SCARB2/LIMP2 deficiency in action myoclonus-renal failure syndrome

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ABSTRACT – Action myoclonus–renal failure syndrome (AMRF) is an autosomal recessive progressive myoclonus epilepsy (PME) associated with renal dysfunction that appears in the second or third decade of life and that is caused by loss-of-function mutations in the SCARB2 gene encoding lysosomal integral membrane protein type 2 (LIMP2). Recent reports have documented cases with PME associated with SCARB2 mutations without renal compromise. Additional neurological features can be demyelinating peripheral neuropathy, hearing loss and dementia. The course of the disease in relentlessly progressive. In this paper we provide an updated overview of the clinical and genetic features of SCARB2-related PME and on the functions of the LIMP2 protein.

Key words: progressive myoclonus epilepsy, myoclonus, cerebellar syndrome, photosensitivity, SCARB2, LIMP2, lysosome

In 1986, Andermann *et al.* reported four French Canadian patients from three sibships with a condition characterized by the appearance of tremor in the fingers and hands and proteinuria at 17-18 years of age. Severe progressive action myoclonus, dysarthria, ataxia, infrequent generalized seizures, and renal failure ensued between 19 and 23 years of age. Despite severe neurological disability, due mainly to action myoclonus, intelligence remained normal in all four patients. They labelled this condition 'action myoclonus-renal failure (AMRF) syndrome'. Since this first description, it has been pointed out that the neurological picture was not caused merely by a metabolic encephalopathy due to renal failure, but rather was the result of a pathophysiological process that appeared to involve primarily both the brain and the kidneys (Andermann *et al.*, 1986). This syndrome was not recognized prior to the advent of dialysis and renal transplantation because of its rapidly fatal course if renal failure is untreated.

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Discovery of *SCARB2* as the causative gene for AMRF

Three unrelated Australian families with a single proband were used to identify *SCARB2* as the causative gene for AMRF. Case A was of Turkish-Cypriot origin; her parents were first cousins. The ancestors of families B and C came from different regions of Britain and no inbreeding loops were known for either family. Case C was deceased, but stored brain tissue in paraffin blocks was available for DNA extraction.

A critical region of 5.3 cM (equivalent to 6.6 Mb) on chromosome 4 (4q13-21) was narrowed down by identifying an overlap of regions in which Case A and Case C were homozygous by descent. The region was reduced slightly in size by excluding a region in family B in which the affected individuals shared a segment identical by descent with an unaffected sibling.

The critical region on chromosome 4 contained 66 annotated genes of which approximately half are expressed in both the brain and kidney. In order to prioritize candidate genes for sequencing, it was hypothesized that the mRNA of the causative gene would be downregulated in affected subjects, possibly because of mutations causing RNA instability or removal by the process of nonsense-mediated decay. RNA from lymphoblastoid cell lines derived from two living affected subjects and from a healthy gender-matched sibling of each (families A and B) was analysed with Affymetrix U133 Plus2 arrays to look for RNAs with decreased abundance in the affected individuals in comparison to their unaffected sibling. RNA analysis was confined to the probe sets in the 6.6 Mb chromosome 4 region, defined by homozygosity mapping. *SCARB2* (*Scavenger Receptor B2*) emerged as a possible candidate gene. The amount of mRNA was reduced approximately 2-fold in the affected subjects compared to their healthy siblings.

The protein-coding regions of the *SCARB2* gene were analysed for mutations by direct Sanger sequencing. A homozygous splice-site mutation (c.1239+1G>T) in Case A was identified. RT-PCR analysis showed that this mutation leads to retention of intron 10 and the subsequent insertion of 20 amino acids, and premature truncation of the protein at residue 433. A homozygous mutation in Case C (c.435_436insAG W146SfsX161) was identified resulting in a frameshift predicted to truncate the SCARB2 protein to 160 amino acids. Case B was a compound heterozygote, carrying two different mutations in the *SCARB2* gene: a frameshift mutation (c.296 delA N99IfsX34) predicted to shorten the protein to 131 amino acids and a splice site mutation c.704+5G>A. These findings confirmed *SCARB2* as a causative gene for AMRF (Berkovic *et al.*, 2008).

The *SCARB2* gene encodes a 478 amino acid glycoprotein located in lysosomal membranes in a range of tissues including the brain and kidney. The function of *SCARB2* is not well understood, but it is thought to play a role in the biogenesis and maintenance of endosomal and lysosomal compartments. The human and mouse SCARB2/Limp2 proteins share 85 per cent amino acid identity.

