, the type and severity of the underlying mutation and whether the gene product is cell autonomous or non-autonomous. A gene product is defined as cell autonomous, if it functions independently of other cells within the tissue or organism, and defined as cell non-autonomous, if it functions in cooperation with gene products from other cells. This review aims to discuss these considerations in several X-linked metabolic disorders including defects of energy metabolism, defects of cholesterol biosynthesis, disturbance of phosphate metabolism and lysosomal storage disorders.

Defects of Energy Metabolism

Pyruvate Dehydrogenase Deficiency

The pyruvate dehydrogenase complex (PDC) is a large mitochondrial multienzyme complex, which converts pyruvate to acetyl-CoA, which enters the tricarboxylic acid cycle (TCA) leading to complete oxidation of pyruvate to carbon dioxide and maximum energy production via oxidative phosphorylation. PDC contains three catalytic components, pyruvate dehydrogenase (E1), dihydrolipoamide acetyltranferase (E2) and dihydrolipoamide dehydrogenase (E3) and the non-catalytic E3-binding protein. Pyruvate-dehydrogenase (PDH) consists of two α and two β subunits, whereby the gene encoding the E1α subunit, namely *PDHA1*, could be mapped to the X-chromosome (Xp22.12). The other components of the pyruvate dehydrogenase complex are encoded by autosomal genes.[8]

PDH deficiency leads to decrease of ATP generated from carbohydrates, accumulation of pyruvate and subsequently to lactic acidosis. The effect of PDH depends on the capacity of the cell to undertake aerobic oxidation of carbohydrates, whereby most organs can use alternative energy sources such as amino acids and fatty acids. However, since the central nervous system is a very energy-demanding organ, which is absolutely dependent on using glucose as an energy supplier, brain function is primarily affected in patients with PDH deficiency.

Mutations of the X-chromosomale *PDHA1* gene, encoding the E1α-subunit of pyruvate dehydrogenase, lead to different clinical consequences in males and females. In males, severity depends on the level of residual enzyme activity; males carrying mutations that result in complete loss of enzymatic function die in utero. Other clinical manifestations in boys include neonatal lactic acidosis and encephalopathy, infantile or childhood-onset Leigh syndrome and childhood-onset milder disorder with several neurological symptoms. Females with PDH E1α-deficiency have one normal and one mutated *PDHA1* gene: Cells with the normal, active allele can metabolize lactic acid released by the enzyme-deficient cells, but they cannot provide ATP to these cells; this means that regarding lactic acid level cells are non-autonomous, but regarding ATP production they are autonomous. For this reason, females carrying a severe E1α mutation, for example a frameshift mutation, and having an unfavourable (skewed) X-chromosome inactivation pattern in which predominantly the normal allele is inactivated, will have considerable neurological symptoms and structural brain abnormalities, but only slightly elevated or even normal lactate levels.[9] Females with a mild E1α mutation (leading to a significant residual PDH acitivity) and a favourable X-inactivation with the mutant allele predominantly inactivated, will have only a few, if any, neurological symptoms and almost normal residual ATP levels. The hypothesis that in heterozygotes of E1α-deficiency the degree of clinical manifestation depends not only on the kind of mutation, but also on the skewing of X-inactivation has been supported by observations in many cases, most recently in a report of twins who showed differences in the disease expression: Horga *et al*. described female monozygotic twin females with a missense mutation of the *PDHA1* gene, who presented with a similar clinical phenotype such as developmental delay, seizures and other neurological symptoms, they differed, however, in the severity of clinical manifestation: In the less severely affected twin, a significant imbalance in the X-inactivation with a ratio of 75:25 (normal:mutant allele) was found, whereas this ratio was about 50:50 in the other twin.[10]

Creatine Transporter Defect

Creatine has an essential function in energy metabolism in many organs, particularly in the brain. It plays an important role in storage and transmission of high-energy phosphate (ATP) via its reversible conversion into phosphocreatine, catalyzed by creatine kinase. In addition, creatine mediates the intracellular uptake of neurotransmitters.[11]

Creatine is derived from the diet and endogenously synthesized from arginine and glycine, mainly in kidneys, pancreas and liver. Creatine synthesis takes place also in the central nervous system; the main proportion of brain creatine, however, is taken up from the blood against a large concentration gradient by a specific Na^+/Cl^- dependent transporter (CrT). The CrT transporter is encoded by the *SLC6A8* gene which is mapped to chromosome Xq28.[12]

In a retrospective study of clinical, biochemical and molecular genetic data of 101 hemizygous males with X-linked creatine transporter deficiency van de Kamp *et al*. have demonstrated that in these patients primarily the central nervous system is affected, but other organs may also be involved. The patients` phenotype was characterized by mental retardation, severe speech delay, behaviour problems and seizures. One third of the patients have been reported to suffer from gastrointestinal symptoms consisting of failure to thrive, vomiting or chronic constipation. Magnetic resonance spectroscopy, which was performed in 66 patients, demonstrated an almost total absence of creatine.[13]

There are many reports describing the clinical phenotype of females heterozygous for creatine transporter deficiency, they may be asymptomatic or present as index patients with mild or moderate intellectual disability, behaviour disturbance and even

seizures.[14–16] Van de Kamp and co-authors investigated the clinical phenotype and X-inactivation pattern of eight female heterozygotes.[17] Neurological examination revealed mild cerebellar symptoms in two females, in six patients the IQ scores ranged from the mental retardation range (IQ < 70) to borderline intellectual functioning (IQ 70-85). In six heterozygotes, in whom X-inactivation studies in cultured skin fibroblasts could be performed, a severely skewed pattern, either in favour of the wild-type or of the mutated allele, was detected. In fibroblasts with a normal creatine uptake the wild-type was the most active allele, and vice versa the mutant allele was the most active in cells with creatine uptake deficiency. There was, however, no clear correlation between degree of skewed X-inactivation and severity of symptoms.

