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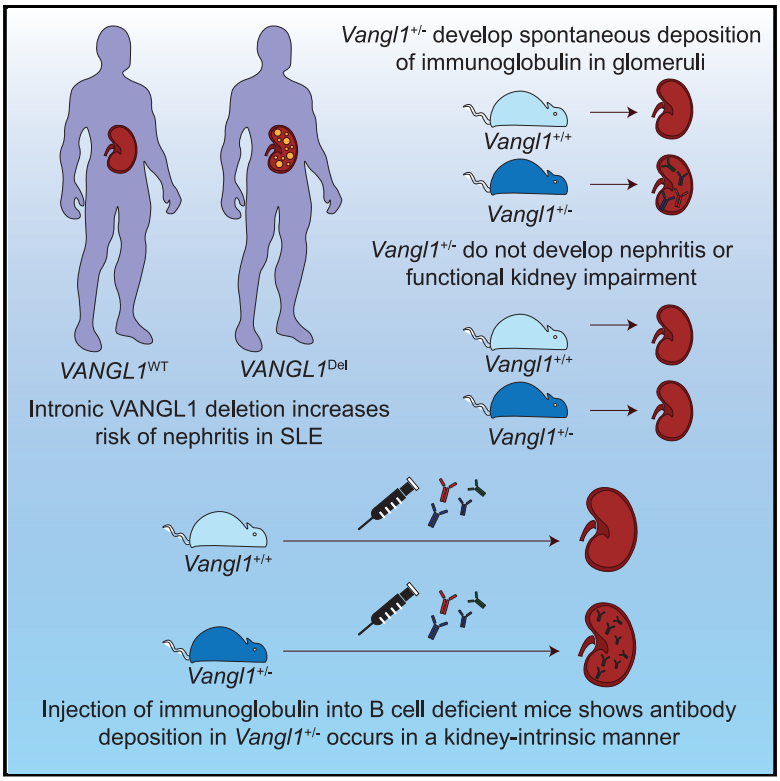
Deletions in *VANGL1* are a risk factor for antibody-mediated kidney disease

Graphical abstract



Highlights

- Intronic deletions in *VANGL1* are associated with risks of glomerulonephritis in SLE
- *Vangl1*$^{+/-}$ mice develop spontaneous deposition of immunoglobulin in glomeruli
- *Vangl1*$^{+/-}$ mice do not develop glomerulonephritis despite antibody deposition
- Immunoglobulin deposition in *Vangl1*$^{+/-}$ occurs in a kidney-intrinsic manner

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In brief

The organ specificity risks of lupus nephritis are unclear. Jiang et al. identify a common intronic deletion in *VANGL1* that increases risks of nephritis in SLE patients. *Vangl1*$^{+/-}$ mice develop spontaneous deposition of immunoglobulin in the kidney without glomerulonephritis in a kidney intrinsic manner.

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Deletions in *VANGL1* are a risk factor for antibody-mediated kidney disease

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SUMMARY

We identify an intronic deletion in *VANGL1* that predisposes to renal injury in high risk populations through a kidney-intrinsic process. Half of all SLE patients develop nephritis, yet the predisposing mechanisms to kidney damage remain poorly understood. There is limited evidence of genetic contribution to specific organ involvement in SLE.[1,2] We identify a large deletion in intron 7 of *Van Gogh Like 1* (*VANGL1*), which associates with nephritis in SLE patients. The same deletion occurs at increased frequency in an indigenous population (Tiwi Islanders) with 10-fold higher rates of kidney disease compared with non-indigenous populations. *Vangl1* hemizygosity in mice results in spontaneous IgA and IgG deposition within the glomerular mesangium in the absence of autoimmune nephritis. Serum transfer into B cell-deficient $Vangl1^{+/-}$ mice results in mesangial IgG deposition indicating that Ig deposits occur in a kidney-intrinsic fashion in the absence of *Vangl1*. These results suggest that *Vangl1* acts in the kidney to prevent Ig deposits and its deficiency may trigger nephritis in individuals with SLE.

Table 1. Demographics of patient cohorts

Clinical	HC n = 11	SS n = 11	SLE 1 n = 55	SLE 2 n = 177	SLE 3 n = 281
Age (years) (IQR)	57 (56-66 years)	48 (45-51 years)	50 (35-60 years)	47 (35-60 years)	
Gender (female) (%)	6 (55%)	11 (100%)	49 (89%)	148 (83%)	253 (90%)
Oral ulcers (%)	0	0	13 (24%)	53 (30%)	73 (26%)
Arthritis (%)	0	0	38 (69%)	122 (69%)	204 (72%)
Raynaud's (%)	0	0	19 (35%)	60 (34%)	Unknown
Cutaneous (%)	0	0	30 (55%)	99 (56%)	153 (54%)
Nephritis (%)	0	0	23 (42%)	62 (35%)	113 (40%)
Serositis (%)	0	0	15 (27%)	41 (23%)	91 (32%)
Alopecia (%)	0	0	7 (13%)	39 (22%)	Unknown
Seizure (%)	0	0	9 (16%)	20 (11%)	9 (3%)
Sicca (%)	0	11 (100%)	15 (27%)	62 (35%)	Unknown
Myositis (%)	0	0	0	7 (4%)	Unknown
Cytopaenia (%)	0	0	28 (51%)	76 (43%)	178 (63%)
Antiphospholipid Ab (%)	0	0	27 (49%)	78 (44%)	Unknown
ANAs (%)	0	10 (91%)	55 (100%)	177 (100%)	257 (91%)
dsDNAs (%)	0	0	44 (80%)	112 (63%)	159 (56%)
Anti-Sm (%)	0	0	8 (15%)	21 (12%)	31 (11%)
Hypocomplementemia (%)	0	0	36 (65%)	76 (43%)	Unknown

HC: healthy control, SLE: systemic lupus erythematosus, SS: Sjogren's syndrome. The SLE cohort (n = 281) has been described previously.[36,37]

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease and end-organ damage is thought to result from deposition of autoantibodies. Although clinical presentation varies considerably between individuals,[3,4] kidney involvement is strongly associated with adverse effects on mortality and morbidity among SLE patients.[5,6] Lupus nephritis (LN) occurs in 21%–58%[7,8] of SLE patients and manifests as several distinct though often overlapping histopathologic lesions.[9] The mechanisms promoting different organ involvement between SLE individuals remain unclear although ethnicity,[7,8] specific autoantibodies,[10,11] autoantigens within affected organs[12,13] and HLA[14–16] have been implicated.

Genetic variation is a potent risk factor for development of SLE[17] and may also influence organ-specific involvement[18,19]. Most reported variants predisposing to SLE are either single nucleotide variations[20] (SNV) or deletions[21,22] in genes with important roles in the immune system. It is increasingly recognized that relatively large structural genetic changes comprising insertions, deletions, and duplications of >1,000 base pairs called copy number variants (CNVs) represent a sizable proportion of individual genetic variability.[23] Unsurprisingly, CNV in genes primarily involved in immune function have also been increasingly implicated in SLE pathogenesis.[24–26]

Van Gogh Like 1 (*VANGL1*) and *Van Gogh Like 2* (*VANGL2*) are genes essential in the establishment of planar cell polarity (PCP). Both genes are highly conserved in vertebrates and influence PCP through interaction with several core pathway PCP genes such as *Dishevelled* (DVL1), *Flamingo/Starry night* (FMI/STAN), *Prickle* (PK) and *Diego* (DGO).[27] Like most PCP genes, the *VANGL* are important for neural tube development.[28] SNV in the murine ortholog *Vangl2* results in the loop-tailed mouse model of neural tube defects (NTD),[29] and SNV in *VANGL1* and *VANGL2* are associated with human NTD.[30,31] In addition to their role in neural development, *VANGL* genes also regulate kidney development. Deficiency of *Vangl2* in mice impairs kidney organogenesis,[32] glomerular maturation, and development and repair response to glomerular injury.[33] Podocyte-specific deletion of *Vangl2* enhanced injury in experimental nephritis.[34] Despite this known contribution of *VANGL2* to kidney development, neither *VANGL1 nor VANGL2* have yet been associated with human kidney disease. Here we identify a recurrent deletion in *VANGL1* that predisposes to Ig deposition in the glomerulus and is associated with lupus nephritis.

