EDITOR'S CHOICE

Dosage compensation of the mammalian X chromosome influences the phenotypic variability of X-linked dominant male-lethal disorders

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ABSTRACT

In mammals females inactivate one of the two X chromosomes during early development to achieve an equal gene dosage between sexes. This process, named X chromosome inactivation (XCI), usually occurs randomly. However, in a few instances, non-random XCI may take place, thus modulating the phenotype observed in female patients carrying mutations in X-linked genes. Different aspects related to dosage compensation contribute to explain the influences of XCI on the phenotypic variability observed in female patients. The study of two X-linked dominant male-lethal disorders, such as the microphthalmia with linear skin lesions (MLS) syndrome and the oral–facial–digital type I (OFDI) syndrome, offers the opportunity to discuss this intriguing topic. In addition, recent data on the characterisation of a murine model for OFDI provide the opportunity to discuss how differences in the XCI between *Homo sapiens* and *Mus musculus* can justify the discrepancies between the phenotypes observed in OFDI patients and the corresponding murine model.

Transcripts from mammalian sex chromosomes have evolved specific controls of gene expression in order to compensate for the dosage difference between sexes, as females display two copies of the X chromosome while males are hemizygous for X-linked traits. In females, this is achieved through inactivation of one of the two X chromosomes in each somatic cell at the blastocyst stage.[1] Of particular interest for geneticists is the fact that the chimerism that typically occurs for X-linked genes in somatic tissues (where 50% of cells in females inactivate one X, whereas 50% inactivate the other X) can be skewed in specific genetic conditions. With respect to X chromosome inactivation (XCI), non-randomness can occur as a result of cell selection effects that arise downstream of the actual X-inactivation process. In this scenario, the genetic composition of either X chromosome may cause a selective advantage, which subsequently results in clonal survival and proliferation. In the context of hereditary diseases, a negative selection normally occurs against clones carrying a mutated gene on an active X, thus decreasing the severity of the phenotypes in females which have X-linked dominant mutations. However, in some cases, the opposite is true: skewed X-inactivation acts against the wild type allele in the heterozygous carrier female, resulting in an increased severity of the disease pathology.[2] XCI pattern is usually analysed using the polymorphic androgen receptor gene assay, or chromosome replication studies, using accessible tissues, such as blood.[3]

Interestingly, not all genes undergo X inactivation in females, and therefore behave as autosomes, which are present in two copies in somatic tissues and are generally expressed from both alleles. Some "escapees" have retained a Y-linked paralogue, although many genes have lost their Y copy.[4] In humans, about 15% of genes escape X inactivation, while in mice, only six genes are known to display this characteristic.[4 5] Mice thus seem to have fewer escapees although a systematic and rigorous study has not been performed yet to address this question.

Recent data have demonstrated that most of the transcripts escaping XCI are not fully expressed from the inactive X (Xi), demonstrating that escape from inactivation is partial and incomplete. Moreover the same authors also demonstrated that X-linked transcripts display different levels of expression from Xi chromosomes in different cell lines, indicating that females have considerable heterogeneity in levels of expression of X-linked transcripts.[5]

To draw these conclusions, the expression profile for the assayable genes on the human X chromosome was determined using a combination of mouse–human somatic cell hybrids and allele specific assay in primary human fibroblast cell lines.[5]

To obtain a complete picture of the variability of the expression of X-linked genes, one must also take into account that the XCI pattern could vary among the different tissues, although this has not been formally demonstrated. If this possibility will prove to be true, the accompanying variability will represent an additional element to explain the phenotypic variability of X-linked genetic diseases.[6]

Autosomal transcripts display a diploid expression, while X-linked genes are characterised by monoallelic expression; thus, a potential problem for genes that undergo X-inactivation may be that of haploinsufficiency. To overcome this problem, several mammalian species developed a mechanism of dosage compensation, resulting in doubling of the global expression levels of X-linked transcripts to maintain a balanced expression between X chromosomes and autosomes. Proof of this mechanism was provided by Nguyen and colleagues who demonstrated, by microarray analysis, up-regulation of genes from the X chromosome in diploid cells.[7] Interestingly, X-linked transcripts were found to be expressed, but not up-regulated, in spermatids and secondary oocytes, thus preserving balanced expression in these

haploid cells. Furthermore, the global transcription output from the X chromosome was comparable in adult female and male tissues, and no apparent differences were noted for genes escaping XCI.[7 8]

In this review, we discuss different mechanisms to achieve dosage compensation in mammals (X inactivation, X escaping and X up-regulation) which can influence the phenotypic variability observed in X linked dominant male-lethal disorders. The microphthalmia with linear skin defects (MLS) syndrome and the oral–facial–digital type I (OFDI) syndrome will be used as clarifying examples.