Defects of Cholesterol Biosynthesis

Cholesterol fulfils multiple biological functions in cellular as well as in developmental processes. Cholesterol represents the structural lipid in membranes and myelin and is the precursor for the synthesis of bile acids, steroid hormones, neuroactive steroids and oxysterol. In addition, cholesterol is essential for the modification and function of several hedgehog signaling proteins that control embryonic development.[18]

A series of enzymatic reactions is necessary for the conversion of lanosterol - the first sterol formed after cyclization of squalene - to cholesterol, and several genetic defects within these synthetic pathways are known to cause disorders that have in common some clinical features such as congenital skeletal malformations, dysmorphic features and psychomotor retardation.[18] Whereas most of these disorders are inherited in an autosomal-recessive manner, some are X-linked and will be discussed here.

Conradi-Hünermann-Happle and MEND Syndrome

Conradi-Hünermann-Happle syndrome, also named chondrodysplasia punctata 2, X-linked (CDPX2), is caused by pathogenic variants of the emopamil binding protein gene *EBP,* which encodes sterol D8,D7 isomerase, an enzyme involved in the biosynthesis of cholesterol. This disorder is normally associated with male intrauterine lethality. Affected females present at birth with mostly asymmetric skeletal deformities and punctate calcification of cartilaginous structures (chondrodysplasia punctata) and ichthyotic skin lesions, which follow the lines of X-inactivation in the skin, called the lines of Blaschko.[19] In addition, dysmorphic facial features are characteristic for this condition. Asymmetric cataracts and sensorineural hearing loss have also been observed in heterozygous CDPX2 patients. Only a few females have renal or cardiac malformations, intelligence is almost always normal. One has to state, however, that the phenotype of affected females is extremely variable, ranging from early death just after birth to much more mildly affected individuals. The variation of disease expression within the same mutation and the same family can be explained by differences in Lyonization, but also somatic mosaicism and modifier genes may play a role in intrafamilial variation.[20]

Although Conradi-Hünermann-Happle syndrome is usually a lethal prenatal disorder in hemizygous males, cases of patients with Klinefelter syndrome (46,XXY) or somatic mosaicism for a normal *EBP* allele and an apparent postzygotic *EBP* mutation have been identified with features of CDPX2, similar to those seen in heterozygous females.[21]

In addition, hemizygous patients with non-mosaic *EBP*-mutations are known, who show a clinical phenotype different from that of males with a somatic mosaicism: In these males neurological symptoms such as developmental delay, learning difficulties, seizures and severe behavioural difficulties represent the predominant clinical features, but also digital abnormalities, short stature, microcephaly, scoliosis and cerebellar and renal hypoplasia have been observed.[22–25] At birth the patients may show transient erythematous skin lesions or even present as collodion baby.[25,26] It has been proposed to denote these males by the acronym MEND syndrome, for *m*ale *E*BP disorder with neurological defect.[26] Mothers of patients with MEND syndrome are considered to be asymptomatic, but careful examination of carriers often reveals subtle symptoms such as hyperpigmented skin lesions or focal cataract, characteristic of CDPX23.[25,27]

Summarizing published genetic and clinical data, *EBP* mutations can lead to a phenotypic spectrum, probably related to differences in total sterol D8,D7 isomerase activity and zygosity. At one end of this spectrum null mutations lead to male lethality and a severe phenotype of CDPX females, and at the other end hemizygous *EBP* mutations with residual enzyme activity result in the milder phenotype of male patients with MEND syndrome. The most severe cases of MEND syndrome may show phenotypic features characteristic of CDPX, such as skin alterations, skeletal abnormalities or unilateral cataract; they often die in early childhood.[23,26–28]

Because the clinical outcome and prognosis may be different it is important to distinguish between males with Conradi-Hünermann-Happle syndrome that represents a mosaic phenotype, and those with MEND syndrome, that is a non-mosaic trait.

CHILD and CK Syndrome

*C*ongenital *h*emidysplasia with *i*chthyosiform erythroderma and *l*imb *d*efects (CHILD syndrome) is caused by pathogenic variants of the *NSDHL* gene, which encodes the NAD(P)H steroid dehydrogenase-like protein. This protein is a C4-demethylase, an essential enzyme in the cholesterol biosynthesis pathway. CHILD syndrome is lethal in males; in females the disorder is characterized by unilateral inflammatory skin lesions, ipsilateral limb and visceral abnormalities. Skin abnormalties, which affect the right side twice as often as the left side, are present at birth and persist throughout life. The cardiovascular, respiratory, nervous

and renal system may also be involved in females. Intelligence is usually normal, unless there are CNS malformations.[29]

A male with symptoms of CHILD syndrome was described at first by Happle *et al*.[30] and later again by Fackler *et al*.:[31] At birth the patient had a hypoplastic right leg, which required above-the knee amputation, and an erythematous ichthyosiform naevus on the right side. At the age of 24 years the man was in good health, the skin abnormality remained relatively stable over the years, other organs such as the heart, kidneys, brain or eyes were not affected. He had a normal chromosome constitution 46,XY and carried the pathogenic *NSDHL* variant c.262C>T (p.R88X). The authors assume that the occurrence of CHILD syndrome in a male with normal chromosomes could be caused by an early postzygotic mutation that may have occurred between days 22 and 55 of development.[31]

Besides this exceptional case of a man with CHILD syndrome, more males have been observed who as well carry a mutation of the *NSDHL* gene, but have a different phenotype, called CK syndrome (according to the initials of the original proband). Characteristic features of these patients are intellectual disability, seizures, cerebral malformations and behaviour problems such as aggression and attention deficit hyperactivity.[32] Skin abnormalities or other symptoms of CHILD syndrome are absent. Mutation analysis of patients with CK syndrome revealed these pathogenic variants: A missense mutation p.Gly152Asp,[33] a 3-bp deletion (c.696_698del,p.Lys232del)[32] and the frameshift variant 1098_1099insT (R367fs*31).[34] The 3bp deletion and the frameshift mutation are temperature-sensitive variants that lead to almost normal enzyme activity at 30° C, but diminish enzyme stability at 37° C. This may explain why males carrying one of these mutations survive, whereas other deletions, nonsense and frameshift mutations result in CHILD syndrome with male lethality.[32] Mothers of a male with CK syndrome apparently have no physical abnormalities.[35]