RESULTS

Copy number variation in *VANGL1* associates with nephritis in SLE

To determine the role of CNV in SLE we performed a genome-wide SNV association using Affymetrix 5.0 SNP chip arrays in a cohort of SLE patients selected according to disease severity (n = 55), Sjogren's syndrome (n = 11) and healthy controls (n = 11) (Table 1). SLE patients were classified according to the 1997 American College of Rheumatology guidelines,[3] and qualitatively chosen for quantity or severity of system involvement. The Affymetrix 5.0 array has ~500,000 SNP probes and ~420,000 CNV probes. Array data were analyzed using R statistical language package CRNA (v2).[35] All 77 arrays passed QC checks. After filtering, a total of 982 CNVs were retained for all 77 samples (Supp Data). Several CNVs were detected in more than one individual, including three SLE individuals with CNVs in *VANGL1*. Of the three SLE patients, two had nephritis. No Sjogren's Sd

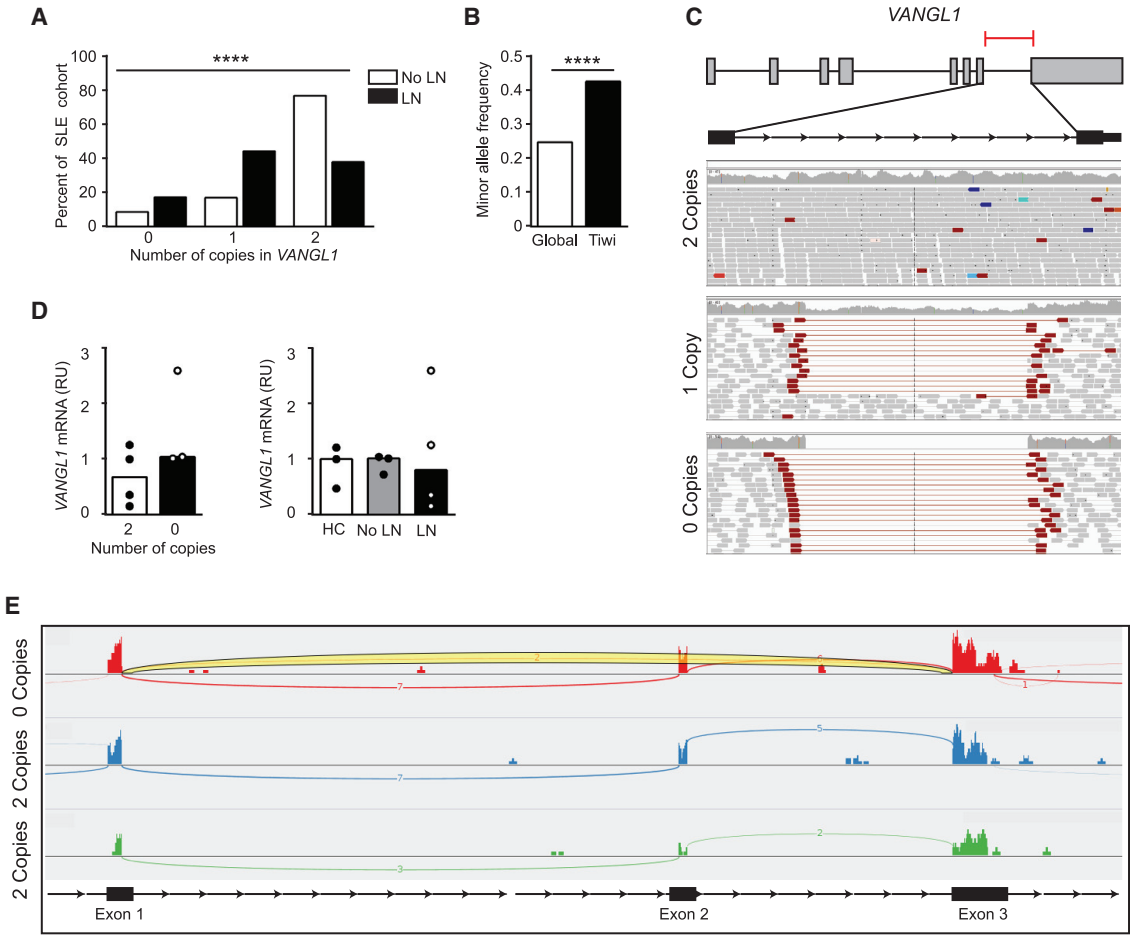

Figure 1. Intronic deletions in *VANGL1* associate with kidney disease

(A) Association of copy number variation in *VANGL1* detected by qPCR with the presence or absence of nephritis in SLE (n = 177); χ^2 2d.f., p < 0.0001 (LN = lupus nephritis).

(B) Comparison of MAF of *VANGL1* CNV in esv3587290 in gnomAD global and Tiwi Islander populations (n = 120); Fisher's exact, p < 0.0001).

(C) Representative whole genome sequencing reads of intronic deletion in *VANGL1* of the maximum length reported for the CNV.

(D) Comparison of relative *VANGL1* expression in PBMCs from (i) SLE patients with 0 or 2 copies of *VANGL1*, (ii) Healthy controls or SLE patients with or without lupus nephritis.

e) RNaseq read alignment demonstrating skipping of exon 2 in a patient homozygous for the *VANGL1* CNV with read covering exon 1 and exon 3 highlighted in yellow.

patients or healthy controls had the CNV. The initial array analysis suggested all three SLE patients had the same double deletion of an estimated 3.17 kb of this gene spanning chr1:116,030,911–116,034,081 (NCBI36/hg18 assembly) within intron 7. This finding was supported by only two SNPs on the array but was consistent with the small size of the CNV. To confirm these findings, we performed Taqman qPCR targeting the CNV region identified in intron 7 of *VANGL1* in a larger cohort of SLE patients (n = 177) (Figure 1A). In this larger SLE cohort, 18 were homozygous, 41 were heterozygous, and 119 did not have the CNV. Overall, the MAF of the CNV in the SLE cohort was similar to that of the global gnomAD frequency (MAF 0.28 versus 0.25, χ^2 p = 0.7). However, we observed an association between the *VANGL1* CNV and the presence of nephritis in SLE patients (χ^2 = 27.06, 2 d.f., p < 0.0001). Furthermore, the correlation between 0, 1 or 2 *VANGL1* copies and nephritis suggested a gene-dose increase in nephritis risk with the *VANGL1* CNV. Indeed, the *VANGL1* 0 copies CNV was observed at twice the frequency in patients with nephritis compared to patients without nephritis (minor allele frequency (MAF) of 0.39 versus 0.17 respectively). The qPCR was also repeated in a third cohort (n = 281) and this revealed a possible, albeit non-statistically significant, trend between CNV in *VANGL1* and nephritis in SLE patients (Figure S1) (χ^2 = 2.1, 1 d.f., p = 0.14).

Gene variants with a large difference in risk allele frequency between populations are strong candidates to explain variations in disease.[38] We hypothesized that if the *VANGL1* CNV predisposes to kidney disease, it may be more prevalent in populations at high risk of kidney disease. The Tiwi Islanders are an Australian Aboriginal group with rates of kidney disease 10 times greater than the non-Aboriginal population.[39] In a large Tiwi Island cohort (n = 120) with an even gender distribution of 50% males and females and a median age of 40.8 years (IQR 12.7 years) who underwent

whole genome sequencing (WGS), a large CNV in intron 7 of *VANGL1* (esv3587290) similar to the CNVs found in patients with SLE nephritis, was identified at a frequency significantly higher compared to the global gnomAD frequency (Figure 1B, MAF 0.43 versus 0.25, χ^2 $p < 0.0001$) and all other racial groups (Figure S2A). We explored the association between the CNV and severity of kidney disease in Tiwi. We did not observe a significant trend in later stages of CKD within patients with the CNV compared to those without (Figure S2B). When comparing Tiwi Islanders with the *VANGL1* CNV compared to those without, there was no significant difference in serum creatinine (72.0 versus 65.0 μmol/L, p value 0.28) or urinary albumin-to-creatinine ratio (31.4 versus 0.9 mg/mmol, p value 0.56). Histological data are unavailable for this cohort and therefore the extent of immunoglobulin deposition or autoimmunity as a cause of chronic kidney disease could not be determined. Together, this suggests the intronic CNV in *VANGL1* may predispose to the development of kidney disease, but not impact the progression of kidney damage.

