Research article

Possible effects of ancestry-related oxysterol-binding protein-like 10 genetic polymorphisms on dengue virus replication and anti-dengue immune response

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A B S T R A C T

Purpose: Oxysterol-binding protein-like 10 (OSBPL10) gene has been associated with reduced susceptibility to severe dengue in individuals of African descent. The aim of this study was to determine the possible effect of OSBPL10 on dengue virus (DENV) replication as well as the impact of African and European haplotypes of six OSBPL10 small nuclear polymorphisms (SNPs) on dengue multiplication and innate immune response.

Methods: We conducted gene knockdown experiments targeting OSBPL10 in THP-1 and Huh-7D12 cell lines, followed by a DENV-2 replication assay. Extracellular viral load was determined using qRT-PCR. To investigate the impact of SNPs haplotypes on viral replication and gene expression we cultured peripheral blood mononuclear cells (PBMC) from individuals with homozygous African and European haplotypes of OSBPL10 with DENV-2. Individual genotyping was performed using High Resolution Melt (HRM) analysis. The level of viral replication was assessed through plaque assay, while RT-PCR was employed to determine the expression levels of RXR-α, IFN-γ, IL-10 and IL-8 genes.

Results: In vitro OSBPL10 knockdown significantly reduced DENV-2 replication. Individuals carrying European haplotypes showed higher DENV titers along with elevated levels of RXR-α and IL-8 mRNA compared to those carrying African haplotypes, who exhibited lower viral loads alongside increased IFN-γ and IL-10 expression.

Conclusions: Our findings further explore the role of OSBPL10 in DENV multiplication, immune response to infection. The European haplotypes of OSBPL10 appear to increase DENV replication and promote RXR-α and IL-8 mRNA expression which correlates with the suppressive effect of these mediators on type I IFN, promoting viral replication and a deficient antiviral response. In contrast, the African haplotype showed a reduction in DENV replication and enhanced IFN-γ and IL-10 mRNA expression, which could be related to the better management of dengue infection and the low frequency of severe disease in this ethnic groupe.

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Abbreviation list

OSBPL10   Oxysterol-binding protein-like 10
DENV   Dengue virus
SNP   Small nuclear polymorphism
PBMC   Peripheral blood mononuclear cells
RXR-α: Retinoid X receptor alpha
IFN-γ: Interferon gamma
IL-10   Interleukin 10
IL-8   Interleukin 8
DSS   Dengue Shock Syndrome
DF   Dengue Fever
OSBP   Oxysterol-binding proteins
PFU   Plaque forming units
HPI   Hours post infection
LDL: Low density lipoproteins
HMG-CoA   β-Hydroxy β-methylglutaryl-CoA
MAPKs   Mitogen-activated protein kinases
JNK   c-Jun N-terminal kinases
LXR   Liver X receptor
RXR   Retinoid X receptor

1. Introduction

Dengue is an arthropod-borne flavivirus disease caused by infection with any of the four dengue viruses (DENV-1 to DENV-4) [1]. The virus is transmitted to humans by female mosquitoes of the species *Aedes aegypti*, less commonly *Aedes albopictus*, and a few *Aedes* species [2]. DENV infection may result in a wide spectrum of clinical manifestations, from mild flu-like syndrome, referred to as dengue fever (DF), to potentially life-threatening dengue shock syndrome (DSS). Only a small proportion of infected individuals develop the severe form, whereas the vast majority suffer from asymptomatic infection or mild disease [3].

Differential susceptibility to disease severity indicates that host genetics may influence infection outcomes, acting in a complex interplay with viral and environmental factors [4]. An interesting research field in the study of DENV pathogenesis is the exploration of the interconnection between the immune system and the vir us [5], level at which lipids appear to perform relevant functions [6]. DENV infection activates pro-inflammatory gene patterns at the interface of innate immunity, inflammation, and host lipid metabolism [7]. In this regard, six polymorphisms in the oxysterol-binding protein-like 10 (OSBPL10) gene, which encodes a protein that functions in pathways that link lipid metabolism with immune response, have been associated with reduced susceptibility of Cuban people of African descent to severe dengue. Interestingly, individuals homozygous for the African SNPs haplotype of the OSBPL10 gene have been shown to have significantly reduced OSBPL10 mRNA expression when compared with individuals homozygous for the European haplotype [8].

