Mitochondria are the energy powerhouses of cells and play an important role in kidney function. Not surprisingly, mitochondrial dysfunction is becoming increasingly recognized as contributing to renal glomerular and tubular diseases (1, 3, 7, 12, 13, 28). A rare genetic form of focal segmental glomerulosclerosis (FSGS) is due to mitochondrial dysfunction (26). Lesions were accompanied by an increase of reactive oxygen species (ROS) production and lipid peroxidation in isolated glomeruli, and oxygen radical scavengers were shown to ameliorate the renal damage (2). When subsequently bred onto a C57BL/6 background, the renal lesions in MPV17−/− mice changed from early onset renal failure and FSGS to late onset proteinuric nephropathy preceded by glomerular mtDNA depletion (25).

Here we demonstrate that Mpv17 protein was consistently reduced in glomeruli from mice and human biopsies manifesting glomerulosclerosis. Loss of Mpv17 increased susceptibility for proteinuria and renal failure in mice subjected to experimental nephritis. Mpv17 protein is localized to mitochondria in podocytes and is shown here to play a critical role in protecting podocytes against ROS injury and apoptosis in vivo and in vitro. This study provides new insights into an essential protective role of mitochondrial Mpv17 in podocytes.

MATERIALS AND METHODS

Animal models and cell culture. Transforming growth factor-β1 (TGF-β1) transgenic (18) and CD2AP−/− mice (10) were killed at 3 or 4 wk, and disease severity was assessed by measurement of albuminuria and periodic acid-Schiff (PAS) staining of kidney tissue with consecutive histopathological analysis.

Nephotoxic serum nephritis (NTSN) was induced in 8- to 10-wk-old C57BL/6 wild-type (WT) and Mpv17−/− littermates by a single intraperitoneal injection as previously described (22). Then, 0.05 or 0.02 ml of nephotoxic sheep serum/g body wt were used to induce severe and mild nephritis, respectively. In the severe model, mice were killed 1 or 7 days after injection. In the mild model, mice were killed 3 days postinjection. All animal protocols and procedures were approved by the Institutional Animal Care and Use Committee of Icahn School of Medicine at Mount Sinai.

Murine podocytes were kindly provided by Dr. Peter Mundel and cultured as described previously (19). Transfections were performed with Effectene (Qiagen, Valencia, CA) according to the manufacturer’s protocol. Isolation of immortalized podocytes was performed as previously reported from WT and Mpv17−/− C57BL/6

**CONCLUSION**

Our study demonstrates that Mpv17 is a abundant mitochondrial protein, whose loss in podocytes results in increased susceptibility to mitochondrial dysfunction. This is shown here to play a critical role in protecting podocytes against ROS injury and apoptosis in vivo and in vitro. Therefore, Mpv17 serves as a crucial mitochondrial protein in podocytes and may be a potential therapeutic target for the treatment of renal diseases.
mice crossed with Immortomouse transgenic mice (Charles River, Wilmington, MA).

**Serum creatinine, serum albumin, albuminuria, and urinary 8-oxoguanine measurement.** Serum creatinine was measured by acetonitrile deproteinization, followed by isocratic, cation exchange HPLC previously described by Yuen et al. (29). Serum albumin was tested by the Clinical Chemistry laboratory at Icahn School of Medicine at Mount Sinai. Urinary albumin, creatinine, and 8-oxoguanine (8oxoG) were quantified as previously reported (4).

**Histopathology.** PAS was performed on paraffin-embedded tissue using standard protocols. Glomerular injury score was based on blind histopathological evaluation of PAS-stained slides considering both glomerular sclerosis and podocyte apoptosis based on a 1–4 scale.

**Human samples.** Renal biopsies were obtained after written consent and approval of the ethics committee and in the frame of an international multicenter study, the European Renal cDNA Bank-Kroener-Fresenius biopsy bank, approved by the specialized subcommittee for internal medicine of the Cantonal Ethics Committee of Zurich.

