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Linear Skin Defects with Multiple Congenital Anomalies (LSDMCA): an unconventional mitochondrial disorder

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Abstract

Mitochondrial disorders, although heterogeneous, are traditionally described as conditions characterized by encephalomyopathy, hypotonia and progressive postnatal organ failure. Here we provide a systematic review of Linear Skin Defects with Multiple Congenital Anomalies (LSDMCA), a rare unconventional mitochondrial disorder which presents as a developmental disease; its main clinical features include microphthalmia with different degrees of severity, linear skin lesions, and central nervous system malformations. The molecular basis of this disorder has been elusive for several years. Mutations were eventually identified in three X-linked genes, i.e., *HCCS*, *COX7B*, and *NDUFB11*, which are all endowed with defined roles in the mitochondrial respiratory chain. A peculiar feature of this condition is its inheritance pattern: X-linked dominant male-lethal. Only female or XX male individuals can be observed, implying that nullisomy for these transcripts is incompatible with normal embryonic development in mammals. All three genes undergo X-inactivation that, according to our hypothesis, may contribute to the extreme variable expressivity observed in this condition. We propose that mitochondrial dysfunction should be considered as an underlying cause in developmental disorders. Moreover, LSDMCA should be taken into consideration by clinicians when dealing with patients with microphthalmia with or without associated skin phenotypes.

Keywords: MLS/MIDAS/LSDMCA, X-inactivation, HCCS, COX7B, NDUFB11, mitochondrial disorders, mitochondrial respiratory chain, microphthalmia, linear skin defects

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Introduction: an historical perspective

In the early 90s, a number of females were observed with a specific phenotypic combination of short stature with developmental abnormalities, including microphthalmia and linear skin defects of the face and neck. In these cases, terminal or interstitial deletions and unbalanced translocations of the short arm of the X-chromosome resulted in monosomy of the Xp22.3 region [1]. In males, the lack of distal Xp results in contiguous gene syndromes characterized by the appearance of recessive traits underlain by a number of genes disrupted by the X-chromosome abnormalities. However, apart from 8 males showing a 46 XX karyotype and a translocation Xp/Yp (see below), no males with the typical ocular and skin phenotype have been described to date. The condition defined by the clinical features observed in females was denominated Microphthalmia with Linear Skin defects (MLS) syndrome (MIM # 309801). Given the presence of aborted fetuses in familial cases and the absence of males with X chromosomal rearrangements and a classical MLS phenotype, it was defined as an X-linked dominant male-lethal trait. An alternative name proposed for the disorder was Microphthalmia Dermal Aplasia and Sclerocornea (MIDAS) syndrome. This condition was subsequently demonstrated to be genetically heterogeneous and following identification of three responsible genes (see below), this rare genetic disease has been renamed Linear Skin Defects with Multiple Congenital Anomalies (LSDMCA) 1, 2 and 3 (OMIM # 309801, OMIM # 300887 and OMIM # 300952), respectively.

A deletion mapping approach combined with a positional candidate gene strategy allowed the identification of the first disease gene, *HCCS* (holocytochrome c synthase; OMIM # 300056) from the Xp22.2 region in 2006 [2]. Subsequently, in 2012, a candidate gene strategy led to the identification of mutations in *COX7B* (cytochrome c oxidase subunit 7B; OMIM # 300805) localized to Xq21.1 [3]. Finally, in 2015, whole exome sequencing facilitated detection of point mutations in the *NDUFB11* (NADH-Ubiquinone oxidoreductase 1 beta, subcomplex 11; OMIM # 300403) gene from the Xp11.3 region [4], thus placing all the causative genes so far identified for this disease on the X-chromosome. The disorder is quite rare; to date a total of 82 affected individuals, plus four aborted and molecularly diagnosed fetuses, have been described. The majority of cases account for LSDMCA1 and are associated with abnormalities of distal Xp (70 cases with Xp22 translocation/terminal deletion/small or large interstitial deletions). In addition, 4-point mutations in the *HCCS* gene have also been described. The remaining cases consist of 3-point mutations in *COX7B* and 2-point mutations in *NDUFB11* (see Table 1).

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Notably, studies have indicated that there is still room for additional causative genes as mutation analysis has demonstrated that not all reported cases can be explained by the causative genes so far described (BF, unpublished).