OSBPL10 is a member of the oxysterol-binding protein (OSBPs) family, which is a cytosolic receptor for oxysterols. OSBPs have been implicated in signal transduction, vesicular transport, lipid metabolism [9–11] and viral infection and replication [12–14]. OSBPs are important for the formation of membrane-bound viral replication organelles (RO), which are formed at the ER-Golgi interface [15]. During DENV infection, modulation of host cholesterol levels facilitates viral entry, replicative complex formation, assembly, egress, and control of interferon response [16]. In this study, we investigated the influence of OSBPL10 knockdown on DENV-2 replication as well as the impact of the African and European SNPs haplotypes of the OSBPL10 gene on DENV-2 replication and the innate immune response.

2. Materials and Methods

2.1. Cells and viruses

Cell lines Huh-7D12 (ECACC 01042712), THP-1 (ECACC 88081201), and BHK-21 clone 15 (Syrian golden hamster kidney cells) were grown in Minimum Essential Medium, RPMI-1614 Medium and Dulbecco's Modified Eagle's Medium respectively. The media were supplemented with 10 % fetal bovine serum, sodium bicarbonate, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, L-glutamine, and non-essential amino acids. All cells were maintained at 37 °C with CO_2.

Cell infections were carried out using DENV-2 strain A-15 (isolated in Cuba, 1981) with three passages on mouse brain and four passages on *Aedes albopictus* mosquito cell line C6/36HT. We utilized DENV-2 in all our experiments as multiple studies have indicated that DENV-2 is associated with more severe disease; however, additional research is required to explore the other viral serotypes.

2.2. OSBPL10 knockdown and viral infection in Huh-7D12 and THP-1 cell lines

OSBPL10 knockdown by transient transfection using small interfering RNA (siRNA) was performed in Huh-7D12 and THP-1 cell lines. We selected these cell lines, THP-1 and Huh-7D12, as they are derived from monocytes and hepatocytes, respectively, which are known to be cellular targets of the virus during natural infection. Transfection of siRNA into Huh-7D12 and THP-1 cell lines was conducted using HiPerfect Transfection Reagent (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The siRNA specific for OSBPL10 was purchased from FlexiTube GeneSolution (Qiagen, Hilden, Germany). 24 h prior to transfection, TPH-1 and Huh-7D12 cells were subcultured in 24-wells plates at a density of 2×10^5 cells/well. The transfection reagents were used as follows, 35.7 ng of siRNA and 3 μl of HiPerFect Reagent for Huh-7D12 cells and 750 ng of siRNA and 6 μl of HiPerFect Reagent for THP-1 cells. AllStars Negative Control siRNA (Qiagen, Hilden, Germany) was used as the negative control. Cells were incubated overnight under normal growth conditions. The efficacy of transfection was evaluated using qRT-PCR.

After the OSBPL10 knock-down, silenced-treated, non-treated and negative silenced Huh-7D12 and THP-1 cells were infected with DENV-2 with a multiplicity of infection (MOI) = 1. The cellular supernatant was collected at 24 h post-infection (hpi) for use in viral load determination.

2.3. Viral load determination

For determining whether DENV-2 replication was affected by OSBPL10 gene knockdown transfected and non-transfected cells were infected with a MOI = 1 and the viral load in supernatants was determined 24 hpi by qRT-PCR. Cell lines treated with negative control siRNA were included in this experiment to rule out the possible influence of the reagents used for gene silencing on virus replication. RNA was extracted using the QIAamp Viral RNA Extraction Kit (Qiagen, Hilden, Germany) and amplified using the TaqMan Assay [17]. Standard curves were obtained using titrated DENV-2 supernatants serially diluted from 106 to 10 PFU/ml.

2.4. Subjects and samples

Peripheral blood samples from 40 healthy adult volunteers selected according to ethnic background and were obtained using acid citrate dextrose vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA). The study was conducted according to the Helsinki Declaration as a statement of ethical principles for medical research involving human subjects and was approved by the Institutional Ethical Review Committee of the Institute of Tropical Medicine "Pedro Kourí" (IPK) and by the Ethical Committee of the Cuban National Academy of Sciences. Reference code: CEI-IPK 26-17. Written informed consent was obtained from all the participants.

