Review

Generalised and conditional inactivation of Pex genes in mice

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Abstract

During the past 10 years, several Pex genes have been knocked out in the mouse with the purpose to generate models to study the pathogenesis of peroxisome biogenesis disorders and/or to investigate the physiological importance of the Pex proteins. More recently, mice with selective inactivation of a Pex gene in particular cell types were created. The metabolic abnormalities in peroxisome deficient mice paralleled to a large extent those of Zellweger patients. Several but not all of the clinical and histological features reported in patients also occurred in peroxisome deficient mice as for example hypotonia, cortical and cerebellar malformations, endochondral ossification defects, hepatomegaly, liver fibrosis and ultrastructural abnormalities of mitochondria in hepatocytes. Although the molecular origins of the observed pathologies have not yet been resolved, several new insights on the importance of peroxisomes in different tissues have emerged.

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1. Introduction

The metabolic role of peroxisomes has been extensively studied during the last 30 years mostly by using the rat liver as a source of peroxisomes [1]. The identification of human diseases that are due to peroxisomal dysfunction underscored the physiological importance of peroxisomal metabolism in different tissues. However, the molecular mechanisms leading to anomalies such as neuronal migration defects, hypotonia, dys- and demyelination, kidney, eye and testicular defects, abnormalities in bone development and facial dysmorphism remain unresolved. Since naturally occurring peroxisomal disease models are lacking, it was necessary to generate such models by gene manipulation to study the pathogenesis of these diseases. The newly discovered Pex genes and the novel insights in the mechanism of peroxisomal matrix protein import offered a unique opportunity to apply gene targeting techniques. Depending on the mutated gene, mice were created in which either PTS1- and PTS2-dependent protein import (Pex5, Pex2, Pex13), or only PTS2-dependent import (Pex7), or peroxisome proliferation (Pex11) were affected. More recently, mice with conditional inactivation of peroxisome biogenesis in specific cell types became available i.e. mice with selective inactivation in hepatocytes and in Sertoli cells.

2. Generalised inactivation of Pex genes involved in PTS1- and PTS2-dependent protein import: models for Zellweger syndrome?

2.1. Phenotype of the mice

Three different Pex genes were inactivated in the mouse i.e. Pex5, Pex2 and Pex13 [2–4]. In view of the complete block of PTS1- and PTS2-dependent matrix enzyme import in each case, the three knockout lines were expected to constitute a model for Zellweger syndrome, the most severe peroxisome biogenesis disorder [5]. All knockout mice displayed severe hypotonia and growth retardation at birth and they died within a few days. A
notable exception were the Pex2 knockout mice bred in a specific genetic background i.e. Swiss Webster ×129SvEv a fraction of which was less hypotonic and survived 1, 2 or rarely 3 weeks [6]. As discussed below, all knockout strains displayed abnormalities in the formation of the brain. However, other typical hallmarks present in fetal Zellweger patients were not found, including renal cysts and facial dysmorphism. At birth the mice did not display major liver pathology, a feature that only develops in the postnatal period in patients.

At the subcellular level, embryonic fibroblast cultures derived from Pex5, Pex2 and Pex13 knockout mice contained peroxisomal remnants which were reduced in number but increased in size as compared to regular peroxisomes [2–4]. This is very similar to findings in Zellweger patient fibroblasts. Recently, it was demonstrated that in cultured Pex13 null mouse fibroblasts (like in Pex1 deficient human fibroblasts), remnant peroxisomes displayed an altered distribution as compared to normal peroxisomes in wild type fibroblasts (Fig. 1A, B) [7]. The peroxisomal ghosts were only present in the cell centre but not in the periphery [7]. However, the peroxisomal ghosts were still associated with microtubuli, as previously shown for normal peroxisomes in different cell lines like HepG2 and Cos cells [8]. Likewise, in cultured Pex13 null neurons and astrocytes peroxisomes were clustered in the cell soma.

Ultrastructural examination of the liver of the three Pex knockout models revealed severe abnormalities of the mitochondria in hepatocytes, in particular at the level of the inner mitochondrial membrane [2,4,9,10] (see also 5.1). Such mitochondrial anomalies in hepatocytes were also described in some early papers on Zellweger patients [11–13].

Overall, it can be concluded that several but not all features of human Zellweger patients are mimicked in the mouse models. This might be related to the shorter timeframe of murine as compared to human fetal development. It is in fact quite remarkable that within the short period in which the mouse brain is formed, the absence of functional peroxisomes has such an important impact, as will be further discussed below.

