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ORIGINAL ARTICLE

Focused panel sequencing points to genetic predisposition in non-cirrhotic intrahepatic portal hypertension patients in India

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Abstract

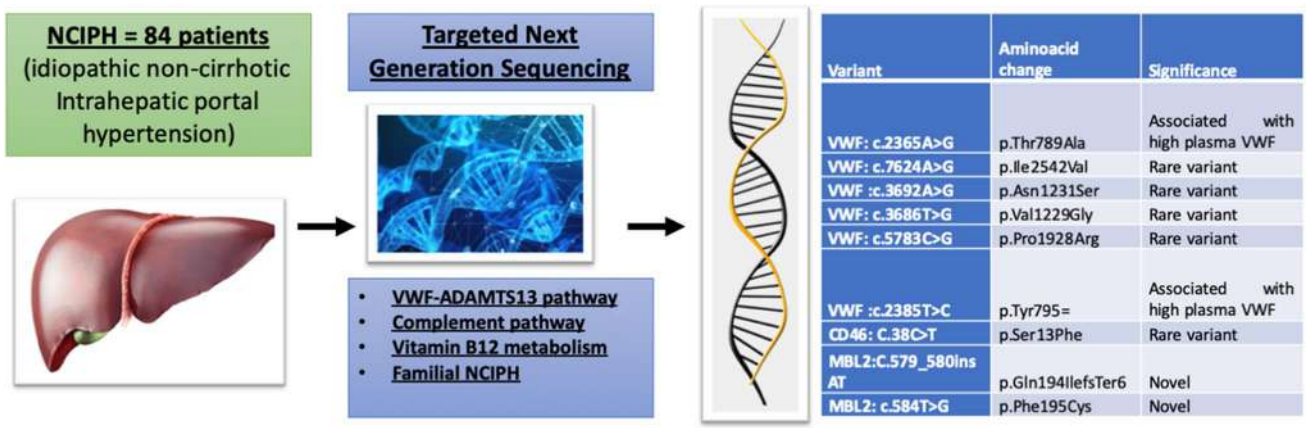
Objective Non-cirrhotic intrahepatic portal hypertension (NCIPH), a portal microangiopathy affecting small portal vein radicles, is a disease of Indian sub-continent. NCIPH appears to be a complex disease with interactions between inherited and acquired factors, though the exact pathophysiological mechanism is unknown. We aimed at investigating the genetic variants that might contribute to susceptibility to NCIPH.

Methods In this case-control study, we analyzed genes associated with microangiopathy—VWF-ADAMTS13 (von Willebrand factor and its cleavase enzyme — a disintegrin and matrix metalloprotease with thrombospondin type-1 motifs member 13) and alternative complement system vitamin B_{12} metabolism and with familial NCIPH.

Result Eighty-four Indian patients with liver biopsy–proven NCIPH (cases) and 103 healthy controls (matched for residential region of India) were included in the study. Targeted next-generation sequencing (NGS) panel, comprising 11 genes of interest, was done on 54 cases. Genotyping of selected variants was performed in 84 cases and 103 healthy controls. We identified variants in *MBL2*, *CD46* and *VWF* genes either associated or predisposing to NCIPH. We also identified a single case with a novel compound heterozygous mutation in *MBL2* gene, possibly contributing to development of NCIPH.

Conclusion In this first of a kind comprehensive gene panel study, multiple variants of significance have been noted, especially in ADAMTS13-VWF and complement pathways in NCIPH patients in India. Functional significance of these variants needs to be further studied.

Graphical abstract



Keywords ADAMTS13 · MBL2 · NCIPH · Targeted panel · Variants · VWF

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Introduction

Idiopathic non-cirrhotic intrahepatic portal hypertension (NCIPH), portal microangiopathy affecting small portal vein radicles, is a disease of Indian sub-continent [1]. This under-recognized clinical entity is often mis-labeled as cryptogenic cirrhosis and liver biopsy is required for a diagnosis. Various studies estimate that in India, NCIPH constitutes 10% to 30% of patients with portal hypertension [2]. In a prospective study, 16 of the 39 patients labeled as "cryptogenic cirrhosis" (41%) who underwent liver biopsy were diagnosed to have NCIPH [3].