The molecular basis of LSDMCA

As previously mentioned, the majority of LSDMCA1 patients carry rearrangements of the Xp region and are females. Exceptions to this observation are represented by the description of 8 male individuals showing 46 XX karyotypes and translocations involving the short arms of the X and Y chromosomes and resulting in Xp monosomy in one of two X chromosomes [5]. A complete list of chromosomal abnormalities associated with LSDMCA1 is reported in Supplementary Table 1. The LSDMCA1 minimal critical region was initially defined by a combination of cytogenetic analysis and breakpoint mapping on somatic cell hybrids obtained using patients with deletions and translocations involving the short arm of the X chromosome [6,7]. The critical region spans approximately 610 Kb in Xp22.2 and contains 3 genes: *MID1*, which has been shown to be responsible for X-linked Opitz syndrome [8]; *HCCS*, which encodes for the mitochondrial holocytochrome c-type synthase (also known as heme lyase) that catalyzes the covalent attachment of heme to both apo-Cytochrome (Cyt) c and c1 [9,10] and *ARHGAP6*, a gene encoding for a Rho GTPase–activating protein (Rho GAP) that functions as a GAP for the small GTPase RhoA [11]. In 2002, it was shown that deletions involving the syntenic LSDMCA1 critical region in the mouse led to embryonic lethality early in development. In addition, it was also demonstrated that this lethality could be rescued by overexpression of the human holocytochrome c-type synthase, indicating *HCCS* as the most convincing candidate gene for LSDMCA1 [12].

However, the most conclusive evidence derived from analysis of the few LSDMCA cases not associated with chromosomal abnormalities identified in the last 15 years [2–4]) (Table 1 and supplementary Table S1). First, point mutations and a small deletion were identified in *HCCS*, thus providing the evidence that this gene is indeed responsible for LSDMCA1 [2]. Specifically, *de novo* heterozygous point mutations, i.e., a nonsense mutation (c.589C>T/p.R197*) which was subsequently identified in an additional case [13] and a missense mutation (c.649C>T/ p.R217C) were identified in two patients showing a normal karyotype [2]. Later, a novel missense mutation (c.475G>A/p.E159K) was identified in a sporadic female patient with bilateral microphthalmia and sclerocornea without skin lesions, indicating that the phenotypic variability described in LSDMCA1 is not correlated to the extent of the Xp-terminal deletion [14]. Finally, a mosaic 2-bp *HCCS* deletion, (c.[=/524_525delAG]

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(p.[=/E175Vfs*30]), was identified in a patient with unilateral ocular anomalies and no skin defects [13]. This patient showed a variable degree of mosaicism in different tissues that may have contributed to her mild phenotype. However, a patient with a mosaic X-chromosomal rearrangement showed the classical LSDMCA1 phenotype [15], indicating that other mechanisms are responsible for the high clinical variability in patients with *HCCS* mutations.

HCCS is a highly conserved nuclear-encoded mitochondrial protein, located on the outer surface of the inner mitochondrial membrane where it catalyzes the covalent attachment of heme to both Cytc and Cytc1 [9,10]. Cytc1 is an integral component of the mitochondrial respiratory chain (MRC) complex III and transfers electrons to Cytc, which, in turn, shuttles them from complex III to IV. In *Saccharomyces cerevisiae*, two heme lyases exist, Cyc3 and Cyt2, responsible for heme incorporation into Cytc and Cytc1, respectively [16]. Inactivation of either Cyc3 or Cyt2 results in loss of respiratory growth [17,18]. Conversely, in higher eukaryotes a single heme lyase, HCCS, is instead sufficient for maturation of both Cytc and Cytc1 [9,16]. Functional studies have demonstrated that the point mutations identified in LSDMCA1 patients interfere with the role of HCCS in mitochondrial function and exert their pathogenic effect via oxidative phosphorylation (OXPHOS) impairment [19]. Moreover, conditional inactivation of *Hccs* in the murine heart and its downregulation in medakafish result in severe OXPHOS defects, thus definitively demonstrating a key role for this protein in the formation and function of the MRC [19,20].

Although HCCS is a ubiquitous protein, increased expression levels have been detected in the heart, skeletal muscles, and the Central Nervous System (CNS), including the eye, suggesting a tissue/cell type-specific requirement for this protein. In injured adult rat motor neurons, Hccs migrates from mitochondria to the cytosol under apoptotic stimuli resulting in suppression of the X-linked inhibitor of apoptosis (XIAP) protein and activation of cell death [21]. Hearts of *Hccs*$^{+/-}$ mouse females show decreased cardiomyocyte proliferation during embryonic development [20]. Interestingly, downregulation of *hccs* in medaka fish leads to increased cell death via apoptosome-independent caspase-9 activation, which occurs in the mitochondria and is triggered by OXPHOS defects and overproduction of reactive oxygen species (ROS). Notably the activation of this pathway specifically occurs in the brain and eyes and underlies the development of microphthalmia and microcephaly observed in LSDMCA1 [19].