DNA was extracted from peripheral blood using QIAamp DNA Blood Mini kits (Qiagen, Hilden, Germany), according to the manufacturer's instructions. All DNA samples were quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA).

2.5. SNP Selection and genotyping

The SNPs used (rs4600849, rs975406, rs11129475, rs6419811, rs7639637, and rs11718700) were chosen according to a previous study by Sierra et al. [8], who identified several polymorphisms in the OSBPL10 gene associated with African ancestry, lower OSBPL10 mRNA expression levels and protection against severe dengue forms (See Table 1). SNPs entries for the gene studied are in the National Center for Biotechnology Information Single Nucleotide Polymorphism database, which is publicly available. We designed primers using primer3 online tool (http://primer3.ut.ee/).

DNA amplification followed by HRM was performed on a Rotor-Gene (RG6000, Corbett & CO, Teddington, United Kingdom). HRM was performed and optimized according to the protocol described by Ref. [18]. Each reaction consisted of a 1X Type-it HRM PCR kit (Qiagen, Hilden, Germany), 0.35 μM forward and reverse primers (see Table 2), and RNase-free water in a final volume of 15 μL, using 30 ng of genomic DNA. The reactions were incubated at 95 °C for 5 min and then subjected to an initial step at 95 °C for 10 s, an SNP-specific incubation time and annealing temperature, and a final step at 72 °C for 10 s. Melting-curve data were generated by increasing the temperature with a SNP-specific ramp at 0.1 °C/s. HRM curve analysis was performed using the provided software and HRM algorithm (Rotor-Gene, version 1.7.27). Normalization regions and a confidence threshold of 90 % were applied, and profiles giving an identity of <90 % were considered as distinct profiles. Prior to the HRM assay, identification of positive controls was performed by Sanger sequencing. Sequencing data also served to validate the accuracy of HRM genotyping results.

Table 1
OSBPL-10 SNPs location and Allele haplotype for the African and European variants.

Haplotype SNP	Position	African Haplotype Allele	European Haplotype Allele
rs4600849	32027672	C	T
rs11129475	32030544	T	C
rs6419811	32031135	G	A
rs11718700	32033248	C	A
rs975406	32035587	C	T
rs7639637	32036042	C	A

Table 2
SNPs genotyping primers for High Resolution Melt analysis.

SNP	Primer forward	Primer reverse
rs4600849	5′-GAGAAGCAGTACAGGTGTTTGG-3′	5′-ATTGTGTTCAGGCCCTGTG-3′
rs975406	5′-CCCTCAACTGGGGAATGAAT-3′	5′-CTGGCTTCTTTCACTGAACAT-3′
rs11129475	5′-CAGATCGCCTCAGTGAAGGT-3′	5′-TCCTCTTCTGAGCTGCTTCC-3′
rs6419811	5′-CAGACCAAAGGGAAAGTTGG-3′	5′-TGGATTTTGGGCCTAGAAGA-3′
rs7639637	5′-GGATGGATGGATGGTCTGC-3′	5′-CTGCACACCTACCCAAAAGA-3′
rs11718700	5′-AATACAGATGCCTACCCTGAAA-3′	5′-TGGTAGTGAGAAAGATCCCAAAA-3′

2.6. PBMC isolation and viral infection

Dengue virus enters blood stream via lymphatics and peripheral blood mononuclear cells (PBMC), especially monocytes, support virus infections. Thus, study of dengue virus - PBMC interaction is important to understand the pathogenesis of dengue virus infections [19]. Peripheral mononuclear cell (PBMC) samples were isolated from the blood of individuals with the homozygous haplotype for the European and African variants of the OSBPL10 gene using Ficoll-Histoplaque density gradient centrifugation. Cells were then infected with DENV-2 at a MOI = 1. Freshly isolated PBMCs were then infected in triplicates for 90 min at 37 °C and 5 % CO_2. The medium was replenished and incubated for 24 h at 37 °C in an incubator with 5 % CO_2, followed by harvesting of cells and supernatants for gene expression analysis and viral quantification by plaque assay.