2.2. Metabolic changes

Most of the major metabolic changes observed in Zellweger patients were recapitulated in the mouse models. An important advantage of the latter models is that metabolite levels can be measured not only in body fluids but also in the diseased tissues at several stages. It was striking that C26:0 was already 2-fold increased measured not only in body fluids but also in the diseased tissues patients were recapitulated in the mouse models. An important impact, as will be further discussed below.

This might be related to the shorter timeframe of murine as compared to the development of steatosis and cholestasis [10]. In 10-day-old Pex2 knockout mice, cholesterol levels of plasma and liver were also 40% reduced but they were normal in brain and other tissues. The reduced cholesterol levels were accompanied by a concerted increase in expression and activity of cholesterol synthesizing enzymes was either normal or slightly increased [18]. This is not consistent with the 40% reduction of plasma cholesterol described in newborn Pex2 knockout mice [10]. In 10-day-old Pex2 knockout mice, cholesterol levels of plasma and liver were also 40% reduced but they were normal in brain and other tissues. The reduced cholesterol levels were accompanied by a concerted increase in expression and activity of cholesterol synthesizing enzymes in liver homogenates probably mediated by the induction of SREBP2. Because it is well known that the brain is self sufficient with regard to cholesterol synthesis, the normal cholesterol content in brain of Pex5 and Pex2 knockout mice strongly suggests that the absence of import competent peroxisomes has no direct negative effects on the cholesterol genesis. The aberrant cholesterol homeostasis in liver of Pex2 knockout mice might be a consequence of the metabolic perturbations that occur in the liver as exemplified by the development of steatosis and cholestasis [10].

2.3. Cortical neuronal migration

The disturbed lamination of the cortical plate associated with medial pachygryria and lateral polymicrogyria is a major characteristic of Zellweger patients [19–21]. It is ascribed to a unique defect of the neuronal migration process that is clearly distinguishable from other migration disorders such as those seen in lissencephaly or double cortex syndrome. In all three Pex knockout models an altered distribution of cortical neurons was observed [2–4] (Fig. 1C, D). For Pex5 and Pex2 knockout mice, a migration disorder was demonstrated by performing 5′,3′-bromo-2′-deoxyuridine (BrdU) birthdating experiments. Furthermore, a delay in the differentiation of neurons, increased apoptotic cell death but a normal distribution of radial glial cells were reported for Pex5 knockout pups.

Fig. 1. Pathology in generalised Pex knockout mice. (A–B) Immunofluorescent staining of α-tubulin (red) and Pex14p (green) to visualise microtubuli and peroxisomal membranes in cultured fibroblasts. In wild type fibroblasts (A) peroxisomes are associated with microtubuli throughout the cell whereas in Pex13−/− fibroblasts (B) peroxisomes are clustered in the cell centre but still aligned with microtubuli. (C–D) Cresyl violet staining of frontal sections of the cortex of newborn Pex5 knockout mice reveals increased cell densities in the intermediate zone (IZ) of the knockout, the prospective white matter. CP=cortical plate, GZ=germinative zone. (E–F) Immunofluorescent staining of Purkinje cells in the cerebellum of 7-day-old wild type (E) and Pex2−/− mice (F) using the anti-calbindin D28k antibody. In the knockout, these cells remain polydendritic and the size of the dendritic arbor and the degree of branching are markedly reduced. (G–H) Whole mount skeletal staining of cartilage (blue) and bone (red) of newborn wild type (G) and Pex7−/− pups (H). A lack of ossification in the middle phalanges of the hindpaws of the knockout is visible (arrows). Reprinted from [7,2,6,29] with permission from the respective publishers.
It was further investigated whether reduced levels of DHA or increased levels of VLCFA could play a causative role in these defects. Supplementation of pregnant dams with DHA normalised the levels of this PUFA in brain but did neither improve hypotonia, nor the migration disorder [16]. In mice with a selective defect of peroxisomal β-oxidation by inactivating multifunctional protein 2 (MFP-2), levels of C26:0 were increased to the same extent as in Pex5 knockout mice but no migration defect could be observed [22]. This is in variance with the human situation where MFP-2 deficiency is often associated with brain malformations which are very similar to those present in Zellweger syndrome [23]. These observations rule out that alterations in fatty acid levels on their own induce cytotoxic abnormalities in mouse brain.