**Microscopy.** Immunofluorescence was conducted on frozen kidney sections using anti-Mpv17 (Proteinitech, Chicago, IL) and anti-synaptopodin (kindly provided by Dr. Peter Mundel). For DNA oxidation we used mouse anti-8oxoG monoclonal antibody (N45.1; Japan Institute for the Control of Ageing), with anti-synaptopodin or anti-transcription factor A, mitochondrial (TFAM; Santa Cruz Biotechnology, Dallas, TX) followed by the appropriate secondary IgG-Alexa antibodies (Sigma-Aldrich, St. Louis, MO). Images were taken in the Icahn School of Medicine at Mount Sinai Microscopy Core Facility with a Zeiss Axiosplan2 or 2iE, equipped with a Q-imaging MP3.3 RTV color camera running QED capture software. Super resolution images (see Fig. 3) were taken with a Zeiss ELYRA PS.1 microscope.

**RNA isolation, RT-PCR, and mtDNA depletion analysis.** Total RNA was prepared from cell lysates using Qiagen RNeasy mini columns (Qiagen, Valencia, CA) and then reverse transcribed with Superscript II reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA) following commercial protocols. Quantitative amplification of the cDNA using 2 × SYBR-Green PCR Master Mix (Applied Biosystems, Foster City, CA) was performed in an ABI-Prism 7900HT Sequence Detection System (Applied Biosystems). For mtDNA depletion analysis, total DNA was extracted from podocytes by using the DNeasy blood and tissue kit (Qiagen), following the vendor’s protocol. mtDNA was quantified by RT-PCR as described previously (20).

**Apoptosis detection, determination of ROS generation, and measurement of inner mitochondrial membrane potential.** Apoptosis, ROS, and inner mitochondrial membrane potential (mATP) were assessed using a BD FACSCanto cytometer (BD Biosciences, San Jose, CA). Detection kits were as follows: annexin V with propidium iodide or 7AAD (BD Pharmingen) for apoptosis; MitoSOX (Invitrogen) for mitochondrial superoxide; and tetramethyl rhodamine (TMRE; Invitrogen) for mitochondrial membrane potential. Cells were treated with 10 μM rotenone (Sigma-Aldrich, St. Louis, MO), mitotempo 5 μM (Enzo Life Sciences, Farmingdale, NY), or a combination of both for 24 or 48 h.

**Mitochondrial function.** For oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) quantification, WT and Mpv17−/− podocytes were plated in collagen-I-coated XF24-well microplates (Seahorse Bioscience, MA). OCR and ECAR were analyzed with the Seahorse Bioscience XF24 Extracellular Flux Analyzer in basal conditions following a 24-h incubation with rotenone 10 μM plus or minus 18-h recovery in untreated medium as indicated in RESULTS.

Citrinate synthase activity was measured as indicated in (24), and colorimetric reaction was measured with a Victor3 1420 Multilabel Plate Counter (Perkin Elmer, Waltham, MA).

**Statistics.** Statistical analysis was performed by using ANOVA and Student’s t-test. All results are means ± SE.

**RESULTS.**

Mpv17 is expressed in podocytes and glomerular Mpv17 protein and mRNA levels are decreased in murine models of glomerular disease and in glomeruli of human biopsies with FSGS but not minimal change disease. By using immunofluorescence analysis on kidney sections, we analyzed Mpv17 protein expression patterns. In healthy kidneys, Mpv17 was most prominently expressed in glomeruli, as previously described (2) and only to a lesser extent in tubular segments (Fig. 1A). Glomerular Mpv17 protein expression colocalized in part with the podocyte-specific marker synaptopodin, but there was also some nonpodocyte staining, consistent with mitochondrial staining of other glomerular cells (Fig. 1B).