2.7. Gene expression analysis

DNase-treated total RNA was isolated from PBMC using a RNeasy Mini Kit (Qiagen, Hilden, Germany). The mRNA expression levels of the cytokines IFN gamma, IL-8, IL-10 and the lipids mediators RXR-alpha and OSBPL-10 were determined by RT-PCR (LightCycler 2.0 instrument, Roche), using a LightCycler RNA Master SYBR Green I kit (Roche Diagnosis, Basel, Switzerland) and designed primers. Each sample was duplicated and the mean Ct values were normalized to the average Ct value of the housekeeping gene. Assay
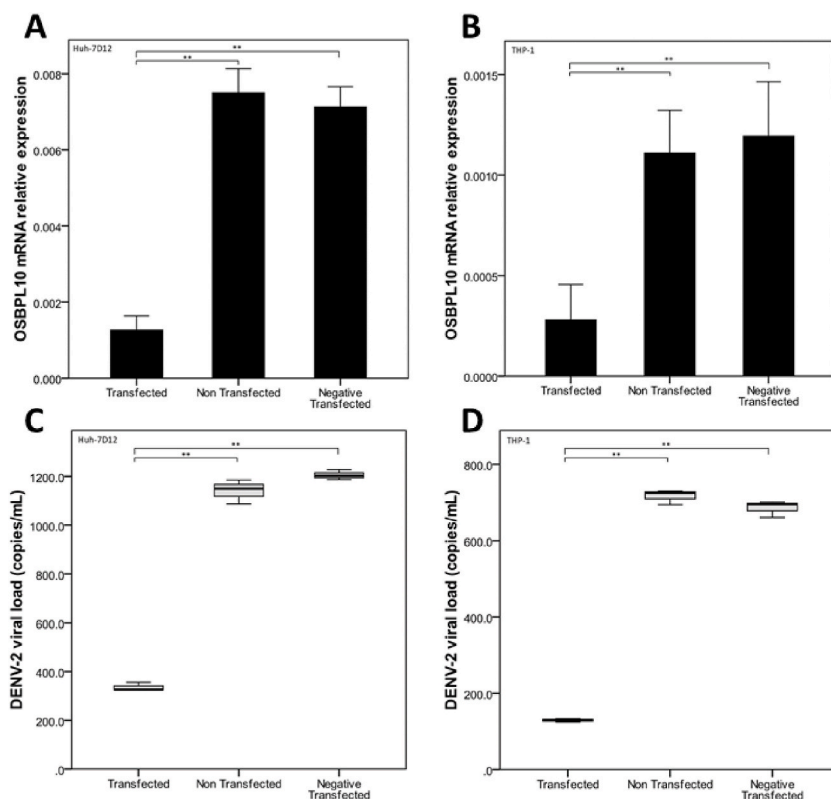
Fig. 1. OSBPL10 knockdown and its effect on DENV-2 replication. OSBPL10 mRNA expression in the transfected, non-transfected and negative transfected Huh-7D12 **(A)** and THP-1 **(B)** cell lines. Viral load (copies/ml), measured extracellularly at 24 hpi in the transfected, non-transfected and negative transfected Huh-7D12 **(C)** and THP-1 **(D)** cell lines. The detection limit of the qPCR assay was 110 copies per mL. The ** symbol indicates that the two-tailed t-test p-values for comparisons are significant.

specificity was evaluated using a melting curve analysis.

2.8. Viable virus Titration using plaque assay

Virus titer was quantified as viable virus in the supernatant using plaque assay method. Briefly, 24-well plates were seeded with BHK21 cells in RPMI complete medium at a density of 2×105 cells/well, and incubated overnight at 37 °C in an incubator with 5 % $CO2$. Cell monolayers were infected with 100 μl of the tenfold serially diluted supernatant from the PBMCs infection assay, and the virus was allowed to adsorb for 60 min at 37 °C and 5 % $CO2$. The inoculated wells were then aspirated and replenished with the addition of 0.5 ml of 1 % carboxymethylcellulose (CMC, Merck) overlay. The plates were incubated for five days at 37 °C and 5 % $CO2$. The cells were then fixed and visualized by crystal violet staining. Plaques were counted and calculated by incorporating the dilution factor and titer then expressed as PFU/ml.

