International Journal of Infectious Diseases 96 (2020) 136–138

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Short Communication

A new genetic variant of dengue serotype 2 virus circulating in the Peruvian Amazon

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ARTICLE INFO

Article history:
Received 19 March 2020
Received in revised form 29 April 2020
Accepted 29 April 2020

Keywords:
Dengue virus
Genotype
Lineage
Envelope protein
Domain III

ABSTRACT

We sequenced the envelope gene of dengue virus serotype 2 (DENV-2-E) in samples from an outbreak reported in 2018, in Yurimaguas, Peru. The strain belongs to lineage 2 of the American/Asian genotype. We report a variant with two novel mutations (I379T and V484I) located in domain III of DENV2-E.

In Peru, 80% of the population is at risk of dengue virus infection due to the spread of its main vector, *Aedes aegypti*, which is now present in 20 out of the 24 departments. The four dengue serotypes have been reported in Peru, with a different serotype typically dominating each epidemic season (Williams et al. 2014).

During October 2018 and March 2019, a DENV-2 outbreak was reported in Yurimaguas, a city located in the Loreto region of the Peruvian Amazon. Interestingly, the incidence rate during this outbreak reached around 30 per 100 000 inhabitants, three times the rate of previous years. 348 dengue cases were registered at the Hospital Santa Gema Yurimaguas (HSGY), of which 101 cases (~29%) required hospitalization. This number of hospitalizations is alarming when compared with the rates of 9% and 17% registered in 2016 and 2017, respectively (Unidad de Epidemiologia y Salud Ambiental, HSGY, personal communication) (Figure 2A). We hypothesized that, during this latest outbreak, aside from environmental, demographic, and host factors, viral determinants may have contributed to the increased viral spread and pathogenesis.

We sequenced the envelope gene (1485 bp) from 30 DENV-2 cases, collected between August 2018 and July 2019 in HSGY. Four additional samples collected in April 2018 in Iquitos, a city also located in the Loreto region, 397 km away from Yurimaguas, were also included. Sequences were aligned and edited with Codon Code Aligner and submitted to GenBank (accession Nº MT379579-MT379612).

Phylogenetic analysis based on nucleotide sequences showed that samples from the recent outbreak were clustered within the American/Asian (AM/AS) DENV-2 genotype, lineage II (Figures 1). The AM/AS lineage II displaced lineage I in Peru in 2000, and its appearance in Loreto resulted in the largest DHF epidemic in that region so far (Williams et al. 2014). Similarly, the AM/AS lineage I, displaced the American genotype in 2010, and coincided with the first reports of severe dengue in the country, with fatal cases.

The flavivirus envelope gene is essential for viral attachment and membrane fusion (Bennett et al. 2006). It encodes the major type-specific epitopes targeted by neutralizing antibodies (NAbs) (Weaver and Vasilakis 2009; Hsieh et al. 2010; Wong et al. 2018), among which those directed against domain III (E-DIII) can be the

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https://doi.org/10.1016/j.ijid.2020.04.087

