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ORIGINAL ARTICLE

Interspecies transcriptomic comparison identifies a potential porto-sinusoidal vascular disorder rat model suitable for in vivo drug testing

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Abstract

Background: Porto-sinusoidal vascular disorder (PSVD) involves a group of rare vascular liver diseases of unknown aetiology that may lead to the development of portal hypertension and its life-threatening complications. Its pathophysiology is not well understood, and animal models described to date do not fully recapitulate human disease.

Methods: We developed three different PSVD rat models by either immunosensitization (repetitive intraportal LPS or intramuscular spleen extract injections) or toxic (Selfox: combination of FOLFOX and a selenium-enriched diet) treatment and characterized them at haemodynamic, histological, biochemical and transcriptional levels. We compared these results to human data.

Results: All three models developed significant portal hypertension, while only the LPS and the Selfox models displayed PSVD-specific and nonspecific histological

Abbreviations: 5-FU, 5-fluorouracil; CAF, cancer-associated fibroblast; DEG, differentially expressed gene; FDR, false discovery rate; GSEA, gene set enrichment analysis; HSC, hepatic stellate cell; ISC, incomplete septal cirrhosis; MAP, mean arterial pressure; NRH, nodular regenerative hyperplasia; OPV, obliterative portal venopathy; PH, portal hypertension; PP, portal pressure; PSVD, porto-sinusoidal vascular disease; RIN, RNA integrity number.

Genís Campreciós, Marina Vilaseca, and Dinesh M. Tripathi contributed equally to this work.

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alterations in the absence of cirrhosis. Transcriptional comparison between rat models and human data showed that both LPS and Selfox models recapitulate the main transcriptional alterations observed in humans, especially regarding haemostasis, oxidative phosphorylation and cell cycle regulation. Reproducibility and feasibility was higher for the Selfox model.

Conclusions: The Selfox rat model faithfully reproduces the main alterations described in PSVD. Its use as a preclinical model for drug testing in progressing PSVD can be a significant step forward towards the development of new therapeutic targets for this rare condition.

KEYWORDS
animal model, interspecies comparison, porto-sinusoidal vascular disease (PSVD)

1 | INTRODUCTION

Porto-sinusoidal vascular disorder (PSVD) involves a group of rare vascular liver diseases of unknown aetiology that affect intrahepatic vessels at the level of portal venules and/or sinusoids in the absence of cirrhosis.[1,2] PSVD typically affects young individuals, and the progression of the disease leads to the development of portal hypertension (PH) and its associated life-threatening complications, which are associated with high morbidity and mortality worldwide.[3–6]

Information on the pathophysiology of PSVD is scarce, and although several pro-thrombotic, immunologic, toxic, infectious and/or hereditary alterations have been suggested to play a role in the pathogenesis of PSVD,[1] none of them have been clearly confirmed. This lack of knowledge is directly responsible for the inexistence of pathophysiological-oriented treatments for the disease, which, clinically, implies that PSVD patients' medical treatment is limited to the management of PH-related complications when they appear.[7] Therefore, there is an urgent need to develop new therapeutic strategies able to stop or halt the progression of the disease.

Recently, we reported the first transcriptional analysis of liver samples from PSVD patients with PH where we integrated transcriptomic and clinical data. This analysis uncovered a distinct transcriptomic profile with altered pathways pointing towards a role of haemostasis and platelet aggregation, lipid metabolism and oxidative phosphorylation.[8] However, the therapeutic potential of modulating these altered pathways has not been evaluated so far.

Animal disease models are a great research tool for unravelling the pathophysiology of human diseases and for drug testing in preclinical studies. Several different strategies have been previously attempted to reproduce PSVD: repeatedly immunizing animals with serum albumin,[9] immunosensitizing them with splenic extract[10] or with *Escherichia coli*,[11,12] chemoembolization of portal tracts with small particles,[13,14] administration of oxaliplatin[15] or feeding animals with a selenium-enriched diet.[16,17] Finally, some animal models such as Notch1KO[18,19] and JAK1$^{S645P+/-}$[20] have also been described with features resembling PSVD. Most of these models, however, were mostly characterized only at haemodynamic and histological levels, but it has never been shown whether these models reproduce the core molecular signalling pathways altered in livers from PSVD patients. Additionally, some models were produced in rabbits, a costly and difficult-to-manage model. Altogether, these issues have hindered its use as relevant PSVD preclinical models.

Lay summary

Porto-sinusoidal vascular disorder (PSVD) is a rare vascular liver disease that leads to the development of portal hypertension and its life-threatening complications. Its pathophysiology is not well understood, and no treatments are currently available. We have developed a new easy-to-implement rat model that recapitulates PSVD at haemodynamic, histological, biochemical and transcriptional levels to be used for future preclinical studies.

Therefore, the aim of the current study was to develop a PSVD murine model that highly mimics the biochemical, haemodynamic, histologic and transcriptomic liver profile observed in patients with PSVD and PH.