To characterize the CNV in *VANGL1*, we first examined the Database of Genomic Variants which identifies 15 of 26 (57%) reported *VANGL1* CNV located within intron 7 of *VANGL1*.[40] These CNVs were predominantly large deletions of variable size indicating that this is a recurrent CNV region. We performed WGS in SLE patients with and without the deletion, confirming the presence and variability in size of the CNV (Figure 1c). We first tested if this CNV influenced *VANGL1* expression in peripheral blood mononuclear cells (PBMCs) from SLE patients by RT-qPCR. We found no difference in exon 3 and 4 *VANGL1* expression between those that had been identified with 0 CNV and 2CNV (Figure 1D) nor between healthy controls and SLE patients with or without nephritis (Figure 1D).

To detect additional splicing abnormalities potentially associated with the deletion that would not result in changes to the coding region of *VANGL1*, we performed RNaseq from PBMCs of patients homozygous for the *VANGL1* deletion (n = 6) or lacking the deletion (n = 4). Interestingly, in two of the six patients homozygous for the deletion we observed several reads skipping or alternatively splicing exon 2, and therefore predicted to result in loss of the native *VANGL1* start codon (Figure 1E). We hypothesized that this may be due to presence of a cryptic splice site within intron 7. This could either lead to translation from the next in-frame ATG start site generating a truncated protein lacking the first 98 amino acids or use an alternative out-of-frame ATG start site that would putatively encode for a different peptide and thus reduce the amount of VANGL1 protein expressed. VANGL1 protein is expressed at low levels in PBMCs, and therefore, we were not able to test for differences in the molecular weight of VANGL1 that may occur with loss of exon 1. Therefore, kidney biopsies from patients heterozygous (n = 4) or wild-type (n = 4) for the *VANGL1* CNV were stained for VANGL1 using a polyclonal antibody raised against either the c-terminus (Thermofisher PA5-98739) or n-terminus (Thermofisher PA5-55231) of VANGL1 and scored by a nephropathologist for tissue expression. No obvious difference in protein expression intensity or pattern using the available polyclonal antibody was observed in heterozygous individuals (Figure S3). VANGL1 was detected primarily in tubular epithelium with some glomerular staining (Figure S3). Unfortunately, we did not have biopsy samples from patients homozygous for the CNV to measure significant impact on protein expression. Thus, recurrent intronic deletions in *VANGL1* are associated with the predisposition to nephritis in SLE patients, potentially affecting RNA splicing in some patients.

Vangl1$^{+/-}$ mice develop spontaneous deposition of immunoglobulin in the kidney

Mice deficient in *Vangl2*, the other *Vangl* family member, have reduced glomerular numbers and size, and impaired kidney morphogenesis.[32] Recent work has demonstrated a role for *Vangl2* in directing podocyte repair and *Vangl2* deficiency within podocytes was associated with impaired recovery after acute glomerular injury and a predisposition to focal segmental glomerulosclerosis.[33] *VANGL1* is highly expressed in human CD34+ and CD105+ cells, which includes endothelial cells, some T cells, and monocytes.[41] We hypothesized that given the role of *Vangl2* in kidney development and repair, and the functional interaction between the *Vangl1* and *Vangl2* in other tissues,[42] the observed *VANGL1* CNV may predispose to SLE nephritis in a kidney-intrinsic manner. We first examined expression of *Vangl1* in murine kidney. Using immunohistochemistry, we stained kidney sections from 10-week-old adult C57BL/6 mice. Similar to the observations made of Vangl2, Vangl1 was detected largely within tubules but not within the glomeruli (Figure 2A). One possibility is that *Vangl1* and *Vangl2* are expressed transiently in glomeruli during development and repair.[33] To determine if *Vangl1* had a prominent role in glomerular development similar to *Vangl2*, we stained sections of D18 fetal kidneys from C57BL/6 mice but did not detect Vangl1 in these sections (data not shown).

To test the consequences of *Vangl1* deficiency *in vivo,* we obtained *Vangl1* knockout mice (KOMP repository) and rederived them onto a C57BL/6 background. *Vangl1* deficiency was embryonically lethal between D15 and D18 due to neural tube defects (Figure 2B). This was surprising, as published observations indicated that mice homozygous for a gene-trapped *Vangl1* allele did not develop NTD, but additively worsened NTD associated with *Vangl2* deficiency.[42] We examined kidneys from mice lacking a single allele of *Vangl1 (Vangl1*$^{+/-}$*).* Given the association of *Vangl2* deficiency with impaired kidney morphogenesis we investigated if *Vangl1* deficiency affects organogenesis. By indirect immunofluorescence, however, we observed spontaneous deposition of IgG and traces of IgA in glomeruli (Figure 2C), but not IgM, C3 or C4 (data not shown). Electron microscopy demonstrated significant Ig deposits within the mesangium and mild mesangial expansion (Figure 2D). We compared the IF patterns in the *Vangl1* mice with biopsy reports that were available from SLE patients (who comprised part of the n = 177 SLE cohort) with biopsy-confirmed lupus nephritis and in whom we confirmed were wild-type or homozygous for the CNV but did not detect a significant difference in IF profiles (Table 2). Interestingly, by light microscopy, *Vangl1*$^{+/-}$ glomeruli appeared normal despite immunoglobulin (Ig) deposition (Figure 3A). We did not observe a significant difference in kidney size, glomeruli size, or glomeruli numbers/light field between *Vangl1*$^{+/-}$ and *Vangl1*$^{+/+}$ littermates (Figure 3B). To ascertain the clinical consequences of Ig deposition, serum creatinine and albuminuria were measured at 6 and 9 months of age and no difference between *Vangl1*$^{+/+}$ or *Vangl1*$^{+/-}$ mice was detected (Figure 3C). Considering the association of *Vangl2* with tissue

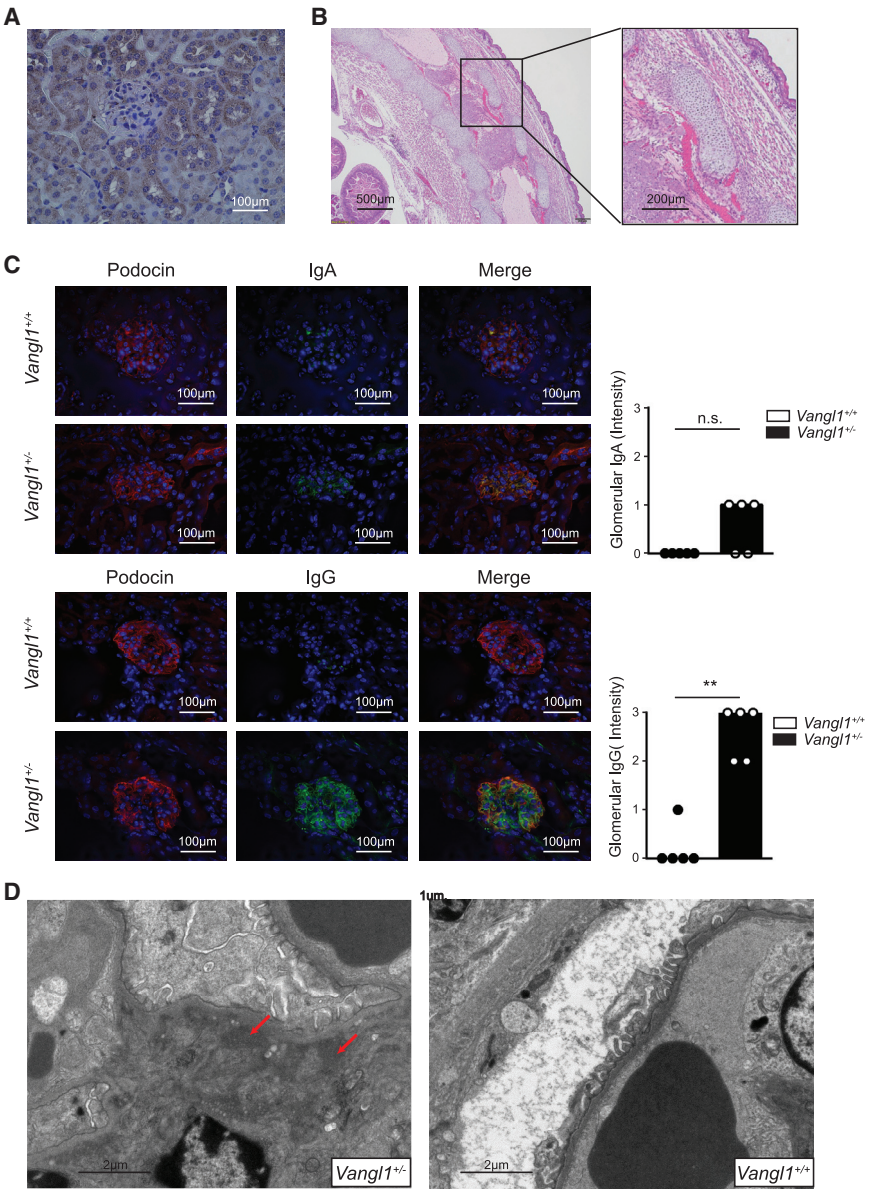

Figure 2. *Vangl1* deficiency causes immunoglobulin deposition

(A) Immunohistochemistry demonstrating Vangl1 (brown) in kidney sections from 3-month-old wild-type C57BL/6 mice (n = 5).