Since deficiency of Mpv17 in mice results in progressive glomerulosclerosis (2, 14, 25, 26), we analyzed Mpv17 mRNA and protein levels in TGF-β1 transgenic mice and CD2AP−/− mice, two different models of glomerular injury (10, 18). By immunohistology, glomerular Mpv17 expression was markedly decreased in TGF-β1 transgenic mice with advanced glomerulosclerosis, compared with nontransgenic control mice (Fig. 1C). Similarly, Mpv17 mRNA levels were decreased in glomeruli of TGF-β1 transgenic mice, compared with nontransgenic mice (Fig. 1D). Mpv17 protein and mRNA levels were also reduced in glomeruli of CD2AP−/− mice, another model of proteinuria and glomerulosclerosis induced by podocyte injury, when compared with WT CD2AP+/− (Fig. 1, C and D). The possibility has to be considered that part of the loss of MPV-17 could be due to podocyte losses. Podocyte numbers in the TGF-β1 transgenic mice have been reported to be reduced by 30–40% (19) at the 3-wk time point examined in our present study and by 40–50% at 4 wk in the CD2AP−/− mice (10). Thus the reduction in mRNA for Mpv17 at the corresponding time points for TGF-β1 transgenic mice of 50% and for CD2AP−/− mice of 50–60% could to a considerable extent be due to podocyte loss. However, this is unlikely the only explanation, as by immunohistology (Fig. 1C) the decrease in signal for Mpv17 is diffuse rather than focal, as would be expected if podocyte loss were the sole factor accounting for the reduction. Mpv17 mRNA expression levels were also significantly reduced in kidneys of WT mice with a severe form of NTSN associated with renal insufficiency but were unchanged in mice with mild NTSN associated with transient albuminuria (Fig. 1E). At the studied time points, podocyte apoptosis in NTSN was in fact present at a much lower extent (no more than 15%) than in TGF-β1 transgenic and CD2AP−/− mice, while reduction of Mpv17 mRNA expression was comparable at ~60% (Fig. 1E).

We extended these data from murine models to human glomerular disease by examining microdissected glomeruli from patients with minimal change disease (MCD) and primary FSGS. Glomeruli from pretransplant living donor kidneys were used as controls (CTRL). Patients with FSGS and MCD manifested comparable degrees of proteinuria and serum creatinine levels (Table 1). Interestingly, as shown in Fig. 1F, Mpv17 mRNA expression was significantly reduced in glomeruli isolated from human renal biopsies with FSGS (n = 17), but not with MCD (n = 8), when compared with living donors (n = 9). These results suggest that, similar to the downregulation of Mpv17 in murine models of FSGS, Mpv17 mRNA expression is reduced in human FSGS but...
not in MCD with comparable degrees of proteinuria but without sclerosis.

Again podocyte loss may contribute to the reduction in mRNA for MPV17 in the glomeruli from biopsies with FSGS but is unlikely to be the single explanation in view of the normal serum creatinine levels in these patients and the fact that markedly sclerosed glomeruli were excluded from analysis.

Fig. 1. Localization of Mpv17 in murine kidney and alterations of Mpv17 levels in experimental and human kidney disease. A: immunofluorescence for Mpv17 in kidney cryosections showing predominant glomerular localization (arrow). B: immunofluorescence double staining of Mpv17 and podocyte-specific marker synaptopodin in mouse kidney cryosections showing nonexclusive localization of Mpv17 in podocytes. Mpv17-immunostaining in kidney cryosections from 4-wk-old transforming growth factor-β1 (TGF-β1) transgenic (TG) mice (C, left) and 3-wk old CD2AP−/− mice with glomerular disease compared with age matched wild-type (WT) mice (C, right). Bar graphs show average ± SE of normalized, relative mRNA levels for Mpv17 in whole kidneys of TGF-β1 transgenic mice (n = 4) and WT mice, in kidneys of CD2AP−/− mice (n = 4) compared with WT mice (D) and in kidneys of WT C57BL/6 mice with a mild (n = 7) or severe (n = 6) form of nephrotoxic serum nephritis (NTSN; E). For normalization, actin mRNA or 18S rRNA levels were used. F: MPV17 mRNA expression in microdissected glomeruli from biopsies of living kidney donors (CTRL; n = 9), focal segmental glomerulosclerosis (FSGS) patients (n = 17), and minimal change disease (MCD) patients (n = 8). **P ≤ 0.01, ***P < 0.005.