Almost all LSDMCA cases (with and without chromosomal abnormalities) show skewed X-chromosome inactivation, which suggests that there is a selective disadvantage for cells carrying the mutated allele on their active X chromosomes [22] (see below). Moreover, the increased cell death observed in the CNS [19] and the reduced proliferation of *Hccs*-deficient

cardiomyocytes [20] suggest that a tissue-specific activation of different molecular pathways may cause some of the phenotypes observed in LSDMCA patients, explaining the specificity of the defects observed in the disease. On the other hand, the influence of X-inactivation remains the best explanation for the high degree of clinical variability observed in LSDMCA patients (see below).

After the discovery of *HCCS* mutations in LSDMCA patients, more recent data have also implicated the X-linked *COX7B* and *NDUFB11* genes in the pathogenesis of this genetic disorder. Interestingly, these genes are key components of the MRC complexes IV and I, respectively.

COX7B is a small gene comprising 3 exons on Xq21.1. It is ubiquitously expressed and encodes an integral component of the cytochrome c oxidase (COX), the MRC complex IV [23,24]. In humans COX is composed of 3 proteins encoded by the mitochondrial DNA (mtDNA) (COX1, COX2, and COX3) that assemble with 10 nuclear-encoded proteins (COX4, COX5A, COX5B, COX6A, COX6B, COX6C, COX7A, COX7B, COX7C, and COX8) to form the mature holo-complex [25].

In 2012, pathogenic point mutations in the *COX7B* gene were found in LSDMCA patients with normal karyotypes and no mutations in *HCCS* [3]. In particular, a heterozygous 1-bp deletion in exon 3 (c.196delC/p.L66Cfs*48), a heterozygous splice mutation in intron 1 (c.41-2A>G/p.V14Gfs*19), and a heterozygous nonsense mutation in exon 2 (c.55C>T/p.Gln19*) were identified [3] (see Table 1). These mutations result in a truncated COX7B protein, which is predicted to lack the functional domain necessary for interaction with other subunits of the COX complex [3]. Although the MRC complex IV had been extensively studied, the function of COX7B within this complex has only been characterized after the discovery of the pathogenic mutations leading to LSDMCA2. Notably, it has been shown that the small COX7B subunit is necessary for COX activity, COX assembly, and mitochondrial respiration [3]. Moreover, downregulation of *cox7B* in medakafish resulted in increased cell death leading to microcephaly and microphthalmia, thus resembling the phenotype observed in *hccs*-defective fish [3,26]. These data indicate an essential function for complex IV activity in vertebrate CNS development [3].

NDUFB11 is located on Xp11.23 and comprises 3 exons. Also this gene is ubiquitously expressed and encodes for one of 30 poorly characterized supernumerary subunits of NADH:ubiquinone oxidoreductase, the MRC complex I [27,28]. This complex is the largest within the MRC and is composed of about 45 subunits in mammals, 7 of which encoded by

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mtDNA. Only 14 proteins represent the core subunits and are essential for energy transduction, whereas the roles of the 30 supernumerary subunits are still poorly understood [28].

In 2015, a heterozygous nonsense mutation (c.262C>T/p.Arg88*) and a heterozygous 1-bp deletion leading to a frameshift (c.402delG/p.Arg134Serfs*3) in *NDUFB11* were described in LSDMCA patients [4] (see Table 1). The authors also showed that NDUFB11 is necessary for the assembly of complex I membrane arm, for the maturation of the holocomplex, and for complex I-dependent mitochondrial respiration [4]. Interestingly, *NDUFB11* knockdown in HeLA cells caused impaired cell growth and increased apoptosis [4], also shown in *in vivo* models of *HCCS* and *COX7B* downregulation [19,26].

Table 1. Summary of point mutations identified to date in LSDMCA

GENE	GENE OMIM#	NUCLEOTIDE CHANGE	TYPE OF MUTATION	PREDICTED PROTEIN	DISEASE	DISEASE OMIM#	REF
HCCS	300056	c.589C>T	Nonsense	p.R197*[a]	*MLS/ MIDAS MCOPS7 LSDMCA1*	309801	[2,13,14]
		c.649C>T	Missense	p.R217C			
		c.475G>A	Missense	p.E159K			
		c.[=/524_525delAG]	Frameshift	p.[=/E175Vfs*30]			
COX7B	300885	c.196delC	Frameshift	p.L66Cfs*48	*LSDMCA2*	300887	[3]
		c.41-2A>G	Frameshift	p.V14Gfs*19			
		c.55C>T	Nonsense	p.E19*			
NDUFB11	300403	c.262C>T	Nonsense	p.Arg88*	*LSDMCA3*	300952	[4]
		c.402delG	Frameshift	p.Arg134Serfs*3			