Founder mutations of SCARB2 in the French-Canadian and Scottish populations

A number of families were identified from Quebec, Canada, to have probands clinically diagnosed with AMRF. The Quebec population is known to have a high degree of consanguinity which results in a higher incidence of recessive disorders than other parts of the world. Molecular analysis of SCARB2 found that all but one of the Quebec cases of AMRF were homozygous for the mutation c.862C>T, Q228X. Haplotype analysis using microsatellite markers on affected members and carriers was employed to determine whether SCARB2 Q288X was a founder mutation inherited from a common ancestor. A shared haplotype spanning 0.6 cM encompassing the SCARB2 mutation indicated the presence of a founder Q288X mutation for AMRF in the Quebec population. One family from Quebec was found to carry a second SCARB2 mutation, c.1197+3insT.

Two subjects with AMRF from Scotland were found to be homozygous for the SCARB2 mutation c.435_436insAG (W146S fs X161). This mutation is the same as that found in an Australian patient (Case C above) and in a Canadian patient (not French-Canadian). Haplotype analysis of the Australian, Canadian and the two Scottish cases, which were not previously known to be related, showed a shared haplotype of 0.6 cM, indicating that the mutation was inherited from a shared ancestor. The Scottish population, therefore, also contains a SCARB2 founder mutation, W146S fs X161, causing AMRF.

Further cases of AMRF due to SCARB2 mutations

Given that demyelinating neuropathy is seen in the mouse model deficient in functional SCARB2/Limp2 (Gamp *et al.*, 2003), a patient from the USA presenting with PME and asymptomatic neuropathy was hypothesized to carry a SCARB2 mutation. This patient was found to be a compound heterozygote, carrying two different AMRF-causing mutations: the Q288X (Quebec) mutation on one chromosome and the

second Quebec mutation c.1187+3insT on the other (Dibbens *et al.*, 2011). Since renal dysfunction is usually seen in AMRF, this patient is now undergoing tests for kidney function. Further cases of AMRF from Argentina, Turkey, Portugal and Spain (Balreira *et al.*, 2008; Perandones *et al.*, 2012) have been found to be caused by mutation of SCARB2, suggesting the syndrome is likely to be found worldwide.

Clinical features of action myoclonus renal failure

Following the initial report by Andermann *et al.* (1986), several studies confirmed that the predominant clinical manifestations of AMRF are progressive myoclonus epilepsy (Minassian *et al.*, 2016) and renal failure (Badwhar *et al.*, 2004; Vadlamudi *et al.*, 2006; Balreira *et al.*, 2008; Perandones *et al.*, 2012). Disease onset is typically in the late teens or early twenties, and the neurological features can be seen before, after, or simultaneously with the renal features. The neurological picture may present as a tremor, which is typically first noted in the fingers and hands, present at rest and exacerbated by fine motor activities. The tremor can later involve the head, trunk, lower extremities and sometimes the tongue and voice. As the disease progresses, involuntary spontaneous action-activated myoclonic jerks are seen, as well as asynchronous involuntary spontaneous myoclonic jerks at rest. A reflex myoclonus which is sensitive to touch on the extremities is also present. Action myoclonus, refractory to antimyoclonic drugs, is the most debilitating feature of the disease and, in the final stages, it renders the patients bedridden or wheelchair-bound with lap, trunk and leg belts. Diurnal or nocturnal generalized tonic-clonic seizures occur in the majority of patients. Badhwar *et al.* (2004) reported that the convulsive seizures start with a generalized clonic phase with preserved consciousness proceeding to unconsciousness with tonic-clonic features. Antiepileptic drugs can control convulsive seizures without affecting ongoing active myoclonic jerks. Other common features appearing during the course of the disease include ataxia and dysarthria due to cerebellar dysfunction. Remarkably, despite the progression and severity of the neurological picture, cognitive function is preserved or only slightly affected until the final stages of the disease.

A demyelinating peripheral neuropathy has been reported in a number of patients (Dibbens *et al.*, 2011; Hopfner *et al.*, 2011; Rothdach *et al.*, 2001), while electrophysiological findings indicating a predominantly axonal neuropathy have been observed in a patient without clinical evidence of involvement of the peripheral nervous system (Badhwar *et al.*, 2004). Auditory defects ranging from abnormal brainstem auditory evoked potentials without clinical expression to severe hearing loss have been reported by Perandones *et al.* (2012, 2014) in a patient and two siblings with SCARB2 mutations and clinical features of AMRF. Interestingly, both these latter neurological manifestations correlate with the phenotype of the Limp2 knock-out mice whose neurological alterations consist of deafness and peripheral neuropathy, without features of progressive myoclonus epilepsy (Gamp *et al.*, 2003). Finally, a dilated cardiomyopathy has been described in two patients (Hopfner *et al.*, 2011).