Defect of Phosphate Metabolism

Hypophosphatemic Rickets

X-linked hypophosphatemic rickets (XLH) is caused by pathogenic variants of the *PHEX* gene (phosphate regulating endopeptidase homolog X-linked), which is located on chromosome locus Xp22.1 and contains 22 exons. It encodes a membrane-bound endopeptidase that is expressed primarily in osteoblasts of bone and teeth and has the function of regulating the level of the systemic phosphaturic factor FGF-23 (fibroblast growth factor 23). And inactivating *PHEX* variants lead to increased concentration of serum FGF-23, impairing renal phosphate reabsorption on proximal tubular cells cells via FGFR1 (fibroblast growth factor receptor 1) and its co-receptor KLOTHO.[36] The action of FGF-23 is also to reduce the systemic levels of 1,25-dihydroxyvitamin D by directly inhibiting expression of 25-hydroxyvitamin D3 1- alpha-hydroxylase and stimulating the catabolic 24-hydroxylase, resulting in inadequate production of 1,25(OH)2D, which is necessary for optimal enteral calcium and phosphate absorption and to inhibit parathyroid hormone (PTH) secretion.

The clinical manifestation of *PHEX* mutations can be explained by the impaired renal phosphate reabsorption, which results in low serum phosphate levels and elevated alkaline phosphatase.

Leading clinical signs of hypophosphatemic rickets in males and females are skeletal and teeth abnormalities.[37] First symptoms such as bowing of the legs and abnormal walking may become apparent already in the first years of life. Rachitic rosary, dolichocephaly, bone pain and growth retardation are further symptoms in children. Dental abnormalities such as malposition of teeth and enamel hypoplasia may cause frequent abscesses. Radiological examination of the skeleton shows typical features of rickets without the significant bone resorption that characterises rickets secondary to vitamin D-deficiency. Adult patients with *PHEX* mutations usually show symptoms of defective bone mineralization (osteomalacia), resulting in bone and joint pain and frequently to fractures. Clinical manifestations include short stature and musculoskeletal pain, too. In later life, often calcification of tendons and ligaments develops causing enthesopathy. Severity of the disease and specific clinical manifestations are variable even among members of the same family.

In an analysis of *PHEX* mutations in 50 patients with XLH (males and females) Holm *et al*. did not detect a correlation between the severity of the disease and the type or location of the mutation.[38] They found, however, a trend toward more severe dental disease in males in comparison with females. A similar observation was made by Shields *et al*.:[39] These authors determined the severity of dental manifestation by measuring the pulp profile area (PRATIO [= pulp area/tooth area]), whereby higher PRATIO values mean less mineralization of secondary dentin. And their study revealed that males had significantly higher PRATIO values than females, indicating a gene dosage effect. Hemizygotes and heterozygotes, however, did not differ in serum phosphate levels. According to this finding Reid *et al*. did not observe a significant sex difference in renal phosphate reabsorption, that means that in XLH renal phosphate transport does not exhibit gene dosage.[40] This can be explained by results of experiments that have been performed in animals: It is known, that the proximal nephron has a low-affinity phosphate transport function with high capacity and an high-affinity transport function with low capacity.[41] And studies in mice carrying a mutation of X-linked hypophosphatemic rickets (*Hyp* mice), have demonstrated that the mutation affects only the high-affinity system that has a low capacity.[42] One may speculate that the capacity of this transporter is exhausted even at half of the gene dosage, leading to a dominant phenotypic expression. However, the effect on tubular phosphate resorption could also be explained by the increased FGF23 serum concentration which is an indirect mechanism, and not because of a direct effect related to the X-gene linked PHEX mutation.

In order to prove whether the apparent lack of gene dosage is due to skewed X inactivation, Ørstavik *et al*. have examined the X-inactivation pattern in 12 females affected by XLH and found that their patterns did not differ significantly from that in 30 healthy females. Based on this result it is unlikely that a ubiquitous preferential inactivation of the X chromososome carrying the wild type allele is the explanation for lack of dosage effect on the renal defect in XLH.[43]

In summary, clinical and biochemical investigations have revealed that in XLH a gene dose effect seems to be present regarding the dental manifestation, whereas such an effect is absent in the renal phosphate transport system. The phenotype of XLH seems to be fully dominant with respect to phosphate transport, either because it affects only the high-affinity phosphate transport system or is mediated by the (indirect) FGF23 mechanism.[44]

Lysosomal Storage Disorders

There are three lysosomal storage disorders that are inherited in an X-linked manner: Mucopolysaccharidosis type II (Hunter syndrome), Fabry disease and Danon disease. They differ in the phenotypic expression of female heterozygotes, whereby carriers of Hunter syndrome in general (with very rare exceptions) are asymptomatic, in Fabry and Danon disease, however, heterozogytes show clinical manifestations in variable degree. The possible pathogenic mechanisms, that may explain this difference, will be discussed below.

Mucopolysaccharidosis Type II (Hunter Syndrome)

Mucopolysaccharidosis type II (Hunter syndrome) is based on the deficiency of the lysosomal enzyme iduronate-2-sulfatase, which is responsible for the degradation of glycosaminoglycans. A defect of this enzyme, caused by mutations of the *IDS* gene, results in the accumulation of glycosaminoglycans in several tissues and finally to clinical manifestations in multiple organs. Clinical symptoms include hepatosplenomegaly, joint contractures, skeletal abnormalities, cardiomyopathy and in many patients also involvement of the central nervous system, leading to mental retardation,[45] whereby almost exclusively males are affected. Mothers of patients are simply carriers without any clinical signs or symptoms as has been convincingly shown by careful clinical, biochemical and genetic investigations in 22 heterozygous females.[46] Clinical manifestation of Hunter syndrome in females represent exceptions that can be explained by different mechanisms such as homozygosity, abnormalities of the X-chromosome such for example Turner syndrome or by almost totally X-inactivation of the X-chromosome that bears the wild-type *IDS* allele.[47]

Fabry Disease

Fabry disease is a multisystemic disorder, caused by mutations of the *GLA* gene, that encodes the lysosomal enzyme α-galactosidase, in which skin lesions (angiokeratomata), recurrent burning pain (acroparaesthesia), hypohidrosis, progressive impairment of renal and cardiac function and cerebral ischaemia are the leading clinical features.[48] Heterozygous females are not merely carriers, but often show pathological manifestations in very variable degree.[49]

To determine whether preferential X-inactivation affecting the wild type *GLA* locus contributes to the pathological manifestations in Fabry heterozygotes, the validated Mainz severity score values were compared with the X-inactivation status in 39 females. Heterozygotes, in whom predominantly the X-chromosome carrying the wild-type *GLA* allele was inactivated, were more severely affected than those without biased X-inactivation.[50] These results support the hypothesis that the extent of X-inactivation of the wild-type or mutant allele has a substantial influence on the severity of clinical manifestations in Fabry heterozygotes.