NCIPH appears to be a complex disease with interactions between physiological and environmental factors, though the exact pathophysiological mechanism is still obscure [2]. Microangiopathy restricted to intrahepatic small portal vein radicles suggests the central role of the gut, which can either be involved (e.g. celiac disease, small intestinal bacterial overgrowth) or serve as a portal of entry to potential toxins (e.g. arsenicosis, human immunodeficiency virus [HIV] drugs) in the pathogenesis of NCIPH [2].

Studies have documented severe ADAMTS13 (a disintegrin and matrix metalloprotease with thrombospondin type-1 motifs member 13, von Willebrand factor [VWF] cleavase) deficiency in 10% to 28% of NCIPH patients, despite having well-preserved liver functions, suggesting the imbalanced ratio of ADAMTS13-VWF as one of the probable mechanistic pathways triggering intrahepatic portal vein thrombosis [4, 5]. A low level of ADAMTS13 is also known to activate the alternative complement pathway. Unfettered activation of the alternative complement pathway is also associated with thrombotic microangiopathy.

Genetic variations in the VWF-ADAMTS13 pathway and the complement system are known to predispose to thrombotic microangiopathy, including NCIPH [6, 7]. These observations prompted us to explore genetic variations in VWF-ADAMTS13 and complement system in patients with NCIPH.

Additionally, nutritional or genetic vitamin B_{12} deficiency has been reported as a modifiable risk factor for ischemic stroke and also a useful marker to differentiate NCIPH from cirrhosis [8].

Though the familial NCIPH is uncommon, recent studies showed genetic causes in rare familial forms of NCIPH. Sarin et al. reported four families with more than one member afflicted with non-cirrhotic portal fibrosis [9]. Whole exome sequencing in familial NCIPH revealed potential pathogenic mutations in *DGUOK* and *KCNN3* genes in individual familial cases [10, 11].

Thus, pathophysiology of NCIPH (localized thrombotic microangiopathy) appears to be modulated by environmental factors (poverty-linked thrombophilia and gut-related insults) as well as possible genetic factors [2]. In this study, we aimed at investigating the genetic variants and focused on pathways associated with microangiopathy (ADAMTS-13-VWF and alternative complement system), vitamin B_{12} metabolism and rare inherited variants reported in familial NCIPH.

Methods

Patient recruitment

We recruited liver biopsy–proven NCIPH patients ($n=84$) after obtaining informed consent. All patients fulfilled the diagnostic criteria for NCIPH—presence of esophageal and/or gastric varices, patent hepatic inflow and outflow, absence of any known etiology of cirrhosis (e.g. hepatitis B and C, alcohol) and liver biopsy showing the absence of advanced fibrosis/cirrhosis and ruling out competing liver disease etiology [12]. All liver biopsy specimens (≥5 portal tracts) were deemed adequate and reported by trained hepatopathologists. For comparison, we recruited region-matched healthy adult voluntary blood donors ($n=103$) after obtaining informed consent.

Initial next-generation sequencing (NGS) analysis was done on consecutive NCIPH patients' ($n=54$) sample. Based on the sequencing data, potential candidate variants were identified for further analysis. These selected variants were then screened in the entire 84 NCIPH cases and also in the 103 controls with either restriction fragment length polymorphism (RFLP) or Sanger sequencing (Fig. 1).

Biochemical analysis was performed in a subset of NCIPH cases and control groups.

Focused gene panel

1. VWF-ADAMTS13 pathway: *VWF*, *ADAMTS13*
2. Complement pathway: *MBL2*, *CFB*, *CFH*, *C3*, *CD46*, *CFI*
3. Vitamin B_{12} metabolism: *MTHFR*
4. Familial NCIPH: *KCNN3*, *DGUOK*

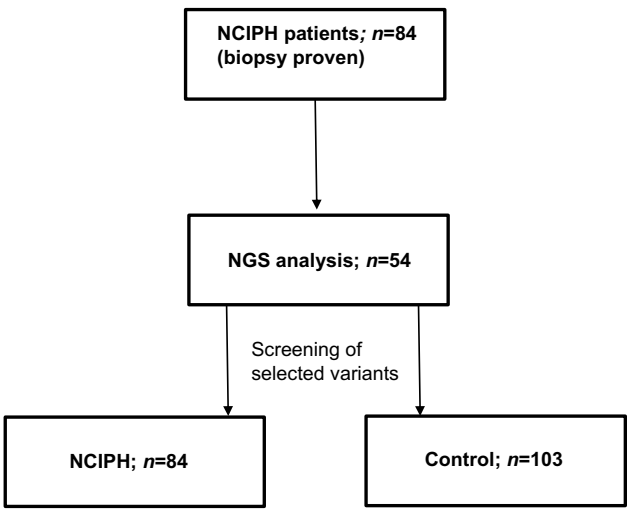

Fig. 1 Flow of selection of patients and genetic analysis done in the present study focusing genetic basis of microangiopathy in idiopathic non-cirrhotic intrahepatic portal hypertension (NCIPH), *NGS* next-generation sequencing