Mpv17 deletion in mice enhanced albuminuria and glomerular injury scores in mild NTSN and increased serum creatinine in severe NTSN models. Local generation of ROS plays a significant role in the glomerular injury of experimental NTSN and of glomerulosclerosis developing in Mpv17−/− mice (2, 30). We therefore hypothesized that administration of NTS might enhance the glomerular injury in Mpv17−/− mice in the C57BL/6 strain, in which MPV17 deletion by itself results in
only mild and late onset glomerulosclerosis (25). The use of a customary dose of NTS (see methods) resulted in severe NTSN with nephrotic syndrome with edema, massive proteinuria, and hypoalbuminemia (about 40% reduction from baseline in WT and Mpv17−/− mice, not shown) in both WT and Mpv17−/− mice (Fig. 2A), starting at day 1 after injection of the NTS and persisting over the 7-day study period. Histological analysis confirmed marked and significant but comparable glomerular injury with apoptosis and sclerosis in WT and Mpv17−/− mice (Fig. 2B). We did not observe any tubule-interstitial changes in either WT or Mpv17−/− mice at 7 days of NTSN. In contrast to the comparable degree of proteinuria and pathological changes in WT and MPV17−/− mice, only the MPV17−/− mice developed a significant reduction in glomerular filtration rate as evidenced by a doubling of serum creatinine at day 7 (Fig. 2C).

As a potential explanation for these differences, we examined glomerular deposition of the sheep NTS and subsequent immune complex formation with mouse immunoglobulin in WT and Mpv17−/− mice. However, both the initial deposition of sheep IgG at day 1 and the subsequent deposition of mouse IgG during the autologous phase at day 7 were comparable in WT and Mpv17−/− mice (not shown).

We considered the possibility that the degree of glomerular injury in the severe NTSN might already be maximal, so that any further glomerular damage in the Mpv17−/− mice could not distinguish itself by proteinuria or histology but only by a decrease in glomerular filtration as indicated by the doubling of serum creatinine. We therefore induced a less severe form of NTSN by reducing the dose of NTS injection by 60%. Compared with high-dose NTS, this dose of NTS resulted in significantly reduced and transient albuminuria, peaking on day 1 in WT mice (Fig. 2D), while albuminuria was significantly prolonged over a 3-day course in Mpv17−/− mice (Fig. 2D). Mpv17−/− mice also showed signs of moderate glomerular injury with sclerosis and podocyte apoptosis 3 days after exposure to reduced dose NTS, while histology of WT NTSN mice were comparable to control mice (Fig. 2E). No interstitial changes were noted in any of the groups at 3 days. Consistent with the mild and transient form of NTSN, serum creatinine levels remained comparable to control levels in WT and Mpv17−/− mice (Fig. 2F). Kidneys of untreated WT and Mpv17−/− appeared normal and indistinguishable (not shown).