X-LINKED DOMINANT MALE-LETHAL DISORDERS

The mammalian X chromosome consists of approximately 160 Mb of DNA and is estimated to contain approximately 1400 genes (including non-coding RNA) (http://www.ncbi.nlm.nih.gov/projects/mapview); 316 phenotypes show a distinctive X-linked inheritance pattern and 187 of these have known molecular bases (Online Mendelian Inheritance in Man, OMIM, available at www.ncbi.nlm.nih.gov/sites/entrez).

Most X-linked disorders show a recessive pattern of inheritance. However, a small group of X-linked phenotypes are represented by dominant disorders that can affect heterozygous females. Years of clinical experience, however, suggest that the boundaries between X-linked dominant and recessive diseases might not be well defined for a number of cases displaying intermediate disease penetrance in heterozygous females. X-linked dominant male-lethal disorders can be more easily recognised as affected patients are only females, with some rare exceptions. Interestingly, for the majority of these disorders, inter- and intrafamilial phenotypic variability has been reported.[9–15] To date, this subgroup of X-linked disorders is represented by 10 genetic diseases, whose main features are summarised in table 1. They represent a group of developmental disorders caused by mutations in genes belonging to different gene onthology classes (http://www.geneontology.org). Causative genes are known for eight of these. Six are not transcribed from Xi, while the remaining two escape X chromosome inactivation: *OFDI*, mutations in which are associated with ODFI (MIM 311200),[5 16 17] and *NEMO* that when mutated causes incontinentia pigmenti (MIM 308300).[5]

THE INFLUENCE OF NON-RANDOM X INACTIVATION ON PHENOTYPIC VARIABILITY

Microphthalmia with linear skin defects syndrome

The microphthalmia with linear skin defects (MLS) syndrome, also known as MIDAS (MIM 309801), is a rare X-linked dominant neurodevelopmental disorder that is lethal in males. Affected females show microphthalmia and localised dermal aplasia. Typically, the skin lesions heal with age, leaving hyperpigmented lesions. Additional features include agenesis of the corpus callosum, sclerocornea, chorioretinal abnormalities, infantile seizures, congenital heart defects, and mental retardation. In the majority of cases, patients carry deletions or unbalanced translocations involving the Xp22.3 region resulting in segmental monosomy of this chromosome.[18] Although most patients display the classical phenotype of MLS, a high phenotypic variability, not correlated to the extent of the deleted region, has been reported. For example, a group of patients has been reported to show the characteristic skin defects without ocular malformation.[19–22] This group also includes a female patient with one of the largest deletions ever described in an MLS case.[22] In contrast, other patients display eye abnormalities with complete absence of skin lesions.[23–25] The same phenotypic variability has also been described in MLS cases with point mutations and small deletion in the *HCCS* transcript (see below).[26 27]

In addition, phenotypic intrafamilial variability has also been previously described in the literature for MLS patients with segmental monosomy of Xp22.3. In 1991, Allanson and Richter described a female patient with typical MLS clinical manifestations. In contrast, her mother was healthy, except for an area of patchy depigmented skin over the shoulder and on the leg, which was recognised after examination with ultraviolet light. However, both mother and daughter showed an identical terminal deletion of Xp.[20] Also, in 1994 Lindsay and colleagues described a woman displaying no phenotype other than scar lesions on her neck, who aborted a female anencephalic fetus. Subsequent analysis determined that both of them carried the same terminal deletion of the short arm of X chromosome.[22] Finally, Mucke and colleagues described a girl and her mother, both of whom showed the same Xp22.3 monosomy associated with different degrees of microphthalmia, dermal aplasia and other clinical features.[28]

It has been proposed that the pattern of X inactivation may influence the development of symptoms in patients with MLS.[29] Indeed, skewed X inactivation has been detected in 16 out of the 17 MLS patients analysed to date.[26 27 30–33] Only one single full-blown MLS patient with an Xpter-p22.3 deletion showed a random X inactivation pattern (patient 2 in Naritomi *et al*[34]), as revealed by chromosome replication studies.