Now the question arises why in Fabry disease heterozygous females often show clinical manifestations (albeit to variable degree), whereas in Hunter syndrome heterozygotes (with a few exceptions) are asymptomatic carriers. One possible explanation could be that the differences in phenotypic expression are due to differences in the biological properties of α-galactosidase and iduronate-2-sulfatase. This hypothesis was confirmed by experiments performed Fuller and co-workers in which they analyzed secretion and re-uptake of α-galactosidase in cultured fibroblasts.[51] In their studies the authors have demonstrated, that unaffected fibroblasts predominantly secrete the mature 46kDa α-galactosidase, which cannot be efficiently endocytosed by affected fibroblasts as the enzyme lacks the mannose 6-phosphorylated moiety; consequently normal cells are unable to cross-correct Faby cells. Also in control plasma primarily the mature 46 kDa enzyme, and not the high uptake, mannose 6-phosphorylated 52 kDa form was detected. Enzyme replacement therapy, however, works, as the recombinant enzyme preparations consist of the 52 kDa high uptake form, containing numerous mannose 6-phosphorylated moieties.

Using an artificial *in cellulo* technique, no complementary functional cross-correction (degradation of the storage material ceramide trihexoside) in the Fabry system was observed, whereas cross-correction (glycosaminoglycan degradation) was seen in Hunter fibroblasts.[51]

These findings indicate that in contrast to the situation in Hunter syndrome, Fabry heterozygotes show clinical manifestation, because *in vivo* the unaffected cells secrete the mature, and not the mannose-6-phosphorylated form of α-galactosidase, that would be able to complement the activity in the population of cells lacking expression of the enzyme. This means that – by using the term that has been introduced by Dobyns *et al*.[7] – in Fabry disease the gene product is operationally cell autonomous, as it cannot be readily complemented in the presence of wild type cells.

As cross-correction is absent in females with α-galactosidase deficiency, the degree of disease expression will preferentially

depend on the degree of X-inactivation - as was unambiguously demonstrated by Echvarria *et al*.:[52] In their study, in which the X-inactivation of 65 females with Fabry disease was investigated using DNA methylation analysis, it could be demonstrated that heterozygous female Fabry patients with skewed X-inactivation profiles differed markedly in the severity of their clinical manifestations and in a manner, that was directly related to the parenteral *GLA* allele, that was most frequently inactivated: Inactivation of the mutant allele leads to a mild phenotype, and inactivation of the wild-type *GLA* allele induces disease with an earlier onset and worse prognosis.[52]

Danon Disease

The lysosomal storage disorder Danon disease is caused by the genetic defect of the lysosome-associated membrane protein LAMP-2, that leads to a block in autophagy resulting in impaired fusion of autophagosome-lysosome and to insufficient lysosomal function.[53] Clinically, Danon disease is characterized by severe cardiomyopathy, skeletal myopathy and intellectual disability. Other symptoms include hepatopathy, retinal dystrophy and pulmonary disease.[54]

Brambatti *et al*. analyzed variability in age of onset and progression of Danon disease in males and females through a systematic review of all published cases. Whereas males showed a multisystemic manifestation including hypertrophic cardiomyopathy, skeletal myopathy and cognitive disability, females showed a more variable phenotypic expression with isolated cardiac disease (either hypertrophic or dilated cardiomyopathy). There was no sex difference regarding the composite outcome of death, heart transplantation or ventricular assist devices, but these events occurred later in heterozygotes (median age 38 years) than in males (median age 21 years). The observation that in the majority of female patients cardiac, and not muscular manifestations are present, can be explained by the fact that in case of random X-inactivation the overlap of nuclear domains can rescue LAMP2 expression in skeletal muscle fibres, but not in cardiomyocytes which do not have the ability of regeneration.[55]

There are several factors that contribute to the variable clinical manifestation observed in females with Danon disease. One factor seems to be the extent of X-inactivation. In Danon disease the gene product LAMP-2 is not a soluble, but a membrane bound protein which cannot be compensated by adjacent normal cells; in heterozygotes the pattern of X-inactivation apparently determines the disease severity. This pathogenetic mechanism has been confirmed in some cases: In a family, studied by Xu *et al*., one female carrier, who had a skewed X-inactivation (in favour of the unaffected chromosome) showed a normal heart size, whereas the other female carrier showed a hypertrophic left ventricle, left ventricular diastolic dysfunction and decreased *LAMP2* expression.[56]

Bottillo described a heterozygous Danon patient who required heart transplantation because of severe hypertrophic cardiomyopathy and rapid progression to heart failure. By immunohistochemical analysis of LAMP2 a mosaic pattern of distribution was detected with discrete groups of either stained or unstained cardiac myocytes. These findings corresponded to X-chromosome inactivation within the myocardium.[57] A mosaic pattern was detected also in the retinal pigment epithelium, indicating a Lionization in the eyes, too.[58]

In the muscle and leukocytes of a female affected by Danon disease, however, random X-inactivation was found, therefore it seems to be unlikely, that the severity of organ involvement in female patients depends solely on the extent of X-inactivation.[59] This is confirmed by the observation of Hashida *et al*., who described two monozygotic twin sisters with identical LAMP2 expression in leucocytes of which one had heart symptoms, whereas the other was asymptomatic.[60]

Based on the reports presented here it seems that in Danon disease there are many pathogenic mechanisms such as the type of mutation, the extent of X-inactivation, genetic modifiers and lifestyle, that are responsible for the variable phenotypic expression in heterozygous females. Further clarification of the pathogenesis underlying Danon disease is required.