Renal involvement in AMRF is heralded by the appearance of proteinuria that can relentlessly progress to a nephrotic syndrome and end-stage renal disease, requiring dialysis or renal transplantation. Detection of proteinuria usually occurs around the age of 20, although onset in childhood has been reported (Badhwar *et al.*, 2004). No correlation has been observed between the ages of onset of proteinuria and tremor, nor between renal failure and onset of myoclonus.

The absence of renal involvement in PME associated with SCARB2 mutations has also been described. In 2009, Dibbens *et al.* reported SCARB2 mutations in 5 of 41 cases considered clinically to be 'Unverricht-Lundborg disease (ULD)-like' (Dibbens *et al.*, 2009). The patients had disease onset between 14 and 26 years of age, with no evidence of renal failure during 5.5 to 15 years of follow-up, although one of them had slight proteinuria in the final stage of the disease. Death ensued in all five patients (the only surviving patient at the time of the report died later). Since this initial report, other cases have been reported and the clinical features of PME without renal failure associated with SCARB2 have been described (Rubboli *et al.*, 2011; Guerrero-López *et al.*, 2012; Higashiyama *et al.*, 2013; Fu *et al.*, 2014; Zeigler *et al.*, 2014). Features seen in these patients included a variable severity of epilepsy: from uncontrolled seizures or status epilepticus with prominent photosensitivity in patients with adolescent onset, to infrequent or no major seizures in patients with a more delayed onset (Rubboli *et al.*, 2011), late onset in adulthood (Higashiyama *et al.*, 2013; Fu *et al.*, 2014), and the occurrence of dementia (Fu *et al.*, 2014). These findings suggest that SCARB2 mutations in patients with PME without renal complications might not be rare and that SCARB2 gene mutations should therefore be evaluated even in the absence of renal involvement.

The course of AMRF is fatal with relentless progression of neurological deterioration and increasing severity of myoclonus and renal impairment leading to death usually within 7 to 15 years after disease onset, due to renal failure, aspiration pneumonia, or septicaemia with multiorgan failure.
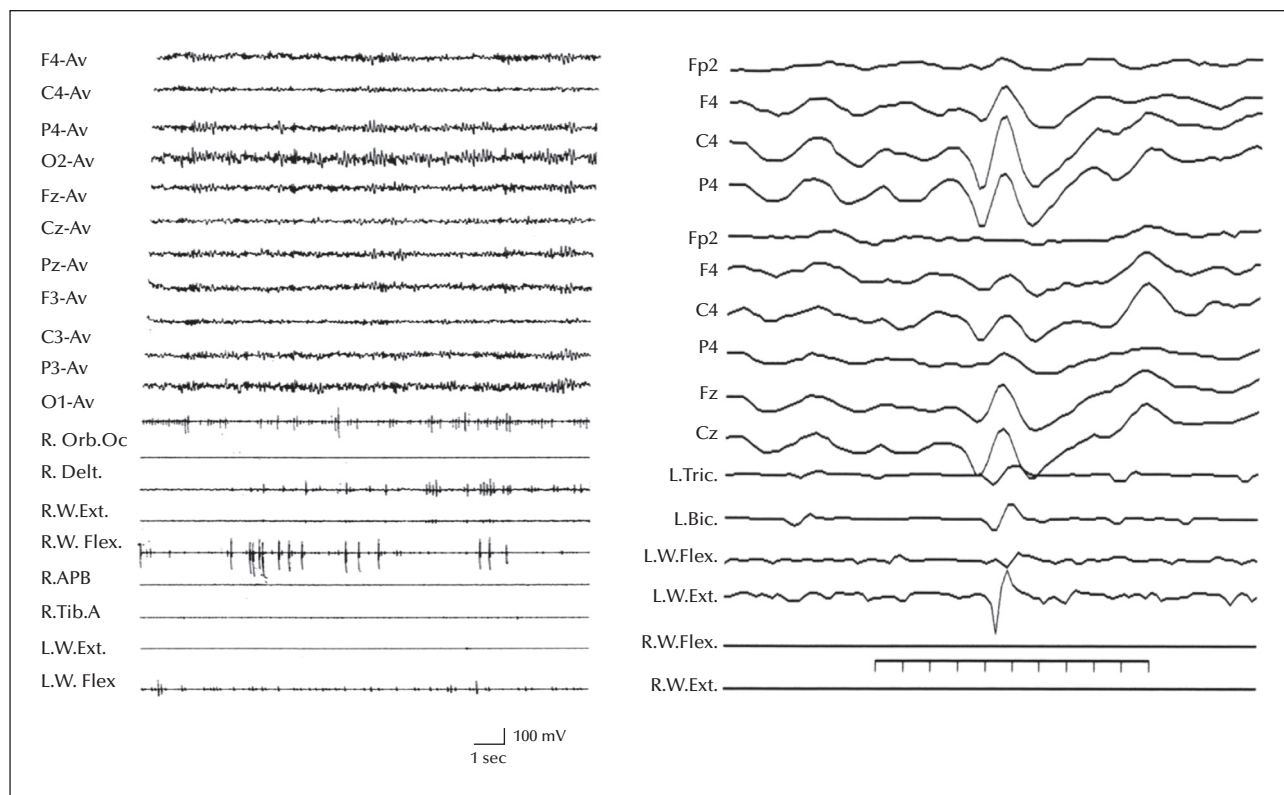
Figure 1. Polygraphic recording in a 32-year-old patient with PME without renal failure associated with SCARB2 mutation. Left panel: the recoding shows preserved EEG background activity and erratic myoclonic jerks at rest without overt EEG correlate. Right panel: back-averaging triggered by myoclonia in the left wrist extensor reveals a cortical spike at the contralateral centroparietal electrodes.