Blood collection and processing

Platelet poor plasma and buffy were separated and stored. The genomic deoxyribonucleic acid (DNA) was extracted from buffy coat using the QIAamp blood mini-column method. DNA was quantitated using Nanodrop and OD 260/280 was documented.

Multiplex PCR

We designed specific primers including all exons and splice site junctions of the 11 genes of interest, using Primer 3 software. The novel multiplex polymerase chain reactions (PCRs) were standardized for the panel of 11 genes (VWF, *ADAMTS13*, *MBL2*, *CFB*, *CFH*, *C3*, *CD46*, *CFI*, *MTHFR*, *KCNN3*, *DGUOK*) in 20 PCRs. This targeted NGS panel testing was performed in 54 NCIPH patient samples.

Next-generation sequencing workflow [13]

a) Target enrichment: We designed novel multiplex PCRs to enrich the target regions using the QIAgen Multiplex PCR kit method, covering 50 bp upstream and downstream of the exons including also 3′ and 5′ untranslated regions.
b) Library preparation: Pooled amplicons (size: 282–362 bp) were fragmented using an ultrasonicator, followed by end repair, adaptor ligation and amplification using the Ion Torrent express library kit. The ligated samples were size-selected using E-gel size select in a 2% precast gel.
c) Template preparation: The libraries were attached to ion spheres and amplified using Emulsion PCR. Each one of the libraries in the microdroplet was clonally amplified followed by Streptavidin Dynabead–based enrichment in the ion one-touch enrichment system before sequencing.
d) Sequencing and bioinformatic analysis: Sequencing was performed on Ion Torrent PGM using Ion PGM™ 200 Sequencing Kit (Ion Torrent, Life Technologies, South San Francisco, CA, USA) using 318 chips. The sequencing data were mapped to the reference hg19 human genome using the Torrent Mapping Alignment Program (TMAP). The Torrent suite software with v2.0 and/or v3.6.2 was used for all analysis. Identification of potentially significant variants was based on prevalence as compared to publicly accessible genomic databases (1000 Genomes, ExAc, gnomAD, ClinVar, and HGMD), in silico predictions (mutation taster, SIFT, PolyPhen, VarSome based on American College of Medical Genetics and Genomics [ACMG] guideline) and literature review.

We further curated the data based on published literature and rarity of the variant and narrowed it down to nine important candidate variants (Table 1). For confirmation of these selected variants, uniplex PCRs were standardized: two variants in the same exon of MBL2 gene, c.579_580ins AT and c.584 T > G; one in CD46, c.38 C > T; and six variants in VWF, c.2365A > G, c.2385 T > C, c.3686 T > G, c.3692A > G, c.5783C > G and c.7624A > G. Of the selected nine variants, we could further analyze only seven variants out of nine in healthy controls ($n = 103$) by RFLP (VWF: c.2365A > G, c.2385 T > C, c.5783C > G, c.7624A > G; CD46: c.38 C > T) or by Sanger sequencing (two variants in MBL2 gene).

Restriction fragment length polymorphism (PCR–RFLP)

For RFLP analysis, a mixture of 6 µL PCR product, nuclease-free water 6.7 µL, 1.5 µL of 10 × buffer and 0.8 µL of specific NEB restriction enzyme (HPYAV for CD4: c.38 C > T; RSA I for VWF: c.2365A > G; CViQI for VWF: c.7624A > G and

Table 1 List of selected variants based on the rarity and functional evidence identified by focused next-generation sequencing in 54 patients with idiopathic non-cirrhotic intrahepatic portal hypertension