2 | MATERIALS AND METHODS

2.1 | Animal models

Animals were housed in polycarbonate cages and maintained in a temperature and light-controlled facility under standard food and water ad libitum. All procedures were performed in accordance with Spanish legislation and approved by the Animal Research Committee of the University of Barcelona and were conducted in accordance with the European Community guidelines for the protection of animals used for experimental and other scientific purposes (EEC Directive 86/609). For in vivo experiments, animals were randomly

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distributed between groups and the experimenters were blinded for liver analysis purposes. Between 4 and 10 animals per group were used. All the experiments were performed in male Sprague Dawley rats 10–14 weeks old at the beginning of the experiments. The spleen extract and LPS models were adapted from Refs. [10,12], respectively. For the selenium-enriched diet plus FOLFOX models, we combined and adapted dosages from Refs. [15,16].

2.1.1 | Spleen extract model

Spleen was extracted from healthy rats under anaesthesia, weighted and homogenized immediately. A 20% homogenate was then prepared by mixing 200 mg of spleen in 1 mL of PBS pH 7.5. The mixture was then homogenized with a polytron homogenizer, centrifuged at 6000 rpm for 30 min and supernatant collected. The protein content of the homogenate was measured by Bradford's method, and 6 mg of splenic protein was prepared after mixing with Freund's complete adjuvant in a 1:1 ratio. Experimental rats were injected the solution intramuscularly twice per week for 3 months. The control group of animals were injected normal saline with Freund's complete adjuvant in an equal ratio. Spleen extract was prepared fresh on injection days. The spleens used to prepare the injections came from a set of rats used only for this purpose and sacrificed right after the splenectomy.

2.1.2 | LPS model

Rats were anaesthetised with isoflurane. Animals were then laparotomized, and permanent access to the portal vein was obtained with the introduction of an indwelling catheter (Venofix® A 27G .4 × 10 mm L:30 cm Luer Lock) to the gastric vein right above the ileocolic vein that would extend to the back of the rat, where it was fixed with a harness, leaving it accessible for successive injections. Rat's abdomen was then sewed. Animals received a bolus of 1 mg/kg of LPS (Escherichia coli O111:B4) twice a week for a period of 8 weeks. Control rats were operated and the catheter introduced the same way, but received a .09% saline solution instead of the LPS bolus.

2.1.3 | Selenium-enriched diet plus FOLFOX (Selfox model)

Rats were fed ad libitum with the selenium-enriched diet during 8 weeks. During this time, rats were also injected intravenously with a solution of oxaliplatin (3 mg/kg) + 5-FU(25 mg/kg) + folinic acid (45 mg/kg) once per week, for a total of eight injections.

Animal survival rates were 90–100% for all groups except for the LPS model, where survival rate dropped to 50% due to technical problems related to the maintenance of the catheter, leading to catheter obstruction, infection or even biting and scratching by rats.

TABLE 1 Effects of indicated treatments on hepatic and systemic haemodynamics.

	Control	LPS	Spleen extract	Selfox
Model	$n=23$	$n=7$	$n=5$	$n=10$
MAP	114 ± 13	103 ± 20	113 ± 22	100 ± 12
HR	341 ± 39	371 ± 65	335 ± 49	342 ± 28
PP	8.4 ± 1.1	11.9 ± 1.5*	10.4 ± 2.3*	11.0 ± 1.3*

Note: Values represent mean ± Standard Deviation. Statistical analysis was performed by ANOVA.

Abbreviations: HR, heart rate; MAP, mean arterial pressure; PP, portal pressure.

*p-value <.05.

Additional Materials and Methods can be found in Supplementary material.

3 | RESULTS

Because PSVD is a very complex disease with unknown aetiology, we opted to reproduce both immunosensitizing and toxic models of PSVD in the rat setting. We discarded Notch1 and JAK1 genetic models because they have not been reported in humans so far, while chemoembolization models do not recapitulate PSVD pathophysiology. The election of rat over mice came motivated for the ease to conduct reproducible haemodynamic studies.

For the immunosensitizing group, we adapted the protocols originally developed in rabbits from Kathayat et al. (repeated splenic extract injections)[10] and Omanwar et al. (repeated infusion of LPS into the portal vein through an indwelling catheter)[12] to Sprague Dawley rats. For the toxic model, we decided to combine the treatment of FOLFOX (a combination of Oxaliplatin, 5-FU and folinic acid, originally described in mice[15]) with a selenium-enriched diet, the only PSVD model developed in rats so far.[16] Both of these treatments have shown to induce some features characteristic of PSVD on their own, and we hypothesized that by combining both regimes the resulting model would incorporate the distinct features from each individual treatment and result in a more complete model for PSVD.

The different models and doses are described in the Materials and Methods section and resumed in Figure S1.

3.1 | Haemodynamic studies

We initially analysed the liver haemodynamics from each model. As observed in Table 1, both the LPS and the spleen extract models showed PH (11.9 ± 1.5 and 10.4 ± 2.3 mmHg, respectively) when compared to control rats (8.4 ± 1.1 mmHg).