The possible involvement of glutamatergic neurotransmission via the NMDA receptor, known to control the speed of migration was also examined [24]. Treatment of Pex5−/− embryos with NMDA antagonists induced embryonic death whereas NMDA agonists partially reversed the migration defect [25]. A deficit in NMDA signal transduction was demonstrated in neuronal cultures derived from Pex5 knockout mice by monitoring calcium influx in response to NMDA. Pex5−/− cells were less sensitive to NMDA than wild type cells but sensitivity could be restored by preincubation with the ether phospholipid platelet activating factor (PAF) [25]. Attempts to prove that the concentration of PAF is reduced in brain of Pex5−/− mice did not succeed probably due to the very low levels and instability of PAF but the content of the degradation product lysoPAF was 3-fold reduced (H. Van Overloop, M. Baes and P. Van Veldhoven, unpublished observations). Although PAF is a well known regulator of neuronal migration [24] and thus a very good candidate to link peroxisome deficiency to migration, the absence of clearcut cortical migration defects in Rhizomelic Chondrodysplasia Punctata (RCDP) patients, who have a selective and severe deficiency of ether lipids, contradicts such a primary role of PAF. To further document a potential involvement of PAF in the neuronal migration defect associated with peroxisome deficiency, it will be of importance to investigate cortical lamination in DHAPAT knockout mice [26], a model with a selective depletion of ether lipids.

A major question is whether the brain malformations of peroxisome deficient mice are caused by the local absence of peroxisomes in the brain or by extraneural deletion of peroxisomal metabolic activity. Pex5−/− mice with a selective reconstitution of peroxisomal function in brain or in liver both exhibited a significant correction of the neuronal migration defect despite an incomplete reconstitution of peroxisomal function in the targeted tissue [27]. These data suggest that peroxisomal metabolism in brain but also in extraneural tissues is necessary for the normal development of the mouse neocortex. Interestingly, despite the improvements of the neuronal migration, both Pex5 rescue models were as hypotonic as the generalised Pex5 knockout mice and died on the day of birth.

Overall, these investigations with the Pex knockout mouse models were important to prove that peroxisome deficiency leads to cortical malformations in another species besides humans. A number of metabolites were excluded as single causative factors but there is still no clear view on the molecular mechanism underlying the migration defect.

2.4. Formation of cerebellum

In contrast to the cortex, the formation of the cerebellum extends into the postnatal period of the mouse. Therefore, the early death of Pex5 and Pex13 knockout mice precluded the analysis of cerebellar development but the longer survival of some Pex2 knockout mice allowed an extensive analysis of cerebellar histogenesis [6]. Abnormalities were reported at the level of granule cells with slower migration from the external granular layer (EGL) to the internal granular layer (IGL) and increased cell death in the EGL. These migratory abnormalities in the postnatally developing cerebellum were more severe than those seen in the prenatally developing cortex [6]. This was attributed to postnatal malnutrition problems, increasing hepatic or renal dysfunction after birth and/or to the fact that maternal metabolism could clear toxic substances from the embryonic circulation [6]. The majority of Purkinje cells aligned under the cerebellar surface and only rare cells mislocalised in the IGL which is very different from the extensive Purkinje cell heterotopias in human Zellweger patients [19,20]. However, the Purkinje cells displayed stunted dendritic trees with abnormal arborization (Fig. 1E, F). A delayed maturation of the olivary climbing fibers and their defective translocation from the perisomatic to the dendritic compartment of Purkinje cells resulted in numerous spines on the soma and dendrites of Purkinje cells. Preliminary studies by Faust et al. with mixed cerebellar cultures further suggested that the abnormal maturation of Purkinje cells might be due to the inactivity of intrinsic peroxisomal metabolism as well as to the elimination of hepatic peroxisomal metabolism [28]. Indeed, also in cultured Pex2 knockout Purkinje cells a deficient branching was observed whereas on the other hand, supplementation of pups with mature bile acids not only improved survival of the mice but also arborization of Purkinje cells.

3. Inactivation of Pex7 involved in PTS-2 dependent protein import: a model for RCDP type 1?

In mammals, the task of Pex7p is restricted to the import of a few PTS2 containing proteins. Thus far only alkyl DHAP synthase, phytanoyl CoA hydroxylase and 3-oxoacyl-CoA thiolase were unequivocally shown to possess such a signal but they take part in three major peroxisomal pathways i.e. ether phospholipid synthesis, α-oxidation and β-oxidation, respectively.