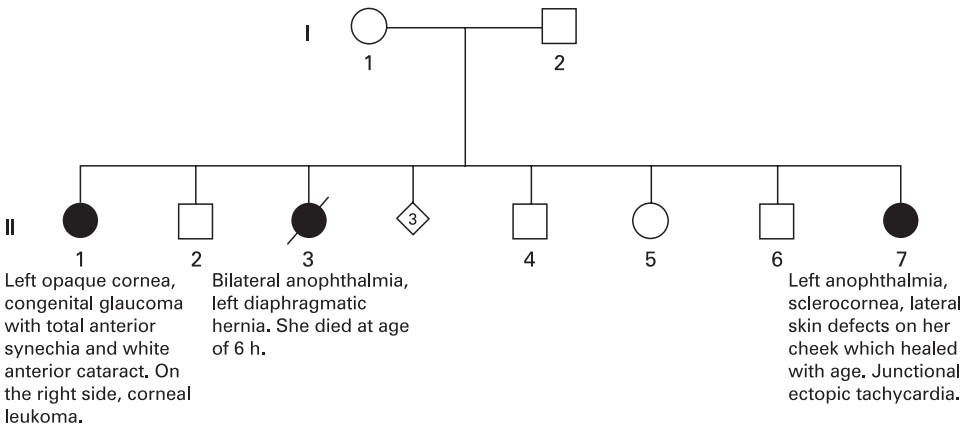
The molecular basis of MLS syndrome has been recently determined after the identification of point mutations in the *HCCS* gene in non-deleted cases.[26 27] This transcript encodes the mitochondrial holocytochrome c-type synthase that functions as a heme lyase by covalently adding the prosthetic heme group to both apocytochrome c and c1. In 2002 Prakash and colleagues demonstrated that *Hccs* null mutation was not compatible with embryonic stem cell (ES) survival. In vivo generated deletions, resulting in complete loss of *Hccs,* led to embryonic lethality that could be rescued by overexpression of the human *HCCS* from a bacterial artificial chromosome (BAC) clone. These data provided the proof that *Hccs* is responsible for the male lethality observed in MLS syndrome.[35] *HCCS* is ubiquitously expressed and it is not transcribed from the Xi.[5 36] Wimplinger and colleagues identified in a familial case an 8.6 Kb deletion in *HCCS* comprising its first three exons, as well as the respective intronic sequences, in different members of an Ashkenazi family displaying a considerable level of phenotypic variability (fig 1, top). The index case, II.7, shows left anophthalmia and typical aplastic skin defects on her cheek (fig 2, panel A). The older daughter (II.1) presents a milder phenotype with opaque cornea, congenital glaucoma, total anterior synechiae, anterior cataract on the left eye and corneal leukoma, which has resolved with time on the right side. The mother (I.1) has no obvious signs of the syndrome, although for religious reasons she refused to undergo complete dermatological examination with an ultraviolet lamp. In the same family, a third daughter (II.3) died at the age of 6 h and presented with a diaphragmatic hernia and bilateral anophthalmia. Three spontaneous abortions were also reported in this family. The same 8.6 kb deletion was identified in both II.1 and II.7. Interestingly the same mutation was also found in the unaffected mother (I.1), while the other members of the family analysed (II.2, II.4, II.5, II.6 and I.1) did not show abnormalities.[26]

All family members carrying the deletion showed an extremely skewed X inactivation in blood cells, in contrast with the other