For the selenium-enriched diet plus FOLFOX model, we initially fed rats with the selenium-enriched diet while receiving weekly intraperitoneal injections of FOLFOX during 8 weeks. FOLFOX dosage

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was scaled up from[15] according to rat weight. These rats developed PH in a similar level to that observed in the LPS and Splenic models (12.1 ± 1.2 mmHg, Supplementary Table S1). However, all these rats presented severe chylous ascites, a clinical manifestation uncommon in PSVD patients. Since this latter protocol seemed to be too toxic for the liver, we decided to try a new approach: to perform a two-hit protocol, where we initially fed rats with a selenium-enriched diet for 4 weeks, which were followed by 8 weeks of FOLFOX treatment while on normal chow, for a total of 12 weeks of treatment. Unfortunately, this new approach did not produce significant PH (9.7 ± 1.4 mmHg, Supplementary Table S1). Finally, we performed a slightly different strategy by changing the FOLFOX administration from intraperitoneal to intravenously and reducing the dose by half for 8 weeks while also feeding the animals with the selenium-enriched diet (Selfox model from now on). With this regime, rats developed PH (11.0 ± 1.3 mmHg, Table 1) but not chylous ascites.

Both the Selfox and the LPS models displayed a significant increase in liver weight and liver-to-body weight ratio. However, only the LPS model displayed an increase in the spleen weight and the spleen-to-body weight ratio but not the Selfox model (Supplementary Table S2).

3.2 | Histological analysis

Figures 1 and S2 and Supplementary Table S3 display the histological abnormalities described in the three murine models evaluated. In control rats, we did not detect any histological abnormalities except for the occasional presence of some herniated portal vessels (Figure 1B–D). On the contrary, most rats from the LPS and spleen extract models displayed some sinusoidal dilatation (Figure S2A,F) and herniated portal veins (Figure S2B,H), while only the LPS model seemed to induce some focal but consistent degree of nodular regenerative hyperplasia (NRH, Figure S2C,D). On the contrary, the Selfox model had some degree of portal vein herniation (Figure 1E) and also induced some degree of NRH (Figure 1F,G), while sinusoidal dilatation and obliterative portal venopathy were present less frequently (Figure 1H,I, Table 2). Incomplete septal fibrosis was only observed in one animal from both LPS and Selfox models (Figures 1J and S2E).

3.3 | Transcriptional comparison between PSVD patients and PSVD animal models

Based on the haemodynamic and histology results described above, all developed models fulfilled the PSVD criteria: presence of PH and/or specific histological signs (e.g. NRH) in the absence of cirrhosis. Additionally, some models also displayed certain nonspecific features of PSVD such as herniated portal vessels and sinusoidal dilatation. At this point, we selected the LPS and the Selfox models for further characterization. We discarded the spleen extract model because of the only slight increase in PH that it exhibited and the less evident histological alterations.

Both the LPS and the Selfox models presented minor alterations in the evaluated liver parameters similar to those found in control rats (Table 2).

To analyse whether these two PSVD animal models mirror the molecular alterations observed in the human disease, we performed RNAseq of liver tissue from control ($n = 6$), LPS ($n = 7$) and Selfox ($n = 10$) rats and compared these results with those obtained in the human cohort (Ref. [8] GEO accession number GSE171248).

We initially looked for the differentially expressed genes in each setting by comparing the two animal models to control rats and comparing livers from PSVD patients to healthy livers. We followed the same pipeline for the analysis of the three datasets and finally considered significantly up- or downregulated those genes with a fold change above 1.5, p-value <.05 and a false discovery rate (FDR) <.2. With these filters, we encountered 469 and 173 up- and downregulated genes, respectively, in livers from PSVD patients in relation to healthy livers (Figure 2A and Supplementary Table S4). These numbers were higher in both animal models, with the LPS model displaying 714 up- and 660 downregulated genes, and the Selfox model having 1131 up- and 792 downregulated genes when compared to control rat livers (Figure 2A).

FIGURE 1 Main histological alterations observed in PSVD animal models. (A) Histological liver analysis for PSVD-specific and unspecific lesions. H&E slides were evaluated for the presence or absence of obliterative portal venopathy (OPV), nodular regenerative hyperplasia (NRH), incomplete septal cirrhosis (ISC), aberrant herniated veins and sinusoidal dilatation. A score of 0 means the absence of a certain histological feature, while scores from .5 to 2 refer to the abundance and distribution of the findings, with .5 being minimal and irregular findings and 2 being obvious findings with a homogeneous distribution. Results are shown as stacked bars for each lesion and animal model. Statistical analysis was performed by Fisher's test (* when $p < .05$) where each model was compared with the control group. (B–D) Control rat liver: H&E, Sirius Red and reticulin stainings. No alterations are seen except for occasional presence of some herniated portal vessels. (E) H&E from Selfox mice displaying aberrant microvasculature or 'herniated' portal veins. The portal veins show abnormal dilatation, with a luminal diameter greater than that of the rest of the portal structures. These veins herniate from the portal tract into the paraportal area, sometimes into the lobules, where they can give a pseudoangiomatous appearance. (F) Reticulin staining of a Selfox model liver showing NRH. Reticulin staining highlights nodular architecture and hepatocyte atrophy. (G) Sirius red staining from the same animal as in F displays no nodular architecture, meaning that there is no collagen deposition in the parenchyma. (H) H&E of a Selfox liver displaying foci of mild sinusoidal dilatation. The black arrow is pointing a dilated sinusoidal space. (I) H&E from a Selfox liver displaying obliterative portal venopathy; see black arrow showing diminished lumen of the portal vein. (J) Sirius Red staining from a Selfox mouse liver displaying some degree of incomplete septal cirrhosis; see black arrow showing minimal fibrous expansion of a portal tract with thin fibrous bridge.