Variant	Amino acid change	MAF (1000G or ExAc)	SNP ID	Mutation taster	Significance
VWF: c.2365A > G	p.Thr789Ala	0.22	rs1063856	Polymorphism	Associated with high plasma VWF [14]
VWF: c.7624A > G	p.Ile2542Val	0.0005	NA	Polymorphism	Rare variant
VWF: c.3692A > G	p.Asn1231Ser	0.009	NA	Disease causing	Rare variant
VWF: c.3686 T > G	p.Val1229Gly	0.004	rs61749367	Polymorphism	Rare variant
VWF: c.5783C > G	p.Pro1928Arg	0.002	NA	Polymorphism	Rare variant
VWF: c.2385 T > C	p.Tyr795 =	0.25	rs1063857	Polymorphism	Associated with high plasma VWF [14]
CD46: C.38C > T	p.Ser13Phe	0.003	rs138843816	Polymorphism	Rare variant
MBL2: C.579_580ins AT	p.Gln194IlefsTer6	Not reported	NA	NA	Novel
MBL2: c.584 T > G	p.Phe195Cys	Not reported	NA	Disease causing	Novel

MAF minor allele frequency based on South Asians, *1000G* 1000 Genome database, *ExAc* the Exome Aggregation Consortium database, *SNP* single-nucleotide polymorphism, *NA* not available/applicable

BSP12861 for VWF: c.5783C > G-BSP12861) was incubated at 37 °C overnight. The digested product was resolved on 2% agarose gel and the results were documented using gel imaging system (Bio-Rad Gel Doc-2000) (Fig. 3).

Sanger sequencing

Genotyping for MBL2 variants was performed by Sanger sequencing. The PCR products were purified using EXOSAP IT (Affymetrix) according to the manufacturer's protocol. Sanger sequencing was performed using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) and analysis on ABI 3500 genetic analyzer.

Biochemical analysis

Plasma mannan-binding lectin serine protease 2 (MASP2) and factor-Ba were measured by ELISA in a subset of NCIPH patients and healthy controls using commercially available ELISA kits from (Hycult Biotech, Uden, Netherlands) and MicroVue (Quidel Corporation, San Diego, USA), respectively.

Statistical analysis

Descriptive analysis was performed for the quantitative data using GraphPad prism version 9.0.0 and SPSS. Statistical Package for the Social Sciences (SPSS), Version 21.0 (IBM Corp, Armonk, NY, USA) The Mann-Whitney *U* test was used for quantitative non-parametric variables and Chi-square was used to compare the genotype frequency between the groups. A two-sided *p*-value of < 0.05 was considered significant. The study was approved by the Institutional Review Board and Ethics Committee (IRB MIN No.: 9534, Date: 22.7.2015).

Results

Patient characteristics

Eighty-four patients (M:F: 54:30, age at diagnosis: 30,10-66 years; median, range) with NCIPH were recruited after biopsy confirmation and consent. Most patients underwent liver biopsy either via percutaneous (40) or transjugular (38) route. A total of two cores (1–10 cores; median, range) were obtained with a maximum size of 12 mm (3–23 mm) and containing 10 (5–26) portal tracts for evaluation. Liver biopsy was obtained peroperatively (5) or during explant (1) in a few patients. None of the liver biopsies showed cirrhosis or any other alternative etiology of pre-sinusoidal portal hypertension (e.g. sarcoidosis). The predominant finding was sinusoidal dilatation (68, 81%) in most of these patients. Portal vein ectasia with or without herniation (41, 49%) and obliterative portal venopathy (7, 8%) were also noted. Fibrosis was absent in 26 (31%) and some patients had mild (35, 42%) to moderate (13, 16%) fibrosis with occasional periportal bridging (10, 12%).

Initial presentation was either splenomegaly/hypersplenism (29), incidental (24), variceal bleed (24), ascites (3) or others (4). Associated disorders included immunological/rheumatological disorders (5, selective immunoglobulin A [IgA] deficiency: 2, common variable immunodeficiency: 1, interstitial lung disease: 1, Guillain-Barre syndrome: 1), celiac disease (3) and glomerulonephritis (2) and arsenicosis (1).

Although five patients had a family history of significant liver disease (sibling: 4, parent: 1), none had biopsy-proven NCIPH in the family.

Both NCIPH patients (southern India: 46% and eastern India: 46%) and healthy controls (southern India: 52% and eastern India: 38%) belonged to a similar geographical location.