Discussion

In order to understand the pathophysiological mechanisms that lead to variable disease expression in X-linked metabolic disorders, one has to take into account that thereby several factors play a role. These include the X-inactivation pattern, the type of mutation and the issue of whether the function of the gene product is cell autonomous or non-autonomous.

In some diseases, the gene product has several functions; one function can be autonomous, and the other part non-autonomous. This is the case in pyruvate-dehydrogenase deficiency, in which the lactate level is non-autonomous, but the ATP production is autonomous. This explains why females with a severe mutation E1α mutation and an unfavourable (skewed) X chromosome inactivation profile, in which predominantly the normal allele is inactivated, have substantial neurological symptoms, but only slightly elevated or even normal lactate levels.[9]

Furthermore, the gene product may function differently in different organs: In carriers of Danon disease the X-inactivation pattern contributes to the degree of clinical manifestation, because the gene product LAMP-2 is a membrane bound, not soluble protein, which means it is autonomous and cannot be compensated by normal cells. However, the overlap of nuclear domains can rescue LAMP2 expression in skeletal muscle fibres, but not in cardiomyocytes, which cannot regenerate, explaining why the majority of female patients show cardiac, and not muscular manifestation.[55]

Although the lysosomal enzymes that are deficient in Hunter syndrome and Fabry disease, namely iduronate-2-sulfatase and α-galactosidase respectively, are soluble proteins, carriers of Hunter syndrome are asymptomatic, whereas heterozygotes of Fabry disease have symptoms to variable degree. This

discrepancy can be explained by the fact that iduronate-2-sulfatase and α-galactosidase differ in their biological properties: In Hunter syndrome an exchange of iduronate-2-sulfatase between unaffected and affected cells can take place, enabling complementation of enzyme activity in females with a skewed X-inactivation favouring the mutant cells. In Fabry disease, however, the unaffected cells do not secrete the mannose-6-phosphorylated, but the mature form of α-galactosidase, which cannot complement the activity in cells lacking expression of the enzyme: Carriers of Fabry disease show clinical manifestation, because the gene product (α-galactosidase) is operationally cell autonomous (although it is a soluble protein) as it cannot be readily complemented in the presence of wild type cells.

Mutations of the *EBP* gene, that encodes the enzyme sterol D8,D7 isomerase, can be responsible for a broad, continuous spectrum of phenotypes: Null mutations lead to skeletal deformities and characteristic skin lesions in females, for male hemizygotes they are lethal. This disorder has been termed X-linked dominant Conradi-Hünermann-Happle syndrome (CDPX2).

Males with the so-called MEND syndrome carry *EBP* mutations that result in residual activity of sterol D8,D7 isomerase, they have predominantly neurological symptoms, but often also dysmorphic features. Not surprisingly, their mothers have only subtle symptoms. MEND syndrome has been designated to be an X-linked recessive disorder. As most severe cases of MEND syndrome, however, show phenotypic features characteristic of CDPX2, a clear differentiation between Conradi-Hünermann-Happle syndrome and MEND syndrome seems not to be possible in some cases; the terms X-linked dominant and X-linked recessive apparently are not appropriate. The same applies to CHILD and CK syndrome: Both disorders are caused by mutations of the *NSDHL* gene, severe mutations (deletions, nonsense and frameshift mutations) are associated with male lethality and dysmorphic features of the females (CHILD syndrome), and milder mutations (missense mutations or temperature-sensitive variants) lead to predominantly neurological manifestations in males and asymptomatic females (CK syndrome). Both disorders are X-linked disorders with a broad spectrum of mutations of the *NSDHL* gene, leading to a great variability of symptoms.

It has recently been detected in X-linked and autosomal-recessive disorders that different mutations of a gene can result in a great diversity of organ manifestations.

Examples are the diverse phenotypes that can arise from different mutations of the ceramidase *ASAH1* gene. The most severe form, named Farber lipogranulomatosis, is characterized by skin nodules and a progressive hoarseness, often also the central nervous system is affected, the patients die in early childhood. In other patients, who carry mutations different from those of Farber disease, neurological symptoms such as spinal muscular atrophy and myoclonus epilepsy dominate the clinical picture (SMA-PME).[61]

Mutations of the genes *GNPTAB* and *GNPTG*, which are responsible for postranslational modification of lysosomal enzymes (attachment of the mannose-6-phosphate moiety), lead to the well known lysosomal storage disorders mucolipidosis II and III, but some mutations have been found in patients who suffered from stuttering, but had no further symptoms.[62]

In X-linked hypophosphatemic rickets pathogenic *PHEX* variants lead to impaired renal phosphate reabsorption, resulting in low serum phosphate levels and clinical manifestation such as skeletal and teeth abnormalities in both, males and females. Whereas a trend toward a more severe dental disease in male in comparison with female patients has been observed, a significant sex difference in renal phosphate reabsorption was not found, probably because the mutation affects only the low-capacity phosphate transport system, which does not exhibit gene dosage, or because the high FGF23 serum concentration inhibits tubular phosphate resorption that would be an indirect mechanism. In summary it seems that X-linked hypophosphatemic rickets acts as an X-linked recessive disorder regarding the teeth manifestation, but to be fully dominant with respect to phosphate transport.

Creatine transporter fulfils an essential function in energy metabolism, it supports the delivery of creatine to many organs, predominantly to the brain. This explains why in male patients the central nervous system is primarily affected, whereas females may be asymptomatic or may show intellectual disability or even seizures. In heterozygotes a correlation between the disease severity and degree of skewed X-inactivation has not been observed, although in fibroblasts of these patients a correlation between the level of creatine uptake and X-inactivation was found. The pathophysiology of clinical manifestation of carriers of creatine-transporter defect apparently needs further investigations.