Table 1 Features of X-linked dominant male-lethal disorders

Disease	Locus name	OMIM	Clinical description	Gene	Gene features	Gene XCI	XCI pattern in patients
Aicardi	AIC	304050	Infantile spasms, CC agenesis and chorioretinal lacunae; microphthalmia; MR, grey matter heterotopia, gyral anomalies, and vertebral and rib defects	–	–	–	Preferentially random
Chondrodysplasia punctata 2	CDPX2	302960	Skin defects, coarse lusterless hair and alopecia, cataracts, skeletal abnormalities and craniofacial defects	*EBP*	Emopamil binding protein involved in cholesterol biosynthesis	Inactivated	Random
Congenital hemidysplasia with ichthyosiform erythroderma and limb defects	CHILD	308050	Unilaterally distributed ichthyosiform nevi, ipsilateral limb defects; brain malformations, kidney and cardiac defects	*NSDHL*	NAD(P)H steroid dehydrogenase-like protein involved in cholesterol biosynthesis	Inactivated	Random in one patient analysed
Goltz	FDH	305600	Atrophy and linear pigmentation of skin, dermic herniation of fat, multiple papillomas; digital, oral and ocular anomalies; MR	*PORCN*	Endoplasmatic reticulum protein involved in secretion of Wnt proteins	Inactivated	Preferentially random
Incontinentia pigmenti	IP	308300	Abnormalities of skin pigmentation; CNS, eye, teeth, skeleton, and heart malformations	*NEMO*	Inhibitor of nuclear factor kB kinase β subunit	Escape	Skewed
Microphthalmia with linear skin defects	MLS	309801	Microphthalmia and dermal aplasia on face and neck; CC agenesis, sclerocornea, chorioretinal abnormalities, infantile seizures, congenital heart defects and MR	*HCCS*	Holocytochrome c-type synthase	Inactivated	Skewed
Oculo–facio–cardio–dental	OFCD	300166	Facial abnormalities, cataract, microphthalmia, teeth abnormalities and cardiac septal defects	*BCOR*	BCL6 co-repressor in DNA transcription	Inactivated	Skewed
Oral–facial–digital type I	OFDI	311200	Facial dysmorphism, limb abnormalities, CNS malformation, and MR; polycystic kidney disease	*OFD1*	Required for primary cilia formation and left–right symmetry	Escape	Skewed in 30% of patients
Rett	RTT	312750	Autism, dementia, ataxia, and loss of purposeful hand use	*MECP2*	Methyl-CpG-binding protein 2 in DNA methylation	Inactivated	Preferentially random
Terminal osseous dysplasia and pigmentary defects	ODPD	300244	Abnormal and delayed ossification of hands and feet, brachydactyly, camptodactyly and clinodactyly; digital fibromatosis; pigmentary skin lesions on the face and scalp, dysmorphic features including hypertelorism, and multiple frenula	–	–	–	Skewed

CC, corpus callosum; CNS, central nervous system; MR, mental retardation.



Family members	I.1	II.1	II.2	II.3	II.4	II.5	II.6	II.7
X inactivation pattern	Skewed (100:0)	Skewed (100:0)	/	NA	/	Random	/	Skewed (100:0)
HCCS deletion	+	+	–	NA	–	–	–	+

NA, not available

Figure 1 Pedigree of the microphthalmia with linear skin defects (MLS) syndrome family carrying deletion in the *HCCS* transcript and summary of clinical description, mutation analysis and X chromosome inactivation studies for all family members.

family members (fig 1, bottom). This strongly suggests that there is a selective disadvantage for cells carrying the *HCCS* mutation on their Xa. Similar results were reported for other MLS patients carrying point mutations in the same gene.[26 27]

We hypothesise that, in heterozygous females, once the X-inactivation process (that is, during early blastocyst stage) initiates, cells having an inactivated normal X chromosome die as a consequence of loss-of-function mutations of *HCCS*. Therefore, the developmental problems present in the MLS syndrome would be the result of the different ability of the various tissues and organs to eliminate these "dying" cells by cell selection mechanisms. This could explain the phenotypic variability observed in both sporadic and familial cases in MLS syndrome, the symptoms of which range from the full bloom phenotype to an apparent absence of clinical manifestation, as described above. We hypothesise that a milder phenotype or the total absence of MLS clinical manifestations may be due to a totally skewed X inactivation that forces preferential activation of the unaffected X, not only in blood cells, but also in tissues such as the eye and skin. On the contrary, the most severe MLS clinical manifestations are observed in female patients whose normal X chromosome is inactivated in the affected tissues, or at a specific time of embryonic development.

As mentioned earlier, all the data available to date on the XCI pattern in patients have been obtained from blood samples; it will be interesting to compare those data to that originated from skin biopsies taken from controls, aplastic skin lesions of a newborn MLS patient, and hyperpigmented areas from an adult case. We hypothesise that in normal and hyperpigmented areas the XCI is totally skewed in favour of normal X chromosome expression, as in blood cells. On the contrary, we expect to find cells bearing *HCCS* mutation on the active X in lesions of a newborn patient. These findings could suggest that the progression of the aplastic lesions to hyperpigmented areas is concurrent with the disappearance of cells expressing the mutated form of the *HCCS* gene, supporting the model that the normal cells have a proliferative advantage. To support this hypothesis experiments have been performed in a different X-linked dominant male-lethal disorder incontinentia pigmenti (IP) for which skin biopsies from affected cases were available. In this case, Parrish and collaborators demonstrated that progression of inflammatory lesions to hyperpigmented skin was concurrent with the disappearance of cells expressing the mutated form of the IP gene.[37]