2.9. Statistical analysis

All the experiments were performed using triplicates. Data are expressed as mean ± SD. P-values were calculated using appropriate statistical tests, including a two-tailed unpaired t-test and one-way ANOVA. The results were analyzed using the SPSS software (version 13.0). $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. OSBPL10 knockdown in Huh-7D12 and THP-1 cell lines

OSBPL10 knockdown was efficient in both cell lines, as measured by mRNA expression (Fig. 1A and B).

No significant differences in the OSBPL10 expression were observed among THP-1/non-transfected and THP1/negative transfected (mean values of 1.10E-3 and 1.19E-3, respectively; $p = 0.35$) as well as in Huh-7D12/non-transfected and Huh-7D12/negative transfected (mean values of 7.50E-3 and 7.13E-3, respectively; $p = 0.121$). A significant reduction in OSBPL10 expression levels was observed in both transfected cell lines, THP1/transfected (mean of 2.79E-4; $p < 0.00001$ compared with THP-1/non-transfected and THP1/negative transfected) and Huh-7D12/transfected cells (mean of 1.27E-3; $p < 0.00001$ compared with THP-1/non-transfected and THP1/negative transfected).

3.2. Quantification of viral RNA load in supernatant of Huh-7D12 and THP-1 cell lines

DENV2 infection assays in these OSBPL10-knockdown cell lines provides evidence that down-modulation of OSBPL10 affects in a direct way DENV replication (Fig. 1C and D). Significant ($p < 0.000001$) viral load reductions were observed in the supernatant of both OSBPL10 knockdown cell lines (mean values of 336 copies/ml in Huh-7D/transfected and 129 copies/ml in THP-1/transfected) in
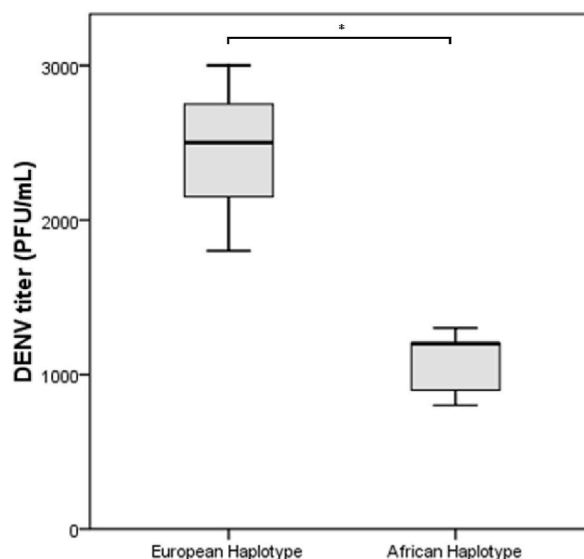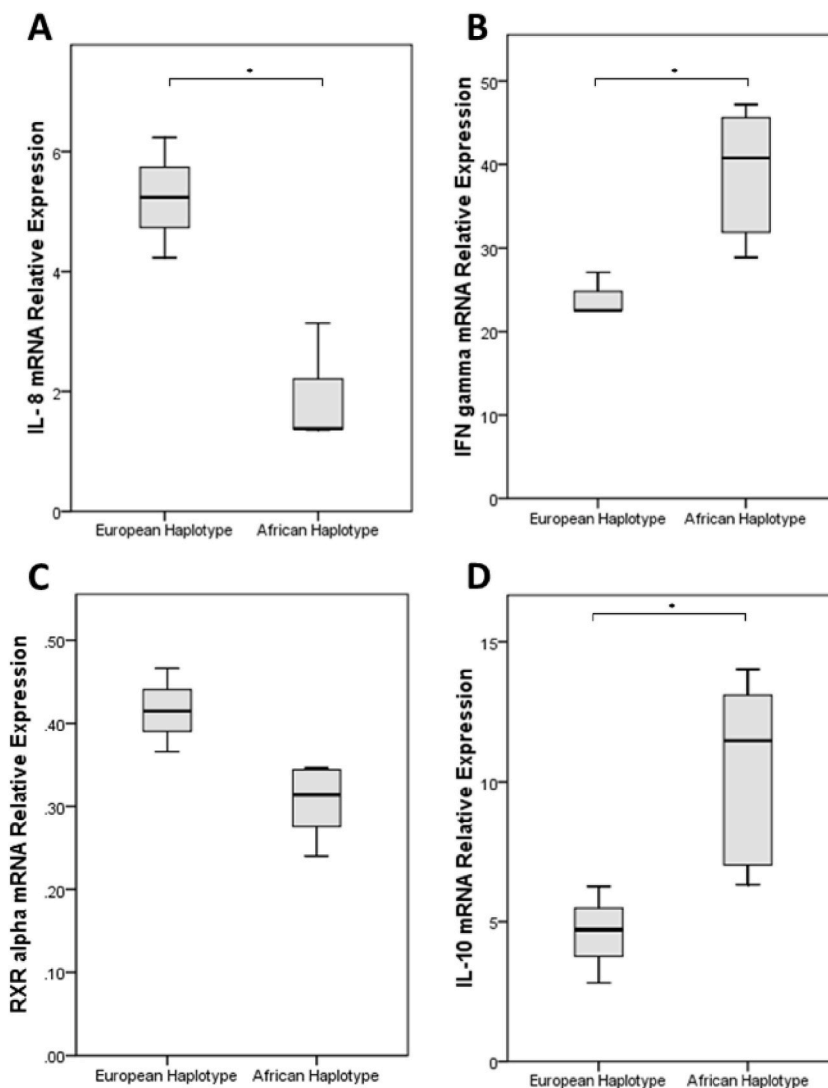