Mpv17 deletion is associated with enhanced glomerular-podocyte oxidative damage. Urinary excretion of 8oxoG as a marker of DNA oxidative injury (9) was increased in both groups but significantly more so in Mpv17−/− mice compared with WT mice at day 7 in the severe NTSN model (Fig. 3A). Consistent with these findings, 8oxoG staining in glomeruli of Mpv17−/− with NTSN was increased compared with that in WT NTSN at day 7 (Fig. 3B). Furthermore, the 8oxoG DNA adduct colocalized in a granular, mitochondrial pattern in podocytes with the cytotoxic marker synaptopodin (Fig. 3B). In less severe NTSN model, urinary excretion of 8oxoG was similar in all groups and comparable to control levels (Fig. 3A). However, despite overall decreased severity, in mild NTSN, increased ROS DNA damage evidenced by 8oxoG accumulation was greater in Mpv17−/− compared with WT mice at day 3 (Fig. 3C). Figure 3D shows colocalization of 8oxoG with mitochondrial transcription factor TFAM confirming accumulation of oxidized mtDNA in Mpv17−/− glomeruli. These results show that genetic deletion of Mpv17 in C57BL/6 mice renders them more susceptible to glomerular injury with increased ROS damage, podocyte dysfunction and apoptosis with worse proteinuria, and reduction of glomerular filtration in severity-graded models of NTSN, respectively.

Mpv17 is localized to podocyte mitochondria and knockdown or deletion of Mpv17 is sufficient to cause mitochondrial oxidative stress with mtDNA depletion, decreased mitochondrial membrane potential (mtΔΨ), and increased apoptosis. Using double staining with cytochrome c as a mitochondrial marker, we confirmed that Mpv17 was localized in mitochondria also in cultured podocytes (Fig. 4A).

As an initial approach to examine the mechanism(s) by which loss of Mpv17 protein alters mitochondrial and podocyte function, we successfully generated several clonal podocyte cell lines with Mpv17 knockdown by stable expression of small interfering Mpv17 RNA (siMpv17) (Fig. 4B). Quantification of mtDNA relative to nuclear encoded 18S DNA demonstrated significant mtDNA depletion in siMpv17 podocyte cell lines, compared with control scrambled siRNA clones (Fig. 4C). Compared with normal mitochondrial distribution in scrambled siRNA transfected cells, in siMpv17-transfected podocytes a mitochondrial pattern with cluster formation was apparent with staining with mitochondrial superoxide-specific MitoSOX (Fig. 4D). Confirming these observations, mitochondrial ROS generation was significantly increased and associated with decreased mitochondrial membrane potential and increased apoptosis in siMpv17 podocytes, compared with control scrambled siRNA transfected clones (Fig. 4E). Thus, reduction of Mpv17 in cultured podocytes by stable transfection with Mpv17 siRNA caused severe mitochondrial dysfunction.

To confirm these results, we generated podocytes with genetic deletion of Mpv17 from WT and Mpv17−/− C57BL/6 mice intercrossed with Immortomouse transgenic mice as previously described (27). Staining with Mitotracker showed increased intensity, condensation and different distribution of the mitochondrial network in the immortalized Mpv17−/− compared with WT podocytes (Fig. 5A) consistent with the changes observed with siRNA knockdown of Mpv17. Quantification of citrate synthase activity as a common marker for mitochondrial content did not show differences between WT and Mpv17−/− podocytes (55.6 ± 5.6 and 56.0 ± 7.8 nmol·min⁻¹·mg⁻¹, respectively; n = 4) suggesting that the morphological differences were not associated with a different content in mitochondria.

Examination of mitochondrial OCR in WT and Mpv17−/− podocytes shows that in unchallenged condition Mpv17−/− podocytes are characterized by a higher OCR compared with WT possibly suggesting mitochondrial uncoupling (Fig. 5B).