Moreover, it must also be noted that the function of the protein encoded by *HCCS* is completely different from that of other genes responsible for severe eye malformations, such as *PAX6*, *SOX2* and *OTX2*. Specifically, these latter examples code for transcriptional factors that regulate different phases of eye development.[38] In contrast, *HCCS* encodes a mitochondrial housekeeping holocytochrome c-type synthase. *HCCS* may not only be responsible for severe functional defects in oxidative phosphorylation (OXPHOS), but may probably also unsettle the balance between necrosis and apoptosis, and direct cell death towards the necrotic path (see also Wimplinger *et al*[26]). Intriguing questions about this disease will be: how such a peculiar phenotype, such as that observed in MLS patients, can be explained as a consequence of mutations in a ubiquitously expressed, housekeeping gene, and which are the mechanisms allowing embryonic cells expressing mutated X chromosomes to survive, ultimately leading to a pathological phenotype? We speculate that, in tissues such as the eye and skin, the XCI pattern of *HCCS* could vary and the gene could be expressed also from Xi, although this has not been formally demonstrated. Due to a basic level of transcription of the normal and functioning allele from Xi, "suffering" cells committed to die could survive and divide in daughter cells. This could cause the degeneration of tissues in specific stages of embryonic development, and explain the peculiar clinical manifestations of this rare neurodevelopmental disorder that affects predominantly the eyes and the skin, as a consequence of mutations of a ubiquitous gene.

Figure 2A displays a schematic representation of XCI in female somatic cells in MLS patients.

DIFFERENT LEVELS OF EXPRESSION OF X-LINKED TRANSCRIPTS MIGHT INFLUENCE PHENOTYPIC VARIABILITY

Oral–facial–digital type I syndrome

Oral–facial–digital type I (OFDI) syndrome (MIM 31200) belongs to the heterogeneous group of oral–facial–digital