(B) Representative H&E section of a D12 *Vangl1*$^{-/-}$ fetus demonstrating neural tube defects (n = 6).

(C) Immunofluorescence of immunoglobulin A or G (green), podocin (red) and DAPI in 8-week-old *Vangl1*$^{+/-}$ and *Vangl1*$^{+/+}$ mice (n = 6/group) median, Mann-Whitney U, $p < 0.05$. (D) Electron microscopy demonstrating electron dense deposits consistent with immunoglobulin in 8-week-old *Vangl1*$^{+/-}$ mice (n = 6/group).

repair, we considered *Vangl1* and *Vangl2* may also be expressed during autoimmune glomerulonephritis. *Lyn*$^{-/-}$ mice develop proliferative glomerulonephritis by as early as 6 weeks of age (Figure S4). We stained kidney samples from 8-week-old lupus-prone *Lyn*$^{-/-}$ C57BL/6 mice with glomerulonephritis for Vangl1 and Vangl2. Interestingly, staining for Vangl1 and Vangl2 in proliferative glomeruli demonstrated scattered positivity in the glomeruli (Figure S4). Therefore, *Vangl1*$^{+/-}$ mice develop spontaneous mesangial immunoglobulin deposition with no evidence of inflammation or impairment of kidney function.

Vangl1$^{+/-}$ immunoglobulin deposition occurs in a kidney-intrinsic manner

To determine the potential cause of immunoglobulin deposition, we first tested whether antibody deposition was related to increased systemic immunoglobulin production or was a manifestation of systemic autoimmunity. Flow cytometric examination of mouse splenocytes did not demonstrate significant differences in lymphocyte populations, including B cell subsets (data not shown). We considered that the Ig deposits could represent either a qualitative or quantitative abnormality of antibodies in *Vangl1*$^{+/-}$ mice, yet at 20 weeks of age there was no difference in detectable anti-nuclear antibodies (ANAs) (Figure 3D) or quantitative difference in any class of Ig (Figure 3E) produced by *Vangl1*$^{+/-}$ mice.

We next hypothesized that glomerular immunoglobulin deposition in *Vangl1*$^{+/-}$ mice is due to a kidney-intrinsic predisposition given detectable expression of Vangl1 protein in murine kidneys and the absence of systemic autoimmunity in *Vangl1*$^{+/-}$ mice. To test this, we crossed *Vangl1*$^{+/-}$ mice to C57BL/6 *CD79a*$^{ken/ken}$ mice. *CD79a*$^{ken/ken}$ have an N-ethyl N-nitrosourea (ENU)-induced premature stop codon in *CD79a*, resulting in impaired B cell signaling with complete failure of mature B cell formation, and are therefore unable to produce circulating immunoglobulin[43]. Murine IgG was injected intravenously daily for 5 days into both *Vangl1*$^{+/-}$*CD79a*$^{ken/ken}$ and *Vangl1*$^{+/+}$*CD79a*$^{ken/ken}$ mice. At day 6, mice were sacrificed and kidneys were examined for immunoglobulin deposition (Figure 4A). IgG was present in *Vangl1*$^{+/-}$*CD79a*$^{ken/ken}$ mouse kidneys but not in those of *Vangl1*$^{+/+}$*CD79a*$^{ken/ken}$ littermates (Figures 4B and 4C). We considered the possibility that the kidney-intrinsic predisposition to immunoglobulin deposition could increase the risk of glomerulonephritis when this is associated with autoreactive antibodies. We transferred serum from *Lyn*$^{-/-}$ mice in whom we confirmed the presence of proliferative nephritis (Figure S4) into *Vangl1*$^{+/-}$ or *Vangl1*$^{+/+}$ littermates and injected at day 0 and day 4 with 200ul of serum as described previously with nephrotoxic serum.[44] Mice were sacrificed at day 7 and kidneys

Table 2. Immunofluorescence reports from kidney biopsies from patients wild type or homozygous for the *VANGL1* CNV

	2 CNV					0 CNV	
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
IgG	2+	Trace	3+	3+	2+	3+	2+
IgM	Trace	1+	1+	Neg	3+	1+	1+
IgA	1+	Trace	2+	1+	1+	2+	1+
C3	Trace	2+	1+	3+	3+	3+	trace
C1q	1+	Neg	2+	3+	1+	1+	neg

preserved for histology. Three of six *Vangl1*$^{+/-}$ mice injected with *Lyn*$^{-/-}$ serum had mild segmental glomerulonephritis, whereas no *Vangl1*$^{+/+}$ mice developed glomerulonephritis, suggesting a vulnerability to nephritis with autoreactive serum (Figure 4D). This result demonstrates that susceptibility to Ig deposition in *Vangl1*$^{+/-}$ is kidney-intrinsic and implies that one of *Vangl1*'s roles is to prevent immunoglobulin deposition in the kidney.

DISCUSSION

While SLE has the capacity to involve most organs, kidney involvement is common, important, and often defines disease outcomes. Here, we demonstrate a kidney-intrinsic genetic disposition to renal involvement in SLE. *VANGL1* and *VANGL2* are important PCP genes that have been implicated in human NTD[30] and *Vangl2* has been shown to play a role in kidney development and repair after kidney injury.[33] Although loss of *Vangl2* in mouse models results in fatal NTD, conditional loss of *Vangl2* in podocytes results in impaired glomerular repair and greater injury.[33] We identified a CNV in intron 7 of *VANGL1,* which in individuals with SLE associates with lupus nephritis in a gene-dose protective manner and is also highly prevalent in Tiwi Islanders who have a high rate of kidney disease. Interestingly, the CNV identified does not represent a single deletion but several deletions within the same intron of differing sizes in the kilobase range. In 2 of 6 patients homozygous for the deletion in intron 7 of *VANGL1* CNV, RNaseq of PBMCs identified read skipping of exon 2 which encodes the start codon. The mechanism by which the *VANGL1* deletion contributes to exon 2 skipping remains unclear in these individuals; however, we hypothesize this is likely to either reduce protein expression or produce a shorter or different protein. It is therefore possible that at least in a proportion of individuals with *VANGL1* CNVs, there is reduced full-length *VANGL1* transcript. Whether *VANGL1* differential splicing varies according to cell type or organ remains unknown.

To determine the contribution of *VANGL1* CNV to the development of nephritis, we examined the *Vangl1*$^{+/-}$ mouse. Although not immediately comparable with the intronic CNV in the SLE cohorts, *Vangl1*-deficient mice permitted examination of reduced expression of Vangl1 in the kidneys. Strikingly, *Vangl1*-deficient mice developed spontaneous IgA and IgG deposition in their mesangium, but IgM and complement were not present. We hypothesize that this pattern of Ig deposition may be due to disturbed endothelial expression of Vangl1 permitting passive diffusion of immunoglobulin across the endothelium of glomerular capillaries. This would explain why the monomeric immunoglobulins IgA and IgG, but not the larger pentameric IgM, are detected in the mesangium of *Vangl1*$^{+/-}$ mice. Furthermore, despite deposition of monomeric immunoglobulins in *Vangl1*$^{+/-}$ mice, there were no complement deposits nor evidence of inflammation or glomerular injury. We attribute this to the immunoglobulins deposited in *Vangl1*$^{+/-}$ mice are not autoreactive or crosslinked, and it is only in the presence of autoantibodies, such as in SLE, that antigen-antibody complexes occur, which activate complement and induce an inflammatory reaction. Indeed, the requirement for autoreactive antibody to cause pathology would permit the relatively common frequency of functional CNVs within *Vangl1* from an evolutionary perspective. We hypothesize that autoreactivity of antibodies depositing in human glomeruli accounts for the difference in complement findings between *Vangl1*$^{+/-}$ mice and human SLE biopsies. In *Vangl1*$^{+/-}$ mice, autoantibodies are not autoreactive, and therefore do not form autoantibody-antigen complexes nor activate the classical complement pathway, whereas autoantibodies depositing in the kidneys of SLE patients are either precomplexed or complex with autoantigen *in situ*, triggering both classical and alternate complement activation with subsequent deposition. *Vangl1* is known to play a role in glomerular development and is expressed in murine podocytes, consistent with glomerular and tubular staining we observed.[32] We hypothesize that deficiency of *Vangl1* impairs either development of glomerular or podocyte function, resulting in impaired clearance of antibody, leading to accumulation. This is consistent with accumulation of antibody in kidneys of B cell-deficient *Vangl1*$^{+/-}$ when immunoglobulin is administered directly. We hypothesize that when the antibodies that accrue are autoreactive or antibody-antigen complex, the inflammatory reaction results in glomerulonephritis.