The examples of X-linked metabolic disorders, presented here, illustrate that several factors contribute to the clinical manifestation of females in these conditions. Careful analysis of the genetic and pathophysiological mechanisms may help to understand why in some X-linked disorders heterozygotes show symptoms in different degree and in others carriers are always asymptomatic. The X-inactivation profile plays an important, but not the unique role in the degree of disease severity in females. The contribution of the X-inactivation pattern to the clinical manifestation is questionable in some X-linked disorders: In fibroblasts of carriers of creatine-transporter defect, for example, a clear correlation of the level of creatine uptake with the X-inactivation pattern was found, whereas a correlation between the disease severity and degree of skewed X-inactivation has not been observed.

A further issue is whether the gene product is cell autonomous or non-autonomous as this has an impact on the clinical manifestation in heterozygotes. A gene product may have different functions in different cells or organs, respectively, meaning, that it may be cell autonomous in one cell type/organ, and non-autonomous in another cell type/organ, as it has been

discussed in pyruvate dehydrogenase deficiency, X-linked hypophosphatemic rickets and Danon disease.

In summary, it can be concluded that it is not possible to assign an X-linked metabolic disorder clearly to a dominant or recessive type. For each X-linked disorder one has to analyse genetic and pathophysiological mechanisms that may explain why and to what degree females are affected. This is in accord with Dobyns *et al*. who declared: "We recommend that use of the terms X-linked recessive and dominant be discontinued, and that all such disorders be simply described as following "X-linked" inheritance".[7]

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References

1. Shvetsova E, Sofronova A, Monajemi R, *et al*. Skewed X-inactivation is common in the general female population. *Eur J Hum Genet*. 2019;27(3):455-465. doi: 10.1038/s41431-018-0291-3
2. Orstavik KH. X chromosome inactivation in clinical practice. *Hum Genet*. 2009;126(3):363-373. doi: 10.1007/s00439-009-0670-5
3. van den Veyver IB. Skewed X inactivation in X-linked disorders. *Semin Reprod Med*. 2001;19(2):183-191. doi: 10.1055/s-2001-15398
4. Migeon BR, Axelman J, Jan de Beur S, *et al*. Selection against lethal alleles in females heterozygous for incontinentia pigmenti. *Am J Hum Genet*. 1989;44(1):100-106. https://www.ncbi.nlm.nih.gov/pmc/articles/pmc1715454/
5. Migeon BR, Moser HW, Moser AB, *et al*. Adrenoleukodystrophy: evidence for X linkage, inactivation, and selection favoring the mutant allele in heterozygous cells. *Proc Natl Acad Sci U S A*. 1981;78(8):5066-5070. doi: 10.1073/pnas.78.8.5066
6. Tukiainen T, Villani A-C, Yen A, *et al*. Landscape of X chromosome inactivation across human tissues. *Nature*. 2017;550(7675):244-248. doi: 10.1038/nature24265
7. Dobyns WB, Filauro A, Tomson BN, *et al*. Inheritance of most X-linked traits is not dominant or recessive, just X-linked. *Am J Med Genet A*. 2004;129A(2):136-143. doi: 10.1002/ajmg.a.30123
8. Pavlu-Pereira H, Silva MJ, Florindo C, *et al*. Pyruvate dehydrogenase complex deficiency: updating the clinical, metabolic and mutational landscapes in a cohort of Portuguese patients. *Orphanet J Rare Dis*. 2020;15(1):298. doi: 10.1186/s13023-020-01586-3
9. Dahl HH. Pyruvate dehydrogenase E1 alpha deficiency: males and females differ yet again. *Am J Hum Genet*. 1995;56(3):553-557. https://www.ncbi.nlm.nih.gov/pmc/articles/pmc1801181/
10. Horga A, Woodward CE, Mills A, *et al*. Differential phenotypic expression of a novel PDHA1 mutation in a female monozygotic twin pair. *Hum Genet*. 2019;138(11-12):1313-1322. doi: 10.1007/s00439-019-02075-9
11. Colas C, Banci G, Martini R, *et al*. Studies of structural determinants of substrate binding in the Creatine Transporter (CreaT, SLC6A8) using molecular models. *Sci Rep*. 2020;10(1):6241. doi: 10.1038/s41598-020-63189-z
12. van de Kamp JM, Mancini GM, Salomons GS. X-linked creatine transporter deficiency: clinical aspects and pathophysiology. *J Inherit Metab Dis*. 2014;37(5):715-733. doi: 10.1007/s10545-014-9713-8
13. van de Kamp JM, Betsalel OT, Mercimek-Mahmutoglu S, *et al*. Phenotype and genotype in 101 males with X-linked creatine transporter deficiency. *J Med Genet*. 2013;50(7):463-472. doi: 10.1136/jmedgenet-2013-101658
14. Valayannopoulos V, Boddaert N, Chabli A, *et al*. Treatment by oral creatine, L-arginine and L-glycine in six severely affected patients with creatine transporter defect. *J Inherit Metab Dis*. 2012;35(1):151-157. doi: 10.1007/s10545-011-9358-9
15. Cheillan D, Joncquel-Chevalier Curt M, Briand G, *et al*. Screening for primary creatine deficiencies in French patients with unexplained neurological symptoms. *Orphanet J Rare Dis*. 2012;7:96. doi: 10.1186/1750-1172-7-96
16. Mercimek-Mahmutoglu S, Connolly MB, Poskitt KJ, *et al*. Treatment of intractable epilepsy in a female with SLC6A8 deficiency. *Mol Genet Metab*. 2010;101(4):409-412. doi: 10.1016/j.ymgme.2010.08.016
17. van de Kamp JM, Mancini GM, Pouwels PJ, *et al*. Clinical features and X-inactivation in females heterozygous for creatine transporter defect. *Clin Genet*. 2011;79(3):264-272. doi: 10.1111/j.1399-0004.2010.01460.x
18. Porter FD, Herman GE. Malformation syndromes caused by disorders of cholesterol synthesis. *J Lipid Res*. 2011;52(1):6-34. doi: 10.1194/jlr.r009548
19. Happle R. Mosaicism in human skin. Understanding the patterns and mechanisms. *Arch Dermatol*. 1993;129(11):1460-1470. doi: 10.1001/archderm.1993.01680320094012
20. Has C, Bruckner-Tuderman L, Muller D, *et al*. The Conradi-Hunermann-Happle syndrome (CDPX2) and emopamil binding protein: novel mutations, and somatic and gonadal mosaicism. *Hum Mol Genet*. 2000;9(13):1951-1955. doi: 10.1093/hmg/9.13.1951