Fig. 2. DENV-2 titers 24 hpi in PBMCs from homozygous individuals for the African and European SNPs haplotypes of OSBPL10 gene. DENV replication assay indicates that there is a diminution in viral titer in the group of individuals with the African haplotype (mean value of 1080 PFU/ml) in comparison with the group with the European haplotype (mean value of 2430 PFU/ml). The * symbol indicates that the two-tailed t-test p-values for comparisons are significant.

contrast with the non-transfected and negative transfected cell lines (mean values of 1140 copies/ml in Huh-7D/non transfected, 1205 copies/ml in Huh-7D/negative transfected and 716 copies/ml in THP-1/non transfected, 685 in THP-1/negative transfected)

3.3. Quantification of viable DENV-2 infective particles in supernatants of PBMC from homozygous individuals for the African and European SNPs haplotypes of OSBPL10 gene

As a result of SNPs genotyping (data not shown), we identified eight individuals homozygous for all the six African and European SNPs haplotypes. Three individuals completely homozygous for the European SNPs variants and five individuals completely homozygous for the Africans SNPs variants. In the PBMCs of these individuals, we performed a DENV-2 replication assay to assess the possible influence of these variants on the dengue virus replication process. Viable DENV-2 particles were quantified in the supernatants of infected PBMCs at 24 hpi using a plaque assay.

Interestingly, the group of individuals with the African haplotype showed lower titers of DENV-2 infective particles (mean value of 1080 PFU/ml (p = 0.003)) compared to the group of individuals with the European haplotype (mean value of 2430 PFU/ml) (Fig. 2).

Fig. 3. Cytokines and RXR-α mRNA expression 24 hpi in DENV-2 infected PBMC from homozygous individuals for the African and European SNPs haplotypes of OSBPL10 gene. Individuals of the group carrying the African haplotype showed higher relative expression levels of IFN-γ (mean value of 38.86) and IL-10 (mean value of 10.38). Individuals of the group carrying the European haplotype showed higher relative expression levels of IL-8 (mean value of 5.23) and RXR-α (mean value of 0.41). The * symbol indicates that the two-tailed *t*-test p-values for comparisons are significant.