Targeted sequencing of 11 genes revealed the presence of novel and rare variants in NCIPH patients

Total 589 variants were identified including coding and non-coding variants from all 11 genes tested. Around 30% of variants were in the coding region accounting for 177 variants. The synonymous variants/silent variants (68%) were not considered for further processing. Based on the allelic frequency, 47/177 (27%) coding variants were classified as rare/very rare (minor allele frequency, MAF < 0.001), whereas 4.5% of them were missense variants. Further curation on this data was done based on published literature and rarity of non-synonymous variants and we identified nine variants of interest (Table 1). There were no significant variants of interest identified in other genes tested, namely, *ADAMTS13*, *CFB*, *CFH*, *C3*, *CFI*, *MTHFR*, *KCNN3* and *DGUOK*.

Additionally, we identified other polymorphisms that have been associated with either NCIPH or various other disorders such as hemolytic-uremic syndrome, Upshaw-Schulman syndrome, thrombotic thrombocytopenic purpura and age-related macular degeneration (Supplementary Table 1).

Sanger/RFLP confirmation of the seven identified variants of interest

To evaluate the significance of these variants detected by targeted NGS in 54 NCIPH cases, we analyzed the frequency of the above-mentioned nine variants against publicly available databases. Seven of these variants were tested in 84 NCIPH cases and geographically matched 103 healthy controls (Table 2).

MBL2 pathogenic and rare variant probable modifier or predisposing factor in NCIPH

A compound heterozygous variant in *MBL2* gene was identified in 31-year-old male who presented with psoriasis

Table 2 Comparison of minor allele frequency of the identified variants between patients with idiopathic non-cirrhotic intrahepatic portal hypertension and geographically matched healthy controls

Variant	Cases (MAF)	Controls (MAF)	OR (*p*-value)	MAF (1000G or ExAc)
VWF: c.2365A > G	84 (0.32)	103 (0.47)	**1.7 (0.02)**	0.22
VWF: c.7624A > G	84 (0.07)	103 (0.009)	**7.8 (0.03)**	0.0005
VWF:c.3692A > G	54 (0.05)	-	-	0.009
VWF: c.3686 T > G	54 (0.03)	-	-	0.004
VWF: c.5783C > G	84 (0.02)	103 (-)	- (0.31)	0.002
VWF:c.2385 T > C*	84 (0.32)	103 (0.47)	**1.7 (0.02)**	0.25
CD46: C.38C > T	84 (0.04)	103 (-)	- (0.04)	0.003
MBL2:C.579_580ins AT	64 (0.007)	103 (-)	NA	Novel
MBL2: c.584 T > G	64 (0.007)	103 (-)	NA	Novel

*VWF: c.2385 T > C was in linkage disequilibrium with VWF: c.2365A > G

Bold signifies significant *p*-value

MAF minor allele frequency, *OR* odds ratio, *1000G* 1000 Genome database, *ExAc* the Exome Aggregation Consortium database

and hypersplenism. His sibling had died due to jaundice at seven years of age.

A heterozygous two base pair insertion c.579_580 insAT in exon 4 resulted in frameshift and premature truncation of protein six amino acids downstream to codon 194 (p.Gln194IlefsTer6). Another heterozygous missense variant, c.584 T > G in exon 4 of MBL2 gene, resulted in substitution of cysteine instead of phenylalanine at codon 195 (p.Phe195Cys) in this patient. These variants were confirmed by Sanger sequencing (Fig. 2). Both of these novel variations were