21. Canueto J, Giros M, Gonzalez-Sarmiento R. The role of the abnormalities in the distal pathway of cholesterol biosynthesis in the Conradi-Hunermann-Happle syndrome. *Biochim Biophys Acta*. 2014;1841(3):336-344. doi: 10.1016/j.bbalip.2013.09.002

22. Barboza-Cerda MC, Campos-Acevedo LD, Rangel R, *et al*. A novel phenotype characterized by digital abnormalities, intellectual disability, and short stature in a Mexican family maps to Xp11.4-p11.21. *Am J Med Genet A*. 2013;161A(2):237-243. doi: 10.1002/ajmg.a.35743

23. Barboza-Cerda MC, Wong LJ, Martinez-de-Villarreal LE, *et al*. A novel EBP c.224T>A mutation supports the existence of a male-specific disorder independent of CDPX2. *Am J Med Genet A*. 2014;164A(7):1642-1647. doi: 10.1002/ajmg.a.36508

24. Hartill VL, Tysoe C, Manning N, *et al*. An unusual phenotype of X-linked developmental delay and extreme behavioral difficulties associated with a mutation in the EBP gene. *Am J Med Genet A*. 2014;164A(4):907-914. doi: 10.1002/ajmg.a.36368

25. de Almeida H Jr., Has C, Rampon G, *et al*. MEND Syndrome: A Case Report with Scanning Electron Microscopy Findings of the Collodion Membrane. *Acta Derm Venereol*. 2017;97(1):110-111. doi: 10.2340/00015555-2477

26. Arnold AW, Bruckner-Tuderman L, Has C, *et al*. Conradi-Hunermann-Happle syndrome in males vs. MEND syndrome (male EBP disorder with neurological defects). *Br J Dermatol*. 2012;166(6):1309-1313. doi: 10.1111/j.1365-2133.2012.10808.x

27. Milunsky JM, Maher TA, Metzenberg AB. Molecular, biochemical, and phenotypic analysis of a hemizygous male with a severe atypical phenotype for X-linked dominant Conradi-Hunermann-Happle syndrome and a mutation in EBP. *Am J Med Genet A*. 2003;116A(3):249-254. doi: 10.1002/ajmg.a.10849

28. Barboza-Cerda MC, Barboza-Quintana O, Martinez-Aldape G, *et al*. Phenotypic severity in a family with MEND syndrome is directly associated with the accumulation of potentially functional variants of cholesterol homeostasis genes. *Mol Genet Genomic Med*. 2019;7(9):e931. doi: 10.1002/mgg3.931

29. Hettiarachchi D, Panchal H, Lai PS, *et al*. Novel variant in NSDHL gene associated with CHILD syndrome and syndactyly- a case report. *BMC Med Genet*. 2020;21(1):164. doi: 10.1186/s12881-020-01094-y

30. Happle R, Effendy I, Megahed M, *et al*. CHILD syndrome in a boy. *Am J Med Genet*. 1996;62(2):192-194. doi: 10.1002/(sici)1096-8628(19960315)62:2<192::aid-ajmg14>3.0.co;2-j

31. Fackler N, Zachary C, Kim DJ, *et al*. Not lost to follow-up: A rare case of CHILD syndrome in a boy reappears. *JAAD Case Rep*. 2018;4(10):1010-1013. doi: 10.1016/j.jdcr.2018.10.001

32. McLarren KW, Severson TM, du Souich C, *et al*. Hypomorphic temperature-sensitive alleles of NSDHL cause CK syndrome. *Am J Hum Genet*. 2010;87(6):905-914. doi: 10.1016/j.ajhg.2010.11.004

33. Preiksaitiene E, Caro A, Benusiene E, *et al*. A novel missense mutation in the NSDHL gene identified in a Lithuanian family by targeted next-generation sequencing causes CK syndrome. *Am J Med Genet A*. 2015;167(6):1342-1348. doi: 10.1002/ajmg.a.36999

34. Tarpey PS, Smith R, Pleasance E, *et al*. A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. *Nat Genet*. 2009;41(5):535-543. doi: 10.1038/ng.367

35. du Souich C, Chou A, Yin J, *et al*. Characterization of a new X-linked mental retardation syndrome with microcephaly, cortical malformation, and thin habitus. *Am J Med Genet A*. 2009;149A(11):2469-2478. doi: 10.1002/ajmg.a.33071

36. Jagtap VS, Sarathi V, Lila AR, *et al*. Hypophosphatemic rickets. *Indian J Endocrinol Metab*. 2012;16(2):177-182. doi: 10.4103/2230-8210.93733

37. Emma F, Cappa M, Antoniazzi F, *et al*. X-linked hypophosphatemic rickets: an Italian experts' opinion survey. *Ital J Pediatr*. 2019;45(1):67. doi: 10.1186/s13052-019-0654-6

38. Holm IA, Nelson AE, Robinson BG, *et al*. Mutational analysis and genotype-phenotype correlation of the PHEX gene in X-linked hypophosphatemic rickets. *J Clin Endocrinol Metab*. 2001;86(8):3889-3899. doi: 10.1210/jcem.86.8.7761

39. Shields ED, Scriver CR, Reade T, *et al*. X-linked hypophosphatemia: the mutant gene is expressed in teeth as well as in kidney. *Am J Hum Genet*. 1990;46(3):434-442. https://www.ncbi.nlm.nih.gov/pmc/articles/pmc1683613/

40. Reid IR, Hardy DC, Murphy WA, *et al*. X-linked hypophosphatemia: a clinical, biochemical, and histopathologic assessment of morbidity in adults. *Medicine (Baltimore)*. 1989;68(6):336-352. doi: 10.1016/0002-9343(91)90507-t