[a]This mutation was identified in two different patients (see also supplementary Table S1). Ref, references

The clinical spectrum of LSDMCA

The identification of mutations in different genes and the characterization of a number of patients has facilitated a more thorough description of the clinical spectrum observed in this rare condition. Supplementary Table 1 reports all the LSDMCA cases reported to date with descriptions of the more commonly observed clinical findings. Table 2 summarizes the percentage of patients displaying the main clinical signs.

Table 2. Summary of clinical findings found in LSDMCA. Extended details can be found in Supplementary Table S1.

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LSDMCA/ Mutation		Skin lesions	EYE		Other	CNS malformations	Intellectual disabilities	Short stature	Cardiac anomalies	Ref
			Micro/ anophthalmia	Corneal abnormalities						
LSDMCA1/ Xp22 R	# cases	54/70	54/70	45/70	31/70	37/65	13/46	23/49	23/65	[1,2,5, 13,15, 29-67]
	%	77	77	64	44	57	28	47	35	
LSDMCA1/ *HCCS* P	# cases	2/5	5/5	5/5	3/5	3/5	3/5	1/5	2/5	[2,13]
	%	40	100	100	60	60	60	20	40	
LSDMCA2/ *COX7B* P	# cases	4/4	0/4	0/4	1/4	3/4	2/4	2/4	2/4	[3]
	%	100	0	0	25	75	50	50	50	
LSDMCA3/ *NDUFB11* P	# cases	2/3	0/4	0/4	2/3	1/3	1/3	1/2	2/3	[4]
	%	67	0	0	67	33	33	50	67	

For each group of patients, the top row indicates the total number of cases, the row below the percentage of patients displaying the indicated clinical sign. Abbreviations: Ref, references; R, rearrangements; P, point mutations.

Linear skin lesions. The most constant and archetypal clinical feature in LSDMCA is represented by the linear skin lesions. As shown in Table 2, linear skin lesions are present in the majority of cases regardless of the underlying causative mutation (77% of LSDMCA1 with Xp22 rearrangements, 40% of cases with *HCCS* point mutations, 100% of LSDMCA2 and 67% of LSDMCA3 patients). These lesions are commonly seen at birth as irregular linear erythematous patches sometimes covered by hemorrhagic crusts. The skin marks are usually located on the face, particularly the cheeks, and the neck with an asymmetric distribution and often extending to the chin and the nose (Figure 1).

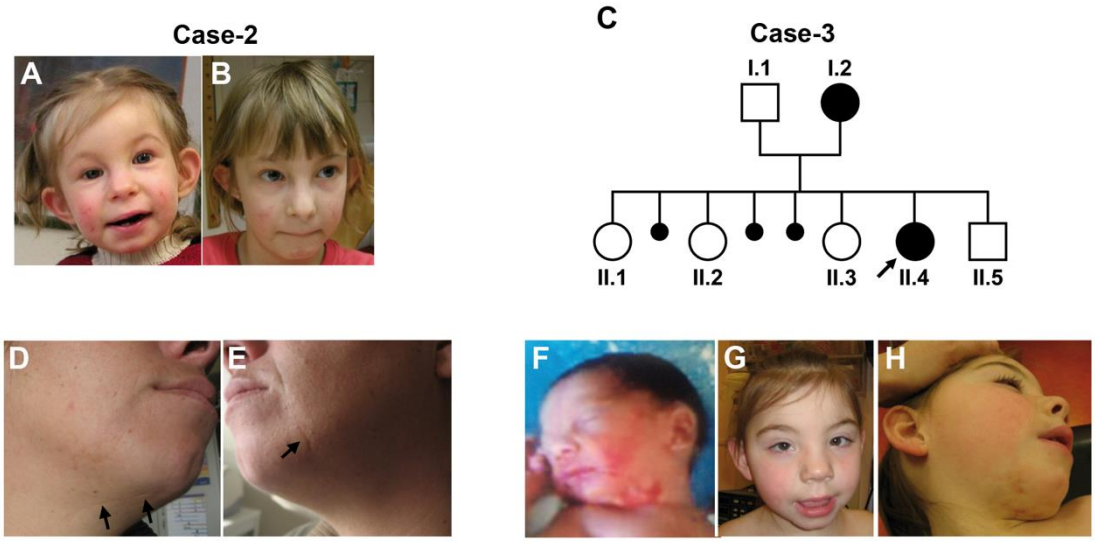

Figure 1. Linear skin lesions in LSDMCA2 patients. (A-B) Case-2. She had asymmetric face with limited