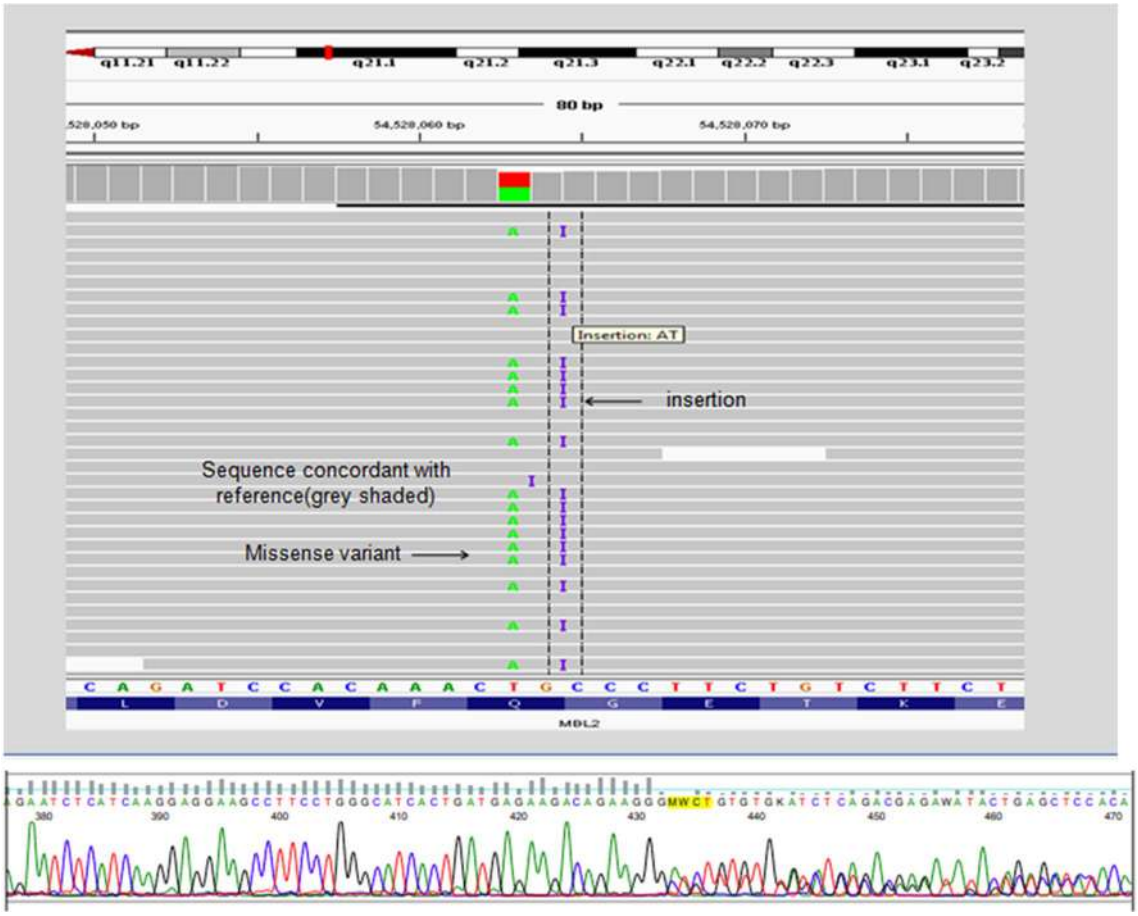

Fig. 2 Next-generation sequencing reads showing compound heterozygous *MBL2* gene variants and corresponding Sanger confirmation

predicted to be damaging and not been reported previously in gnomAD or ExAc databases. Based on this evidence, the variants were classified as variants of uncertain significance (VUS).

To investigate the relevance of these variants, we screened controls and compared the frequency with cases. As the variants in *MBL2* gene c.579_580ins AT (Fig. 2) and the missense variant c.584 T > G (p.Phe195Cys) were detected neither in controls nor in other NCIPH patients, these were reclassified as likely pathogenic. The same patient also had heterozygous Gly54Asp polymorphism in MBL2 gene, which has been associated with low MBL2 level [15].

In addition, MBL2 polymorphisms (Arg52Cys, Gly54Asp and Gly57Glu) were detected in 27% of NCIPH cases ($n = 54$). Four patients had Arg52Cys variant (one homozygous and 3 heterozygous), eight patients were heterozygous for Gly54Asp and three cases had Gly57Glu (one was homozygous). All these polymorphisms have been shown to be associated with low serum MBL level suggesting a probable role of MBL2 variants in pathophysiology of the disease [16, 17].

VWF-ADAMTS13 and CD46 rare variants in NCIPH

In this study on NGS ($n = 54$), the proportion of VWF polymorphic/rare variants were higher (51%; 14/27) in frequency compared to other genes tested that could be attributed to the size of the gene.

The frequency of *VWF* variant c.2365 A > G (p.Thr789Ala) was observed significantly higher in control compared to that in NCIPH patients though four homozygotes were present in the NCIPH cases (Fig. 3). Another *VWF* variant c.2385 T > C, known to be in linkage disequilibrium with c.2365A > G variant, followed the same haplotype (confirmed by sequencing in few samples).

The variant *VWF* c.7624 A > G (p.Ile2542Val) was homozygous in one NCIPH case and the heterozygotes were significantly higher in cases compared to those in controls ($p = 0.05$). Another *VWF* variant c.5783C > G (p.Pro1928Arg) was heterozygous in two NCIPH cases and absent in controls.