EEG and brain imaging

EEG and polygraphic recordings show generalized epileptiform abnormalities that at onset may resemble epileptic activity observed in idiopathic generalized epilepsy (Badhwar *et al.*, 2004; Rubboli *et al.*, 2011). Background activity is preserved at disease onset, slowing progressively over the years. In photosensitive patients, intermittent photic stimulation can trigger bursts of generalized spike-polyspike-wave discharges, often associated with massive myoclonic jerks that can evolve to myoclonic seizures. Polygraphic recordings show action myoclonus and erratic myoclonic jerks at rest, inconstantly associated with contralateral central spikes (*figure 1*). Back-averaging analysis of EEG discharges triggered from myoclonic jerks can reveal a cortical spike at the centroparietal electrodes (*figure 1*). Surface EMG recording of the fine tremor in the upper limbs showed quasirhythmic EMG bursts at a frequency of 12-20 Hz (*figure 2*). Analysis of the EEG-EMG relationship by coherence spectra of the tremor demonstrated a pattern consistent with
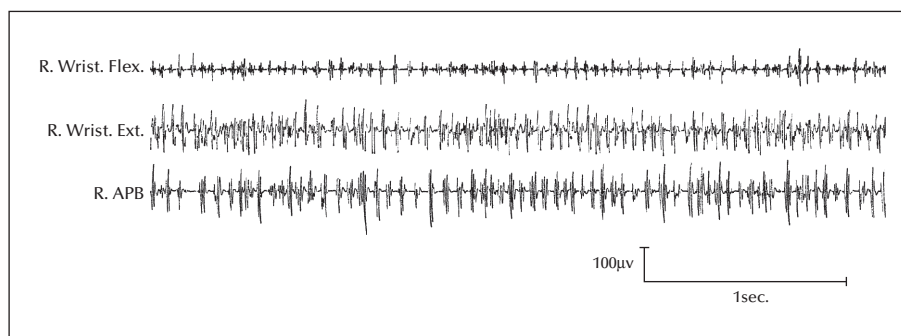

Figure 2. Surface EMG recording of the tremor in the upper limbs shows quasirhythmic EMG bursts at a frequency of 12-20 Hz.

a rhythmic myoclonic phenomenon of cortical origin, as in 'cortical tremor' (Rubboli *et al.*, 2011).

Brain imaging studies are usually unremarkable or display diffuse cerebral atrophy, often associated with cerebrellar atrophy.

Histology

Widespread deposition of abnormal, extraneuronal brown pigment in the brain, with no neuronal loss or significant gliosis, has been reported in AMRF patients (Andermann *et al.*, 1986; Badhwar *et al.*, 2004), and more recently in two patients with PME without renal failure associated with SCARB2 mutations (Fu *et al.*, 2014). Based on the staining characteristics, it has been suggested that the pigment consists of lipofuscin-like oxidized lipid or proteolipid (Badhwar *et al.*, 2004). The deposits of pigment granules are extraneuronal, in astrocytes or in the extracellular space, especially in the cerebellar and cerebral cortices without any increase in intraneuronal lipofuscin (Badhwar *et al.*, 2004; Fu *et al.*, 2014). Interestingly, the patients without renal failure reported by Fu *et al.* (2014) also showed neurodegenerative changes, such as neuronal loss and gliosis in the brain, including the pallidoluysian and cerebello-olivary systems and the spinal cord. The authors speculate that the neuronal loss and gliosis in the pallidoluysian and cerebello-olivary systems may be responsible for the patients' involuntary movements and cerebellar dysfunction. Moreover, degenerative changes observed in the upper and lower motor neuronal systems, as well as in the dorsal root ganglia, strongly suggested that both the motor and sensory neuronal systems were also involved in the disease process.

