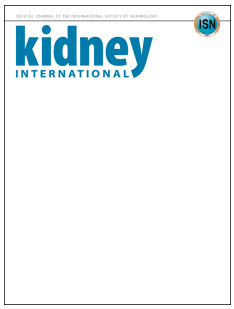
HLA-D and PLA2R1 risk alleles associate with recurrent primary membranous nephropathy in kidney transplant recipients.

Lena Berchtold, Eric Letouzé, Mariam Priya Alexander, Guillaume Canaud, Anne-Els van De Logt, Patrick Hamilton, Christiane Mousson, Vincent Vuiblet, Ann M Moyer, Sylvain Guibert, et al.



HLA-D and PLA2R1 risk alleles associate with recurrent primary membranous nephropathy in kidney transplant recipients.

Lena Berchtold, M.D., Eric Letouzé, Ph.D., Mariam Priya Alexander, M.D., Guillaume Canaud, M.D., Ph.D., Anne-Els van de Logt, M.D., Patrick Hamilton, M.D., Christiane Mousson, M.D., Ph.D., Vincent Vuiblet, M.D., Ph.D., Ann M. Moyer, M.D. Ph.D., Sylvain Guibert, Ph.D, Petra Mrázová, M.D., Charlène Levi, M.D., Valérie Dubois, Josep Maria Cruzado, M.D., Ph.D., Armando Torres, M.D., Ph.D., Manish J. Gandhi, M.D., Nadhir Yousfi, Ph.D., Vladimir Tesar, M.D., Ondrej Viklický, M.D., Maryvonne Hourmant, M.D., Bruno Moulin, M.D., Ph.D., Philippe Rieu, M.D., Ph.D., Gabriel Choukroun, M.D., Ph.D., Christophe Legendre, M.D., Jack Wetzels, M.D., Ph.D, Paul Brenchley, Ph.D., José Aurelio Ballarín Castan, M.D., Hanna Debiec, Ph.D., Pierre Ronco, M.D., Ph.D.

PII: S0085-2538(20)30967-4

DOI: https://doi.org/10.1016/j.kint.2020.08.007

Reference: KINT 2270

To appear in: *Kidney International*

Received Date: 27 April 2020

Revised Date: 30 July 2020

Accepted Date: 13 August 2020

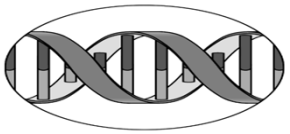
Please cite this article as: Berchtold L, Letouzé E, Alexander MP, Canaud G, Logt AEvd, Hamilton P, Mousson C, Vuiblet V, Moyer AM, Guibert S, Mrázová P, Levi C, Dubois V, Cruzado JM, Torres A, Gandhi MJ, Yousfi N, Tesar V, Viklický O, Hourmant M, Moulin B, Rieu P, Choukroun G, Legendre C, Wetzels J, Brenchley P, Ballarín Castan JA, Debiec H, Ronco P, HLA-D and PLA2R1 risk alleles associate with recurrent primary membranous nephropathy in kidney transplant recipients., *Kidney International* (2020), doi: https://doi.org/10.1016/j.kint.2020.08.007.

during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

HLA-D and *PLA2R1* risk alleles associate with recurrent primary membranous nephropathy in kidney transplant recipients.

Methods

248 Patients with MN



NGS sequencing

HLA-D locus
PLA2R1 locus

MN associated
SNPs
allotypes
haplotypes

Aims and Cohorts

60 pairs with non recurrent MN

45 pairs with recurrent MN

105 pairs recipient /donor NGS sequenced and PLA2R status

Are they associated with MN recurrence after transplantation?

Results

- We identified MN associated SNPs and HLA allotypes
- Seven SNPs located between *HLA-DRB1 and HLA-DQA1* and 3 SNPs in the *PLA2R1* region when present in the donor were associated with disease recurrence after transplantation

Genetic risk score (GRS)

The combination of the two SNPs most predictive of recurrence at each locus identified a group of patients with high risk of recurrence

ROC curve analysis

The addition of the GRS to the clinical variables and PLA2R status increased the prediction performance

CONCLUSION:

Recurrent MN is driven by donor variants in *HLA-D* and *PLA2R1* loci, irrespective of PLA2R status

kidney INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL SOCIETY OF NEPHROLOGY

Berchtold et al, 2020

[QUERY TO AUTHOR: title and abstract rewritten by Editorial Office – not subject to change]

HLA-D and PLA2R1 risk alleles associate with recurrent primary membranous nephropathy in kidney transplant recipients.

Lena Berchtold, M.D.[1, 2], Eric Letouzé, Ph.D.[3, 4], Mariam Priya Alexander, M.D.[5],Guillaume Canaud, M.D., Ph.D.[6], Anne-Els van de Logt, M.D.[7], Patrick Hamilton, M.D.[8], Christiane Mousson, M.D., Ph.D.[9], Vincent Vuiblet, M.D., Ph.D.[10], Ann M. Moyer, M.D. Ph.D.[11], Sylvain Guibert, Ph.D[12], Petra Mrázová, M.D.[13], Charlène Levi, M.D.[14],Valérie Dubois[15], Josep Maria Cruzado, M.D., Ph.D.[16], Armando Torres M.D., Ph.D.[17],Manish J. Gandhi, M.D.[18], Nadhir Yousfi, Ph.D.[1], Vladimir Tesar, M.D.[19],OndrejViklický, M.D.[20], Maryvonne Hourmant, M.D.[21], Bruno Moulin, M.D., Ph.D.[22-24], Philippe Rieu, M.D., Ph.D.[25,26], Gabriel Choukroun, M.D., Ph.D.[27], Christophe Legendre, M.D.[28], Jack Wetzels, M.D., Ph.D[7], Paul Brenchley, Ph.D.[8], José Aurelio Ballarín Castan, M.D.[29], Hanna Debiec, Ph.D.*[1], Pierre Ronco, M.D., Ph.D.[1, 30].*

*These authors contributed equally to this work.

1. Sorbonne Université, Université Pierre et Marie Curie Paris 06, and Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche S1155, Paris, France

2. Service of Nephrology, Department of Internal Medicine specialties, University Hospital of Geneva, Geneva, Switzerland

3. Centre de Recherche des Cordeliers, Sorbonne Universités, INSERM, Paris, Île-de-France, France.

4. Functional Genomics of Solid Tumor, Labex Immuno-Oncology, équipe labellisée Ligue Contre le Cancer, Université de Paris, Université Paris 13, Paris, Île-de-France, France.

5. Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA.

6. Inserm U1151, Necker-Enfants malades hospital; University Paris Descartes, Sorbonne Paris Cité, Department of adults Nephrology and Transplantation, Necker-Enfants malades hospital, 149, rue de Sèvres, 75015 Paris, France

7. Radboud Institute for Health Sciences, Department of Nephrology, Radboud University Medical Center, Nijmegen, The Netherlands

8. Division of Cardiovascular Sciences, School of Medical Sciences, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, United Kingdom.

9. Department of Nephrology and Transplantation, University hospital, F-21000 Dijon, France

10. BioSpec *T* Laboratory, EA 7506 URCA ; Nephropathology Department of Biopathology Laboratory, Reims University Hospital ; Division of Nephrology, Reims University Hospital, Reims, France

11. Department of Laboratory Medicine and Pathology, Personalized Genomics Laboratory, Mayo Clinic, Rochester, MN, USA

12. IntegraGen SA, Evry, France

13. Transplant Laboratory, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

14. Department of Transplantation, Nephrology and Clinical Immunology, Edouard Herriot Hospital, Hospices Civils de Lyon, France

15. Laboratoire HLA, Etablissement Français du Sang Auvergne Rhone-Alpes, Lyon, France

16. Nephrology Department, Hospital Universitari Bellvitge, Bellvitge Research Institute (IDIBELL), and RedInRen, RD16/0009/0031, University of Barcelona, L'Hospitalet de Llobregat, Barcelona, Spain

17. Hospital Universitario de Canarias, Instituto de Tecnologías Biomédicas (ITB)-Universidad de La Laguna, Tenerife, Spain.

18. Division of Transfusion Medicine, Mayo Clinic, Rochester, MN, USA.

19. Department of Nephrology, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic.

20. Department of Nephrology, Transplant Center, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

21. Department of Nephrology, University Hospital, Nantes, France.

22. Strasbourg University, Inserm UMR-S 1109, F-67000 Strasbourg, France.

23. Fédération de Médecine Translationnelle de Strasbourg (FMTS).

24. Department of Nephrology, Strasbourg University Hospital, Strasbourg, France.

25. Université de Reims Champagne-Ardenne, CNRS UMR 7369 (Matrice Extracellulaire et Dynamique Cellulaire, MEDyC), Reims, France.

26. CHU Reims, Division of Nephrology, Reims, France.

27. Department of Nephrology, University Hospital, Amiens, France.

28. Necker hospital, Department of Nephrology, Dialysis, and Renal Transplantation, Université Paris Descartes, F-75743, Paris, France.

29. Department of Nephrology, Fundación Puigvert, Barcelona, Spain

30. AP-HP, Tenon hospital, Department of Nephrology (Day Hospital), F-75020, Paris, France

Address all correspondence to: P. Ronco, Inserm unit UMR-S 1155, Tenon hospital, 4 rue de la Chine, F-75020, Paris, France. Tel: +33 1 56 01 65 08; E-mail: pierre.ronco@upmc.fr

Sources of support: This study was funded by grants from the European Research Council ERC-2012-ADG_20120314 (Grant Agreement 322947), from 7th Framework Programme of the European Community, contract 2012-305608 « European Consortium for High-Throughput Research in Rare Kidney Diseases (EURenOmics) », and the Agence Nationale pour la Recherche ("MN Progress", Programme Blanc SVSE1 (2012) Decision ANR-12-BSE1-0002-01 ; "Genetransnephrose", ANR- 16-CE17-0004-01). PB and PH (Manchester) acknowledge support from Medical Research Council, UK, Project Grant MR/J010847/1 for collection of donor recipient transplant cases. LB was supported by a grant from the Swiss National Foundation (P2GEP3_162179).

Running headline: Recurrence of membranous nephropathy on the graft

Word count: abstract, 259 words; main text, 4095 words

Glossary and abbreviations

PLA2R1 vs PLA2R: PLA2R stands for receptor of phospholipase A2. *PLA2R1* is the name of the gene, PLA2R that of the antigen encoded by *PLA2R1* gene

Locus: In genetics, a locus is a specific, fixed position on a chromosome where a particular gene or genetic marker is located. Here we have sequenced *PLA2R1* and *HLA-D* loci

SNP: Single Nucleotide Polymorphism; it is a substitution of a single nucleotide at a specific position in the genome, that is present in a sufficiently large fraction of the population (e.g. 1% or more)

Variant: A single-nucleotide variant (SNV) is a variation in a single nucleotide

Alleles: When there is a SNP at a specific position, the two possible nucleotide variations – C or A – are said to be the alleles for this specific position

Allotypes: here we use the term allelotype to design the classical alleles of the *HLA-D* locus that code for HLA class-2 molecules responsible for antigen presentation

GWAS: Genome-Wide Association Studies search the genome for SNPs that occur more frequently in people with a particular disease than in people without the disease. GWAS only investigate part of the genome. Here, we sequenced the *PLA2R1* and *HLA-D* loci to provide a detailed analysis of all the SNPs asoociated with membranous nephropathy in those 2 loci.

eQTL : Expression quantitative trait loci (eQTLs) are genomic loci or SNPs that explain variation in expression levels of mRNAs, sometime very distantly in the genome.