Several important lines of further investigation remain. It is unclear how intronic CNVs in *VANGL1* translate to kidney-specific injury in the absence of NTD observed in mice. Complete deficiency of *VANGL1* should result in high rates of neural tube defects and therefore be under evolutionary pressure. However, the relatively high frequency of CNVs in intron 7 of *VANGL1* would suggest limited selectivity against these CNVs, perhaps even balancing selection where the heterozygous deletion may confer some selective advantage for a hitherto unknown trait. Although many antibody-mediated kidney diseases can be associated with specific antibodies and epitopes, the kidney-intrinsic defect from *VANGL1* deficiency may predispose to any antibody-mediated kidney disease including infection associated forms of glomerulonephritis. The precise mechanism through which *VANGL1* deficiency permits this antibody deposition in such a kidney-intrinsic manner requires further

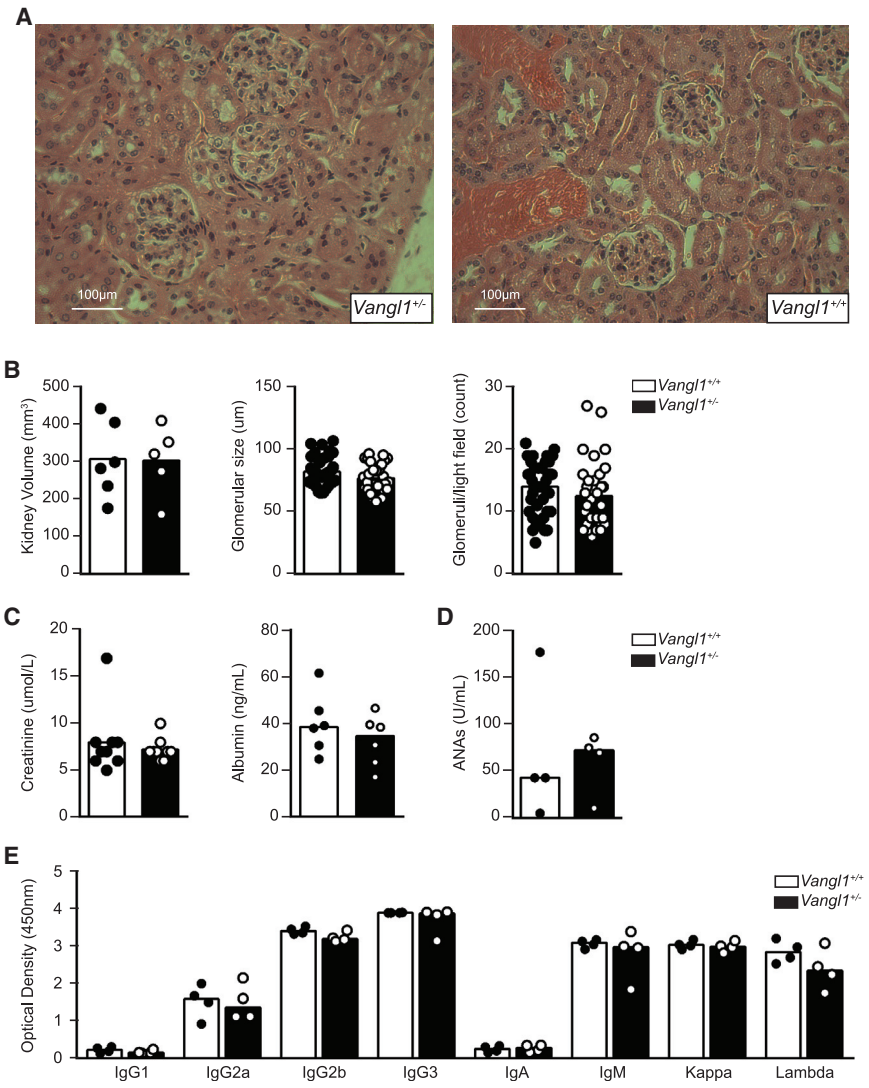

Figure 3. *Vangl1*$^{+/-}$ does not alter glomerular development

(A) Representative H&E stain of glomerular sections from 8-week-old *Vangl1*$^{+/-}$ and *Vangl1*$^{+/+}$ (n = 6/group).

(B) Comparison of kidney volume, glomerular size and glomeruli/light field in 8-week-old mice of indicated genotype.

(C) Serum creatinine and urine albumin in 16-week-old *Vangl1*$^{+/-}$ and *Vangl1*$^{+/+}$ mice.

(D) ANA from 16-week-old mice of indicated genotype

(E) Serum immunoglobulin of 12-month-aged mice of indicated genotype. Data are represented as median throughout.

elucidation.[45] While in our initial cohort we observed a clear gene-dose risk of developing nephritis associated with the *VANGL1* deletion, in our replication cohort we observed only a trend. One explanation may arise from the initial cohort being comprised of majority European ethnicity whereas the replication cohort were largely Spanish and therefore differences in ethnicity may account for the observed discrepancy. This raises the possibility that our findings in human SLE may be an issue predominantly for Caucasian patients compared with other ethnicities. It would be useful to test the effect of the variation in additional cohorts of different ethnicities.

The Tiwi Islanders are a population that suffer one of the highest rates of kidney disease anywhere in the world.[45] The cause of kidney failure in this community may be a combination of immune mediated kidney disease, including antibody mediated postinfectious nephritis, with metabolic disease such as diabetes and hypertension.[46] The exact role of *VANGL1* in the different types of kidney disease and the effect on prognosis remains to be elucidated.

A heritable kidney-intrinsic predisposition to glomerulonephritis also has implications for live-related kidney donation. It may be advisable to perform paired-kidney exchange rather than related donation in circumstances where a heritable predisposition within the kidney itself is present. In summary, we have described how a common CNV in *VANGL1* predisposes toward nephritis in a kidney-intrinsic manner. This offers insights into how SLE may develop predilection for specific tissues, as well as vulnerability to other forms of antibody-mediated kidney disease. The work highlights the evolving role of *VANGL1* and other PCP genes in kidney injury and repair, which may have important implications for therapeutics and transplantation.