Table 1. Patient demographics

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>MCD (n = 8)</th>
<th>FSGS (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>51.9 ± 14.0</td>
<td>36.1 ± 13.8</td>
<td>42.1 ± 15.3</td>
</tr>
<tr>
<td>Sex, %male</td>
<td>50.0</td>
<td>55.6</td>
<td>52.9</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>&lt;1.1</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Proteinuria, g/24 h</td>
<td>&lt;0.2</td>
<td>5.2 ± 1.7</td>
<td>4.2 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; number of patients per group is in parentheses. Demographic data for control, minimal change disease (MCD), and focal segmental glomerulosclerosis (FSGS) patients.
Fig. 2. Glomerular injury in Mpv17−/− mice with severe and mild NTSN. Urinary albumin/creatinine ratio (A; n ≥ 5 per group) and periodic acid-Schiff (PAS) staining (B) of kidneys sections from WT and Mpv17−/− mice at day 7 after NTS injection showing advanced glomerular sclerosis and some tubular casts (×400 top, ×600 middle, and ×1,000 bottom). Bar graph shows glomerular injury scores from kidneys of WT and Mpv17−/− (n = 4) mice at 7 days following NTS injection. C: serum creatinine (n = 10 per group) in WT and Mpv17−/− mice at day 0 (CTRL) and 1 and 7 days following NTS injection. A–C: severe NTSN. Urinary albumin/creatinine ratio for the time-course days 0–4 in WT and Mpv17−/− mice (n = 7 per group) with mild NTSN (D) and PAS staining (E) in WT and Mpv17−/− mice at day 3 after NTS injection showing normal WT and initial sclerosis in Mpv17−/− (×400 top and ×600 middle, and ×1,000, bottom) show a normal WT glomerulus and an Mpv17−/− glomerulus with initial sclerosis. Bar graph shows glomerular injury score in Mpv17 WT (n = 7) and Mpv17−/− mice (n = 5) at day 3 after NTS injection. F: serum creatinine in WT and Mpv17−/− mice at day 0 (CTRL) and after 1 and 3 days following NTS injection (n = 5 per group). D–F: mild NTSN. *P < 0.05, ***P < 0.005, *P < 0.05 vs. other groups.
Fig. 3. Increased glomerular oxidative stress in Mpv17<sup>−/−</sup> mice with NTSN. A: urinary 8-oxoguanine (8oxoG) excretion detected by HPLC in WT and Mpv17<sup>−/−</sup> mice at time 0 (CTRL) and at the indicated time points after induction of severe or mild NTSN (n = 4 per group). Immunofluorescence double staining for 8oxoG and synaptopodin in kidneys cryosections from WT and Mpv17<sup>−/−</sup> mice 7 days following induction of severe nephrotoxic nephritis (B) and kidneys cryosections from WT and Mpv17<sup>−/−</sup> mice three days following induction of mild nephrotoxic nephritis (C) showing colocalization between DNA oxidative marker and podocyte marker. D: double staining for 8oxoG and mitochondrial transcription factor transcription factor A, mitochondrial (TFAM) showing accumulation of oxidized DNA in mitochondria. Microscopy original magnification: ×630. ***P < 0.005 vs. relative CTRL, *P < 0.05 vs. WT day 7.
However, upon challenge with rotenone, a mitochondrial complex I inhibitor and ROS inducer (16), OCR was reduced in both WT and MPV17−/− podocytes. When rotenone was removed and cells were allowed to a recovery period in control medium, the marked reduction in OCR persisted in Mpv17−/− podocytes, while in WT podocytes mitochondrial function returned to control levels (Fig. 5B). Extracellular acidification rate, an index of the glycolytic pathway, was instead reduced by 40% in Mpv17−/− podocytes compared with WT (WT = 0.73 ± 0.05 and Mpv17−/− = 0.47 ± 0.03 μP·min⁻¹; mg⁻¹; n = 4; P < 0.05) indicating that the absence of Mpv17 in podocyte results in an imbalance in the equilibrium between aerobic and anaerobic metabolism. A role of Mpv17 as an important ROS regulator in podocytes was also supported by increased basal superoxide generation in Mpv17−/− compared with WT podocytes (Fig. 5C). Increased mitochondrial superoxide generation in Mpv17−/− podocytes was maintained upon treatment with rotenone (Fig. 5C). Mitochondrial membrane potential was comparable at baseline in WT and Mpv17−/− podocytes and similarly affected by rotenone in both WT and Mpv17−/− podocytes (Fig. 5D). The increased susceptibility of Mpv17−/− podocytes to mitochondrial stress was associated with increased apoptosis following incubation with rotenone for 48 h, consistent with a protective role of MPV17 against mitochondrial dysfunction and the associated apoptotic cell death. The effect of rotenone on apoptosis was partially reversed by MitoTempo, a mitochondrial-specific superoxide scavenger (Fig. 5D) (23). Interestingly, mitochondrial ROS production and OCR dysfunction, detectable already at 24 h (Fig. 5C), appeared to precede apoptosis in Mpv17−/− podocytes suggesting a cause-effect relationship.