ABSTRACT 259 words

Recurrence of primary membranous nephropathy after transplantation occurs in up to 44% of patients and is driven by PLA2R antibody. Here, we asked whether genetic determinants could improve risk prediction. First, we sequenced *PLA2R1* and *HLA-D* loci in 248 patients with primary membranous nephropathy and identified two independent single nucleotide polymorphisms (SNPs) at risk for primary membranous nephropathy at each locus. These were rs9271188 (intergenic between *HLA-DRB1* and *HLA-DQA1*,) and rs9275086 (intergenic between *HLA-DQB1* and *HLA-DQA2*) at the *HLA-D* locus along with rs6726925 and rs13018963 at the *PLA2R1* locus. Then, we investigated whether primary membranous nephropathy at-risk variants were associated with recurrence in a retrospective cohort of 105 donor-recipient pairs and a replication cohort of 40 pairs. Seven SNPs located between *HLA-DRB1* and *HLA-DQA1* in linkage disequilibrium with rs9271188, and three SNPs in the *PLA2R1* region predicted recurrence when presented by the donor, but not when presented by the recipient. The two SNPs in the *HLA-D* region most strongly associated with recurrence (rs9271705 and rs9271550) were confirmed in the replication cohort. A genetic risk score based on the two best predictors at each locus (rs9271705, rs9271550, rs17830558, and rs3828323) identified a group of patients with high risk of recurrence. Thus, our results suggest that the graft contributes to recurrence of primary membranous nephropathy through the disease susceptibility *HLA-D* and *PLA2R1* SNPs in an autoimmune milieu. Further studies are needed before implementation of the genetic testing in donor selection.

Key words: membranous nephropathy, recurrence, transplantation, genetics, HLA-D, PLA2R1, genetic risk score, next generation sequencing

INTRODUCTION

Considerable progress has occurred in the pathomechanisms of membranous nephropathy (MN) with the identification of phospholipase A2 receptor antibodies (PLA2R-Ab) in ~70% of patients with primary MN (pMN)[1] . Development of serological tests has induced a paradigm shift in patient care. Yet, many patients still reach advanced kidney failure[2]. A major threat is recurrence in the graft. Recurrence may occur in the first weeks[3] or later when immunosuppression is tapered[4]. Recurrence rate varies from 7% to 44% depending on biopsy policy[5, 6]. Half of the patients with nephrotic recurrence will lose their graft although prognosis is now improved with anti-CD20 antibody rituximab[6]. Living donor transplantation was the only known but controversial risk factor for recurrence until PLA2R-Ab discovery[7, 8].

Screening for PLA2R-Ab has improved risk prediction and patient monitoring[9, 10]. Recurrence rate is~70% if PLA2R-Ab titers are high at transplantation, while it is~30% in the PLA2R-Ab and PLA2R-antigen negative patients[10]. The positive predictive value of pre-transplantation PLA2R-Ab for recurrence is 83%, while the negative predictive value is 42%[11]. These results indicate there are many outliers[12]. Furthermore, in the largest series from the Mayo Clinic[4], PLA2R-Abs were positive in only 58 % of patients (18/31) pre-transplant. This relatively low percentage of positivity is explained by the fact that pMN is often immunologically inactive at the time of transplantation and that other specificities like THSD7A may be involved[13]. Since PLA2R status is unknown in about 40% of patients at transplantation, additional biomarkers are highly desired.

PLA2R-related pMN is a genetically determined auto-immune disease. Two series of single nucleotide polymorphisms (SNPs) in *HLA-DQA1*and *PLA2R1* loci are strongly associated with pMN through all ethnicities[14, 15]. In addition to those mostly non-coding SNPs, classical *HLA-D* allotypes that code for histocompatibility class II molecules are at risk for pMN. A

recent trans-ethnic GWAS meta-analysis of 3,782 cases of pMN and 9,038 controls reported ancestry-specific effects of three classical *HLA* alleles: *DRB1*15:01* in East Asians, *DQA1* 05:01* in Europeans, and *DRB1*03:01* in both ethnicities[16]. Of note, PLA2R-Ab titers were correlated both with the lead SNPs of *HLA-DQA1*[17] and with the *HLA-D* allotypes *DQA1* 05:01* and *DQB1* 02:01* in Caucasians[9, 18], and *DRB1*15:01* and *DRB3*02:02* in Han Chinese[19]. Furthermore, strong gene-gene interaction was noted between the lead SNP in *PLA2R1* and the allotypes *DRB1*15:01/ DRB1*03:01,* which was suggestive of a role in PLA2R epitope presentation [20]. *S*tructural models further suggested that amino acids at positions 13 and 71 in the MHC-DR beta1 chain might confer susceptibility to pMN by facilitating presentation of T cell epitopes on PLA2R[20] .

Therefore, we hypothesized that genetic variants in *PLA2R1* and *HLA-D* loci could contribute to recurrent MN when expressed by the donor or the recipient.. Because in all previous studies, risk alleles were identified by genome-wide meta-analysis (GWAS), we sought to finely map the *HLA-D* and *PLA2R1* loci by next-generation sequencing (NGS), then to test whether the identified risk alleles for pMN would be able to predict recurrence when present in the recipient and/or the donor kidney.

RESULTS

Identification of pMN-associated SNPs and *HLA-D* allotypes

The first step of our study was to identify pMN-associated variants. Genotyping was performed according to established procedure (Supplementary material) in 248 Caucasian pMN cases recruited at Tenon hospital[21] or referred for the current study on recurrence (i.e. the 105 recipients of the discovery cohort), and in 192 ethnically matched controls from the *Etablissement Français du Sang Auvergne Rhone-Alpes, Lyon, France* (Fig 1A). Written

consent was obtained before sample collection. Diagnosis of pMN relied on a kidney biopsy. Secondary MN was excluded in the absence of malignancy, auto-immune disorder, infectious disease and a toxic cause.

At the *HLA-D* locus, the SNP most significantly associated with disease was rs9271188 ($P=8.1\times10^{-18}$, odds ratio [OR], 3.45; 95% confidence interval [CI], 2.6-4.6), localized between *HLA-DRB1* and *HLA-DQA1* genes. This SNP is in low LD ($r^2 = 0.33$) with the lead SNP rs2187668 located in intron 1 of *HLA-DQA1* previously identified as disease-associated in the first GWAS[14], and in high LD ($r^2 = 0.71$) with the lead SNP rs9271541 located between *HLA-DRB1* and *HLA-DQA1* genes identified in Europeans by the latest GWAS[16], (Table S1). Logistic regression analysis revealed a second SNP, rs9275086, upstream from *HLA-DQB1* that was also associated with disease risk ($P=2.1\times10^{-8}$, OR 2.43; CI 1.8-3.3). No additional SNP was significant when conditioning on the first 2 SNPs (Fig.2A).

At the *PLA2R1* locus, the SNP most significantly associated with disease risk was rs6726925 ($P=5.7\times10^{-11}$, OR, 2.48; CI, 1.9-3.3), localized in intron 4. This lead SNP is in low LD ($r^2 = 0.30$) with the lead SNP rs4664308, located in intron 1, previously identified as disease-associated in the first GWAS[14] and with the lead SNP rs17831251, also located in intron 1 identified in both East Asian and European ethnicities by the latest GWAS[16], (Table S2). Logistic regression analysis revealed a second SNP, rs13018963, intergenic between *PLA2R1* and *ITGB6* that was independently associated with disease risk ($P=3.4\times10^{-10}$, OR, 1.84; CI, 1.3-2.5). No additional SNP was significant when conditioning on the first 2 SNPs (Fig. 2B).These two lead SNPs are located in different haplotype blocks, separated by a recombination hotspot in intron 1. These results obtained by NGS of the *PLA2R1* locus are suggestive of a second risk haplotype that has not been revealed by large GWAS studies[16].

Because the cohort of 248 patients included 105 patients that had to have renal transplantation and represented a higher proportion with very advanced renal failure than in the usual cohorts, we asked whether the top signals in these 105 patients compared to the remaining patients who did not develop severe renal failure. To answer this question, we redid the association studies considering only the 105 transplanted patients, or only the 143 other cases, compared with the same control series used for the whole cohort. We obtained globally consistent results in both comparisons, with top *HLA* signals in the *DQA1/DQB1* region, and top *PLA2R1* signals in the 5' or upstream region of *PLA2R1* gene (not shown).

These results were in agreement with the observation that all lead SNPs identified by GWAS in previous studies popped up in our NGS with highly significant P values (Table 1)[22-24]. They indicated that the profiling of genetic at-risk variants was not skewed by enrolling more severe patients and thus could be used in recurrence studies.

The PHLAT software was used to predict the classical *HLA-D* allotypes from the raw sequence data. We found that the allotypes *HLA-DQA1*05:01*, *DQB1*02:01* and *DRB1*03:01*, defining a common haplotype *HLA-DR3-DQ2*, were significantly enriched in cases (Table S3). None of the risk allotypes remained significant after controlling for the 2 *HLA-D* lead SNPs (not shown). These results are consistent with the recent analysis of classical *HLA-D* allotypes showing that *HLA-DQA1*05:01* was the most strongly associated risk allotypes in Europeans, followed by *DRB1*03:01* that remained genome-wide significant after conditioning on *HLA-DQA1*05:01*[16].

Recurrence of MN in the discovery and the replication cohorts

The second step of our study was to identify recurrence associated variants. To this end, we collated a discovery cohort and a replication cohort. For the discovery cohort, 138 donor-recipient pairs were retrieved from archival records from 1982 through 2015, 105 were

available for the present study (Fig 1B). Fifteen centers in Europe and one in Canada participated (See Supplementary material). The replication cohort enrolled all 40 donor-recipient pairs with biopsy-proven primary MN and available DNA, recruited at the Mayo Clinic, Rochester, MN and in Manchester, UK, from 1998 through 2017 (Fig. S1**).** Baseline characteristics of the discovery and the validation cohorts are shown in Table 2 and Table S4, respectively. The discovery cohort was characterized by an age at transplantation of 50.4± 12.8 years, a large male (81.0%) and Caucasian (95.0%) preponderance, a high rate of PLA2R- status positivity (76.9%), and of Caucasian (97.7%) and deceased (82.7%) donors. The replication cohort had similar age at transplantation of 53.7± 11.5 years, large male (80%) and Caucasian (82.5%) preponderance, but compared to the discovery cohort, a lower rate of PLA2R-status positivity (46.7%) and deceased (40 %) donors. The PLA2R1 status overall and the PLA2R-Ab status at transplantation are detailed in the legend of Table 2.

In the discovery cohort, median follow-up time from transplantation was 72 months (IQR: 20-118). Diagnosis of recurrence was established by biopsy (44 patients) or suspected on undetermined nephrotic syndrome (1patient). Recurrence occurred after a median time of 7 months (IQR: 3-14). No recurrence was established by protocol biopsy, usually performed at 3 and 12 months (38 patients), or was suspected if proteinuria was <0.5g/day on repeated measurements after >1 year (22 patients). Median follow-up in these non-recurrent patients was 75.5 months (IQR: 25.5-121). Because of missing data of proteinuria and long-term outcome, we opted for a histological definition of recurrence, while being aware that weakly proteinuric recurrence cases were likely missed.

In the replication cohort, median follow-up time was 81 months (IQR: 57-227). Diagnosis of recurrence was established by biopsy in 9/9 patients after a median time of 9 months (IQR: 5-34) ; no recurrence was established by protocol biopsy in 14/31 patients or on the same clinical criteria as above (17 patients) after a median follow-up of 137 months (IQR: 69-243).