LIMITATIONS OF THE STUDY

The SLE cohorts in whom the *VANGL1* deletion was identified were predominantly of European ethnicity, and thus the prevalence and contribution of the deletion to nephritis in non-European groups requires exploration. Variants in *VANGL1* are linked

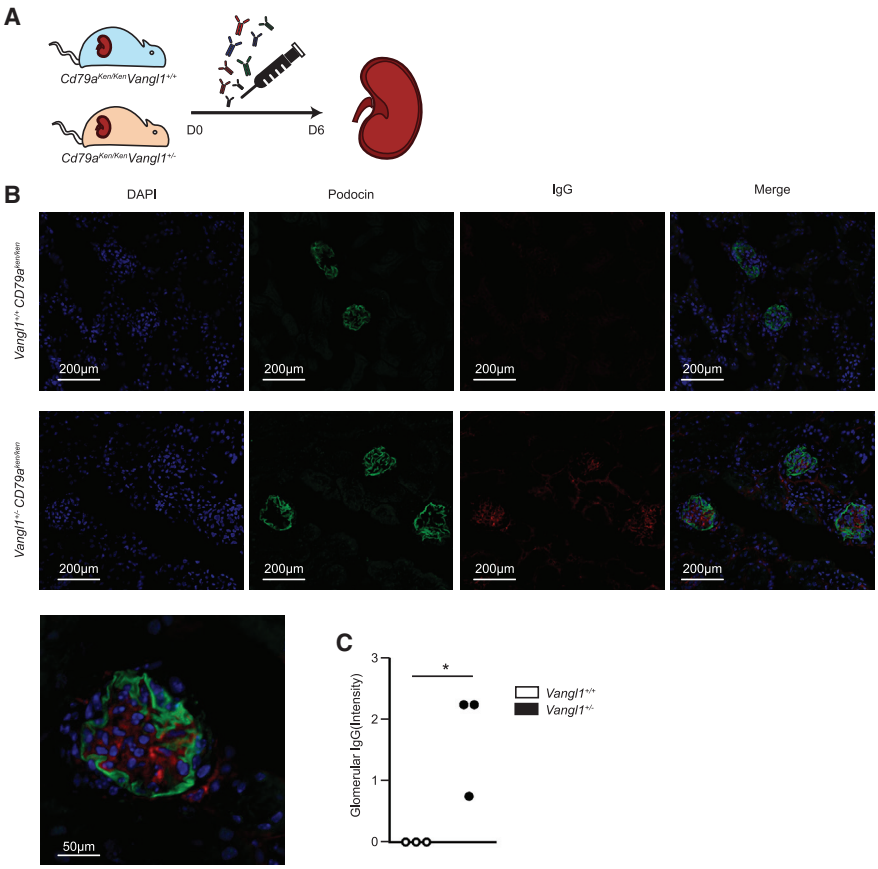

Figure 4. Immunoglobulin occurs in a kidney intrinsic manner

(A) Schematic demonstrating experiment design testing kidney-intrinsic predisposition to kidney disease.

(B) Immunofluorescence of immunoglobulin G (red), podocin (green) and DAPI in cryosections from 8-week-old mice of B cell deficient *Vangl1*$^{+/-}$ and *Vangl1*$^{+/+}$ mice injected with IgG.

(C) Scoring of immunofluorescent IgG deposition in mice post injection. Data are represented as median, Mann-Whitney U, p = 0.02.

to NTD and *Vangl1*$^{-/-}$ mice are embryonically lethal from NTD, and thus it remains unclear why homozygotes for the *VANGL1* deletion do not develop these defects. The precise mechanism through which *Vangl1* deficiency permits antibody deposition also requires elucidation. Further, we did not correlate the deletion with specific forms of glomerulonephritis, and it would be useful to determine the effect of the VANGL1 deletion on specific histopathologic forms of glomerulonephritis.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

S.H.J.: all aspects of study including conceptualization, investigation, and original draft writing; S.M.: conceptualization and methodology investigation; I.P.: methodology investigation and review writing and editing; M.M.: methodology investigation, software, and review writing and editing; G.D.W.: methodology and review writing and editing; M.K.: investigation and review writing and editing; M.F.: investigation and review writing and editing; M. Stanley: investigation and review writing and editing; T.L.-H.: investigation and review writing and editing; A.C.: investigation and review writing and editing; J.E.: investigation and review writing and editing; B.M.: resources, formal analysis, and review writing and editing; M. Sundaram.: resources and review writing and editing; R.T.: software and review writing and editing; P.F.C.: investigation and review writing and editing; W.H.: conceptualization, resources, and review writing and editing; H.H.: investigation and review writing and editing; M. Srivastava: investigation; K.M.: investigation and review writing and editing; I.F.: investigation and review writing and editing, R.C.: investigation and review writing and editing, R.F.: investigation and review writing and editing. S.D.: investigation and review writing and editing, M.G.: investigation and review writing and editing, V.A.: investigation and review writing and editing; M.F.: software, investigation, and review writing and editing; J.M.: conceptualization, investigation, and review writing and editing; E.C.: software, investigation, and review writing and editing; T.D.A.: software, investigation, and review writing and editing; A.R.K.: investigation and review writing and editing; M.C.C.: resources and review writing and editing; M.A.R.: investigation and review writing and editing; M.B.: software, investigation, formal analysis, and review writing and editing; C.G.V.: all aspects of study including conceptualization, methodology, resources, and review writing and editing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

Reagent or resource	Source	Identifier
Antibodies		
IgG	Invitrogen	A28175; RRID:AB_2536161
IgA	Southern Biotech	1040-02; RRID:AB_2794370
IgM	BD PharMingen	555782; RRID:AB_396117
C3	MP Biomedicals	SKU 0855500; RRID:AB_2334931
Vangl1	Sigma	SAB4503254; RRID:AB_10748043
VANGL1	ThermoFisher	PA5-55231; RRID:AB_2649388
VANGL1	ThermoFisher	PA5-98739; RRID:AB_2813352
VANGL2	ThermoFisher	PA5-23207; RRID:AB_2540733
Biological samples		
Human PBMCs	This study	N/A
Human kidney samples	This study	N/A
Chemicals, peptides, and recombinant proteins		
MiScript RT Kit	QIAGEN	218160
SYBR Green	QIAGEN	204143
Tissue-Tek O.C.T.	Sakura	4583
2% osmium tetroxide	Electron Microscopy Sciences, USA	**19100**
Cacodylate buffer	This study	N/A
TAAB epoxy	TAAB Laboratory and Microscopy, U.K	E049
Critical commercial assays		
Affymetrix 5.0 SNP Chip	Affymetrix USA	#901167
Taqman CN Assay	Applied Biosystem, USA	Hs05725015_cn
ANA ELISA	Alpha diagnostic	5210
Total serum immunoglobulin	BD Bioscience	550487
Indiko Creatinine-detect	ThermoFisher	10015638
Albumin ELISA kit	abcam	Ab108792
Deposited data		
RNA sequences	This study	GEO: GSE188480
Experimental models: Organisms/strains		
C57BL/6 mice	Charles River	C57BL/6
Vangl1+/−	KOMP	EPD0164_3_G07
$CD79a^{Ken}$	Australian phenomics facility	N/A
Lyn−/−	Australian phenomics facility	N/A
Oligonucleotides		
Vangl1 exon three F'	Integrated DNA Technologies	GACACAAGTCACCCCGGAATA
Vangl1 exon three R'	Integrated DNA Technologies	TCCTCTGTCCGAGTAGAATCATT
Vangl1 exon four F'	Integrated DNA Technologies	CCGATCCTGTGGAGGGATGA
Vangl1 exon four R'	Integrated DNA Technologies	AAACACCCGTGGCATGTCA
Software and algorithms		
Graphpad Rism 8.0	GraphPad Software CA, USA	N/A
FlowJo	Tristar Inc, Stanford, US	N/A
RNA sequencing annotation	M Field	10.1371/journal.pone.0143199

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Carola Vinuesa (carola.vinuesa@crick.ac.uk).

Materials availability

This study did not generate new unique reagents.

Data and code availability

RNA sequences are deposited in GEO and accession numbers are found in the STAR table.

- This paper does not report original code
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Vangl1$^{-/-}$ mice were created from the ES cell clone (EPD0164_3_G07) obtained from the supported KOMP Repository (www.komp.org) and generated by the Wellcome Trust Sanger Institute using CSD targeted alleles as described previously.[47] *Vangl*$^{+/-}$, *Lyn*$^{-/-}$, and *CD79a*$^{-/-}$ mice were re-derived onto a C57BL/6 background and housed in sterile conditions. Equal numbers of male and female 12 week old mice were used unless otherwise specified and there was no gender bias in *Vangl*$^{+/-}$ mice. All mouse experiments were approved by the Institutional Ethics Committee at the Australian National University. Human subjects had written informed consent obtained as part of the Australian Point Mutation in Systemic Lupus Erythematosus study (APOSLE). The study was approved by the Australian National University and ACT Health Human Ethics Committees. Age and demographics are described in Table 1.