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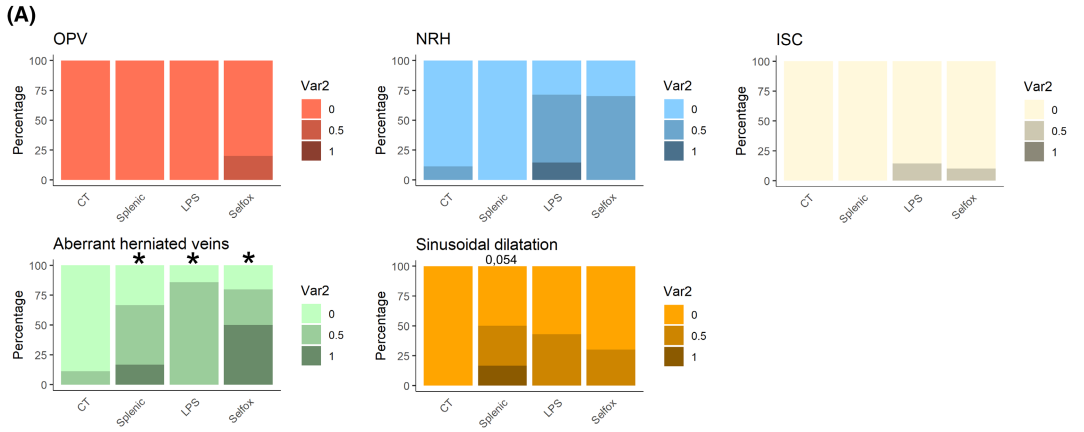