Renal biopsy specimens have shown extensive tubular abnormalities with isometric vacuolization in distal and collecting tubules, the presence of granular material in cortical tubules without inflammatory infiltration (Chaves *et al.*, 2011), and focal glomerulosclerosis, with features of collapsing glomerulopathy (Badhwar *et al.*, 2004; Berkovic *et al.*, 2008).

Differential diagnosis

Differential diagnosis of AMRF primarily concerns other PMEs without dementia. At onset, since they share various clinical features, the correct diagnosis may be difficult on clinical grounds, particularly when renal impairment has not yet appeared or has not been diagnosed. Unverricht-Lundborg disease (ULD) is the paradigmatic example of PME with action myoclonus, ataxia, generalized seizures, and preserved intellect. The clinical suspicion of AMRF should be raised when a fine postural tremor in the upper limbs, which is generally not present in ULD, is detected on neurological examination at disease onset. Other rare causes of PME without dementia that should be considered in the differential diagnosis include sialidoses, distinguishable by macular cherry red spots, and PME, caused by mutations in PRICKLE1, the onset of which is generally earlier than that in AMRF and associated with early ataxia.

Concluding clinical remarks

The molecular analysis of patients clinically diagnosed with AMRF showed that mutations in SCARB2 have been identified in many patients, confirming that the likelihood of identifying a SCARB2 mutation in a patient presenting with PME and renal complications is fairly high. The French Canadian and Scottish populations have been shown to have founder mutations in SCARB2 causing AMRF. Different SCARB2 mutations have now been found in patients in many different countries, suggesting that SCARB2-associated PME is a world-wide disorder that is presently under recognized. Onset of PME due to mutations in SCARB2 occurs in teenagers or young adults, and diagnosis is important in terms of providing counselling for the patient and family, particularly as the prognosis is worse than for classic ULD. Being informed about the carrier status of family members is particularly relevant in terms of the prevention of future disease in populations of known high consanguinity. In addition, in AMRF patients, an early diagnosis is of utmost importance as renal dysfunction can cause premature death in childhood or adolescence. Treatment with kidney dialysis or renal transplantation has been shown to prolong life by at least 10 years (Badhwar *et al.*, 2004). Furthermore, SCARB2 mutations in patients with PME in the absence of renal complications are probably not rare, therefore mutations of the SCARB2 gene should be considered in undiagnosed PME without renal involvement.

The LIMP-2 protein

The lysosome is the major degradative compartment of the cell. Its limiting membrane fulfils multiple functions, such as acidification of the interior, sequestration of active lysosomal enzymes, and transport of degradation products from the lysosomal lumen to the cytoplasm (Saftig & Klumperman, 2009). The lysosomal membrane contains several highly N-glycosylated proteins, the functions of which remain largely unknown (Eskelinen *et al.*, 2003).
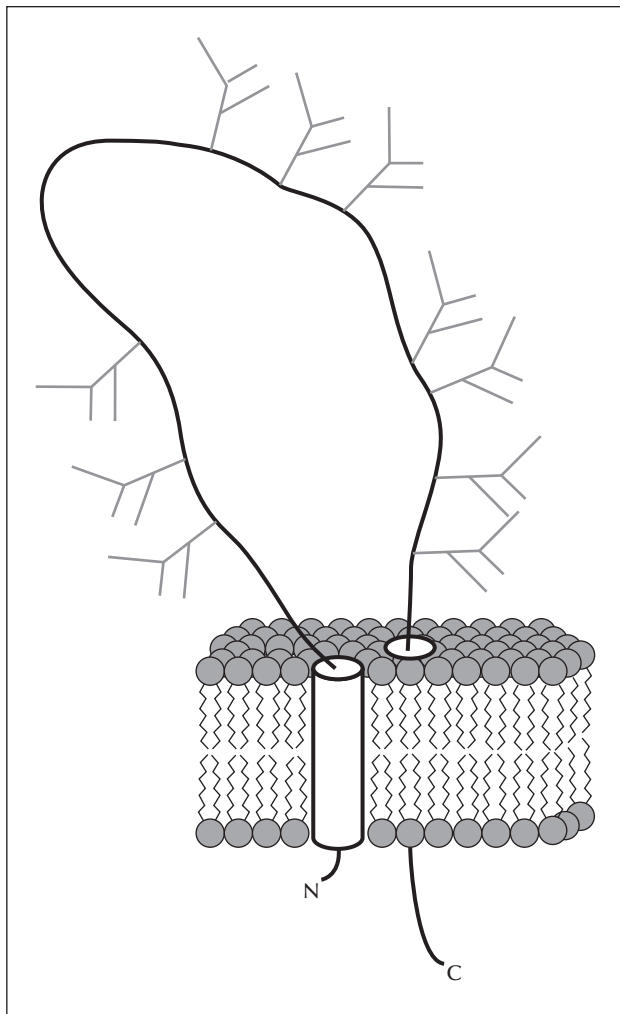
Figure 3. Topology of the lysosomal membrane protein LIMP-2. LIMP-2 is a heavily N-glycosylated type III transmembrane protein with N- and C-termini located in the cytoplasm. The coiled-coil domain, which is necessary for GC binding, is located within the lumenal domain of LIMP-2.