In Pex7 knockout mice [29] the inactivity of mislocalised alkyl DHAP synthase causes the expected depletion of plasmalogens in brain and erythrocytes. Due to the mislocalisation of phytanoyl-CoA hydroxylase a slight accumulation of phytanic acid was seen in newborn mice. Supplementation of adult Pex7 knockout mice with phytol triggered a massive increase in plasma and tissue levels of this branched chain fatty acid. Based on data of RCDP type 1 patients in which straight chain peroxisomal β-oxidation substrates are not accumulating [5,30],

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probably because of the alternative thiol activity exerted by SCPx, it was surprising to find that C26:0 levels were elevated in plasma and tissues of newborn Pex7 knockout mice.

Given this impaired functioning of all major peroxisomal pathways, it is not surprising that the phenotype of Pex7 knockout mice is very similar to that of Pex5 knockout mice although the penetrance is very variable [29]. About 50% of the Pex7 knockout mice were very hypotonic at birth and died postnatally, 20% died before weaning and the others survived past 18 months. All knockout mice displayed a neuronal migration defect that was less severe than in Pex5 knockout mice. At birth several cartilage based structures that depend on endochondral ossification for their formation were not ossified or showed a marked delay in ossification e.g. distal bone elements of the limbs, parts of the skull and the vertebrae (Fig. 1G, H) [29].

Since abnormalities in bone formation are a major hallmark of RCDP patients, this mouse model should allow for the further investigation of the precise role of peroxisomes in the ossification process. Other features observed in Pex7 knockout mice that need further study are cataract and male infertility [29].

4. Inactivation of Pex11α and β

In contrast to the other knocked out peroxins, Pex11 proteins, encoded by three different genes in mammals, do not have a function in the import of peroxisomal matrix proteins [31]. They are peroxisomal membrane proteins that seem to be involved in the division of peroxisomes since overexpression of Pex11β leads to peroxisome proliferation whereas inactivation causes reduced peroxisome abundance [32,33]. Pex11αβ seems to be responsible for peroxisome proliferation in response to external stimuli whereas Pex11β/β is required for constitutive peroxisome biogenesis [31]. A recently reported function of Pex11β/β is to recruit the dynamin like protein DLP1 to the peroxisomal membrane [34].

Pex11α knockout mice did not have an obvious phenotype suggesting that its function can be taken over by other Pex11 proteins [35]. In tissues of Pex11β knockout mice only minor metabolic alterations were found i.e. a 20% depletion of plasmalogens levels in brain and a 1.4-fold elevation of C26:0 in liver [36]. Moreover, studies in fibroblasts documented normal import of PTS1 and PTS2 containing proteins. It was therefore unexpected that the phenotype of Pex11β deficient mice was very similar to that of Pex5, Pex2 and Pex13 knockout mice [36] i.e. these mice were all very hypotonic and growth retarded at birth, they displayed a neuronal migration defect and died in the postnatal period. However, no alterations of mitochondrial structure in hepatocytes were noted. Although it could be envisaged that the migration defect and hypotonia are caused by metabolic abnormalities different from peroxisomal β-oxidation or ether phospholipid synthesis defects, it seems inexplicable that such a severe phenotype is present despite intact import of PTS1 and PTS2 proteins. In order to reconcile these discrepancies an alternative model of pathogenesis was proposed in which the disease phenotype correlates with the abundance and distribution of peroxisomes rather than with their metabolic activity [7]. Indeed, Pex11β deficient fibroblasts and hepatocytes have a reduced peroxisome abundance and increased clustering and elongation of peroxisomes in common with Pex13 knockout fibroblasts and neurons [7]. For Pex13 knockout fibroblasts and neurons it was shown that the remnant peroxisomes were associated only with centrally located microtubuli, and they were apparently not able to travel to the cell periphery (see also 2.1). It is not unlikely that the abnormal distribution of peroxisomes on these microtubuli hinders the dynamic activity of the cytoskeleton which is necessary for normal migration to occur [37,38].