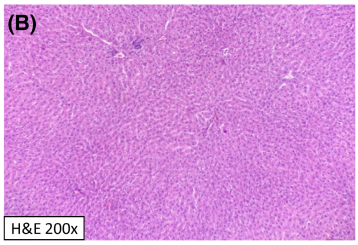

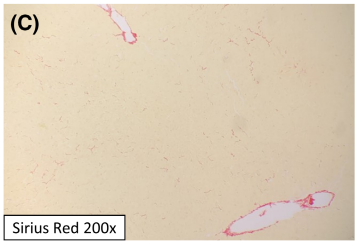

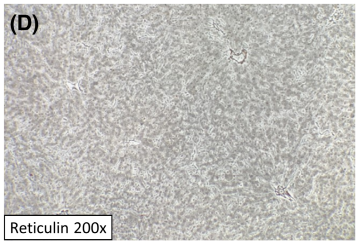

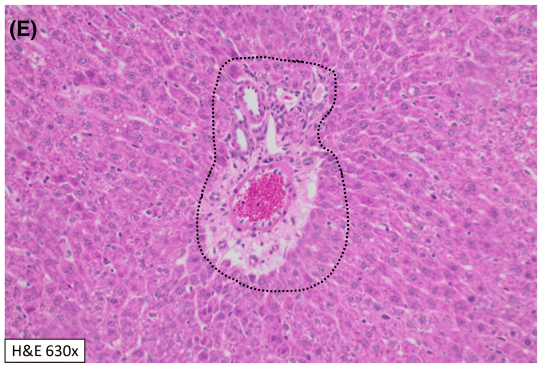

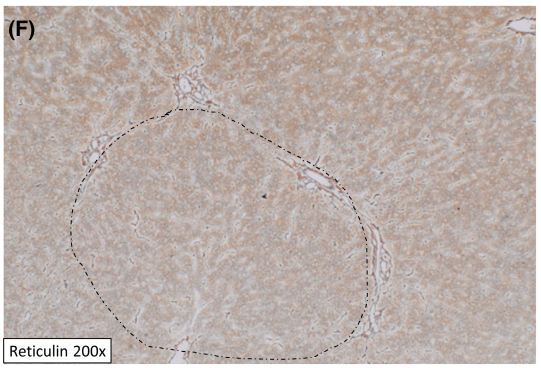

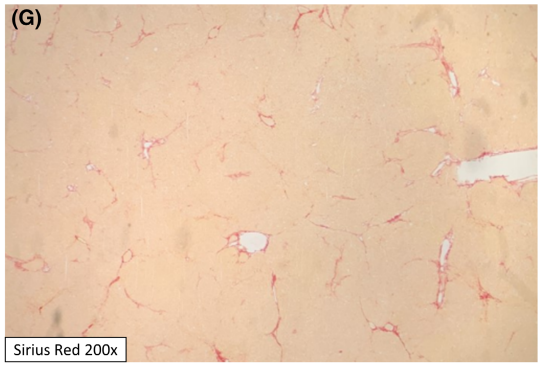

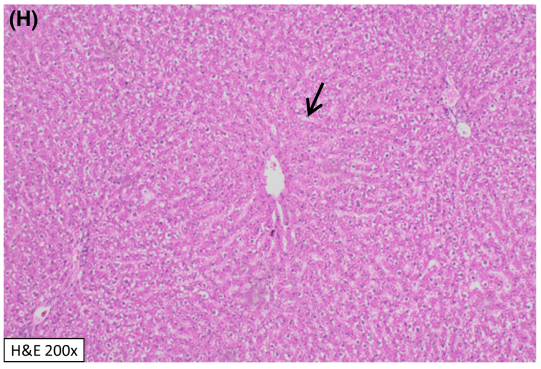

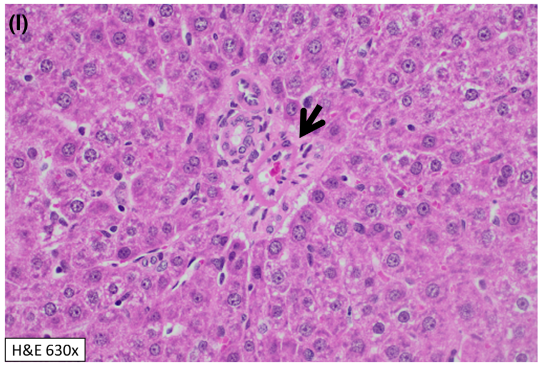

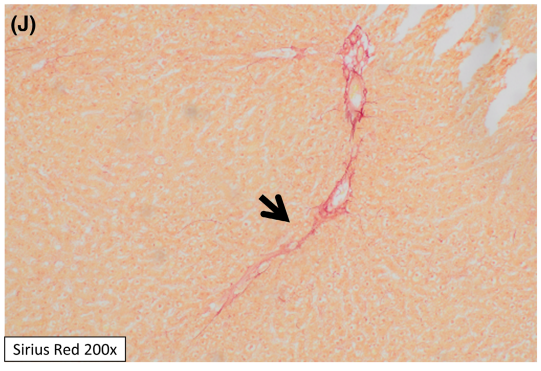

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TABLE 2 Effects of indicated treatments on biochemical parameters.

	Control	LPS	Selfox
Model	***n*=6**	***n*=7**	***n*=10**
Albumin (g/dL)	2.6±.1	2.5±.1	2.4±.2
ALT (U/L)	42±5	40±9	50±19
AST (U/L)	103±17	104±23	98±20

Note: Values represent mean±Standard Deviation.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

As shown in Figure 2B, the LPS model shared with livers of PSVD patients 18 upregulated and 7 downregulated genes while the Selfox models shared with the PSVD livers 52 upregulated and 6 downregulated genes.

We next complemented the gene-level analysis by checking whether the directionality of the deregulated genes (upregulated or downregulated) was consistent between the human PSVD dataset and our two rat models, and for that purpose, we used the approach proposed by Holland et al.[21] We took the 469 significantly up- and 173 downregulated genes from the human PSVD dataset and used them as gene signatures to perform gene set enrichment analysis (GSEA) on both rat datasets. Figure 2C shows how PSVD directionality is only significantly preserved in the Selfox model for upregulated genes, with the LPS model displaying just a nonsignificant trend. Overall, these results indicate that the Selfox model resembles human PSVD better than the LPS model at gene level.

Because similar biological processes might be regulated by different molecular actors in different species, we then performed GSEA in our three datasets (LPS, Selfox murine models and PSVD patients) to analyse these models at pathway level. We grouped pathways by processes based on the Reactome hierarchical pathway classification.[22] We then carefully looked to identify those processes that were recapitulated by either LPS or the Selfox model and those that were not, paying special attention to those already described in human PSVD[8] (Supplementary Table S5). Livers from patients with PSVD and from LPS and Selfox models showed 173, 148 and 176 upregulated and 229, 272 and 252 downregulated pathways, respectively. There were 12% and 17% overlap in the upregulated pathways between PSVD patients vs the LPS and the Selfox model, respectively, while downregulated pathways presented a higher overlap of 34% and 31% for the LPS and the Selfox models, respectively (Figure 3A). Interestingly, although generated by different mechanisms, the shared up- and downregulated pathways between the two experimental models (LPS and Selfox models) were of roughly 40% and 70%, respectively (Figure 3A).

Remarkably, when looking at specific processes, both proposed rat models showed concordance with human PSVD samples in some important pathways such as upregulation of oxidative phosphorylation, mitochondrial activity or G protein-coupled receptor signalling, while displaying downregulation of cell cycle, metabolism of RNA and response to stress (Figure 3B). Platelet activation, a hallmark in human PSVD, was only observed in the Selfox model, while the LPS model had only mild platelet activation but more activation of the intrinsic pathway of fibrin clot formation. Similarly, livers of PSVD patients showed increased extracellular matrix reorganization and elastic fibre formation, which followed a nonsignificant trend in the Selfox model but not in the LPS model. In contrast, the general upregulation of the adaptive immune system and lipoprotein metabolism was only modestly observed in the LPS model, while most of these processes were actually downregulated in the Selfox rat model (Figure 3C).