Clinical predictors of recurrence for the discovery cohort are shown in Table S5 and Fig. S2. By univariable analysis, PLA2R1 positive status at any time was associated with recurrence (Table S5). By multivariable analysis in a model without PLA2R status, the recipient gender (male) was associated with recurrence. When PLA2R status was added (40 missing values), PLA2R status was the only variable associated with recurrence (P=0.009; HR, 4.5; CI, 1.5-13.9).

We also looked at the correlations with PLA2R-Ab positivity rate at the time of transplantation. These results show that the level of implementation of the PLA2R-Ab assay developed since 2011 was low until 2015. Among the 45 recurrent patients, 12 received a transplant between 2011 and 2015: 4 were tested at transplantation (all positive), 5 were tested after (all positive), 3 have not been tested. Among the 60 non-recurrent patients, 16 received a transplant between 2011 and 2015: 5 were tested at transplantation (all negative), 1was tested after (negative), 10 have not been tested.

Predicting recurrence from genotypes

The 105 recipients were part of the cohort of 248 patients with pMN that had been genotyped in the case control study to determine pMN associated risk alleles (Fig. 1). *HLA-D* and *PLA2R1* loci were genotyped by NGS in the 105 donor samples on the same platform as for the recipients ("Integragen Genomics", Evry, France). We reasoned that the allele variants that were the most at risk for the development of pMN would also be at risk for disease recurrence on the graft. We tested 15 SNPs of the *HLA-D* region and 9 SNPs of the *PLA2R1* region. For the *HLA-D* region, we focused on the lead SNP (rs9271188) and the second lead SNP (rs9275086) identified by logistic regression in our study, the lead SNPs identified by Stanescu et al (rs2187668)[14] and by Xie et al (rs9271541)[16], and SNPs with highly significant

P values representative of each haplotype group (Fig.3, Tables 1&S1). For the *PLA2R1* region, we selected the lead SNP (rs6726925) and the second lead SNP (rs13018963) identified by logistic regression in our study, and all lead SNPs previously reported in the literature (Fig. 4, Tables 1&S2).

The classical *HLA-D* allotypes *HLA-DQA1*05:01, DQB1*02:01* and *DRB1*03:01* were also tested for association with recurrence. We only tested those 3 allotypes because they were the only ones identified at the *HLA-D* locus as at-risk for pMN in Caucasians in previous reports[16, 22] as well as in the current study.

All the selected SNPs and classical *HLA-D* allotypes were tested both in the donor and the recipient kidneys.

Whole discovery cohort

Among the 15 SNPs tested, our lead SNP rs9271188 and 7 SNPs in LD ($0.57 \leq r^2 \leq 0.89$) in the haplotype block 1 (Fig. 3) were associated with recurrence when present in the donor ($2\times10^{-4} < P < 0.025$), (Fig. 5A) but they did not predict recurrence when present in the recipient (Fig. S3&S4). Fig. 6 shows Kaplan-Meier curve for SNPs rs9271550 and rs9271705 that were the best predictors of recurrence in the discovery cohort (rs9271550, $P=2.5x10^{-4}$; rs9271705, $P=1.1\times10^{-3}$) and were confirmed as at risk for recurrence in the replication cohort (see below). By contrast, the other SNPs (including the second lead SNP rs9275086) in the haplotype block 2 that did not show significant LD with rs9271188 (Fig. 3), were not associated with recurrence (Fig.5A). None of the risk *HLA-D* allotypes conferred risk of recurrence when present in the donor or the recipient (Fig.5A & S5).

To confirm that the donor SNPs at risk for pMN conferred risk of recurrence irrespective of whether the donor has the classical haplotype (*HLA-DQA1*05:01_HLA-DQB1*02:01_HLA-DRB1*03:01*), we redid the analyses of disease recurrence for the most predictive SNPs after

restricting the cohort to (1) pairs for which both the donor and recipient share the classical at-risk haplotype (n=35), (2) pairs for which neither the donor nor the recipient has the classical at-risk haplotype (n=45) and (3) pairs for which only the donor or the recipient has the classical at-risk haplotype (n=25). Results suggest that the SNPs we identified are associated with relapse risk in absence of the at-risk haplotype, and at least for the top *HLA-D* SNP rs9271550, when the donor and the recipient share the at-risk haplotype (not shown). Because this stratified analysis is underpowered, larger studies will be needed to unravel with confidence the potential interactions between predictive SNPs and at-risk haplotype.

Nine *PLA2R1* SNPs most associated with pMN, including the 2 lead SNPs rs6726925 and rs13018963, were tested for association with recurrence. Three *PLA2R1* SNPs (rs3828323, rs17830558, rs3749117) out of the 9 tested were associated with recurrence only when present in the donor kidney ($0.012 < P < 0.035$ (Fig.5C & S3). These SNPs were in low to moderate LD ($0.24 \leq r^2 \leq 0.49$) with the pMN risk-associated lead SNP rs6726925 (Fig.4). No other SNP, including the second lead SNP rs13018963, conferred risk of recurrence in the donor or the recipient (Fig.5C, S3&S4). Fig. 6 shows Kaplan-Meier curves for SNPs rs17830558 and rs3828323 that were the best predictors of recurrence in the donors of the discovery cohort (rs17830558, P=0.012; rs3828323, P=0.015).

PLA2R-positive population

PLA2R status could be established in 65 recipients, based on identification of PLA2R-Ab in serum and/or PLA2R antigen in immune deposits at any time. Fifty patients were positive, 15 were negative. In PLA2R-positive patients, no *HLA-D* SNP was associated with recurrence, while more *PLA2R1* SNPs (n=5) than in the discovery cohort (n=3) were associated with risk, including rs1684475, rs4664308 and rs3749119 (Fig.5 B&D). Those 3 SNPs are in strong LD, while they are in low or no LD with the 3 SNPs (rs3828323, rs17830558, rs3749117)

identified as predictors in the whole cohort (Fig. 4). None of the SNP in the recipient predicted recurrence (Fig. S3). Fig.6 shows Kaplan-Meier curves for the two SNPs that were the most significantly associated with recurrence in the donors (rs17830558, P= 0.016; rs3828323, P=0.05) but were not predictors in the recipients (Fig. S4).

Overall, these results suggest that recurrence is driven by the donor *HLA-D* and *PLA2R1* variants in the whole cohort, and only by the donor *PLA2R1* variants in the PLA2R-positive population. However, interpretation of the data should be cautious given the small size of the PLA2R-positive population.

Replication cohort

In the replication cohort, we genotyped both in the recipients and the donors, the SNPs most associated with recurrence in the discovery cohort (Table S6). Fifteen out of the 16 SNPs tested for recurrence in the discovery cohort were successfully genotyped by PCR in the replication cohort (Table S6). We first verified that the pMN-associated SNPs tested for at risk of recurrence in the discovery cohort were risk alleles for pMN in the replication cohort (Table S6). Then we tested whether they predicted recurrence. The 2 non-coding *HLA-D* SNPs that were the best predictors in the discovery cohort were also associated with recurrence, albeit with large CI, when present in the donor (rs9271550, P=0.038; rs9271705 P=0.042), (Fig. S6). None of the *PLA2R1* SNPs was at risk of recurrence most likely because of small size of the cohort and low number of recurrence events. None of the disease-associated *HLA-D* allotypes predicted recurrence, in the donor or the recipient.

Genetic Risk Score (GRS)

We built a genetic risk score based on the combination of the two SNPs most predictive of recurrence at the *HLA-D* locus (rs9271550 and rs9271705) and at the *PLA2R1* locus (rs17830558 and rs3828323). When donors were divided into low (GRS 0 = 0-2 alleles, n=41

patients), intermediate (GRS 1 = 3-5 alleles, n=40 patients) and high (GRS 2 = 6-8 alleles, n=24 patients) genetic risk categories, the risk of recurrence was strongly associated with the score both in the discovery (GRS1, HR, 4.6; GRS 2, HR, 5.3, $P=6.4\times10^{-5}$) (Fig. 7A) and in the replication (GRS 1, HR, 1.9; GRS 2, HR, 10.4, P= 0.005) (Fig. 7B) cohorts.

In the 65 patients with informed PLA2R status, the GRS score remained predictive (GRS1, HR, 2.9; GRS 2, HR, 3.0, P=0.016), (Fig. 7C). The risk of recurrence at 24 months was 25.3% (3.4-42.3%), 62.4% (39.7-76.5%) and 62.5% (29.4-80.1%) for GRS of 0 (n=20), 1 (n=29) and 2 (n=16), respectively. By comparison, the risk was 62.9% in PLA2R positive patients (n=50) and 13.8% in the negative ones (n=15), (Fig.7D). By multivariable analysis, the high genetic risk category and PLA2R status independently predicted recurrence (GRS 0, reference; GRS1, HR, 2.2 (0.91-5.24), P=0.081; GRS2, HR, 3.1 (1.22-8.04), P=0.017; PLA2R positive status, HR, 4.05 (1.4-11.8), P=0.010). To see if the genetic risk score increased performance, we plotted receiver operating characteristic (ROC) curves that include models with clinical, laboratory and genetic predictors, and compared the areas under the curve (AUC). Results are shown in Fig. 7E. The ROC curve based on clinical variables previously reported to predict recurrence (including recipient gender, donor gender, type of donor, number of transplants and PLA2R status), gave an AUC of 0.72, the genetic ROC curve based on GRS gave a similar AUC (0.71), while the addition of the GRS to the clinical variables increased the AUC to 0.81.

This finding was corroborated in PLA2R positive patients (n=50) in whom recurrence was still driven by the GRS (GRS1, HR, 2.1; GRS 2, HR, 3.1, P=0.022); the risk of recurrence at 24 months being 39.4% (5.3-61.3%), 65.8% (41.4-80.0%) and 81.8% (36.3-94.8%) for GRS of 0 (n=13), 1 (n=26) and 2 (n=11), respectively (Fig. 7F).

DISCUSSION

As recently suggested by a group of expert investigators, genetic factors such as *PLA2R1* or *HLA* polymorphism might contribute to recurrent MN, and this genetic susceptibility could enhance the risk of recurrence in the case of living-related donors[5]. Our findings first provide strong clues to the unexpected implication of the *HLA-D* and *PLA2R1* loci of the donor, which suggests that the donor kidney plays a major role in antigen presentation.. Furthermore, our results suggest that recurrence is driven by SNPs localized to non-coding *HLA-D* region of the donor but not by the *HLA-D* allotypes typically associated with MN in native kidneys. Third, we provide arguments that recurrence prediction can be improved by the GRS both in recipients with unknown PLA2R status and in those with PLA2R-related MN.

A prerequisite to this study was the fine mapping by NGS of the *HLA-D* and *PLA2R1* loci identified by GWAS[14, 16, 22]. At the *HLA-D* locus, stepwise conditional analysis revealed 2 independent pMN- associated SNPs (rs9271188and rs9275086) which accounted for the entire signal at this locus. We confirmed a strong association with *HLA-DQA1*05:01*, *DQB1*02:01* and *DRB1*03:01*allotypes that define the common haplotype *HLA-DR3-DQ2*[22]. These findings are consistent with the most recent GWAS data in Europeans in whom the lead SNP rs9271541was in strong LD ($r^2 = 0.71$) with our lead SNP rs9271188 (and with a group of SNPs located in the same haplotype block (Fig.3), and *DQA1*05:01* and *DRB1*03:01*were at risk for the development of MN[16]. Of note, the fact that none of the 3 classical *HLA-D* alleles remained significant after controlling for rs9271188 and rs9275086 (the second lead SNP after logistic regression analysis) suggests that non-coding *HLA-D* regulatory variants may play an important functional role. As shown by eQTL analyses in the human glomerulus[25], these variants may regulate *HLA-D* transcripts (haploblocks 1 and 2), and the complement component *C4A* and *RNF5* (haploblock 2) which codes for a membrane bound ubiquitin ligase involved in autophagy, a process that plays a key role in podocyte

biology[26,27]. These findings are in keeping with the potential role of podocytes as antigen-presenting cells[28]. Thus although we do not provide evidence for a direct implication of *HLA-D* allotypes in recurrence of pMN, our results suggest that their level of expression might be regulated by SNPs located in the non-coding *HLA-D* region of the donor.