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eyelid closure, linear skin defects on face and neck, which became less obvious with age (A: age $1^{10/12}$ years; B: age 7 years). (C) Pedigree of Case-3. Parents are healthy and unrelated. Three pregnancies ended with abortions. (D-H) Photographs of Case-3 (II.4) and her mother (I.2). Individual I.2, presented with linear skin defects, which healed with scaring (arrows) (D-E). Individual II.4 had facial dysmorphism with telecanthus, long upslanting palpebral fissures, short nose, mild retrognathia and posteriorly rotated ears (G). Linear and patchy erythrodermia on cheeks and neck, which were more pronounced at birth (F) compared to the age of 5 years (H). Figure from [3] used with permission.

The same lesions, however, can also be seen more rarely on the hands and other parts of the body. The cutaneous signs observed in all genetic forms of LSDMCA follow Blaschko lines and tend to improve over time leaving minimal to no residual scars. Histopathological investigation of the lesions revealed a thin, atrophic epidermis lacking rete ridges with a significant infiltrate of lymphocytes. In addition, irregular bundles of smooth muscle were observed in the deep dermis while adnexal structures were missing [50]. In a different study, dermatoscopic examination of the lesions demonstrated erythematous areas with telangiectasias accompanied by absence of sebaceous glands and vellus hairs thus confirming the histopathological findings [65]. Notably, all the investigations performed so far on the cutaneous wounds were done on patients with Xp22 rearrangements and thus represent LSDMCA1. However, in all three genetic forms of the disease, the linear skin lesions observed are similar in appearance, evolution, and localization suggesting a similar patho-mechanism. In line with the ectodermal nature of this condition, affected individuals may also present nail dystrophy and spared hair.

Ocular findings. Microphthalmia, the ocular feature that initially defined the MLS syndrome, is only observed in LSDMCA1 and can affect one or both eyes. It is observed in 77% of LSDMCA1 with Xp22 rearrangements and 100% of cases with *HCCS* point mutations (Table 2). The microphthalmic phenotype can be very severe and can progress to anophthalmia. Additional ocular findings include sclerocornea (unilateral or bilateral), corneal opacity, prolapsed iris, orbital cysts, cornea plana, hypoplasia of the optic nerve, aphakia, anterior eye chamber defects, coloboma, microcornea, hypopigmented and disorganized retinal pigmented epithelium, cataracts, choroidal thickening, chorioretinopathy, glaucoma, lens abnormalities, aniridia, pale optic disk and altered visual-evoked potential. As shown in Table 2, some additional ocular signs can be seen also in LSDMCA2 and 3 although they are more common in LSDMCA1 (MLS syndrome). In particular, among patients with point mutations in *NDUFB11*, subject 1 displayed lacrimal duct atresia and subject 2 presented myopia, nystagmus, and strabismus [4].

Central nervous system (CNS) involvement. Regardless of the underlying genetic cause, the CNS is frequently implicated in this condition (in ~60% of LSDMCA1 cases, in the majority

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of LSDMCA2 patients and in 1/3 LSDMCA3 cases) with a variety of clinical signs and symptoms. CNS anomalies include agenesis or hypoplasia of the corpus callosum, abnormal myelination, colpocephaly, seizures, hydrocephalus, ventriculomegaly, cystic cerebral malformation, malformation of the septum pellucidum, and occult spinal dysraphism. Anencephaly has been described in a few cases (mainly aborted fetuses). Microcephaly can also be observed as well as some degree of psychomotor developmental delay/intellectual deficits. Autistic behavior was described in addition to intellectual deficits in a case with a 12.9Mb Xp terminal deletion. Attention-deficit/hyperactivity disorder (ADHD) was diagnosed in addition to intellectual impairment in a child with a point mutation in COX7B (See Supplementary Table S1).