LIMP-2, a major component of the lysosomal membrane

LIMP-2 (lysosomal integral membrane protein type 2), also known as SCARB2, is an abundant, highly glycosylated lysosomal membrane protein and belongs to the CD36 family (Calvo *et al.*, 1995). All family members share a common topology, transversing the membrane twice with an N-terminal transmembrane domain, a large luminal domain, and a second membrane-spanning domain preceding a 20-amino-acid cytoplasmic tail at the C-terminus (*figure 3*). Whereas in most tissues LIMP2 is found in lysosomes, other members of the CD36 superfamily are localized and function at the cell surface.

LIMP2 is a ubiquitously expressed protein with highest expression in the liver and spleen (Tabuchi *et al.*, 1997). It has a molecular weight of about 74 kDa, which includes a 54-kDa polypeptide backbone of 478 amino acids. The luminal domain contains 10 to 11 putative glycosylation sites (*figure 3*). The degree of the complex glycosylation of LIMP-2 depends on the species, tissue, and cell type. A leucine-isoleucine motif, within the C-terminal cytoplasmic tail, interacts with the heterotetrameric adaptor-complex 3 (AP3) and has been proposed to be responsible for the lysosomal localization of LIMP-2 (Honing *et al.*, 1998). In addition, based on antibody uptake experiments, a recycling of LIMP-2 between lysosomes and the plasma membrane has been described (Akasaki *et al.*, 1994). Furthermore, it has been demonstrated that distinct phosphatidylinositol 4-kinases influence the trafficking of LIMP-2 (Jović *et al.*, 2012).

Overexpression of LIMP-2 leads to altered cellular membrane trafficking

Overexpression of LIMP-2 causes an enlargement of early and late endosomes/lysosomes, which has not been observed for other abundant lysosomal membrane proteins. LIMP-2 overexpression impairs the endocytic membrane traffic out of these enlarged compartments and leads to an accumulation of cholesterol in these vacuole-like structures. Co-transfection of LIMP-2 and the dominant-negative form of Rab5b inhibits the formation of enlarged vacuoles, suggesting that Rab5b function is necessary for the formation of such vesicles (Kuronita *et al.*, 2002). Mutation experiments suggest that the N-terminal transmembrane and proximal lumenal domains of LIMP-2 are essential for the generation of the enlarged vesicular structures (Kuronita *et al.*, 2005). These findings support the idea that LIMP-2 plays a role in the biogenesis and maintenance of the endo-lysosomal system.