We finally wondered to what extent did the cellular composition in our two rat models resemble human disease. For that purpose, we performed computational deconvolution on the RNAseq data from our three datasets to extract the proportions of individual cell types in each condition. We used the Cellanneal software,[23] a powerful deconvolution algorithm based on simulated annealing that uses reference signature expression tables curated from single-cell human atlases that have been established by the field.[24]

As observed in Figure 3D, computational deconvolution results showed that, mostly, both human PSVD and animal models produced similar alterations in the cellular components of the liver, as observed by subtle increases in B cells, cholangiocytes and T cells or decreases in the proportion of pericytes, or cancer-associated fibroblasts (CAF). Of special interest is the increased number of vascular endothelial cells and stellate cells in human PSVD (Figure 3D). Both the LPS and the Selfox models displayed an increased number of vascular endothelial cells, representing the induction of endothelial injury, while no changes in the number of hepatic stellate cells (HSC) were detected in neither model, therefore suggesting that the lack of significant alteration in extracellular matrix reorganization and elastic fibre formation might be caused by the lack of sufficient activation of HSC in neither rat model (Figure 3D).

Overall, these results support our hypothesis that both LPS and the Selfox rat models, although very distinct in nature, are able to recapitulate some important aspects from human PSVD specifically involving the hepatocyte and endothelial compartment, while other aspects such as immune function or extracellular matrix remodelling seem to be model-dependent.

4 | DISCUSSION

Historically, PSVD was often diagnosed in advanced stages, with patients arriving at the hospital due to PH-related complications like oesophageal varices, leaving limited room for intervention. More recently, patients are being diagnosed at earlier stages when PH signs and symptoms are less apparent or absent.[4] Unfortunately, effective treatments to alter the disease's natural course remain elusive.

Despite notable recent advances and the discovery of critical deregulated pathways in PSVD, there is still a lack of in vitro tools or animal models that faithfully replicate the disease and can be used for assessing its manipulation in preclinical studies. While 3D organoids and assembloids offer a promising opportunity to work with patient-derived material, incorporating affected cellular components,

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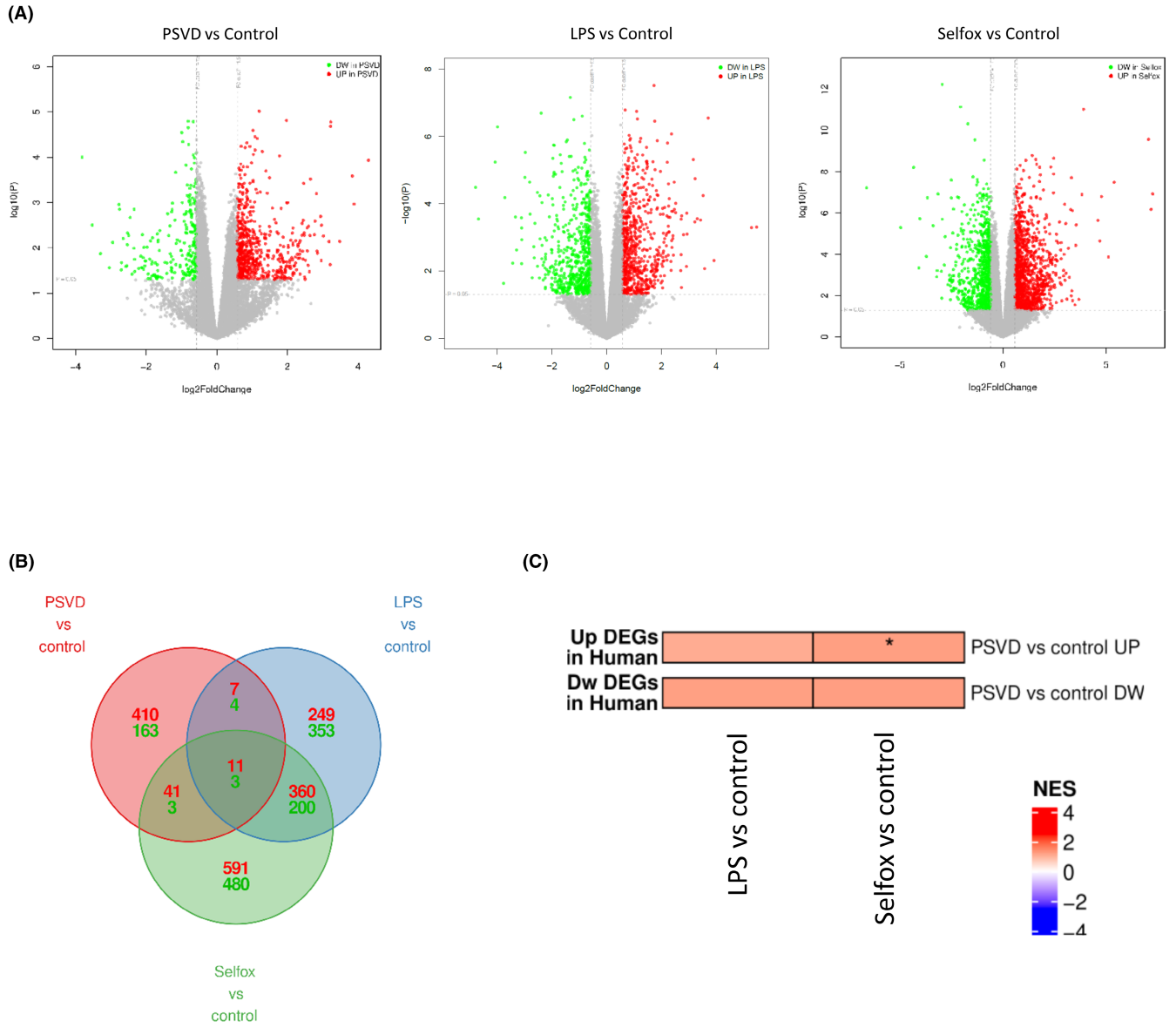