Cardiac findings. As shown in Table 2, heart abnormalities are observed in all the genetic forms of LSDMCA. They have occurred in 36% of cases with Xp22 rearrangements or point mutations in *HCCS*, in 2 individuals with point mutations in *COX7B*, and in 2 cases with point mutations in *NDUFB11*. Clinical features involving the heart include atrioventricular septal defects, patent ductus arteriosum, coarctation of the aorta, patent foramen ovale, ventricular tachycardia, and atrioventricular block. One of the patients with point mutations in *HCCS* presented histiocytoid cardiomyopathy, poor contraction of the left ventricle and eosinophilic cell infiltration. The two patients with point mutations in *COX7B* displayed tetralogy of Fallot (case I.2) and ventricular hypertrophy, pulmonary hypertension and atrial septal defect (case 2) [3]. Finally concerning the patients bearing point mutations in *NDUFB11*, subject 1 presented with histiocytoid cardiomyopathy and subject 2 with dilated cardiomyopathy which needed heart transplantation at the age of 6 months [4]. Notably, mutations in *NDUFB11* have also been described in patients affected only by histiocytoid cardiomyopathy without features of LSDMCA [68,69], in a male infant with lethal mitochondrial complex 1 deficiency [70] and in sideroblastic anemia [71].

Other clinical findings observed in this condition include diaphragmatic hernia, which may also result in respiratory distress, and short stature. Facial dysmorphisms, genitourinary defects (intersexual genitalia, hypoplastic genitalia, imperforate or displaced anus and polycystic ovary syndrome), and skeletal abnormalities are mainly seen in LSDMCA1 cases with Xp22 rearrangements possibly due to the involvement of other genes responsible for additional X-linked disorders. An exception to this observation is represented by a patient displaying a point mutation in *COX7B* (case 2) presenting with an asymmetric face with limited eyelid closure, a small chin, left renal agenesis and ureteral duplication of the right kidney [3]. Figure 1 depicts the typical linear skin lesions and some of the facial dysmorphism observed in LCDMCA.

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Variable expressivity in LSDMCA. In the three genetics forms described, extensive variability in the phenotypic expressivity, ranging from very mild or no phenotype to severe clinical manifestations, has been described between individuals and even within the same family. Several examples of this variability are available at this regard for LSDMCA1. Allanson and Richter described a female patient with typical skin and ocular clinical manifestations. In contrast, her mother was healthy, except for areas of depigmented skin on the shoulder and the leg, which were recognized only after examination with ultraviolet light. In this case, both mother and daughter showed the same terminal deletion of the Xp region [33]. More recently, Vergult et al., reported a familial case in which both the mother and the daughter presented a submicroscopic deletion of 185-220 kb on chromosome band Xp22.2. Both patients presented microphthalmia that in the mother was severe and required eye enucleation. Instead, the skin lesions were only observed in the mother, who also suffered three spontaneous abortions of unknown sex within the first trimester [29]. Moreover, it should be noted that in LSDMCA1 the severity of the phenotype is not strictly related to the extent of the Xp-chromosome deletion that represents the underlying genetic cause in the majority of cases. Molecular characterization indeed demonstrated that patients with very large deletion of distal Xp such as patient 2 (M.S.) from Lindsay et al., show a mild phenotype [1] while patients with point mutations in HCCS display the full phenotype [2,13] (Supplementary Table 1). A specific patient (M.S.) only displayed the typical linear skin defects, presented a 46,X,del (X)(pter-p22.2) karyotype and was referred to the genetics clinic following the abortion of an anencephalic female fetus with the same karyotype [1]. Concerning LSDMCA2 only four cases have been reported to date. However, in the described familial case (family 1), a heterozygous nonsense mutation in the *COX7B* transcript was identified in individual II.4 and her mother (individual I.2). The former presented the classical skin lesions, microcephaly, facial dysmorphisms, tetralogy of Fallot, clinodactyly of the fifth finger, intellectual disabilities, CNS malformation, poor vision, and ophthalmologic findings. While the mother only displayed the skin phenotype, a mild myopia, and had reported three pregnancies that ended with spontaneous abortions of unknown sex within the first trimester [3]. Lastly for LSDMCA3, again only 4 cases have been reported and 3 are from a familial case including an aborted fetus that was not used to calculate the % of main findings illustrated in Table 2. This familial case involves a frameshift mutation in *NDUFB11*, subject 2 showed the typical linear skin lesions, ocular findings not including microphthalmia nor sclerocornea, severe developmental delay, short stature, microcephaly, severe hypotonia, delayed dentition, brain malformations and dilated cardiomyopathy that required heart transplantation. In this familial case the mother in which the same mutation has

been identified was completely asymptomatic. In the next pregnancy ultrasound examination diagnosed a female fetus with a severe cardiological and neurological phenotype and intrauterine growth retardation. Molecular studies identified the same frameshift mutation found in both the mother and subject 2; the pregnancy was terminated at 24 weeks [4].