LIMP-2 receptor functions

Similar to CD36, LIMP-2 appears to display a role as a multifunctional receptor at the plasma membrane. By using LIMP-2-GST fusion proteins and labelled thrombospondin, an interaction between both proteins was shown (Crombie & Silverstein, 1998).
In addition, LIMP-2 appears to be a cellular receptor for enterovirus 71 (EV71) and the coxsackie virus A16 (CVA16), which are most frequently associated with hand, foot and mouth disease (HFMD) (Yamayoshi *et al.*, 2009). Although HFMD is considered to be a mild infection, it can progress to a severe neurological disease, associated with fatal encephalitis, aseptic meningitis, and acute flaccid paralysis.
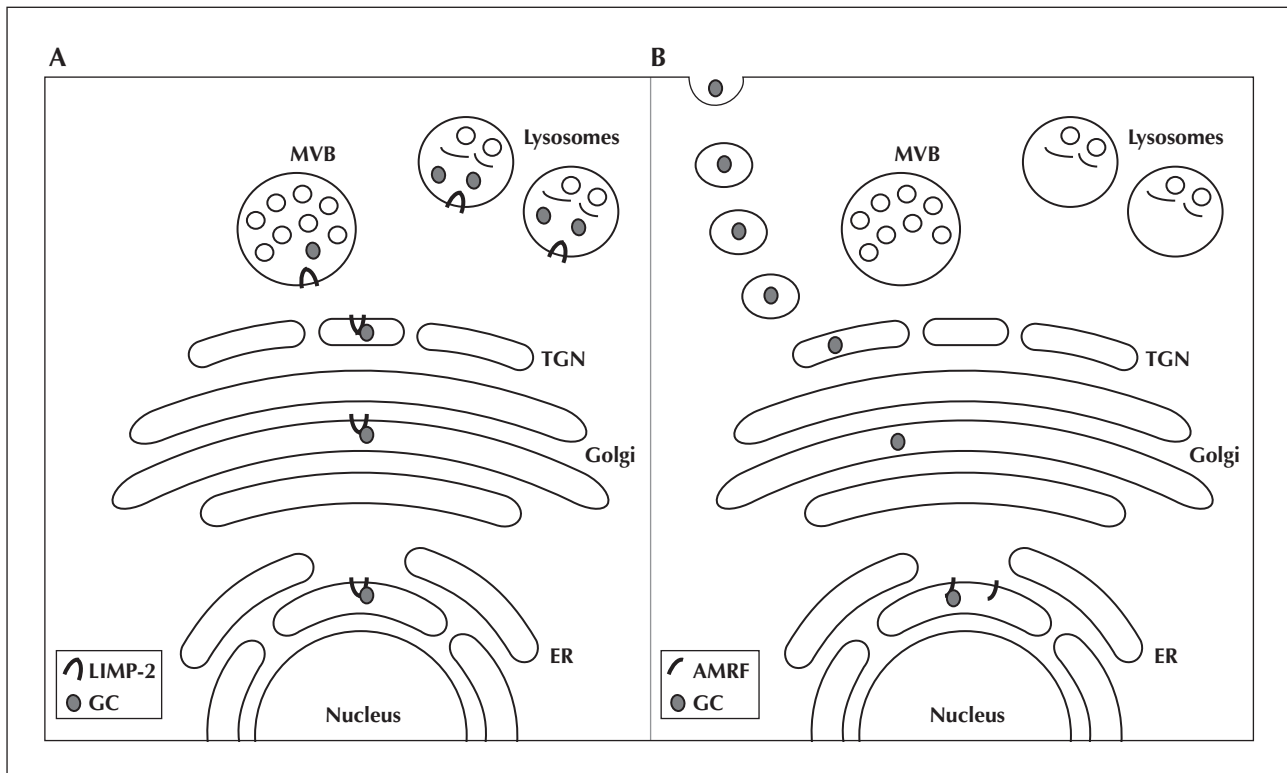
Figure 4. LIMP-2-dependent targeting of GC to the lysosome. (A) In wild-type cells, GC is transported to the lysosome by LIMP-2. After binding between LIMP-2 and GC in the ER, GC leaves the receptor-ligand complex in the ER and moves via the Golgi apparatus to multivesicular bodies and lysosomes, where they dissociate due to the acidic pH. (B) All clinical AMRF-causing mutations described for LIMP-2 thus far have led to retention of the mutated protein in the ER. Thus, in cells derived from AMRF patients, GC is retained in the ER and/or secreted to the extracellular space, depending on the effect of the AMRF-causing LIMP-2 mutation on binding to GC.

It is well established that LIMP-2 has another important receptor function. It acts as a receptor for the lysosomal delivery of acid hydrolase β-glucocerebrosidase (GC) (Reczek *et al*., 2007). Mutations in the gene encoding this enzyme have been shown to cause Gaucher Disease, the most common lysosomal storage disorder, which is due to lysosomal accumulation of the glycosphingolipid glucosylceramide. LIMP-2 binds the enzyme, involving a helical domain in the luminal domain, early in the endoplasmic reticulum and transports it all the way to the lysosome (Reczek *et al*., 2007; Neculai *et al*., 2013, Blanz *et al*., 2015; Zunke *et al*., 2016) (*figure 4a*). Due to the acidic pH of the lysosome, the LIMP-2 receptor and its ligand dissociate (Reczek *et al*., 2007; Zachos *et al*., 2012), leading to the active lysosomal enzyme. All analyzed clinical AMRF-causing mutations described for LIMP-2 thus far have led to retention of the mutated protein in the endoplasmic reticulum (*figure 4b*). However, the binding to GC is differentially affected by the various AMRF-causing mutations (Blanz *et al*., 2010). It will be interesting to study whether the lack of lysosomal transport of GC contributes to the pathology of AMRF syndrome. Interestingly, the structure of the extracellular domain of LIMP-2 was recently revealed, showing an exposed helical bundle where GC binds. In addition, a cavity within the protein suggests a possible lipid transport function (Neculai *et al*., 2013).

LIMP-2-deficiency in mice reveals major roles in the inner ear, kidney and myelinisation of peripheral nerves.

The different roles of LIMP-2 as a receptor may also contribute to the phenotype of mice engineered with a deletion of the mouse LIMP-2 gene. LIMP-2-deficient mice are characterized by the development of deafness, a unilateral or bilateral hydronephrosis, proteinuria, and a peripheral demyelinating neuropathy (Gamp *et al*., 2003).