41. Walker JJ, Yan TS, Quamme GA. Presence of multiple sodium-dependent phosphate transport processes in proximal brush-border membrane. *Am J Physiol*. 1987;252(2 Pt 2):F226-231. doi: 10.1152/ajprenal.1987.252.2.F226

42. Tenenhouse HS, Klugerman AH, Neal JL. Effect of phosphonoformic acid, dietary phosphate and the Hyp mutation on kinetically distinct phosphate transport processes in mouse kidney. *Biochim Biophys Acta*. 1989;984(2):207-213. doi: 10.1016/0005-2736(89)90218-6

43. Orstavik KH, Orstavik RE, Halse J, *et al*. X chromosome inactivation pattern in female carriers of X linked hypophosphataemic rickets. *J Med Genet*. 1996;33(8):700-703. doi: 10.1136/jmg.33.8.700

44. Scriver CR, Tenenhouse HS. X-linked hypophosphataemia: a homologous phenotype in humans and mice with unusual organ-specific gene dosage. *J Inherit Metab Dis*. 1992;15(4):610-624. doi: 10.1007/bf01799618

45. Martin R, Beck M, Eng C, *et al*. Recognition and diagnosis of mucopolysaccharidosis II (Hunter syndrome). *Pediatrics*. 2008;121(2):e377-386. doi: 10.1542/peds.2007-1350

46. de Camargo Pinto LL, Maluf SW, Leistner-Segal S, *et al*. Are MPS II heterozygotes actually asymptomatic? A study based on clinical and biochemical data, X-inactivation analysis and imaging evaluations. *Am J Med Genet A*. 2011;155A(1):50-57. doi: 10.1002/ajmg.a.33770

47. Semyachkina AN, Voskoboeva EY, Zakharova EY, *et al*. Case report: a rare case of Hunter syndrome (type II mucopolysaccharidosis) in a girl. *BMC Medical Genetics*. 2019;20(1):66. doi: 10.1186/s12881-019-0807-x

48. Miller JJ, Kanack AJ, Dahms NM. Progress in the understanding and treatment of Fabry disease. *Biochim Biophys Acta Gen Subj*. 2020;1864(1):129437. doi: 10.1016/j.bbagen.2019.129437

49. Whybra C, Kampmann C, Willers I, *et al*. Anderson-Fabry disease: clinical manifestations of disease in female heterozygotes. *J Inherit Metab Dis*. 2001;24(7):715-724. doi: 10.1023/a:1012993305223

50. Dobrovolny R, Dvorakova L, Ledvinova J, *et al*. Relationship between X-inactivation and clinical involvement in Fabry heterozygotes. Eleven novel mutations in the alpha-galactosidase A gene in the Czech and Slovak population. *J Mol Med*. 2005;83(8):647-654. doi: 10.1007/s00109-005-0656-2

51. Fuller M, Mellett N, Hein LK, *et al*. Absence of alpha-galactosidase cross-correction in Fabry heterozygote cultured skin fibroblasts. *Mol Genet Metab*. 2015;114(2):268-273. doi: 10.1016/j.ymgme.2014.11.005

52. Echevarria L, Benistan K, Toussaint A, *et al*. X-chromosome inactivation in female patients with Fabry disease. *Clin Genet*. 2016;89(1):44-54. doi: 10.1111/cge.12613

53. Rowland TJ, Sweet ME, Mestroni L, *et al*. Danon disease - dysregulation of autophagy in a multisystem disorder with cardiomyopathy. *J Cell Sci*. 2016;129(11):2135-2143. doi: 10.1242/jcs.184770

54. D'Souza R S, Levandowski C, Slavov D, *et al*. Danon disease: clinical features, evaluation, and management. *Circ Heart Fail*. 2014;7(5):843-849. doi: 10.1161/circheartfailure.114.001105

55. Brambatti M, Caspi O, Maolo A, *et al*. Danon disease: Gender differences in presentation and outcomes. *Int J Cardiol*. 2019;286:92-98. doi: 10.1016/j.ijcard.2019.01.020

56. Xu J, Wang L, Liu X, *et al*. A novel LAMP2 p.G93R mutation associated with mild Danon disease presenting with familial hypertrophic cardiomyopathy. *Mol Genet Genomic Med*. 2019;7(10):e00941. doi: 10.1002/mgg3.941

57. Bottillo I, Giordano C, Cerbelli B, *et al*. A novel LAMP2 mutation associated with severe cardiac hypertrophy and microvascular remodeling in a female with Danon disease: a case report and literature review. *Cardiovascular Pathology*. 2016;25(5):423-431. doi: 10.1016/j.carpath.2016.07.005

58. Prall FR, Drack A, Taylor M, *et al*. Ophthalmic manifestations of Danon disease. *Ophthalmology*. 2006;113(6):1010-1013. doi: 10.1016/j.ophtha.2006.02.030

59. Fanin M, Nascimbeni AC, Fulizio L, *et al*. Generalized lysosome-associated membrane protein-2 defect explains multisystem clinical involvement and allows leukocyte diagnostic screening in Danon disease. *Am J Pathol*. 2006;168(4):1309-1320. doi: 10.2353/ajpath.2006.050646

60. Hashida Y, Wada T, Saito T, *et al*. Early diagnosis of Danon disease: Flow cytometric detection of lysosome-associated membrane protein-2-negative leukocytes. *J Cardiol*. 2015;66(2):168-174. doi: 10.1016/j.jjcc.2014.09.011

61. Yu FPS, Amintas S, Levade T, *et al*. Acid ceramidase deficiency: Farber disease and SMA-PME. *Orphanet J Rare Dis*. 2018;13(1):121. doi: 10.1186/s13023-018-0845-z

62. Raza MH, Domingues CE, Webster R, *et al*. Mucolipidosis types II and III and non-syndromic stuttering are associated with different variants in the same genes. *Eur J Hum Genet*. 2016;24(4):529-534. doi: 10.1038/ejhg.2015.154