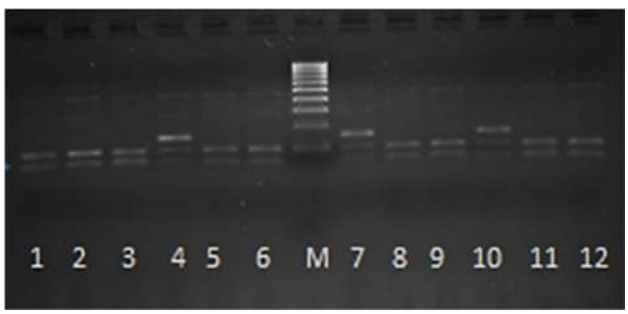

Fig. 3 RFLP using RsaI enzyme for VWF variant c.2365 A > G (p.Thr789Ala); wild-type—two bands of 100 and 55 bp; and heterozygous—three bands of 155, 100, and 55 bp (1–12 samples, M-100 bp size ladder)

The variant *CD46* c.38C > T (p. Ser13Phe) was found to be present only in NCIPH cases (three heterozygous and one homozygous) and not in controls.

Two rare variants (in one patient each) in *ADAMTS13* c.3829C > T and c.1382C > A were noted on NGS of NCIPH cases ($n = 54$). ADAMTS13 c.3829C > T variant was reported in the gnomAD database with a frequency of 0.00002 and classified as VUS [14]. The other heterozygous missense variant (c.1382C > A [p.Ser461Tyr]) identified in ADAMTS13 gene for one NCIPH patient was novel and predicted to be likely pathogenic. This patient also carried other two ADAMTS13 polymorphic variants, rs1055432 and rs2301612, which have been previously reported in association with macular degeneration. However, the significance of these polymorphic variants along with likely pathogenic variant has not been investigated further.

Segregation of multiple rare variants

We identified two NCIPH cases with six VWF variants, which included two rare variants (p.Asn1231Ser and p.Pro1928Arg). Another NCIPH case had eight VWF variants, including two rare VWF variants (p.Asn1231Ser and p.Pro1928Arg). Two VWF variants were present in one patient (p.Asn1231Ser and p.Val1229Gly) along with other six common VWF variants. The aggregation of multiple variants and its effect on plasma VWF levels need to be studied further.

Plasma levels of MASP2 and factor-Ba in cases and controls

MASP2 and factor-Ba levels were measured in plasma samples from 35 NCIPH patients and 15 adult healthy volunteers. The median plasma MASP2 levels were significantly lower in NCIPH patients (661.80 [225.43–1953.20] ng/mL) compared to those in healthy controls (1608.75 [337.02–3514.45], $p < 0.01$). No significant difference was seen in plasma factor-Ba levels between NCIPH patients (666.16 [359.66–2648.40] µg/mL) and healthy controls (720.60 [490.84–1538.86] µg/mL).

Discussion

NCIPH is a microangiopathy affecting portal microvasculature, possibly driven by a variety of gut-related factors. In this study, we investigated the association of genetic variants in microangiopathy-related pathways (VWF-ADAMTS13 and complement pathways). In this first of a kind comprehensive gene panel study, we identified important variants in *MBL2*, *CD46* and *VWF* genes associated with NCIPH.

In addition, NGS-based genetic analysis undertaken on large series of NCIPH patients (none had significant family history of NCIPH) was negative for previously described mutations

in KCNN3 and DGUOK genes [10, 11]. This suggests that KCNN3 and DGUOK mutations, shown to be associated with familial NCIPH, are not associated with sporadic NCIPH.

NCIPH appears to be a complex disorder with both environmental and genetic factors involved in its pathogenesis. Recent data showed the presence of rare *ADAMTS13* and *VWF* variants in association with deep vein thrombosis and further studies are needed to delineate the role of genetic variants and implication for therapeutic intervention [18]. Previous studies have shown that VWF variants, including rs1063856, are associated with high VWF antigen level in coronary heart disease [19].

In a study, 1/3rd of the NCIPH patients developed complement-mediated glomerulonephritis and alternative complement pathway activation after splenorenal shunt [20]. Mutations in proteins that regulate complement or promote amplification of its alternative pathway are known to have a predisposing effect to various thrombotic microangiopathies [21].