Apart from the amount of class II expression, other differences between recurrent and non-recurrent disease may regard the way relevant PLA2R peptides are loaded into class II, or transported/expressed on the cell surface of the antigen presenting cells, or there is a non-*HLA* related mechanism (see below the discussion on Ly75).

At the *PLA2R1* locus, we identified 2 independent pMN-associated SNPs, rs6726925 localized in intron 4, and rs13018963 in *PLA2R1* regulatory region that both explained the entire signal at this locus. These 2 SNPs are located in different haplotype blocks, separated by a recombination hotspot in intron1. Although these results differ from the findings of Xie et al[16] who identified a unique common haplotype in both East Asians and Europeans, it must be noted that their lead SNP rs1783251 was in complete LD ($r^2 = 1$) with a group of SNPs located in the same intron 1 (rs16844715, rs4664308), (Fig.4). Intron 1, particularly rs1783251, plays an important role in *PLA2R1* gene expression[16], which is probably a key to the pathogenesis of the disease since under normal conditions expression of PLA2R at the podocyte surface is low. This intron might be involved in epigenetic regulation of PLA2R1. On the other hand, eQTL analysis showed that the SNP rs3828323, associated with pMN recurrence, increases Ly75 transcripts in the glomerulus[25] . Ly75 (DEC-205) belongs to the mannose receptor family (like PLA2R) and functions in dendritic cells as an antigen uptake receptor targeting its cargo to intracellular compartments where it is processed for presentation to T cells. We suggest that by analogy with the immune system[29], Ly75 might play a role in antigen presentation in the podocyte[28]. Thus rs3828323 might act in concert with intron-1 SNPs to enhance immune response to PLA2R.

It is, however, conceivable that in the post-transplantation state, host antigen presenting cells may continue to present self-antigen to host T cells and provide help to host B cells to produce anti-PLA2R antibodies. At-risk SNPs in the donor, in some indirect way besides antigen presentation, might render the donor kidney more liable to disease recurrence from deposition of host-derived anti-PLA2R antibodies. SNPs recently identified in other loci such as NFKB1 and IRF4[16] may render the podocyte more susceptible to immunological attack.

Because pMN has a slow evolution and is often immunologically inactive in patients with advanced CKD, PLA2R status is unknown in about 40% of the recipients at transplantation[4]. We anticipated that *PLA2R1* and *HLA* risk variants might help with donor selection. We found that recurrence was driven by the donor SNPs only. Unexpectedly, none of the *HLA-D* classical allele was associated with recurrence, while individual SNPs located in the *HLA-D* region were. These results suggest that recurrence might be fostered by the level of expression of transcripts regulated by a group of *HLA-D* and *PLA2R1* SNPs acting in concert to enhance antigen presentation. However, considering the relatively small size of the cohort, one cannot exclude that associations with *HLA-D* alleles might be revealed by larger size studies. Of note, when analysis was restricted to PLA2R-positive patients, the predictive value of *HLA-D* SNPs lost significance although we cannot exclude a size effect.

Our study was limited to *HLA-D* and *PLA2R1*loci. We cannot exclude that SNPs in other genetic regions in the recipient can contribute to recurrence. This might be the case for the newly identified genetic risk loci, including NFKB1 and IRF4 (interferon pathway), reported in the recent trans-ethnic GWAS[16]. These two pro-inflammatory pathways might contribute to clinical expression of the recurrence rather than to the recurrence risk. A recent study by Batal et al[30] based on serological typing showed that the only potential predictor of recurrence was the recipient HLA-A3 antigen, but failed to identify classical risk *HLA-DR/-DQ* allotypes in

keeping with our data. Future studies should analyze more loci in the donor as well as in the recipient.

We built a 4-SNP (rs9271705, rs9271550, rs17830558, and rs3828323) GRS that predicted recurrence independently of PLA2R status. In all populations including the replication cohort, recurrence was associated with increased burden of risk alleles. By ROC curve analysis, we showed that GRS increased the prediction performance. Even in the PLA2R-positive population, the recurrence rate was dependent on allele dosage and the GRS defined a subgroup of patients with high risk of recurrence. These results indicate that the GRS might provide an added value to serology. These data may be particularly helpful when more than one donor is available. The finding that the graft contributes to recurrence of pMN, possibly through antigen presentation involving *HLA-D* and *PLA2R1* loci and associated SNPs in an autoimmune milieu, is of great conceptual interest. This concept may apply to transplantation in other situations and to other organs, and thus may be important for transplantation in other autoimmune diseases.

This paper has several limitations. The relatively small size of the cohorts is explained by the rarity of the disease, with only 30% of the patients going to advanced CKD. However, there are still many patients who require a transplant, sometimes in the context of a living donation, for whom one must decrease the risk of recurrence. We are aware that the study was under powered, particularly the replication cohort, and we consider our prediction data as exploratory, but they have the merit to show the direction in future studies. A kidney biopsy was not mandatory for the diagnosis of no recurrence. Despite very strict criteria (daily proteinuria repeatedly <0.5 g/day, follow-up >12months), a few cases of latent recurrence might have been misclassified as "no recurrence". However, the rate of recurrence in our series of 105 patients (42.8%) was close to that reported in the Mayo Clinic series with protocol biopsies (48% of 63 patients)[4], which suggests that we have missed only a few per

cent of recurrent cases. This is quite reassuring because due to the retrospective nature of the study over 3 decades, protocols were not harmonized. In an ideal world, that is in a prospective study, where all patients benefit from a protocol biopsy at 3 months and one year and are systematically followed from a clinical point of view, it would be possible to have a histological end-point and a clinical end-point for the diagnosis of recurrence. Serologic status was missing in 40/105 patients but the study started in 1982, that is 27 years before the discovery of PLA2R and a majority of biopsies was not accessible. Another limitation is the small number of PLA2R-negative patients among those with informed PLA2R status but their prevalence (23%) is the same as in our general population.

In conclusion, this study provides the first analysis of the *HLA-D* and *PLA2R1* loci by NGS. It strongly suggests that recurrence of pMN is driven by the genotype of the donor, and it provides an exploratory GRS that should prompt further studies aimed to choose the best donor.

METHODS

We declare that the manuscript adheres to the Declaration of Istanbul. The source of donor kidneys was accidental or natural death. Informed consent was obtained from the families or the living donors according to the national legislations.

Genotyping of pMN-associated *PLA2R1* and *HLA-D* SNP variants and *HLA-D* allotypes in the discovery cohort (see Supplementary material)

Genotyping of SNPs in the replication cohort (see Supplementary material)

Statistical analyses

*Clinical data (*see Supplementary material*)*

Association with recurrence and genetic risk score

To test the hypothesis that pMN-associated risk alleles could predict recurrence, we performed log-rank tests for trend comparing for each SNP the risk of recurrence after transplantation according to the number of risk alleles (0, 1, or 2) in the donor and the recipient. We defined a 4-SNP genetic risk score (GRS) based on the combination of the two SNPs most associated with recurrence at each *HLA-D* and *PLA2R1* locus. The scores were "0" for zero to two risk alleles, "1" for 3 to 5 risk alleles, and "2" for 6 to 8 risk alleles. A Cox proportional-hazards regression model that included GRS and PLA2R status was used to assess adjusted associations with recurrence.

The discriminative performance of clinical parameters (PLA2R status, recipient gender, donor gender, type of donor, number of transplant) and GRS to predict recurrence of GEM was assessed by plotting receiver operating characteristic (ROC) curves and calculating area under the curve (AUC) values.

Data availability statement

The data supporting the findings of this study will be openly available under restricted conditions in EGA: The European Genome-Phenome Archive

ACKNOWLEDGEMENTS

We would like to thank Sophie Caillard (Nephrology, CHU de Strasbourg, France), Marie-Noelle Péraldi (St Louis hospital, Paris, France), Cécile Vigneau (Nephrology, CHU de Rennes, France), Dominique Guerrot (Nephrology, CHU de Rouen, France), Tewfik Nawar

(University of Sherbrooke, Canada), Jean-Pierre Venetz (Nephrology, CHUV de Lausanne, Suisse), Eric Daugas (Nephrology, Bichat hospital, Paris, France), François-Xavier Glowacki (Nephrology, CHU de Lille, France), Nassim Kamar (Nephrology, CHU de Toulouse, France), Emmanuel Morelon (Nephrology, CHU de Lyon, France), Antonij Slavcev (Department of Immunogenetics, IKEM, Prague, Czech Republic), Will Allebes (Laboratory of Immunology, Radboud UMC, Nijmegen, The Netherlands), and Julia Hofstra (Nephrology, Radboud UMC, Nijmegen, the Netherlands) for providing samples and medical records of their patients. We also thank Christophe Combescure (Clinical Research Centre and Division of Clinical Epidemiology, Geneva University Hospitals, Geneva, Switzerland), Fernando Fervenza (Mayo Clinic, Rochester, MN**)** for helpful advice.

This study was funded by grants from the European Research Council ERC-2012-ADG_20120314 (Grant Agreement 322947), from 7th Framework Programme of the European Community, contract 2012-305608 « European Consortium for High-Throughput Research in Rare Kidney Diseases (EURenOmics) », and the Agence Nationale pour la Recherche ("MN Progress", Programme Blanc SVSE1 (2012) Decision ANR-12-BSE1-0002-01 ; "Genetransnephrose", ANR- 16-CE17-0004-01). PB and PH (Manchester) acknowledge support from Medical Research Council, UK, Project Grant MR/J010847/1 for collection of donor recipient transplant cases. LB was supported by a grant from the Swiss National Foundation (P2GEP3_162179).

SUPPLEMENTARY MATERIAL

Supplementary Text

Supplementary Figures

Fig S1 Flow chart of the replication cohort

Fig S2 Clinical determinants of outcome in the discovery cohort

Fig S3 Hazard ratio and 95% CI of SNPs of the recipients associated with recurrence in the discovery cohort

Fig S4 Kaplan-Meier curves of recurrence-free survival by *HLA* and *PLA2R1* SNPs ofthe recipients in the discovery cohort

Fig S5 Kaplan-Meier curves of recurrence-free survival by *HLA-D*allotypes in the discovery cohort

Fig S6 Hazard ratio and 95% CI of SNPs associated with recurrence in replication cohort

Supplementary Tables

Table S1 Significant SNPs at *HLA-D* locus

Table S2 Significant SNPs at *PLA2R1* locus

Table S3 Top allelotypes and haplotypes at the *HLA DQ-DR* locus

Table S4 Baseline characteristics of the patients in the replication cohort

Table S5 Clinical predictors of recurrence by univariable and multivariable analysis

Table S6 List of pMN-associated SNPs genotyped in the replication cohort

Supplementary References

REFERENCES

1. Beck LH Jr, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic MN. N Engl J Med 2009;361:11-21.
2. Ronco P, Debiec H. Pathophysiological advances in membranous nephropathy: time for a shift in patient's care. The Lancet 2015; 385:1983-92.
3. Stahl R, Hoxha E, Fechner K. PLA2R1 autoantibodies and recurrent membranous nephropathy after transplantation. N Engl J Med 2010;363:496-8.
4. Grupper A, Cornell LD, Fervenza FC, Beck LH Jr, Lorenz E, Cosio FG. Recurrent Membranous nephropathy after kidney transplantation: Treatment and long-term implications. Transplantation 2016; 100: 2710-6.
5. Leon J, Pérez-Sáez MJ, Batal I, etal. Membranous Nephropathy Post-Transplantation: An Update of the Pathophysiology and Management.Transplantation. 2019; 103: 1990-2002.