3.4. Quantification of cytokines and nuclear receptor RXR-alpha relative expression in DENV-2 infected PBMC from homozygous individuals for the African and European SNPs haplotypes of OSBPL10 gene

To determine whether there was any correlation between the SNPs haplotypes and the differential expression of immune response genes during DENV-2 infection, cytokine quantification by qRT-PCR in the DENV-2 infected PBMCs from individuals with the African and European SNPs haplotypes of OSBPL10 was performed, including the expression of the nuclear receptor RXR-α. The group with the African haplotype showed higher relative expression levels of IFN-γ (mean value of 38.86 (p = 0.025)) and IL-10 (mean value of 10.38 (p = 0.021)) in comparison with the other group (IFN-γ mean value of 24.04 and IL-10 mean value of 4.59) (Fig. 3A and C). In contrast, the group with the European haplotype showed higher relative expression levels of IL-8 (mean value of 5.23 (p = 0.011)) and RXR-α (mean value of 0.41 (p = 0.035)) when compared with the African haplotype group (IL-8 mean value of 1.89 and RXR-alpha mean value of 0.30) (Fig. 3B and D). In the remaining cytokines assessed, no statistically significant variances were observed (data not shown).

4. Discussion

Oxysterol-binding proteins play crucial roles in the replication processes of various viruses, as indicated by previous studies [12–14]. Notably, OSBPs are known to be involved in the maturation, assembly, and secretion of hepatitis C virus within the Flaviviridae family [12]. Additionally, it has been reported that OSBP plays a role in dengue virus multiplication by regulating lipid homeostasis and facilitating proper distribution of cholesterol for the formation of dengue virus replication complexes [14].

In our study, upon silencing OSBPL10 in THP-1 and Huh-7D12 cell lines, we noted a significant decrease in the multiplication of DENV-2 as a consequence of reduced OSBPL10 expression levels. Previous research has suggested that ORP10, the product of the OSBPL10 gene, plays a role in influencing the production of triglycerides and cholesterol, as well as the formation of low-density lipoprotein (LDL), specifically in *in vitro* settings [20]. The requirement for cholesterol in DENV replicative cycle has been documented for several cell types. For example, it has been reported that lipid rafts, membrane microdomains rich in cholesterol and sphingolipids, play an important role in DENV entry in macrophages, either in the absence or in the presence of facilitating antibodies [21–23]. Furthermore, the inhibitory effect of an intracellular cholesterol transport inhibitor on DENV entry and replication in BHK-21 cells has also been reported [24]. When considering these collective findings alongside our own, it is plausible to suggest that decreased levels of OSBPL10 expression, and consequently lower levels of LDL and cholesterol, could impact the replication of DENV.

We further examined the impact of six SNPs located in weak enhancers on the regulatory region of the OSBPL10 gene on DENV replication. Our findings revealed a significant decrease in DENV replication among individuals carrying the African haplotype (low expression) compared to those with the European haplotype (high expression). In vitro evidence has demonstrated that OSBP overexpression leads to an increase in the mRNA levels of several sterol-regulated genes (LDL receptor, HMG-CoA reductase, and HMG-CoA synthase), along with enhanced cholesterol biosynthesis [25]. The increased HMG-CoA reductase activity, leading to elevated cholesterol levels in the endoplasmic reticulum, is essential for the formation of replicative complexes, thereby facilitating DENV replication [26]. Also, during the early stages of DENV replication, the activation of MAPKs such as JNK, depend on the presence of intact lipid rafts and cholesterol. Inhibition of JNK by the disruption of lipid rafts resulted in a notable decrease in viral protein synthesis and viral yield [27]. Taking into consideration the aforementioned evidence, one might speculate that the group of individuals with elevated OSBPL10 expression (European Haplotype) may possess more optimal conditions for an efficient DENV replication compared to the group with diminished expression (African Haplotype). Our findings are in line with previous research, which has shown lower levels of DENV multiplication in individuals of African ancestry compared to those of European ancestry [28]. They also support epidemiological observations indicating a low incidence of severe dengue fever among individuals of African descent in regions with black populations such as Cuba, the Caribbean, and Africa [29–31].