5. Conditional Pex5 KO mice

Because of their early postnatal death, the generalised Pex2, Pex5 and Pex13 knockout mice do not allow to study the functional importance of peroxisomes in adult tissues. Fortunately, thanks to a second wave of technological developments a gene can now be conditionally inactivated in the mouse. The technique is based on the use of two mouse lines, one in which two LoxP sites are introduced by homologous recombination in introns flanking an essential part of the gene of interest and another line expressing Cre recombinase. In the presence of Cre recombinase, the gene fragment encompassed by the two loxP sites will be deleted, given that these sites have the same orientation [39]. By mating the loxP containing mice with mice expressing Cre recombinase under the control of a cell type specific promoter, the target gene will be inactivated from the moment that the promoter driving Cre expression becomes active in the target cells. At this point two mouse lines with floxed Pex genes are available i.e. Pex5-loxP [40] and Pex13-loxP mice [41].

5.1. Mice with selective inactivation of peroxisomes in hepatocytes in the postnatal period

As already mentioned, the only defect observed in liver of newborn Pex2, Pex5 and Pex13 knockout mice was a change in the ultrastructure of the mitochondrial inner membrane. In order to investigate hepatic changes developing at later ages, Pex5-loxP mice were bred with mice expressing Cre under the control of the albumin promoter [42]. The resulting mice with hepatocyte specific elimination of peroxisomes will be denoted further as L-Pex5 knockout mice [43].

Functional peroxisomes were eliminated from the liver between the first and second postnatal week, as shown by a number of biochemical parameters. Electronmicroscopic analysis of 10-week-old mice further confirmed the virtual absence of catalase containing peroxisomes from hepatocytes but not from endothelial cells and Kupffer cells [43]. Only a few small clusters of hepatocytes with catalase-positive peroxisomes were found. Surprisingly, plasmalogens and C26:0 levels were normal in the peroxisome deficient livers suggesting that peroxisomes present in other tissues provided precursors for plasmalogens and degraded C26:0. Other parameters i.e. the concentration of branched chain fatty acids phytanic acid and pristanic acid and the ratio of C27/C24 bile acids were increased in L-Pex5 knockout livers, as expected.
Two features that are common in postnatal Zellweger patients also occurred in L-Pex5 knockout mice. First, they displayed a severe hepatomegaly which was due to hypertrophic and hyperplastic hepatocytes, particularly in the pericentral region (Fig. 2A, B). Furthermore, fibrosis developed in these livers from the age of 20 weeks on (Fig. 2C, D). Throughout the lifetime of the mice (>15 months), they looked healthy, were fertile and hepatocellular integrity was unaffected as judged by normal plasma values of alanine and aspartate aminotransferase. However, all the L-Pex5 knockout mice developed extensive liver tumours from 12 months on [43].

Severe changes in mitochondrial ultrastructure were observed in 60–70% of the mitochondria in peroxisome deficient hepatocytes. The most common finding was the proliferation of pleomorphic mitochondria with rarefication of cristae or with abnormally curled or stacked cristae. Strikingly, mitochondria were normal in the few hepatocytes in which catalase-positive peroxisomes were present, indicating that this is a cell autonomous phenomenon. Additional ultrastructural changes in hepatocytes lacking peroxisomes were the proliferation of smooth endoplasmic reticulum (sER) and the appearance of lipid droplets and large groups of lysosomes with electron-dense deposits around dilated bile canaliculi. The ER proliferation was associated with the induction of the cytochrome P450 ω-hydroxylation enzyme CYP4A1 [43].

Analyses of mitochondrial functions demonstrated that the complexes I, III and V of the respiratory chain that are embedded in the inner membrane were severely inactivated whereas several matrix enzymes including citrate synthase and mitochondrial β-oxidation were induced. Given the importance of the complexes to generate ATP, it was surprising that no significant depletion of ATP levels were found [43]. However, a compensatory increase in glycolysis as an alternative source of ATP was demonstrated. Another consequence of the impaired functioning of the respiratory chain was a collapse of the inner mitochondrial membrane potential (Fig. 2E, F). Because dysfunction of the electron transport chain is often accompanied by the generation of oxygen radicals that in turn can cause mitochondrial damage, the hypothesis that reactive oxygen species were increased in the peroxisome deficient livers was investigated. However, neither oxidative damage to proteins or lipids, nor increased peroxide production in cultured hepatocytes or elevation of oxidative stress defence mechanisms were found, indicating that the mitochondrial alterations were not related to an excessive production of oxidative radicals [43].