In a single young patient with previous history of psoriasis, we found compound heterozygous mutation in MBL2 gene, absent in other NCIPH patients and controls. Mannose-binding lectin recognizes and binds mannose that is found on the surface of microorganisms and activates the complement system as the first-line innate immune mechanism. Individuals with low mannose-binding lectin are prone for recurrent infections [16]. Omanwar et al. described in a rabbit model the development of NCIPH after repeated injections of *E. coli* into portal system [22]. A quarter of NCIPH patients had MBL2 polymorphisms (Arg52Cys, Gly54Asp and Gly57Glu) previously shown to be associated with low mannose-binding protein. This reaffirms a probable role of MBL2 in disease pathogenesis and needs to be explored further.

In the present study, MASP2 levels were significantly reduced in NCIPH patients compared to healthy controls. Low levels of MBL/MASP2 signaling have been associated with increased risks of infection and poor outcome in liver diseases [23, 24]. It is also reported that if MASP-2 is non-functional/low, the complement cascade would be interrupted and it can contribute to microbial persistence or predisposed to infections. In contrast to the above finding, factor-Ba, which is a component of the alternative pathway, was found to be similar in patients as compared to healthy controls.

There is evidence suggesting that ADAMTS13 deficiency may be a primary event involved in the pathogenesis of NCIPH [25]. Additionally, the release of pro-inflammatory cytokines in response to the chronic inflammatory stimulus from the intestine can activate the endothelium to release VWF into the portal circulation. Mutations in ADAMTS13, which results in low enzyme activity, lead to thrombotic thrombocytopenic purpura (TTP) and are also shown to be associated with higher risk of pediatric stroke, chronic thromboembolic pulmonary hypertension, etc. [26].

In contrast, commonly described mutations in VWF are associated with bleeding symptoms causing von Willebrand disease, a common inherited bleeding disorder. We report a series of variants in VWF gene which is associated with NCIPH, a microangiopathy of portal venous radicles. The frequency of VWF variants c.7624A > G, c.3692A > G and c.3686 T > G is found to be higher in patients compared to that in control, suggesting the importance of investigating such variants in disease modulation and pathogenesis.

We reported a single NCIPH patient with ADAMTS13 missense variant (c.3829C > T [p. R1277W]), resulting in severe persistent ADAMTS13 deficiency and high VWF [5, 25]. Immuno-staining of his liver biopsy revealed globules of ADAMTS13 within stellate cells explaining defective ADAMTS13 secretion and increased VWF in this case [7].

In this study, the proportion of VWF polymorphic/rare variants were higher (51%; 14/27) in frequency compared to other genes tested that could be attributed to the size of the gene. In addition, clustering of variants in VWF and CD46 (VWF: c.5783C > G, CD46: C.38C > T) that were not observed in controls suggests a synergetic effect and genetic susceptibility to disease. The two VWF variants c.2365 A > G and c.2385 T > C reported to be associated with high circulating VWF level were observed in higher frequency in healthy controls compared to patients.

Although NCIPH is a common cause for portal hypertension in India, its pathogenesis remains unclear. Studies have alluded to the importance of often associated enteropathy and other environmental factors in pathogenesis, but the role of genetic susceptibility in Indian population has not been studied. In this comprehensive and targeted genetic study, we identify a possible role of genetic variants in *MBL2*, *ADAMTS13*, *VWF* and *CD46* genes either individually or synergistically in predisposing or modulating the clinical sequel in NCIPH.

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Author contribution RA, AC, UZ, EE, JJ, KAB, SCN, NT, CEE and AG contributed to the study concept and design. UZ, AG and CEE recruited the patients and obtained informed consent. RA, KP, AC, BV, DD, JJ, KAB, SCN and NT performed the laboratory experiments. TAK and BR contributed to the pathology part. All were involved in data collection, interpretation and conclusion, preparation of the manuscript, critical revision and review of the manuscript and approved the final version of the manuscript.

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Declarations

Conflict of interest RA, KP, AC, BV, UZ, EE, TAK, DD, JJ, KAB, SCN, NT, BR, CEE and AG declare no competing interests.

Consent to participate Obtained from all study participants.

Ethics statement The study was performed conforming to the Helsinki Declaration of 1975, as revised in 2000 and 2008 concerning human

and animal rights, and the authors followed the policy concerning informed consent as shown on Springer.com.

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