The role of X-chromosome and X-chromosome inactivation

All the genes so far involved in this rare genetic condition, i.e. *HCCS, COX7B* and *NDUFB11* are localized on the X-chromosome. This segment of our genome has many peculiar features including X-chromosome inactivation (XCI) also known as "lyonization". This phenomenon consists of the transcriptional silencing of one of the two X-chromosomes in female mammals to achieve dosage compensation between sexes [72]. This epigenetic process starts at the blastocyst stage in the early phases of embryo development. In normal conditions, the choice of which of the two X-chromosomes is to be silenced is random but is then maintained in the progeny cells. Thus, in normal conditions, the ratio of the two-cell population (carrying the active and the inactive X, respectively) is about 50:50. As a consequence, normal female individuals are natural mosaic and display organs with a mixed population of cells in which either the paternal or the maternal X has been inactivated. For this reason, women are less susceptible to pathogenic variants on the active X-chromosome as the variant will not be expressed in all cells [73]. This explains why LSDMCA female patients can be observed while nullisomy for these transcripts in the hemizygous males is lethal as affected fetuses do not survive and are aborted. On the contrary, in diseased conditions when one of the X chromosomes displays a mutation, the choice of which of the two chromosomes will be silenced is not random, the normal X-chromosome is favored and the mutated X is preferentially inactivated. In this case we have a divergence from the 50:50 ratio that is known as skewing of the XCI that can occur with variable degrees (Figure 2). The extensive intrafamilial and interfamilial phenotypic variability observed in this condition can be explained by X-inactivation. We hypothesize that in the affected heterozygous females, once the XCI process takes place in the early stages of embryo development, cells inactivating the normal X chromosome will die as a consequence of loss-of-function mutations in *HCCS*, *COX7B* or *NUFB11*, on the transcriptionally active X-chromosome [22,74]. We propose therefore that the clinical signs observed in LSDMCA would be the consequence of the different capability of the diverse tissues and organs to remove the "affected dying" cells by cell selection. According to our hypothesis individuals characterized by a mild phenotype or even the total absence of clinical manifestations are the result of a completely skewed

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inactivation that obligates preferential inactivation of the mutated X chromosome leaving the non-mutated transcriptionally active X in blood cells and tissues (e.g. eyes and skin). Conversely, the most severe clinical manifestations can be found in patients in which the mutated X chromosome is active while the non-mutated one is transcriptionally silenced in tissues affected by the disease and/or at determined developmental stages (Figure 2).