6. El-Zoghby ZM, Grande JP, Fraile MG, Norby SM, Fervenza FC, Cosio FG. Recurrent idiopathic membranous nephropathy: early diagnosis by protocol biopsies and treatment with anti-CD20 monoclonal antibodies. Am J Transplant 2009;9:2800-7.
7. Andrésdóttir MB, Wetzels JF. Increased risk of recurrence of membranous nephropathy after related donor kidney transplantation.Am J Transplant 2012;12:265-6.
8. Kennard AL, Jiang SH, Walters GD. Increased glomerulonephritis recurrence after living related donation.BMCNephrol 2017;18:25.
9. Quintana LF, Blasco M, Seras M, et al. Antiphospholipase A2 Receptor Antibody Levels Predict the Risk of Posttransplantation Recurrence of Membranous Nephropathy. Transplantation 2015;99:1709-14.
10. Cosio FG, Cattran DC. Recent advances in our understanding of recurrent primary glomerulonephritis after kidney transplantation. Kidney Int 2017;91:304-314.
11. Kattah A, Ayalon R, Beck LH Jr, et al. Anti-phospholipase A_2 receptor antibodies in recurrent membranous nephropathy. Am J Transplant. 2015;15:1349-59.
12. Debiec H, Martin L, Jouanneau C, et al. Autoantibodies specific for the phospholipase A2 receptor in recurrent and De Novo membranous nephropathy. Am J Transplant 2011;11:2144-52
13. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. N Engl J Med. 2014 ;371:2277-87.
14. Stanescu HC, Arcos-Burgos M, Medlar A, et al. Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. N Engl J Med 2011;364:616-26.
15. Gupta S, Köttgen A, Hoxha E, et al. Genetics of membranous nephropathy. Nephrol Dial Transplant. 2018;33:1493-1502.

16. Xie J, Liu L, Mladkova N, et al. The genetic architecture of membranous nephropathy and its potential to improve non-invasive diagnosis. Nat Commun. 2020 Mar 30;11(1):1600

17. Lv J, Hou W, Zhou X, et al. Interaction between PLA2R1 and HLA-DQA1 variants associates with anti-PLA2R1 antibodies and membranous nephropathy. J Am SocNephrol 2013;24:1323-9.

18. Kanigicherla D, Gummadova J, McKenzie EA, et al. Anti-PLA2R1 antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. Kidney Int 2013;83:940-8.

19. Le WB, Shi JS, Zhang T, et al.HLA-DRB1*15:01 and HLA-DRB3*02:02 in PLA2R1-Related Membranous Nephropathy. J Am Soc Nephrol2017;28:1642-50.

20. Cui Z, Xie LJ, Chen FJ, et al. MHC Class II Risk Alleles and Amino Acid Residues in Idiopathic Membranous Nephropathy. J Am Soc Nephrol. 2017 ;28:1651-64.

21. Pourcine F, Dahan K, Mihout F, et al. Prognostic value of PLA2R1 autoimmunity detected by measurement of anti-PLA2R1 antibodies combined with detection of PLA2R1 antigen in membranous nephropathy: A single-centre study over 14 years. PLoS One 2017;12:e0173201.

22. Sekula P, Li Y, Stanescu HC, Wuttke M, et al. Genetic risk variants for membranous nephropathy: extension of and association with other chronic kidney disease aetiologies. Nephrol Dial Transplant. 2017;32:325-32.

23. Kim S, Chin HJ, Na KY, et al.Single nucleotide polymorphisms in the phospholipase A2 receptor gene are associated with genetic susceptibility to idiopathic membranous nephropathy. Nephron Clin Pract 2011, 117:253-8

24. Thiri M, Honda K, Kashiwase K et al. High-density Association Mapping and Interaction Analysis of PLA2R1 and HLA Regions with Idiopathic Membranous Nephropathy in Japanese. Sci Rep. 2016, 6 : 38189

25. Qiu C, Huang S, Park J, et al. Renal compartment-specific genetic variation analyses identify new pathways in chronic kidney disease. Nat Med 2018;24:1721-31.

26. Kuang E, Okumura CY, Sheffy-Levin S, et al. Regulation of ATG4B stability by RNF5 limits basal levels of autophagy and influences susceptibility to bacterial infection.PLoS Genet 2012;8:e1003007.

27. Beeken M, Lindenmeyer MT, Blattner SM, et al. Alterations in the ubiquitin proteasome system in persistent but not reversible proteinuricdiseases. J Am SocNephrol. 2014 ;25:2511-25

28. Goldwich A, Burkard M, Ölke M, et al. Podocytes Are Nonhematopoietic Professional Antigen-Presenting Cells. J Am Soc Nephrol. 2013 ; 24: 906-16.

29. Jiang W, Swiggard WJ, Heufler C, et al. The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. Nature. 1995;375:151-5.

30. Batal I, Vasilescu ER, Dadhania DM, et al. Association of HLA Typing and Alloimmunity With Posttransplantation Membranous Nephropathy: A Multicenter Case Series. Am J Kidney Dis. 2020 ;S0272-6386(20)30534-5.

Figure Legends

Figure 1: Flowchart of the discovery cohort and genetic workflow

A. Determination of pMN associated variants: To identify SNPs and classical *HLA* allotypes associated with pMN, NGS sequencing of *HLA-D* and *PLA2R1* loci was performed in a cohort of 248 patients and 192 ethnically matched controls. Patients with pMN were recruited at Tenon hospital for clinical care, or referred for PLA2R-Ab or glomerular antigen determination, or for recurrence studies. pMN-associated SNPs, and *HLA-D* allotypes and haplotypes were identified. One-hundred five patients with end-stage kidney disease were recipients of kidney grafts in the discovery cohort (B).

B. Discovery cohort for the study of variants-associated recurrence: All patients, 18 years of age or older, with biopsy-proven pMN, who received a kidney graft between 1982 and 2015, were eligible. Thirty-three pairs were excluded for the reasons indicated in the boxes.. Finally, 105 patients were included in the study: 45 of themrecurred (42.9%).Donor samples corresponding to the 105 recipients were sequenced on the same platform as the recipients. The *PLA2R1* and *HLA-D* SNPs and the *HLA-D* allotypes most associated with pMN (Fig. 3&4) were tested in donor and recipient samples to identify recurrence risk-associated alleles and build a genetic risk score.

Figure 2: Locus zoom with logistic regression analysis and haploblock reconstruction of the *HLA-D* (A) and *PLA2R1* (B) regions in patients with pMN: identification of pMN-associated variants

Results are from 248 Caucasian patients with pMN and 192 ethnically matched controls sequenced and analyzed on the same platform. They show the SNPs associated with pMN.

The upper panels show the Manhattan plots with the log transformed p-value on the y-axis and the genomic position (in megabases) on the x-axis. The top SNP is indicated in purple, and SNPs in linkage disequilibrium with the top SNP are represented with color code, as indicated in box. The recombination rate across the region is also represented as a red line, and the different *HLA* and *PLA2R1* region genes are indicated below the respective plots. The red horizontal, dotted lines indicate the locus-wide significance level (2.4×10^{-4}). The most significant SNP identifications are indicated in each panel. The corresponding lower panels represent the haploblock reconstructions of the *HLA-D* and *PLA2R1* regions, respectively. A. *HLA-D* region: B; *PLA2R1* region.

Figure 3: Summary of SNPs associated with pMN disease considered for recurrence prediction at *HLA-D* locus: The upper panel presents SNPs and *HLA-D* allotypes most significantly associated with pMN (see Table S1for detail of P values and OR). rs in red bold characters are the lead SNPs associated with pMN before (rs927118) and after (rs9275086) logistic regression analysis. rs2187668 and rs9271541 are the lead SNPs identified by Stanescu et al (13) and Xie et al (18), respectively. LD values are shown by color codes. Note 2 groups of SNPs in LD within each haplotype block group but showing no or weak LD with the other group. *HLA-D* allotypes are in strong LD with the second group defined by rs2187668 rs9272729. rs3129716. rs2647004. rs2856674 and rs1794280. The second lead SNP rs9275086 associated with pMN shows no LD with the other SNPs and *HLA-D* allotypes. The lower panel shows the position of the 15 SNPs in the *HLA-D* locus.

Figure 4: Summary of SNPs associated with pMN disease considered for recurrence prediction at *PLA2R1* locus: The upper panel presents the SNPs most significantly associated with pMN (see Table S2 for detail of P values and OR). rs in bold red characters are the lead SNPs before (rs6726925) and after (rs13018963) logistic regression analysis. rs4664308 and rs17831251 are the lead SNPs identified by Stanescu et al (13) and Xie et al

(18), respectively. LD values are shown by color codes. Note 2 groups of SNPs in LD within each group but showing no or weak LD with the other group. rs13018963 in low LD with the second group is located in a different haploblock. The lower panel shows the position of the 9 SNPs in the *PLA2R1* locus.

Figure 5: Hazard ratio and 95% CI of *HLA-D* and *PLA2R1*SNPs and *HLA-D* classical allotypes of the donor kidneys associated with recurrence in the discovery cohort

The panels show the data for the donor *HLA-D* SNPs and allotypes (A, B) and for *PLA2R1* SNPs (C, D) in the whole cohort (A, C) and in the PLA2R positive population (B, D).The heterozygous (HR1) and homozygous (HR2) state are shown for each SNP and *HLA-D* allotype. Red indicates the risk alleles associated with recurrence. Note the logarithmic scale used for HR.

Figure 6 Kaplan-Meier curves of recurrence-free survival by *HLA-D* and *PLA2R1* SNPs of the donor kidney in the discovery cohort

The upper panel shows the recurrence-free survival in the whole cohort, and the lower panel in the PLA2R positive population for the patients harboring the SNPs of *HLA-D (rs9271550, rs9271705)* and *PLA2R1 (rs17830558, rs3828323)* that are the most associated with recurrence. Color codes refer to 0, 1 (heterozygous), or 2 (homozygous) risk alleles.

Figure 7: Prediction of recurrence by genetic risk score (GRS) in the discovery and replication cohorts

We defined a 4-SNP GRS based on the combination of the two SNPs most associated with recurrence at each locus (HLA: rs9271550, rs9271705; PLA2R1: rs17830558 and rs3828323). The scores are "0" for zero to 2 risk alleles, "1" for 3 to 5 risk alleles, and "2" for 6 to 8 risk alleles. Panels A-C show the Kaplan-Meier curves of survival without recurrence

("relapse free") in the whole discovery cohort (A, n=105), in the replication cohort (B, n=40), and in the patients from the discovery cohort with an informed PLA2R status (C, n=65). The Kaplan-Meier curves according to the PLA2R status (D, n=65) are also shown for comparison (compare with C). Panel E shows the ROC curves of multivariable models of MN recurrence prediction: blue, clinical ROC curve (PLA2R status, recipient gender, donor gender, type of donor, number of transplants); red, genetic ROC curve (GRS); green, combined clinical and genetic ROC curve. Note that the AUC is increased by adding GRS to the clinical variables. Panel F shows the Kaplan-Meier curves of survival without recurrence in the PLA2R-positive restricted population where a high GRS=2 defines a population at high risk of recurrence.