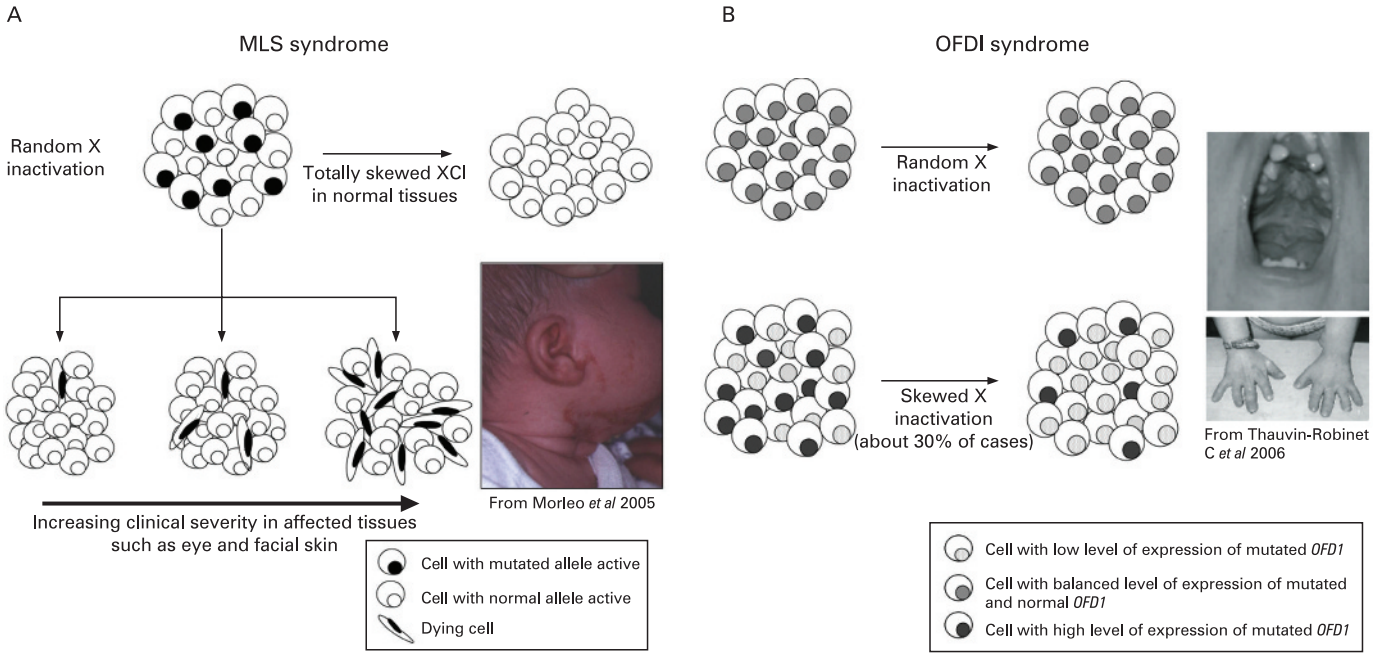


Figure 2 Schematic representation of X chromosome inactivation (XCI) in female somatic cells in two X-linked dominant male-lethal disorders, microphthalmia with linear skin defects (MLS) syndrome and oral–facial–digital type I (OFDI) syndrome. Panel A. In normal condition the expected chimerism results in 50% of cells that inactivate one X, whereas the remaining cells inactivate the other X chromosome. In MLS syndrome, skewing of X inactivation can occur and vary among individuals, tissues and in different stages of development, thus influencing the phenotypic variability observed in this syndrome. Top represents cells undergoing totally skewed X inactivation that forces preferential activation of the unaffected X in normal tissues. Bottom schematises the increasing severity of clinical manifestations depending on the proportion of "suffering" cells whose normal X chromosome is inactivated in the affected tissues, or at a specific time of embryonic development. The picture shows the typical linear skin lesions observed in MLS patients (case II.7). Panel B. Top depicts cells with a random XCI pattern in lymphocytes from OFDI patients, resulting in a balanced biallelic expression of normal and mutated *OFD1* gene in each cell. Bottom displays non-random XCI with a predominant proliferation of cells carrying the normal X chromosome active. We hypothesise that the skewing observed in 30% of patients can be explained by the observation that escaping from XCI is generally partial and incomplete for most genes. For this reason the normal copy of *OFD1* could be expressed at low levels from Xi, creating a disadvantage in the proliferation of these cells. The picture shows some of the oral and digital clinical manifestations observed with a high level of phenotypic variability in *OFDI* patients.

syndromes (OFDS) and is characterised by an X-linked dominant mode of inheritance with lethality in males. OFD type I, similar to all other forms of OFDS, is characterised by malformations of the face, oral cavity and digits with a high degree of phenotypic variability. Polycystic kidney disease, which is typical of OFD type I, and central nervous system (CNS) malformations are commonly associated with this disease.[39]

The syndrome is caused by mutations in the *OFD1* gene, which maps to Xp22.2.[40] This gene codes for a centrosomal/basal body[41–43] protein required for primary cilia formation and left–right symmetry (see Ferrante *et al*[44]). *OFD1* is highly expressed both in human and mouse in tissues affected by this disease, and reverse transcriptase–polymerase chain reaction (RT-PCR) studies using hybrids carrying the active and the inactive X chromosome indicate that this escapes X inactivation in humans.[5 16 17] Most *OFD1* mutations identified to date in patients lead to a premature truncation of the protein, presumably acting with a loss-of-function mechanism.[40 41 45–47]

Clinical variability between sporadic cases is high. It has been observed also in some familial cases reported in the literature. Thauvin-Robinet and colleagues[46] described two families with different mutations in the *OFD1* gene showing extensive intrafamilial phenotypic variability. Family 1 presents an aborted female fetus with full blown OFDI phenotype comprising facial dysmorphism with oral abnormalities, central nervous system malformations and finger abnormalities, while the sister and mother showed a less severe phenotype, represented by oral dysmorphism for the sister, and brachydactyly and clinodactyly in hands and feet for the mother. However, all affected members were found to carry the same mutation in the *OFD1* gene (2349delC). A second family in the same study presented a patient with oral cavity and CNS malformations including mental retardation. The mother of this patient presented only a lobulated tongue, pseudocleft of the upper lip, hypoplasia of the nasal alae and clinodactyly on the hand with a normal mental development.[46] Moreover, intrafamilial variability for additional cases has been described[48–50] (B Franco, unpublished data). All these cases displayed a milder phenotype in the mothers and more severe clinical manifestations in the daughters. Based on our unpublished results, we estimate that 30% of familial cases with mutations in the *OFD1* gene show a high degree of phenotypic variability in the clinical manifestations of the syndrome (B Franco, unpublished data).