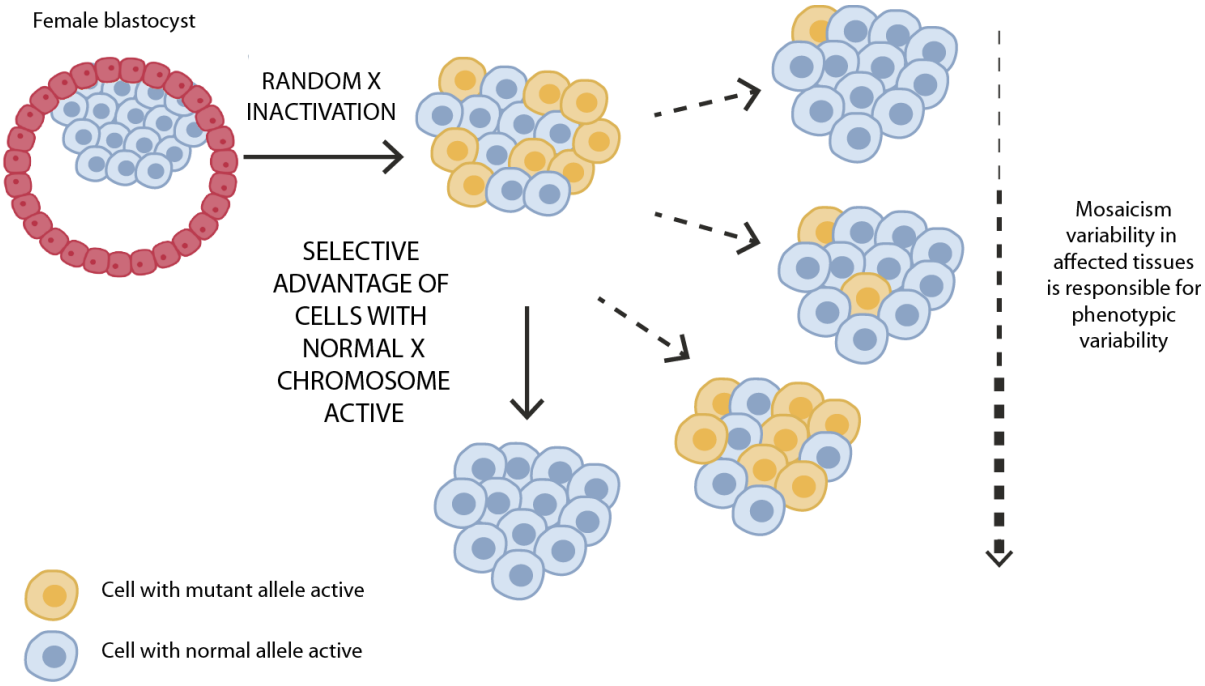

Figure 2. Schematic representation of XCI in female somatic cells. In normal conditions the ratio of the two cell types (carrying the active and the inactive X chromosome) is approximately 50:50, but in females with X linked dominant disorders, this ratio may be different, due to a potential disadvantage for cells expressing a mutant X-linked allele. Divergence from the 50:50 ratio, known as skewing of XCI, can be different in various tissues, in different developmental stages, and may vary among individuals, causing variability to the severity of the phenotype observed. For disorders such as LSDMCA, affected females usually have totally skewed patterns of XCI, in favour of an active wild-type X chromosome.

LSDMCA as an unconventional mitochondrial disorder

Mitochondrial diseases are a clinically heterogeneous group of rare disorders resulting from a MRC dysfunction. *HCCS, COX7B* and *NDUFB11* encode proteins necessary for the proper function of the MRC, thus defining LSDMCA as a mitochondrial disorder.

Although clinically heterogeneous, the typical features of mitochondrial diseases include neuromuscular hypotonia, ataxia, encephalopathy, encephalomyopathy and various myopathies [75–77]. “Canonical” mitochondrial diseases are thus usually characterized by postnatal organ failure. Interestingly, as previously described, LSDMCA patients show diverse phenotypes mainly characterized by developmental defects affecting the eyes and the skin and,

in that regard, LSDMCA represents an unconventional mitochondrial disease. LSDMCA represents a remarkable example of a truly developmental phenotype associated with mitochondrial dysfunction. Although neurological disorders and cardiac defects are common features of mitochondrial disorders, the unique skin lesions mainly affecting the head and neck and the microphthalmia are difficult to assign to deficiency of mitochondrial enzymes. Interestingly, *HCCS* was the first human gene encoding for an MRC protein which causes microphthalmia when mutated. Moreover, the skin involvement observed in mitochondrial diseases is atypical and can include hirsutism and hypertrichosis as described in Leigh syndrome (OMIM # 256000), and twisted hairs as reported in Bjornstad syndrome (OMIM # 262000) [78].

HCCS, *COX7B*, and *NDUB11* are all ubiquitously expressed since they are required for the OXPHOS pathway. However, the phenotypic manifestations observed in LSDMCA-affected females mainly affect the CNS, suggesting that dosage and function of these proteins may be critical for particular tissues.

More recently, patients with *NDUFB11* mutations displaying more classical mitochondrial phenotypes have been described [70,79]. These patients show a combination of neurological symptoms, muscle hypotonia, myopathy, lactic acidosis, histiocytoid cardiomyopathy and sideroblastic anemia. Interestingly these patients are all males carrying a missense or one amino acid in-frame deletion variants [79]. This observation indicates that some residual activity of the NDUFB11 protein may explain the less severe phenotype and is needed for males to be viable.

It is possible that differential tissue sensitivity to mitochondrial ATP depletion (high versus low energy demand) and/or overproduction of reactive oxygen species might elicit different molecular responses in the absence of *HCCS*, *COX7B*, or *NDUFB11* in selected tissues and may therefore induce the blockages to cell replication and/or an increased cell death. In addition, although the main function of the mitochondrion is the production of energy in the form of ATP, it is well known to have a central role in the regulation of the intrinsic pathway of cell death, a key process required for proper development of the CNS. Deregulation of these processes and X chromosome inactivation could function together to select OXPHOS-proficient cells, thus attenuating or abolishing MRC defects in the surviving tissues and individuals.

Nevertheless, additional studies are required to better understand the peculiarity of the LSDMCA phenotype that to date represents a unique example of a mitochondrial disease mainly characterized by an apoptosis-driven developmental phenotype.

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Supplementary material

A table, Supplementary Table S1 is provided as supplementary material. This table describes the chromosomal abnormalities to date reported in LSDMCA1 and the main clinical findings observed in all the LSDMCA patients described up to date.

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Both authors drafted, revised and approved the manuscript

Conflicts of Interest:

The Authors declare no conflict of interest

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