Table 1. Association of previously reported lead SNPs with risk of pMN in the present study

dbSNP ID	Frequency of allele 1 in controls	Frequency of allele 1 in cases	OR	95% OR confidence interval - lower bound	95% OR confidence interval - upper bound	P-value	
***HLA-D* region**							
rs9271188	0.28	0.57	3.4	2.6	4.6	8.13E-18	NGS
rs2187668	0.10	0.33	4.5	3.1	6.7	5.60E-16	Ref 14
rs9272729	0.10	0.33	4.5	3.1	6.7	5.60E-16	Ref 22
rs9271541	0.26	0.51	2.9	2.2	3.9	1.58E-13	Ref 16
***PLA2R1* region**							
rs6726925	0.35	0.58	2.5	1.9	3.3	5.73E-11	NGS
rs17830558	0.46	0.67	2.4	1.9	3.2	1.46E-10	Ref 22
rs3828323	0.50	0.69	2.2	1.7	2.9	2.39E-08	Ref 23
rs4664308	0.60	0.78	2.3	1.7	3.0	3.22E-08	Ref 14
rs3749119	0.68	0.84	2.4	1.7	3.3	5.33E-08	Ref 24
rs17831251	0.60	0.77	2.2	1.7	3.0	4.93E-08	Ref 16
rs16844715	0.60	0.76	2.1	1.6	2.8	3.26E-07	Ref 24

Table 2 : Baseline characteristics of the patients in the discovery cohort

Characteristics	Total (n=105)	Recurrent (n=45)	Non recurrent (n=60)
CLINICAL PARAMETERS			
Recipients			
Age at transplantation. years	50.4 ± 12.8	51.6 ± 12.1	49.5 ± 13.3
Male. n (%)	85/105 (81.0%)	41/45 (91.1%)	44/60 (73.3%)
Caucasian. n (%)*	95/100 (95.0%)	42/44 (95.5%)	53/56 (94.6%)
First transplantation. n(%)	93/105 (88.6%)	42/45 (93.3%)	51/60 (85.0%)
Dialysis prior to transplantation*	83/94 (88.3%)	39/42 (92.9%)	44/52 (84.6%)
Lengths of time on dialysis. months**	16.5 (7-31)	20.5 (7-46)	14.5 (7.5-29.5)
Nbr of HLA mismatch (A-B-DR)***			
- 0 to 2 mismatch	17/83 (20.5%)	9/36 (25.0%)	8/47 (17.0%)
- 3 mismatch	20/83 (24.1%)	6/36 (16.7%)	14/47 (29.8%)
- 4 mismatch	21/83 (25.3%)	6/36 (16.7%)	15/47 (31.9%)
- 5 to 6 mismatch	25/83 (30.1%)	15/36 (41.7%)	10/47 (21.3%)
HLA-antibodies at transplantation***	27/81 (33.3%)	8/37 (21.6%)	19/44 (43.2%)
Anti-HLA donor-specific antibodies***	3/75 (4.0%)	0/37 (0%)	3/38 (7.9%)
PLA2R positive§	50/65 (76.9%)	36/40 (90.0%)	14/25 (56.0%)
Time to recurrence/last follow up. months	24 (12-94)	7 (3-14)	75.5 (25.5-121)
Donors			
Age. years*	49.7 ± 15.1	48.1 ± 15.4	50.9 ± 14.9
Male. n (%)*	57/103 (55.3%)	27/44 (61.4%)	30/59 (50.9%)
Caucasian. n (%)Δ	43/44 (97.7%)	17/18 (94.4%)	26/26 (100%)
Deceased donor transplant. n(%)*	86/104 (82.7%)	35/45 (77.8%)	51/59 (86.4%)
Cold ischemia time. min*	1115 (726-1410)	970 (432-1350)	1170 (900-1436)
TREATMENT at the time of transplantation. n (%)			
Plasma exchange*	2/104 (1.9%)	0/45 (0%)	2/59 (3.4%)
IVIG*	4/103 (3.9%)	0/45 (0%)	4/58 (6.9%)
ATG*	41/104 (39.4%)	18/45 (40.0%)	23/59 (39.0%)
Tacrolimus*	51/104 (49.0%)	24/45 (53.3%)	27/59 (45.8%)
Ciclosporin*	53/104 (51.0%)	21/45 (46.7%)	30/59 (50.9%)
Mycophenolate mofetil*	89/104 (85.6%)	37/45 (82.2%)	52/59 (88.1%)

Corticosteroids*	102/104 (98.1%)	43/45 (95.6%)	59/59 (100%)
Anti-IL-2-R*	42/104 (40.4%)	18/45 (40.0%)	24/59 (40.7%)
Rituximab*	2/104 (1.9%)	0/45 (0%)	2/59 (3.4%)

Values reported as numbers and %. mean±SD. or median (interquartile ranges) as appropriate.*between 1 and 10 missing values;

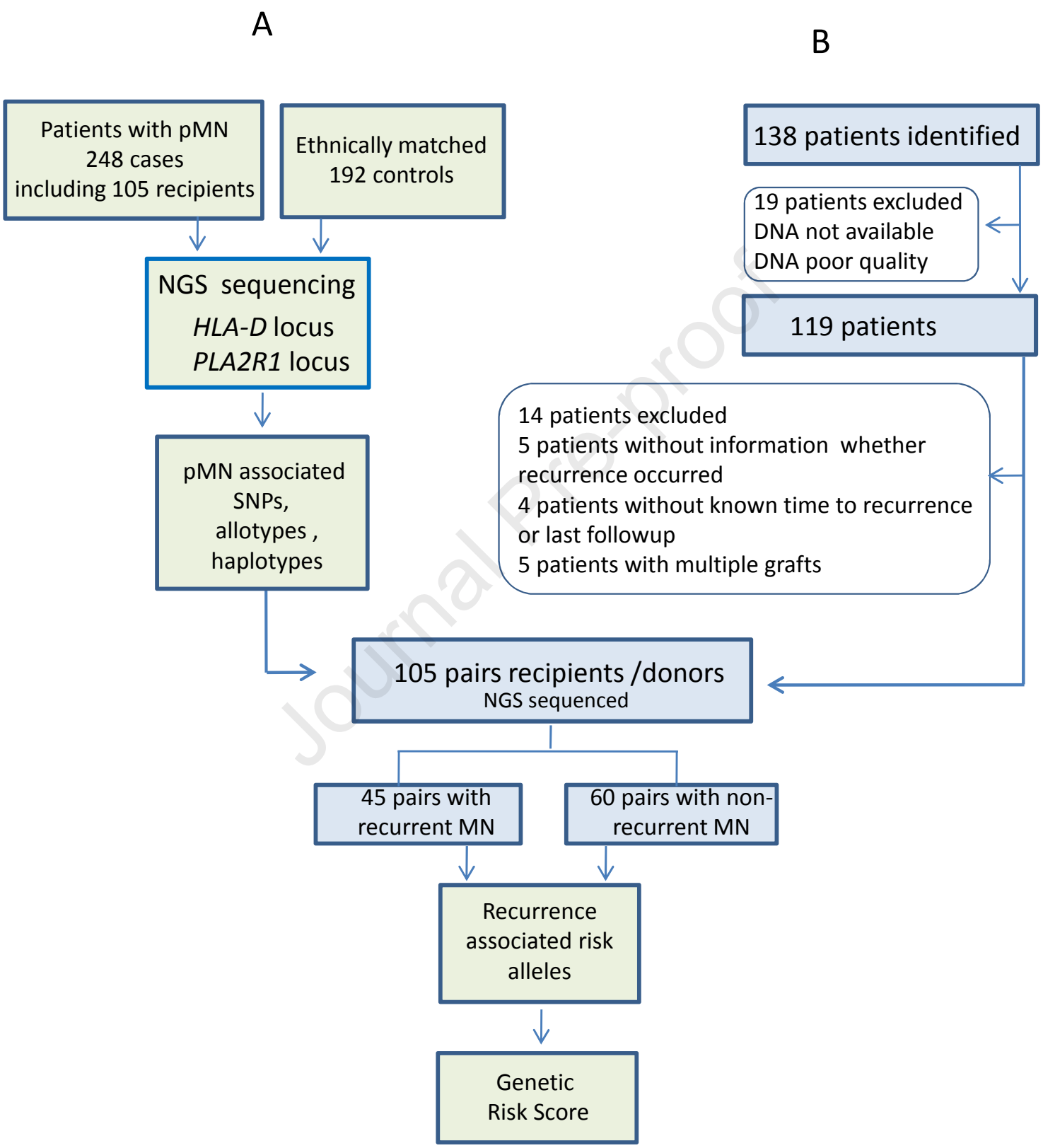
**11-20 missing values;

***21 - 30 missing values;

§31-40 missing values; eGFR (estimated Glomerular Filtration Rate) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation.

§Among the 45 recurrent patients, 12 received a transplant between 2011 and 2015: 4 were tested at transplantation (all positive), 5 were tested after (all positive), 3 have not been tested. Among the 60 non-recurrent patients, 16 received a transplant between 2011 and 2015: 5 were tested at transplantation (all negative), 1was tested after (negative), 10 have not been tested.