We hypothesise that in the OFDI syndrome, as for the other X-linked dominant disorders, X inactivation may affect the phenotype of heterozygous females. Thauvin-Robinet *et al* analysed X chromosome inactivation in lymphocytes from OFDI patients. They showed non-random inactivation for 30% of patients[46] and the same results were obtained after analysis of our cohort of OFDI cases (B Franco, unpublished data).

Since the escaping *OFD1* gene is expressed from both X chromosomes, why should the cell preferentially express one X

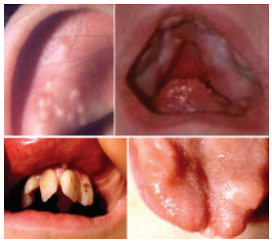
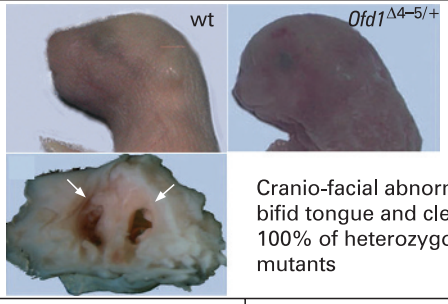
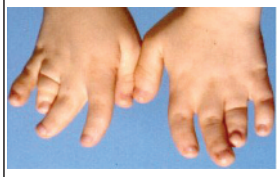
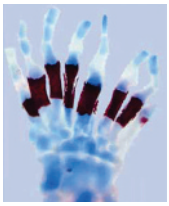
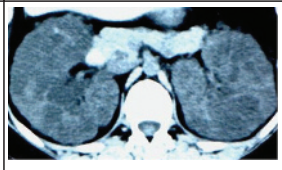
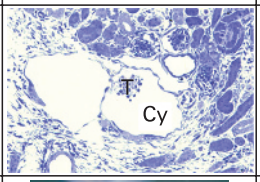
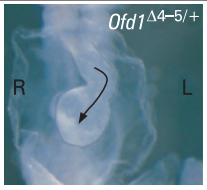
Clinical features	OFDI patients		OFDI mouse model	
Survival	Heterozygous females have normal life expectancy depending on the presence/severity of renal cystic disease		Heterozygous female mutants die at birth	
Cranio-facial abnormalities and oral manifestations		Cranio-facial and oral manifestation are observed in many cases with high phenotypic variability. Examples are facial milia observed in 30% of patients, cleft palate (30–80%), and bifid or lobulated tongue observed in 30–45% of patients		Cranio-facial abnormalities, bifid tongue and cleft palate in 100% of heterozygous female mutants
Limb and skeletal abnormalities		Limb abnormalities are present in a large number of cases and mostly include brachydactyly, syndactyly and clinodactyly. Polydactyly is rarely observed		Limb abnormalities are present in 100% of heterozygous female mutants including polydactyly
Polycystic kidney		Renal cysts are observed in ~40% of cases		Renal cysts in 100% of heterozygous female mutants
Cardiovascular abnormalities	Not described in OFDI patients			Ballooning of the pericardial sac or reversal of the heart loop in 50% of male mutant embryos. Cardiac abnormalities in most females

Figure 3 Comparison between the clinical manifestations observed in oral–facial–digital type I (OFDI) patients and in the murine model for OFD type I. The figure illustrates that the phenotype observed in mice is more severe than that observed in humans: the new born Ofd1$^{\Delta 4\text{-}5/+}$ females die at birth while female patients have normal life expectancy depending on the presence/severity of renal cystic disease. Concerning the cranio–facial–oral abnormalities, these are present in 100% of heterozygous mice analysed (palatoschisis is marked by white arrows), while patients display an evident phenotypic variability. Examples of this are signs such as facial milia (ear), bifid lobulated tongue, teeth abnormalities and cleft palate. Limb and skeletal abnormalities are also very variable among patients while polydactyly is always present in female mutants as revealed by alizarin red (bone) and alcian blue (cartilage) staining. Cystic kidney is present in about 40% of patients while renal cysts were observed in all mutant animals. Finally cardiovascular abnormalities were observed in most mutant animals analysed to date, both female and male embryos, while these anomalies have never been reported for OFDI patients.

chromosome instead of the other? We hypothesise that the skewing observed in 30% of patients can be explained by the recent observation that most genes escaping XCI are not fully expressed from the Xi, demonstrating that escape from inactivation is generally partial and incomplete. It is tempting to speculate that a threshold level of transcription of *OFD1* is required by cells during embryogenesis, thus forcing preferential activation of the X chromosome carrying the normal gene in highly proliferating tissues, such as blood. As a consequence the mutated gene is transcribed from Xi whose levels of expression, however, are incomplete. This model could explain the skewing of X inactivation observed in blood cells for 30% of patients, and this could also justify a non-random inactivation for other tissues.