FIGURE 2 Gene-level comparison analysis between human PSVD and PSVD animal models. (A) Volcano plots showing the number of upregulated (in red) and downregulated (in green) genes for each indicated comparison. (B) Venn diagram showing the number of overlapping up- and downregulated genes among each indicated comparison. (C) Similarity between the human study and the two PSVD animal models by pairwise enrichment analysis of the up- and downregulated genes.

they still face challenges related to vascularization and blood supply. Therefore, we aimed to develop an optimal animal model for testing strategies to halt or delay disease progression while awaiting further advancements in the 3D in vitro field.

The first approaches to obtain animal models for PSVD date from the late 80s and early 90s, when the models based on repeated injections of nonpathogenic *E. coli*[11] in rabbits and the induction of NRH in rats by a selenium-enriched diet[16] were published. In both studies, animals presented with PH in the absence of cirrhosis, and on top, displayed certain histological features suggestive of PSVD. Since then, other models using different strategies: injecting immunosensitizing agents (splenic extract,[10] repeated *E. coli* portal vein administration[12]), introducing veno-occlusive agents (portal venous embolization[14]) or by breeding Notch1$^{-/-}$ mice[18,19] or mice transgenic for Jak1[20] have been evaluated. Unfortunately, none of these models, either for their high cost or because only a partial evaluation of different aspects of PSVD was performed, have neither been validated by other groups nor used for drug testing or pathophysiological studies. Additionally, none of these models performed transcriptomic analysis.

In this study, we presented three rat models that fulfilled the criteria for PSVD diagnosis: LPS, spleen extract and the Selfox models. They all developed PH in the absence of cirrhosis, and additionally, also displayed some specific and nonspecific histological features of PSVD.[25] All three models were conceived based on different strategies using factors that have been involved in PSVD development in patients. In addition, the choice of rats, instead of rabbits, should facilitate their wide use by other labs.

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FIGURE 3 Pathway analysis reveals major conserved pathways between human PSVD and both rat models. (A) Venn diagram showing the number of overlapping up- and downregulated pathways among each indicated comparison. (B, C) Heat map displaying the Normalized Enrichment Score (NES) obtained from the GSEA performed with both human PSVD and rat models gene sets. Significantly up- or downregulated pathways are indicated with an *. (D) Results from computational deconvolution performed in each indicated gene set. Colours are log10 of the estimated frequencies (hepatocytes, which dominate the transcriptional landscape are not shown).

Interestingly, the translation of the LPS and spleen extract models from rabbits to rats by just adapting dosages and catheter sizes to the new species characteristics resulted in a very similar phenotype as those described in their original articles, with all animals presenting PH without cirrhosis.

Likewise, the conversion of the Selfox model, where rats were subjected to both a selenium-enriched diet, known to induce variable levels of NRH,[16] and the administration of the chemotherapeutic agent FOLFOX, known to cause sinusoidal damage,[15] also induced the development of PH, with most of the rats also exhibiting mild NRH, sinusoidal dilation and a widespread presence of herniated vessels. It is worth noting that the degree of NRH induced by the Selfox model was generally less pronounced than that reported by a selenium-enriched diet.[16] However, we believe these differences might stem from variations in tissue collection strategies rather than fundamental differences between the models. Bioulac-Sage described the presence of NRH as highly heterogeneous, with a particular prevalence in the peripheral zones of the lobule, while we standardized our sample collection by resecting the central area of the left lateral lobe from each liver, where NHR is generally less prevalent.

Another noteworthy aspect is that Selfox rats did not develop splenomegaly, a common observation in most PSVD patients with PH. Interestingly, a similar result was reported in the original article by Bioulac-Sage et al.,[16] suggesting that the absence of splenomegaly may be an effect of selenium.