Figure 1



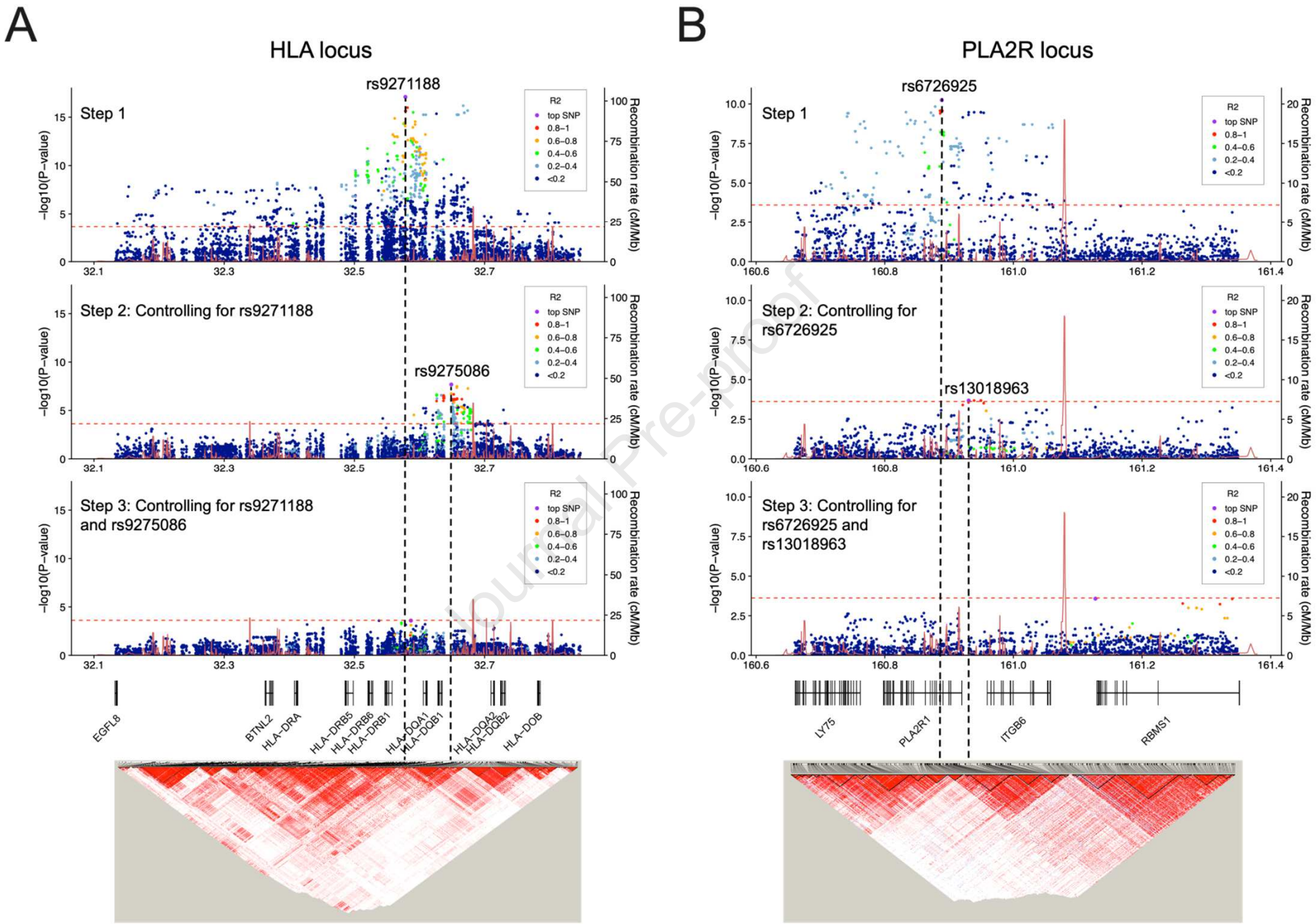
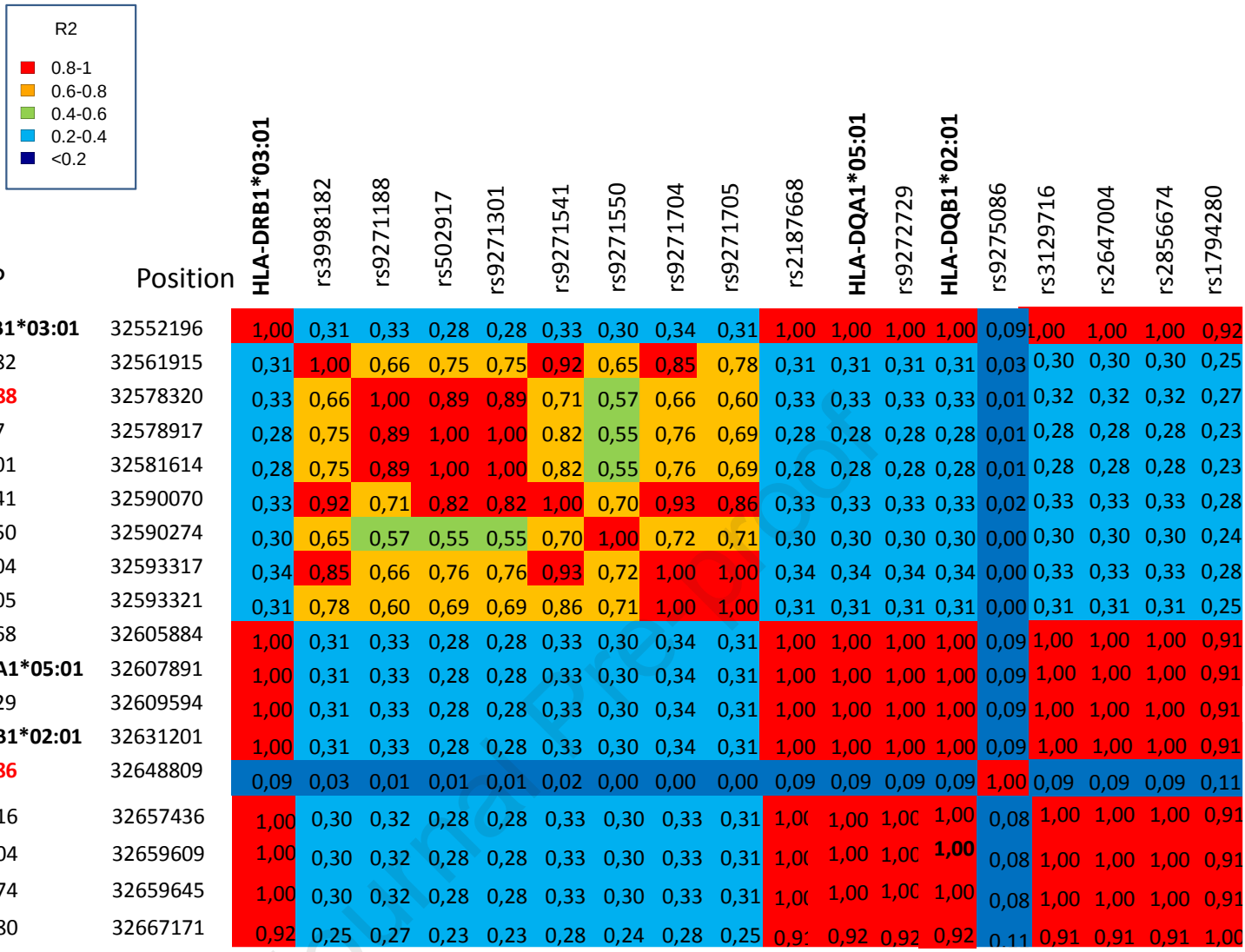
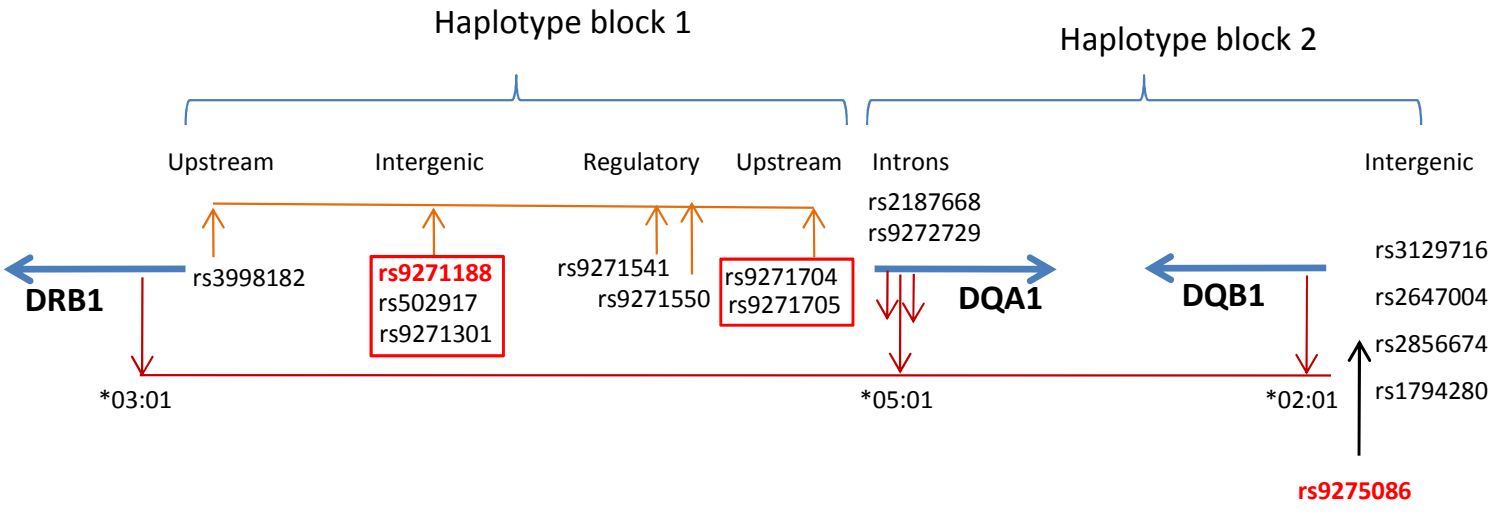
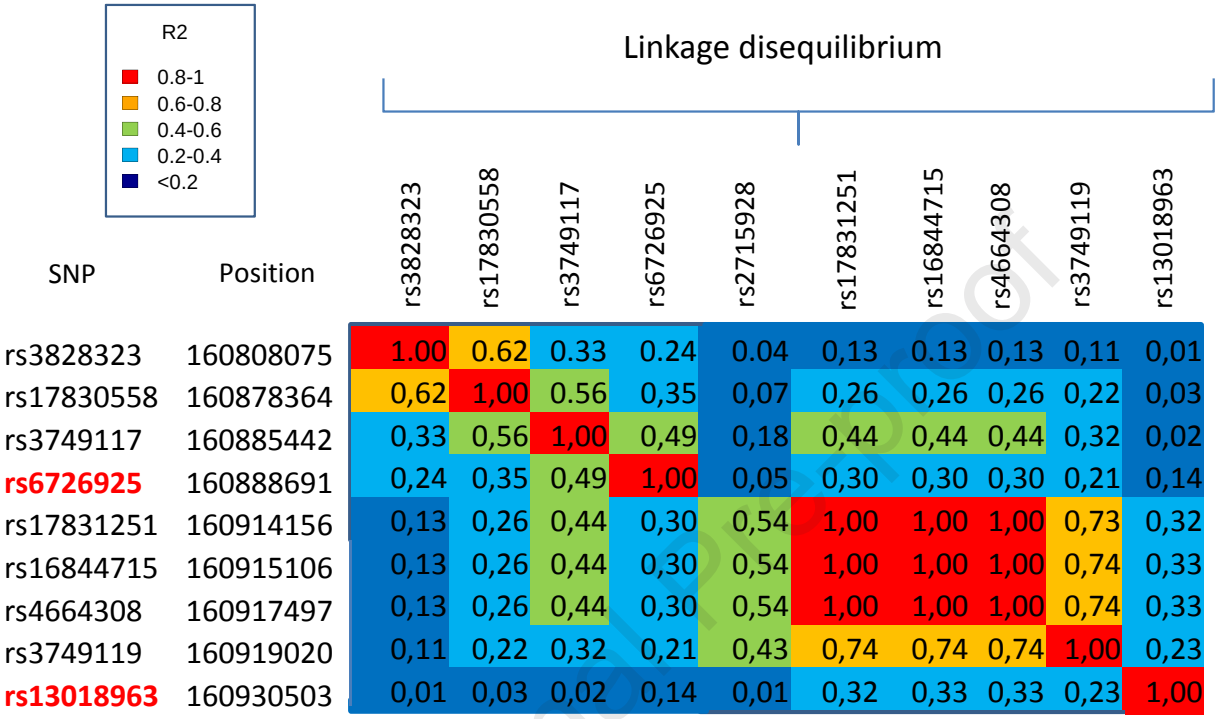