DISCUSSION

In the present report, we demonstrate that Mpv17 specifically protects podocytes against stress-induced mitochondrial dysfunction in vivo and in vitro: 1) in glomeruli, Mpv17 is localized in mitochondria of podocytes; 2) downregulation of Mpv17 protein expression in podocytes in vivo is characteristic of experimental glomerular injury and of FSGS but not MCD patients; and 3) loss of Mpv17 protein function in mice is associated with increased glomerular mitochondrial ROS generation and mitochondrial oxidative DNA damage, demonstrating that loss of a mitochondrial protein in podocytes is associated with modification of glomerular function that is undetectable in physiological condition but clearly emerge under stress. Furthermore, mechanistic in vitro studies using podocytes with knockdown of Mpv17 or podocytes generated from WT or Mpv17−/− mice demonstrated that Mpv17 protects against mtDNA depletion, excessive mitochondrial ROS generation, and apoptosis.

Our findings add substantial new mechanistic insights to a body of evidence implying Mpv17 in glomerular pathobiology over more than two decades (2, 14, 26). Recent findings
suggest that glomerular mtDNA depletion precedes late onset proteinuric nephropathy in Mpv17 mice (25), indicating mitochondrial dysfunction. mtDNA depletion and glomerulosclerosis were aggravated by loss of the DNA repair gene Prkdc in Mpv17 model, suggesting further that the severity of Mpv17 deficiency-associated renal manifestations correlates with the extent of mtDNA depletion and dysfunction (15). Our in vivo work now extends the mitochondrial protective role of Mpv17 in glomeruli to immune-mediated injury models associated with ROS and our in vitro studies using podocyte culture models identify podocytes as glomerular cell culprit, demonstrating that Mpv17 protects podocytes against oxidative stress, mitochondrial respiratory chain dysfunction, and apoptosis. Furthermore, the results of Mpv17 expression in human biopsies suggest a different role of podocyte mitochondria dysfunction in FSGS and MCD pathogenesis.

MPV17 family members have four transmembrane spanning domains and have been localized to the inner mitochondrial membrane (17). Sym1, the yeast orthologue of MPV17, was recently demonstrated to form a channel in synthetic bilayer membranes (17). The predicted pore size of this channel of ∼1.6 nm is sufficient to allow the transport of large molecules such as metabolites across the inner mitochondrial membrane. While the physiological function of the channel remains unknown, hypothetically loss of such an inner mitochondrial membrane channel could underlie the mitochondrial defects characteristic of Mpv17-deficient podocytes in vitro and in vivo. Intriguingly, loss of Mpv17 orthologue function in iridophores of zebrafish is associated with increased cell death, consistent with increased apoptosis in Mpv17-deficient podocytes in our present report (11). Clearly, further detailed molecular mechanistic analyses will be required to define how loss of inner mitochondrial membrane channels formed by Mpv17 multimers underlies the mitochondrial dysfunction and apoptosis susceptibility of podocytes exposed to ROS-mediated stress in vivo and in vitro. However, our results suggest that Mpv17 in podocytes could be part of the buffering system controlling mitochondrial ROS generation and, at same time, have a role in the regulation of respiratory chain activity, expanding on Mpv17 mechanistic clues and on the relevance of mitochondrial metabolism in podocytes physiology.

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GRANTS

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