Moreover recent groundbreaking experiments demonstrated that most of the genes escaping inactivation show substantial differences in the level of expression from the Xi chromosomes in different cell lines, indicating that females have considerable heterogeneity in levels of X-linked gene expression[5]; this could account for the observation that only a small percentage of OFDI patients preferentially express one X instead of the other (skewing of XCI).

This possibility, together with the hypothetical different pattern of XCI in the different tissues, could explain the inter- and intrafamilial phenotypic variability observed in this disease. Figure 2B displays a schematic representation of XCI in female somatic cells in OFDI cases.

We hypothesise that the threshold level of transcription required by cells is responsible for the male lethality observed in the total absence of OFD1 protein. This level of transcription could also influence the phenotype among different species, as shown by the characterisation of the mouse model for OFDI syndrome.

Differences in the severity of the phenotype observed for OFD type I syndrome between patients and animal models

Ofd1-knockout mice have been generated by targeted recombination by deleting exons 4 and 5 of the *Ofd1* transcript, the murine homologue of the *OFD1* gene. Heterozygous females, $Ofd1^{\Delta 4-5/+}$, which do not survive beyond P0 and are consistently smaller than wild-type littermates, reproduced the main features of the human disease, albeit with increased severity, and displayed severe craniofacial abnormalities, limb and skeletal defects and cystic kidneys.[44] The classical orofacial abnormalities such as cleft palate, bifid or lobulated tongue, and teeth abnormalities were observed in 100% of the mice analysed to date, while the same dysmorphic signs are observed in patients in different proportions, in about 30–80%, 30–45%, and 50%, respectively, for cleft palate, bifid or lobulated tongue, and teeth abnormalities. Involvement of the CNS occurs in 40% of patients while CNS malformations are present in all the female mutants analysed to date (B Franco, unpublished data). Limb abnormalities are present in a large number of cases and usually include brachydactyly, clinodactyly and syndactyly, while pre- and/or postaxial polydactyly is rarely observed in these patients. On the contrary, all the mutant animals analysed to date display polydactyly and other skeletal abnormalities[44] (B Franco, unpublished data). In addition, polycystic kidney disease that is present in about 40% of OFDI patients can be detected in 100% of female mutants. Finally, cardiac abnormalities are never observed in OFDI patients while they represent a common feature in $Ofd1^{\Delta 4-5/+}$ mutant mice. Figure 3 displays examples of the differences observed between *Ofd1* mutant animals and OFDI patients.

The majority of mutant male embryos, $Ofd1^{\Delta 4-5}$, die by E12.5, and display early developmental defects, mainly neural tube, heart and laterality defects. Apparently, complete inactivation of *Ofd1* is not compatible with life in hemizygous males and causes male embryonic lethality both in mutant animals and patients.

Most of the genes that escape inactivation in humans are subjected to XCI in mice.[4 51] Also *Ofd1* has been shown to be subjected to XCI and thus displays a different pattern of XCI in the two species.[52] The differences in the XCI pattern could provide an explanation for the differences observed in the phenotypic manifestations between OFDI patients and the mutant mice.

In mice, heterozygous females carrying *Ofd1* mutation on one of the X chromosomes are mosaics for the presence of cells expressing the protein, together with cells completely devoid of Ofd1 function. For the same phenomenon a few cells will be completely devoid of Ofd1 as they carry the mutation on the active X and have the second X inactivated. This situation, which implies that the basal level of expression required for *Ofd1* cannot be reached by each cell, will result in the appearance of more severe clinical manifestations with a consequent perinatal lethality in heterozygous females. Hemizygous male mutants $Ofd1^{\Delta 4-5}$ do not have any active protein, as they carry a mutated *Ofd1* gene on the only X chromosome they have for each cell.

The threshold level hypothesis in this scenario underlies male embryonic lethality both in mice and humans, and perinatal female lethality in mice.

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