Cholesterol metabolism has been confirmed to interact with the immune system [32]. The link between cholesterol metabolism and immunological response and inflammation has been a "hotspot" for research since the millennium, giving rise to the concept of immunometabolism" [33]. Furthermore, OSBPL10 gene have been linked to the LXR/RXR activation pathway which is associated with cholesterol metabolism and cytokine production in macrophages [34–36]. Oxysterols are the classical activators of LXR/RXR [37,38] and studies indicate that LXR/RXR activation upregulates lipid metabolism and inhibits inflammatory response [39]. Interestingly, our study revealed elevated levels of RXR-α and IL-8 and in the European haplotype group. RXR-α has been associated with severe dengue [8] and interacts with OSBPL10 in the LXR/RXR activation pathway [34–36]. The regulation of macrophage immune functions as well as lipid metabolism is controlled by RXRs through processes such as apoptotic cell uptake, β-amyloid clearance, inflammation modulation, pathogen elimination, cholesterol transport, lipid handling and innate inflammatory response enhancement via chemokine up-regulation [40]. The RXRα protein has been found to decrease the immune response against viruses by inhibiting the production of type I interferon and its downstream genes [41]. Additionally, LXR/RXR, agonists have been shown to promote IL-8 production [42]. Therefore, our observation of higher RXR-α in the group of individuals with the European OSBPL10 haplotype are consistent with a higher IL-8 mRNA expression in this same group. IL-8 is a cytokine that plays a significant role in inflammation by inducing the release of lytic enzymes and leukotrienes, which contribute to inflammatory reactions and neutrophil infiltration [43]. Moreover, IL-8 can weaken the inhibitory effect of IFN-α on early viral replication [44]. This is consistent with our study, where we observed that individuals carrying the European haplotype showed higher titers of DENV-2.

In a recent study, it was noted that oxysterols regulate the expression of IL-10 in CD4$^+$ T cells [45]. Furthermore, another recent observation revealed that oxysterols and upregulated genes implicated in cholesterol efflux suppress IFN-γ signaling [46]. These intriguing observations are in agreement with our result as the group of individuals with the lower OSBPL10 expression African

haplotype showed higher IL-10 and IFN-γ expression in comparison with the higher OSBPL10 expression European haplotype. IL-10 primarily exerts immunosuppressive effects by inhibiting proinflammatory cytokines release and modulating macrophage function [47] Moreover, IFN-γ plays a crucial role in host resistance against DENV infection as it regulates nitric oxide synthase-mediated nitric oxide production, which has been shown to inhibit DENV replication [48,49]. These observations validate our results regarding the elevated levels of IL-10 and IFN-γ expression and reduced DENV-2 titers in individuals with the African haplotype. Downregulation of RXRα and OSBPL10 expression appears to be beneficial for a strong antiviral defense mechanism in individuals with the African haplotype.

There are limitations in our study that need to be addressed. Firstly, we were only able to genotype a reduced number of subjects homozygous for the African and European regulatory SNPs haplotypes. Secondly, we still do not fully understand how the functional activities of OSBPL10 are influenced by rs4600849, rs975406, rs11129475, rs6419811, rs7639637 and rs11718700 SNPs. Future research is needed to gain a clearer understanding of OSBPL10 function, regulation of cytokines mRNA expression and viral cycle affectations.

5. Conclusions

In our study, we showed that knockdown of OSBPL10 significantly decreased dengue virus replication. It was also shown that PBMCs from individuals with the European haplotype of the OSBPL10 gene had higher DENV replication and higher levels of RXR-α and IL-8 expression, which is consistent with the suppressive action of these mediators on type I IFN, promoting viral replication and a deficient antiviral response. In contrast, PBMCs from individuals with the African haplotype had lower DENV replication and higher expression levels of IFN-γ and IL-10, which could be related to better management of dengue infection and the low frequency of severe disease in this ethnic group. Our findings provide a better understanding of the genetic and immunological factors involved in dengue pathogenesis.

CRediT authorship contribution statement

Hector Granela: Data curation, Investigation, Writing – original draft, Writing – review & editing. **Ana B. Perez:** Investigation, Supervision, Writing – review & editing. **Luis Morier:** Investigation, Writing – review & editing. **Mayling Alvarez:** Investigation, Writing – review & editing. **Maria G. Guzmán:** Supervision, Writing – review & editing. **Beatriz Sierra:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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