Altogether, it can be concluded that peroxisomes are essential for the maintenance of other subcellular compartments in adult hepatocytes although their absence is not detrimental for the functioning of the cell. The molecular interrelationship between peroxisomes and mitochondria remains to be investigated.

5.2. Mice with selective inactivation of peroxisomes in Sertoli cells

In view of the early postnatal death of patients with peroxisome biogenesis disorders, not much is known about the importance of peroxisomes for testicular function and fertility. Testicular abnormalities were reported in patients with other peroxisomal diseases with longer survival such as X-linked adrenoleukodystrophy [44,45] or X-linked adrenomyeloneuropathy [46]. Male infertility was reported in three different mouse models with single peroxisomal enzyme deficiencies i.e. acyl-CoA oxidase [47], MFP-2 [48] and DHAPAT [26] knockout mice. In acyl-CoA oxidase deficient mice a reduction in the Leydig cell population and hypospermatogenesis were seen whereas in DHAPAT knockout mice an arrest of spermatogenesis before the stage of round spermatids resulting in the complete absence of spermatozoa was described. In contrast, infertility in MFP-2 knockout mice [48] was associated with the accumulation of huge lipid droplets in Sertoli cells followed by a complete fatty degeneration of the tubuli seminiferi while Leydig cell function was preserved. In fact, until recently it was thought that peroxisomes were present only in the interstitial cells of Leydig but according to new data [48,49] they occur also in spermatogonia and in Sertoli cells, both located in the basal compartment of the seminiferous epithelium.

The Cre-loxP technology was recently applied to demonstrate the crucial role of peroxisomes in Sertoli cells. Therefore, Pex5-loxP mice were bred with mice expressing Cre under the control of the Anti-Mullerian hormone (AMH) promoter [48,50]. In the testis extensive accumulations of neutral lipids were observed in Sertoli cells (Fig. 2G, H), beginning in prepubertal mice and evolving in complete testicular atrophy by the age of 4 months. Spermatogenesis was already severely affected at the age of 7 weeks and pre- and postmeiotic germ cells gradually disappeared from the tubuli seminiferi. The AMH-Pex5 knockout mice were completely infertile. Together with analogous Sertoli cell abnormalities in MFP-2 knockout mice [48], these data strongly indicate that peroxisomal β-oxidation is essential to maintain the lipid balance in Sertoli cells which in turn is crucial for male fertility. It needs to be further investigated how the fatty degeneration of the testis relates to the loss of peroxisomal function in Sertoli cells.

6. Conclusions

The phenotypic analyses of mouse models with peroxisome biogenesis disorders have taught us that several but not all changes overlap with those occurring in human diseases. The discrepancies may relate to the huge time difference in developmental period between mice and men and to differences in dietary composition.

In order to understand the importance of peroxisomes for the functioning of different tissues, these animal models still need to be investigated in more detail, including gene expression and more extensive lipidomic analyses. The latter will require sensitive techniques because several of the substrates or reaction products of the peroxisomal pathways are present in very low amounts. It will be further instrumental to compare the metabolic and histological abnormalities of the Pex knockout models with those of single peroxisomal enzyme or transporter knockouts that are already available or being generated (Acox1 [47], MFP-1 [51], MFP-2 [48,52,53], SCPx [54], phytanoyl-CoA hydroxylase, α-methylacyl-CoA racemase [55], DHAPAT [26], ALD [56–58],...
Fig. 2. Pathology in tissue selective Pex5 knockout mice. (A–B) Hematoxylin–eosin staining of livers of 10-week-old control (A) and L-Pex5 knockout mice (B) reveals hypertrophic and hyperplastic hepatocytes in the knockout, in particular in the pericentral region. (C–D) Sirius red stains collagen fibers in 20-week-old L-Pex5 knockout mice (D), compatible with fibrosis, but not in control mice (C). (E–F) Hepatocyte cultures were incubated with the mitotracker JC-1. Mitochondria from control mice (E) fluoresce orange corresponding with a normal inner mitochondrial membrane potential whereas those of L-Pex5 knockout mice fluoresce green indicative of a reduced potential, n = nucleus. (G–H) Oil red O stains neutral lipid droplets in the outer layer of tubuli seminiferi of 9-week-old AMH-Pex5 knockout mice (H) (arrowheads) but not in control mice (G). This is compatible with a Sertoli cell localisation. Leydig cells (arrows) stain in both genotypes because of their high steroid content. (A–F) Reprinted from [43] with permission from the publisher.
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