METHOD DETAILS

Study participants, saliva purification, and CNV analysis

Written informed consent was obtained as part of the Australian Point Mutation in Systemic Lupus Erythematosus study (APOSLE). The study was approved by the Australian National University and ACT Health Human Ethics Committees. Saliva was collected in Oragene DNA collection kits and purified using PrepIT DNA purification kits (Oragene) as per manufacturer's instructions. SLE patients were classified according to 1997 revision of the American College of Rheumatology.[3] Initial patients were qualitatively chosen for quantity or severity of system involvement as reported by referring clinicians but were not assessed by SLEDAI or BILAG scores. Organ involvement was reported by treating clinicians and nephritis was defined as the presence of proteinuria (> 0.5gm/day or > 3+ on dipstick)[3] or biopsy confirmed lupus nephritis consistent with the 2004 revision of the WHO classification.[9]

Samples were analyzed for CNV using Affymetrix 5.0 SNP chip which was hybridized and scanned at the Biomolecular Research Facility (JCSMR, ANU). The Affymetrix 5.0 array has ~500,000 SNP probes and ~420,000 CNV probes. Array data was analyzed using R statistical programming language package CRNA (v2).[35] CNVs were defined in each individual by applying the circular binary segmentation algorithm to the normalized $\log_2$(ratios of case versus pool of controls) signals. The pooled control consisted of all samples and assumed that there are no common pathogenic CNVs shared by the cases. Negative $\log_2$(ratio) values indicate copy number deletion and positive values duplication. Actual copy number was not inferred. Only CNVs supported by two or more probes were called. CNVs were filtered based on their $\log_2$(ratio) signal using an approximate empirical p value of 0.003 per individual. CNVs were further filtered by only retaining CNVs that intersected with known genes, based on the hg18 human genome assembly. Identified CNV were confirmed by qPCR using TaqMan CN Assays (Applied Biosystems, US) using the mean of 6 amplicons from CNV probe Hs05725015_cn compared with housekeeper genes. *Vangl1* expression was measured by primers targeting exon 3 (F:GACACA AGTCACCCCGGAATA, R: TCCTCTGTCCGAGTAGAATCATT) and exon 4 (F:CCGATCCTGTGGAGGGATGA R: AAACACCCGTGGC ATGTCA) of *Vangl1*. cDNA was prepared using miScript RT kit (QIAGEN) and used for qRT–PCR for miRNAs using miScript primers and SYBR Green kit from QIAGEN. Expression was normalized against *HL13* and *UBC* housekeeper genes.

RNA sequencing

Transcriptome data were analyzed using a modified version of an existing variant detection pipeline.[48]

Mice and organ isolation

Vangl1$^{-/-}$ mice were created from the ES cell clone (EPD0164_3_G07) obtained from the supported KOMP Repository (www.komp.org) and generated by the Wellcome Trust Sanger Institute using CSD targeted alleles as described previously.[47] *Vangl*$^{+/-}$, *Lyn*$^{-/-}$, and *CD79a*$^{-/-}$ mice were re-derived onto a C57BL/6 background and housed in sterile conditions. Equal numbers of male and female

12 week old mice were used unless otherwise specified and there was no gender bias in *Vangl*$^{+/-}$ mice. Mice were culled and kidneys set in optimum cutting temperature (OCT, Sakura) cryomolds or formalin and set in paraffin blocks.

Immunofluorescence and immunohistochemistry

Three month-old mice were sacrificed and serum and kidneys were harvested. Bound antibody was detected with anti-mouse IgG, IgA or IgM conjugated with fluorescein isothiocyanate (FITC). Immunoglobulin and C3 deposition analysis was performed on cryopreserved kidney sections. 7 μm acetone fixed sections were blocked with 3% bovine serum albumin and stained with anti-IgG Alexa Fluor 488 (Invitrogen), anti-IgA FITC (Southern Biotech), anti-IgM FITC (BD PharMingen) and anti-C3 FITC (MP Biomedicals). Fluorescence intensity was evaluated with an Olympus XI 71 microscope. Assessment of immunofluorescence, using a semiquantitative scale of 0-3+ was performed by two independent investigators. Formalin-fixed kidneys samples were sectioned and slides were stained with anti-mouse Vangl1 (SAB4503254, Sigma) and polyclonal anti-human VANGL1 (Cat PA5-55231 and Cat PA5-98739, ThermoFisher) for mouse and human samples, respectively.

Transmission electron microscopy (TEM)

Samples were primary fixed in 2% glutaraldehyde overnight, then washed 3 times in 0.1M Cacodylate buffer (pH 7.4) and postfixed with 2% osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA USA) in 0.1 M Cacodylate buffer for 2 h. En bloc staining with 2% uranyl acetate preceded dehydration through a graded series of ethanol steps. Specimens were infiltrated with TAAB low viscosity epoxy resin (TAAB Laboratory and Microscopy, England) (50:50 ethanol:TAAB resin for 2 h followed by TAAB resin for 3 h) embedded and set overnight at 70°C. Multiple levels of thin sections (90 nm) were cut from three blocks of each kidney. Thin sections mounted on copper/palladium grids were stained with Reynold's lead citrate and viewed on a Jeol 1011 transmission electron microscope. Images were captured using a MegaView G2 digital camera and iTEM software package.

Serum injection

Three month old *Vangl*$^{+/-}$ and *Vangl1*$^{+/+}$ mice were culled and whole blood collected. Blood was permitted to clot and centrifuged at room temperature, and serum was collected. Recipient *Vangl*$^{+/-}$.*CD79a*$^{ken/ken}$ and *Vangl1*$^{+/+}$.*CD79A*$^{ken/ken}$ mice were injected daily with 100ul of respective serum for 7 days. At the end of the injection period, kidneys from recipient mice were cryopreserved as previously described. *Lyn*$^{-/-}$ mice in whom we confirmed the presence of proliferative nephritis were bled and allowed to clot. Serum was isolated and 200ul of serum tail-vein injected into *Vangl1*$^{+/-}$ or *Vangl1*$^{+/+}$ recipients at day 0 and day 4. Mice were sacrificed at day 7 and kidneys isolated for histology.

Serum ANA, immunoglobulin and creatinine

Mice were bled at times indicated and serum was collected. ANAs were quantified using ELISA (Alpha Diagnostic). Total serum immunoglobulin was measured by ELISA (BD Bioscience). Serum creatinine measured using the Indiko system as per manufacturer's instructions. Urine was collected weekly at indicated ages and urine albumin measured by ELISA as per the manufacturer's instructions (Ebioscience).

Tiwi Islander study participants

The Tiwi Islands are located off the northern coast of Australia in the Arafura Sea, and its indigenous inhabitants, numbering approximately 2,500,[49] exhibit a distinct genetic ancestry compared to other ethnicities[50] and are considered most closely related to mainland Indigenous Australians (Council 2018).[51] All the participants were self-identifying Tiwi Islanders and consented to collection of blood and DNA for genetic studies. A previous analysis of a separate cohort of self-identifying Tiwi Islanders (n = 73 individuals) indicated low admixture with other populations, including Europeans.[50] Whole genome sequencing was performed on an Illumina HiSeq X Ten System with greater than 50x coverage. The study received the full support of the Tiwi Island Land Council and was approved by the human research ethics committees of The Northern Territory Department of Health (2012-1767),[52] The Australian National University (2014-663), The University of Queensland (2012001146) and The University of Tasmania (H0012832). Chronic kidney disease was defined in the Tiwi according to the KDIGO guidelines.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical comparisons were performed using Graphpad Prism. Statistical details of experiments including tests and numbers are found in the figure legends. All data expressed as medians with primary data presented to demonstrate dispersion in the figures. Significance was defined as a p value < 0.05.