While these results collectively position our three rat models as legitimate PSVD models from a haemodynamic and histological point of view, we discarded the spleen extract model for further analysis because of the low levels of PH, barely above control rats, as well as for the fewer number of typical PSVD histological alterations observed.

In addition to the haemodynamic and histological characterization, our study is the first to perform a molecular characterization of the transcriptomic profile of the two rat models that more closely resemble human PSVD from a histological and haemodynamic point of view. To understand to what extent these models recapitulate the human disease at the transcriptomic level, results obtained were analysed and compared with those found in livers from PSVD patients.

On one hand, the analysis at gene level reported little overlap between livers from either the LPS or the Selfox models and human PSVD. This result, however, was somehow expected considering we were comparing transcriptomes from different species, and only 12 504 out of the 16 266 human genes had a rat orthologous gene, while 2203 rat genes did not have any known human equivalent. Similar results have actually been shown when comparing well-established mouse models for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis and liver cirrhosis (CCl_4) to human data.[26] Of note though, Holland et al. suggested that similarities between gene sets (intra- or interspecies) were generally maintained if gene expression directionality rather than differentially expressed genes were analysed.[21] Indeed, applying this strategy to our analysis revealed that the Selfox model did maintain significant gene expression directionality when compared to human PSVD, but results were slightly inferior for the LPS model.

Along the same line, gene set enrichment analysis, which allows to identify groups of genes that share common biological functions,[27] returned interesting results, with both LPS and Selfox models displaying a percentage of up- and downregulated overlapping pathways with PSVD in the range of 20%–35%. These results are even more meaningful when considering the high heterogeneity of PSVD. Although currently under the same umbrella, it is still not clear whether patients diagnosed with PSVD could be further subclassified according to other histological or etiological factors, thus grouping together entities that despite sharing clinical evolution might as well have distinct altered pathways. Nevertheless, if that was the case, we can hypothesize that our strategy will have identified those processes relevant for the disease that are common in the different heterogeneous patients.

An intriguing question risen by our analysis is the fact that both LPS and Selfox models, despite their essential conceptual and methodological differences, seem to affect quite similarly the different cellular compartments of the liver, with GSEA and computational deconvolution pointing to a specific effect on endothelial cells and hepatocytes, while neither model is able to significantly activate HSCs. One might wonder whether HSC activation might be linked to more advanced stages of the disease, which could explain why our models, which display less severe PH and therefore might represent a less advanced stage of the disease compared with human patients, do not show significant HSC activation.

This study not only has some limitations but also opens new questions and possible directions for the field. On one hand, we have not been able to generate an advanced stage of PSVD in rats that resemble our human cohort due to technical challenges in the LPS model and toxicity issues in rats following the selenium-enriched diet plus FOLFOX regime. While this is probably one of the main reasons which prevented a higher percentage of overlap between PSVD rat models and human data, this still opens the possibility to use this less advanced stage for drug testing (prevention or treatment) and to get pathophysiology insights in a stage where treatment would be extremely relevant to patients, before they start developing PH-related complications. Alternatively, one could consider the usage of additional drugs, for example didanosine, to exacerbate this model and try increasing its resemblance to human

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PSVD, although we have already shown that these regimes are finicky, and such approach would certainly need new adjustments to current food and drug regime to find the correct balance.

Finally, an important aspect when conducting experiments with animal models is their reproducibility and feasibility. In that sense, it is important to notice that the LPS model presented a high rate of technical complications and mortality, discouraging its regular use. On the other hand, the Selfox model is technically easy to implement and did not present such issues, with around 90–100% of animals surviving the treatment. Considering both LPS and Selfox models showed comparable levels of PH, histological features and transcriptomic analysis, we consider that the Selfox model is the one better suited for conducting studies on PSVD (Supplementary Figure S3).

In summary, we have presented the first transcriptional study comparing PSVD animal models to human disease and showed that the easy-to-implement Selfox rat model fulfils the diagnostic criteria for early- to mid-stage PSVD while also displaying key histological and transcriptomic features shared among advanced PSVD patients. While we acknowledge the limitations of this model, we think that it might provide a good basis from which to build upon. Considering there is no treatment for the disorder and scarce information on its pathophysiology, we believe that this model can be a good PSVD preclinical model for drug testing in progressing PSVD while the field deepens in the unravelling of PSVD pathophysiology.

AUTHOR CONTRIBUTIONS

Genís Campreciós designed the research, conceived ideas, performed experiments and wrote the manuscript. Marina Vilaseca and Dinesh M. Tripathi designed research, conceived ideas and performed experiments. Héctor García-Calderó, Rosa Montañés and Aina Anton performed experiments. Carla Montironi and Alba Díaz performed the histological analysis. Virginia Hernández-Gea critically revised the manuscript. Joan Carles García-Pagán designed the research, conceived ideas, wrote the manuscript, obtained funding and directed the study. All authors edited and reviewed the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors who have taken part in this study declare they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

DATA AVAILABILITY STATEMENT

RNAseq data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (GEO; accession numbers GSE229380).

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