Fig. 2

Figure 3







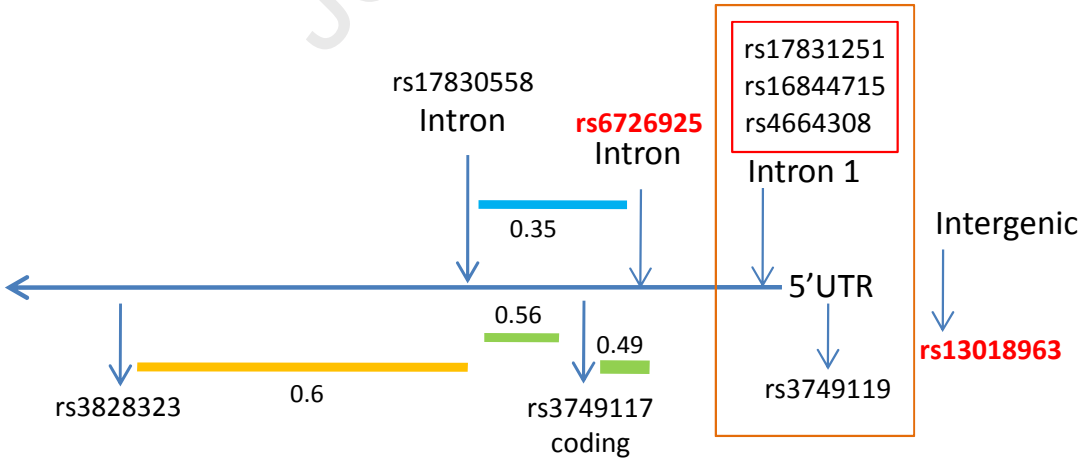
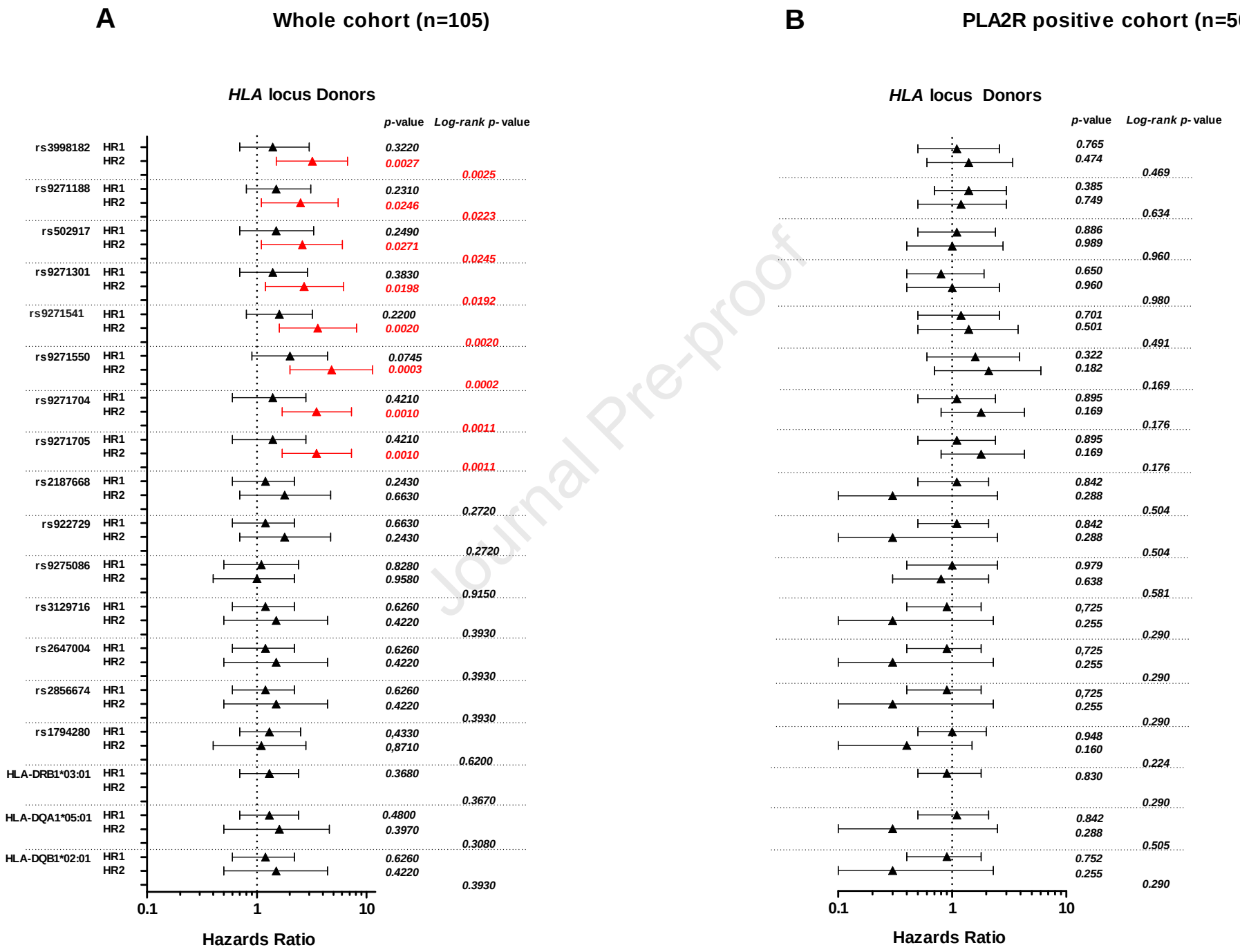


Fig 5



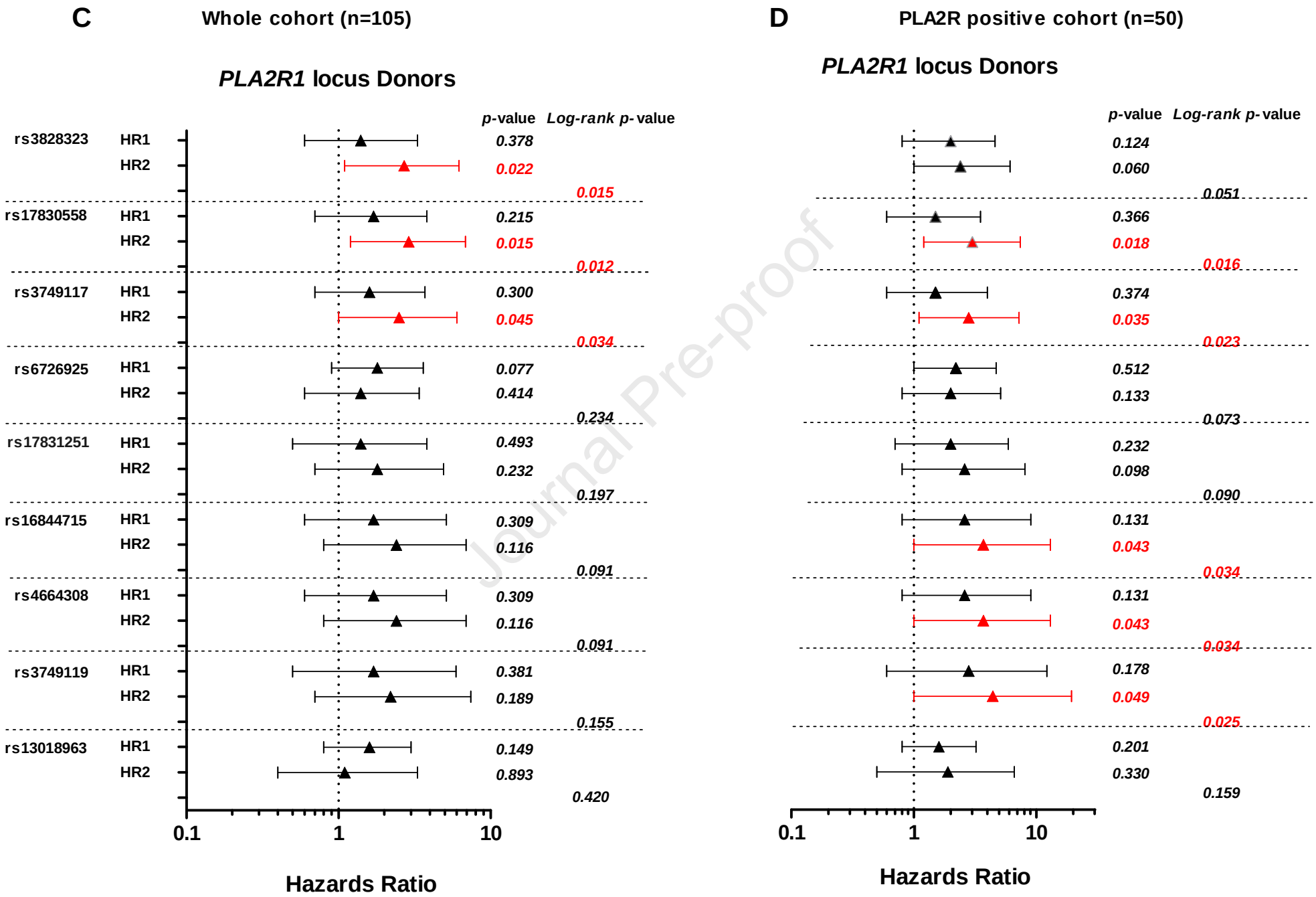
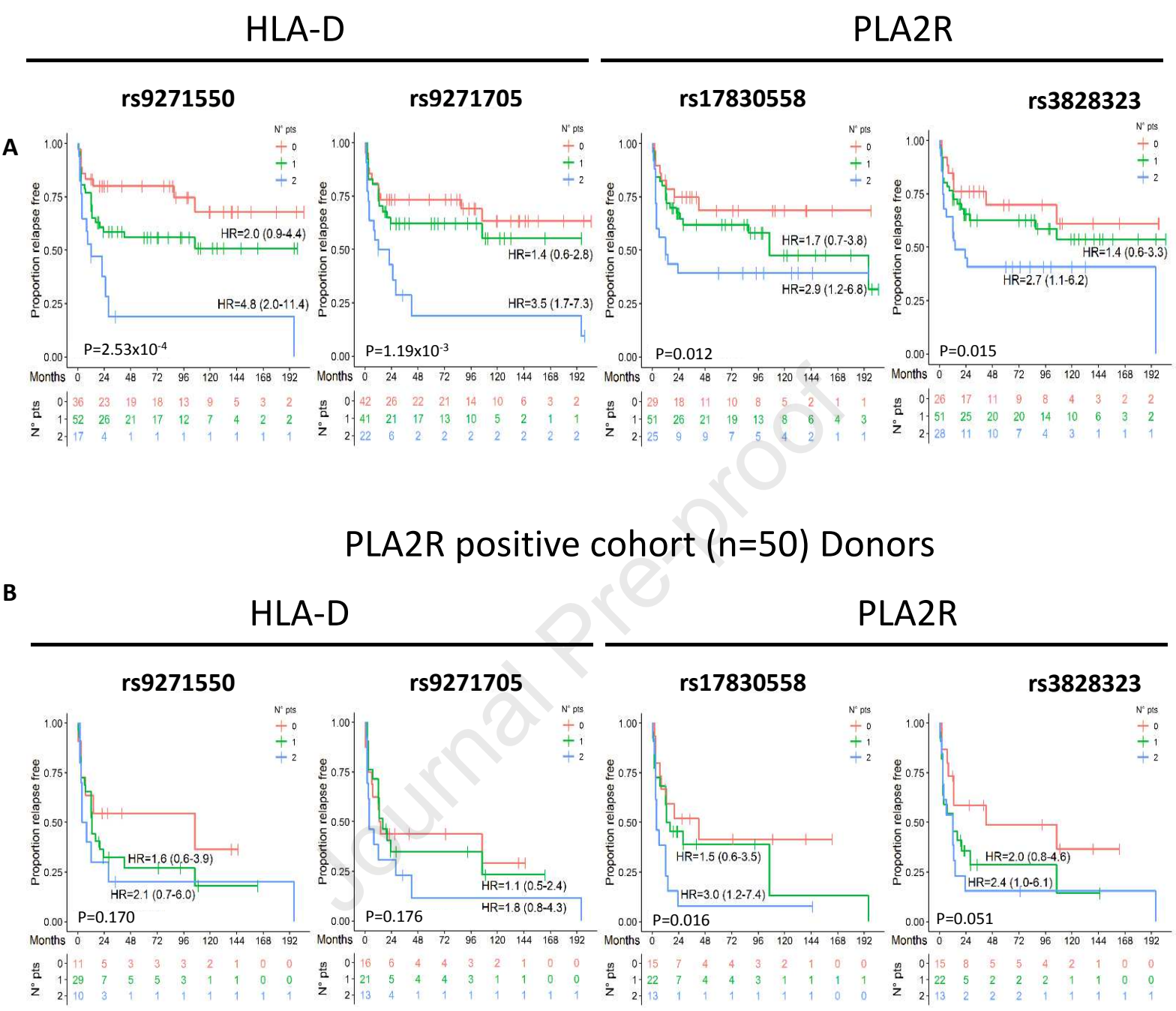


Figure 6



PLA2R positive cohort (n=50) Donors