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Supplemental information

Deletions in *VANGL1* are a risk factor for antibody-mediated kidney disease

Simon H. Jiang, Sevcan Mercan, Ilenia Papa, Max Moldovan, Giles D. Walters, Mark Koina, Mitali Fadia, Maurice Stanley, Tom Lea-Henry, Amelia Cook, Julia Ellyard, Brendan McMorran, Madhivanan Sundaram, Russell Thomson, Pablo F. Canete, Wendy Hoy, Holly Hutton, Monika Srivastava, Kathryn McKeon, Iñigo de la Rúa Figueroa, Ricard Cervera, Raquel Faria, Sandra D'Alfonso, Mariele Gatto, Vicki Athanasopoulos, Matthew Field, John Mathews, Eun Cho, Thomas D. Andrews, A. Richard Kitching, Matthew C. Cook, Marta Alarcon Riquelme, Melanie Bahlo, and Carola G. Vinuesa

Supplementary Figure 1

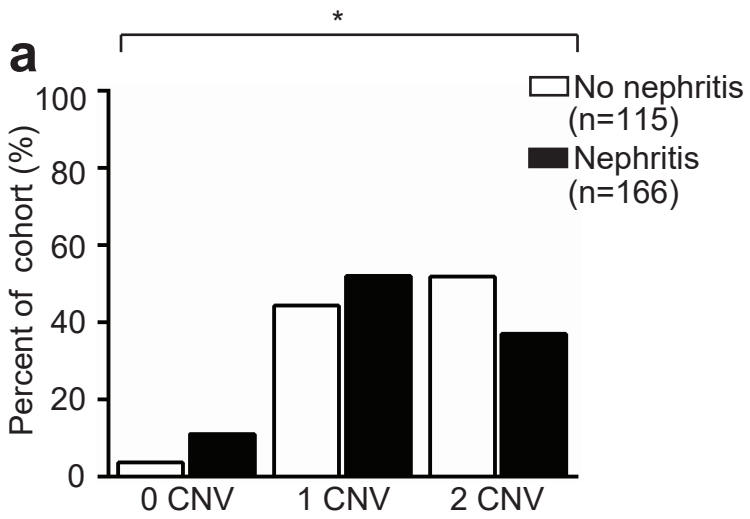

Figure S1. *VANGL1* CNV association with nephritis. Association of copy number variation in *VANGL1* detected by qPCR with the presence or absence of nephritis in SLE; $\chi 2=2.1$, 1 d.f., $p=0.14$ (CNV: Copy number variation). Related to Figure 1

Supplementary Figure 2

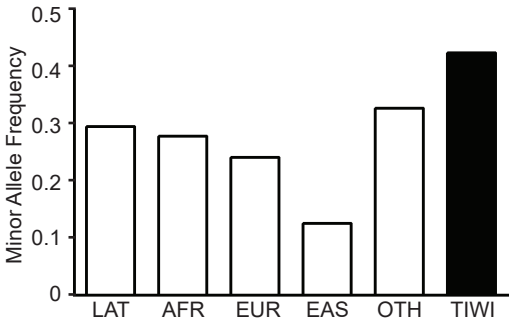

Figure S2. Increased frequency of *VANGL1* CNV in the Tiwi Islands a) Minor Allele Frequency of *VANGL1* CNV in different ethnicities; LAT: Latino, AFR: African, EUR: European, EAS: East Asian, OTH: Other. b) Number of individuals in the Tiwi Islands with a *VANGL1* CNV according to stage of kidney disease. (CKD = chronic kidney disease). Related to Figure 1

Supplementary Figure 3

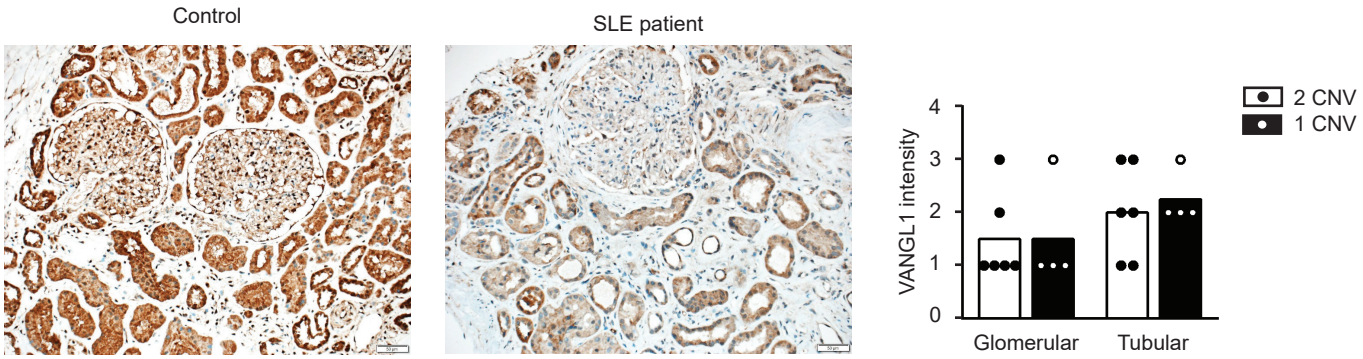

Figure S3. VANGL1 expression in human kidney samples. Representative VANGL1 expression and quantification in healthy and SLE patients with total glomerular and tubular scores. Related to Table 2

Supplementary Figure 4

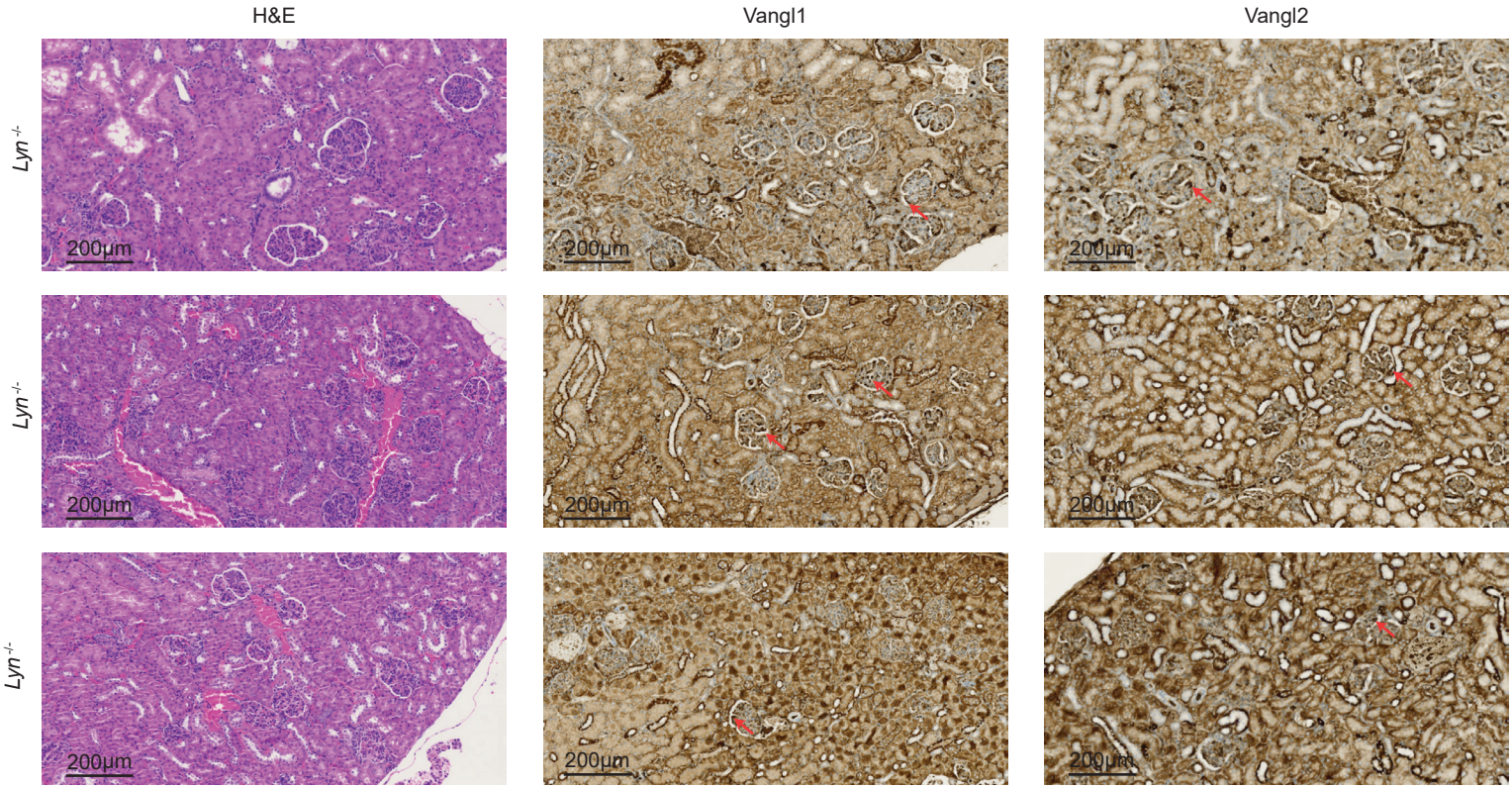

Figure S4. Vangl1 and Vangl2 expression in mouse glomeruli. Kidney sections from 12 week old Lyn-/- mice with glomerulonephritis and corresponding scattered glomerular expression of Vangl1 and Vangl2 (red arrow). Related to Figure 3