Background Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by the expression of antibodies to extracellular vesicles (EVs). These vesicles can arise from dead and dying cells and display nuclear and cytoplasmic molecules. Since EV preparations contain mitochondria, we performed experiments to test directly the binding of SLE antibodies to mitochondria.

Methods Mitochondria were prepared from mouse liver and immobilized on microtiter plates pre-coated with poly-L-lysine at 0.5 μg/mL. Bound antibodies were detected using a peroxidase-conjugated anti-IgG reagent. To determine whether DNA contributed to the antigenicity of the mitochondria, the ability of DNA to inhibit binding was tested. The binding of sera from 211 SLE patients who met 1982 ACR criteria for classification was determined by ELISA and results compared with an ELISA for M2 antimitochondrial antibodies (AMA).

Results Using an ELISA assay with immobilized mitochondria, 60.2% of SLE sera showed positive responses defined as greater than two standard deviations above the mean of control sera. Samples were also analyzed using a commercial AMA ELISA (Euroimmun US, Morris Plains, New Jersey) for IgG antibodies to the M2 antigen. With this kit, 5.7% of the SLE samples tested positive. To determine the relationship of antibodies to anti-DNA, the ability of calf thymus (CT) DNA to inhibit binding was investigated for a subset of samples. In the ELISA, CT DNA at 50 μg/mL inhibited binding for 7 of 8 SLE plasmas, with inhibition ranging from 7.7%–59.3%.

Conclusion These results indicate that blood of patients with SLE contains antibodies to mitochondria. Among these antibodies, some may react to DNA as shown by the ability of soluble DNA to inhibit ELISA binding. While binding mitochondria, these antibodies differ from AMA found in primary biliary cholangitis since few reacted in the ELISA for M2 AMA. Since studies using flow cytometry have demonstrated that IgG positive EVs in the blood of patients contain mitochondria as shown by MitoTracker Deep Red, these results suggest that, like nuclei, mitochondria may be a subcellular organelle that can display autoantigenic determinants to form immune complexes in SLE.

Background Patients with systemic lupus erythematosus (SLE) and anti-ribosomal P (anti-P) antibodies may present lupus psychosis, cognitive impairment and lupus nephritis (LN). Our group provided a molecular mechanism to explain the neurological alterations in SLE identifying the neuronal-surface P-antigen (NSPA) as an anti-P target involved in the regulation of synaptic glutamatergic transmission (Bravo-Zehnder et al. A and R 2015; Segovia-Miranda et al. A and R 2015). There is evidence suggesting that anti-P and anti-dsDNA antibodies have the potential to perturb the function of renal cells in addition to complement activation. The mechanism of anti-P association with lupus nephritis remains unclear. It is possible that direct anti-P interaction with cell surface components activates intracellular signalling pathways resulting in renal cell damage. As a first approach to this hypothesis we study whether and where NSPA is expressed in the kidney.

Methods NSPA expression was assessed by RT-PCR, immunoblot and anti-NSPA immunohistochemistry in C57WT mice, β-galactosidase (β-gal) histochemistry staining in transgenic mice bearing a LacZ gene under the promoter of NSPA gene and anti-P staining in the surface of primary cultures of PTEC and the human kidney cell line HK2. NSPA polarized distribution was studied in Madin-Darby canine kidney (MDCK) cells transfected with NSPA-GFP expression plasmid. The pathogenic potential of anti-P antibodies was analyzed by immunizing C57WT and NSPAKO mice with recombinant P0 ribosomal bearing (P0w) or not the P epitope (P0n) and testing anti-P and anti-dsDNA presence, proteinuria and renal pathology by histochemistry and electron microscopy.

Results NSPA is expressed in PTEC cells and display a mainly apical distribution in vivo and in vitro, including the transfected MDCK cells. Both P0n and P0w generated high levels of anti-P antibodies and some mice also developed anti-dsDNA antibodies. Mice presented mild proteinuria at 6 months of immunization. C57WT and NSPAKO mice immunized with either P0n or P0w also displayed perivenular lymphocytic infiltration, a mild renal pathology change. In addition, NSPAKO mice, even in absence of anti-dsDNA, showed mesangial electron dense-deposits with discrete effacement of foot processes.

Conclusions NSPA is expressed in both PTEC and polarized MDCK epithelial cells with a polarized distribution that precludes direct interaction with circulating anti-P antibodies, which might access to this target only after glomerular filtration. Circulating anti-P can associate with mild kidney damage independently of NSPA expression, at least in non-lupus prone mice.

Acknowledgements CONICYT Basal grant PFB12/2007 and FONDECYT Grant 1160513.
with idiopathic ataxia, but recent studies have indicated that they are also found in systemic lupus erythematosus (SLE). The goals of this study were to determine the frequency of anti-MPP-1 in a local SLE cohort and then identify demographic, clinical, and serologic correlations.

Methods Patients fulfilling the American College of Rheumatology (ACR) or Systemic Lupus Lupus International Collaborating Clinics (SLICC) Classification Criteria for SLE were enrolled in the Southern Alberta Registry for Lupus Erythematosus (STARLET) cohort (Calgary, Canada). Demographic, clinical information (disease activity – SLEDAI-2K; damage – SLICC/ACR DI), and sera were collected at time of enrollment. Antibodies to MPP-1 were determined by an addressable laser bead immunoassay (ALBIA) utilizing an in vitro expressed MPP-1 cDNA construct inserted into a GFP vector (Clontech Laboratories Inc., Saint-Germain-en-Laye, France). ALBIA results were expressed as median fluorescence units (MFU) and a dilution of ≥1500 MFU was considered highly positive. Univariable analysis was performed to determine associations between the prevalence of high positive anti-MPP-1 and demographic (age, sex, race/ethnicity), clinical features (SLEDAI-2K and SLICC/ACR DI total scores and subscales and neurological subscale of the ACR and SLICC Classification Criteria), medications, other autoantibodies (anti-dsDNA, extractable nuclear antigens, and anti-phospholipid antibodies).

Results One hundred and forty SLE patients were included; 89.3% were female with a mean age of 47.3 years (SD 16.3) and disease duration of 13.9 years (SD 11.6). The prevalence of high titer anti-MPP-1 was 15.0% (21/140) respectively. Univariable analysis demonstrated that high anti-MPP-1 positivity was associated with a higher total SLEDAI-2K score (Odds Ratio [OR], 1.1 [95% CI 1.0 to 1.3]), particularly with the serostasis (OR 3.0, [95% CI 1.4 to 6.6]) and immunological subscales (OR 2.0, [95% CI 1.4 to 2.9]). High anti-MPP-1 positivity was also associated with anti-dsDNA (OR 3.5 [95% CI 1.8 to 6.6]), anti-SSA/Ro60 (OR 3.1 [95% CI 1.0 to 8.9]) and anti-phosphorylidyserine/prothrombin complex (aPS/PT)-IgG (OR 3.6 [95% CI 1.1 to 11.5]).

Conclusions High titer anti-MPP-1 antibodies were common in this SLE cohort (15.0%) and may be associated with greater clinical and serologic SLE disease activity. A larger study is currently underway to more clearly delineate its role as a biomarker in SLE.

Acknowledgements The authors are grateful for the technical assistance of Ms. Haiyan Hou and Meifeng Zhang (Mitogen Advanced Diagnostics, University of Calgary).