The development of deafness was indicated by deficits in acoustic startle responses, in brainstem-evoked auditory potentials, and a reduced endochondral potential. A massive decline of spiral ganglia in the cochlea, concomitant with that of the inner and outer hair cells, and a progressive atrophy of the stria vascularis are typical pathological changes which start

shortly after birth (Gamp *et al.*, 2003). Hearing loss is temporally linked to a loss of the potassium channel subunits KCNQ1/KCNE1 and the endocytic receptor megalin in the luminal surface membrane of marginal cells of the stria vascularis (Knipper *et al.*, 2006). A role of LIMP-2 in the regulation of the correct surface expression of these proteins through vesicular transport can be anticipated. This is also supported by the in-depth analysis of the development of a unilateral or bilateral hydronephrosis caused by an obstruction of the ureteropelvic junction. An impairment of the membrane transport processes is suggested by an abnormal accumulation of lysosomes in the epithelial cells of the ureter, adjacent to the ureteral lumen and a disturbed apical expression of uroplakin (Gamp *et al.*, 2003). It is speculated that the pathology in the urothelium leads to the obstruction of the urinary tract between the renal pelvis and the ureter. In addition to this obstruction, kidney functions are affected. Decreased osmolality and altered urine parameters in LIMP-2-deficient mice point towards renal dysfunction. The high quantity of albumin in the urine of LIMP-2 knockout mice may be explained by glomerular filtration damage. Both the hydronephrosis and subtle glomerular changes may explain the kidney pathology in these mice (Berkovic *et al.*, 2008). Interestingly, renin and LIMP-2 are co-regulated in renin-producing cells, although LIMP-2 does not play a role in the direct regulation of renin synthesis or release (Schmid *et al.*, 2013). The development of a peripheral demyelinating neuropathy in LIMP-2-deficient mice is an additional phenotypic hallmark and is most likely caused by a downregulation of peripheral myelin proteins (Gamp *et al.*, 2003). Interestingly, lysosomal enzymes are upregulated in LIMP-2-deficient Schwann cells, suggesting that peripheral myelin proteins are missorted and degraded in the lysosomal compartment. Finally, Berkovic and colleagues observed in the LIMP-2-deficient mice, intracellular inclusions in cerebral and cerebellar cortex neurons accompanied by hyperactivity and ataxic gait behaviour (Berkovic *et al.*, 2008). A recent study also revealed that the loss of LIMP-2 in the brain led to an almost complete loss of neuronal GC. This caused a substrate accumulation, followed by a secondary accumulation of neurotoxic a-synuclein. In cell-based assays, it was shown that an increased expression of LIMP-2 also led to a reduction in a-synuclein, suggesting a putative therapeutic role of LIMP-2 in synucleopathies (Rothaug *et al.*, 2014).

Miscellaneous functions

Additional studies analysing the LIMP-2 homologue in *Dictyostelium discoideum* (DdLIMP) revealed a role as an effective suppressor of the profilin-minus phenotype (Karakesisoglou *et al.*, 1999; Temesvari *et al.*,

2000). Profilin is a ubiquitous G-actin binding protein which is involved in multiple cellular processes, such as cytokinesis, phagocytosis, and micripinocytosis. Profilin-deficient *Dictyostelium discoideum* cells show defects in pinocytosis, macropinocytosis, exocytosis, secretion of hydrolases, and an increased rate of phagocytosis. Interestingly, the infection of mice deficient in LIMP-2 revealed that phagocytosis is also compromised. When such mice were infected with *Listeria monocytogenes* (LM), it was found that they were highly susceptible to infection and displayed defective macrophage activation (Carrasco-Marin *et al.*, 2011). This defect increased the amount of pathogen, which is able to escape to the cytosol, increased the production of early acute phase pro-inflammatory cytokines, and enhanced the ability of LM phagosomes to interact with MIIC vesicles. These experiments suggested that in concert with active Rab5a, LIMP-2 regulates the phagosomal fusion machinery of the late endosomes-lysosomes and the cytosolic levels of the pathogen (Carrasco-Marin *et al.*, 2011).

Conclusions

Although the exact molecular role of LIMP-2 in the various cellular events is still incompletely understood, a central role for this lysosomal membrane protein in health and disease has emerged. Future studies will help to further understand the role of LIMP-2 in various tissues, including the central nervous system. It will be of particular importance to link the multitude of cellular functions with the phenotypic alterations seen in knockout mice and the clinical presentations in human AMRF patients. This will most likely also shed light onto the hitherto poorly understood role of the endocytic pathway in the central nervous system and the development of progressive myoclonus epilepsy disorders.

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