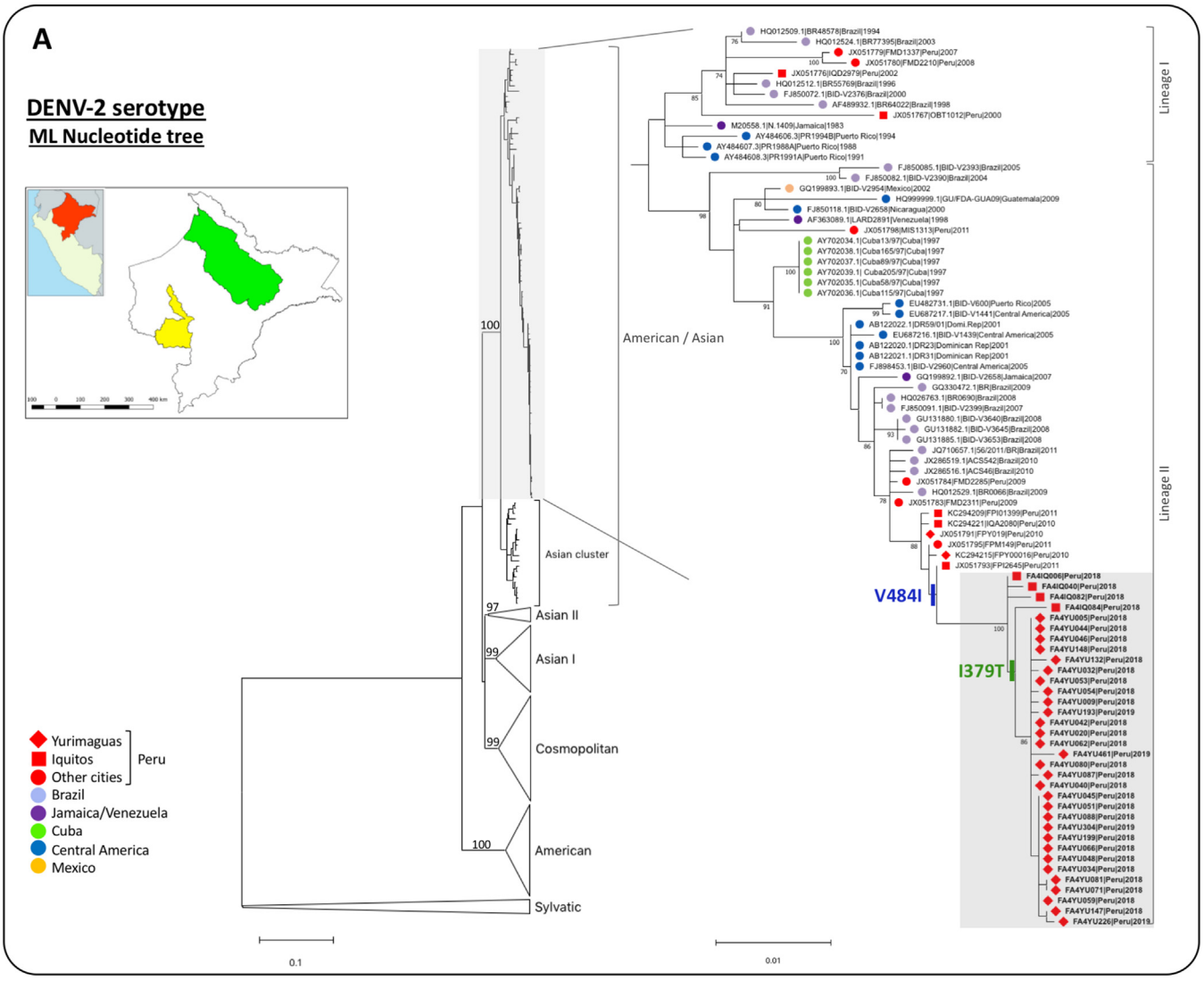


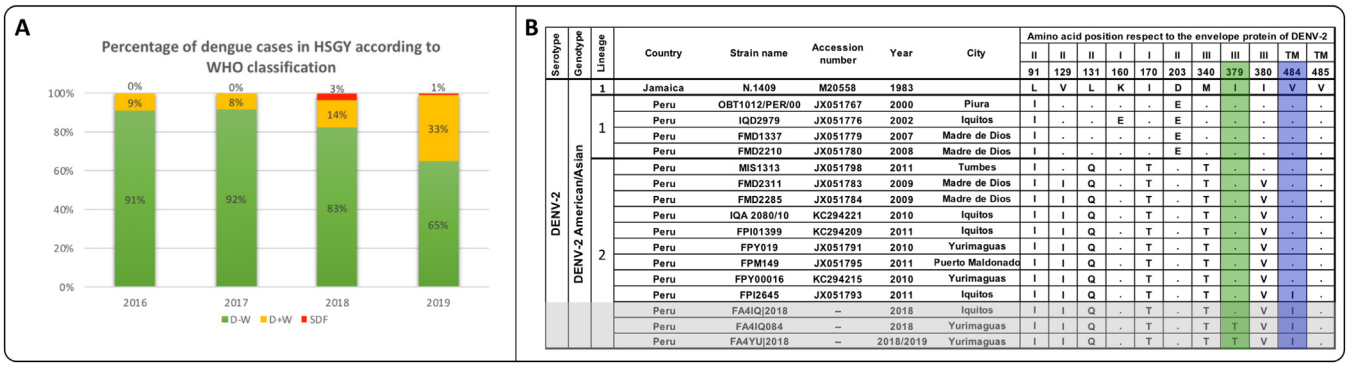
Figure 1. Phylogenetic relationship, based on the envelope gene of the Peruvian isolates, to previously characterized DENV-2 genotypes. The evolutionary relationship analysis was based on the maximum likelihood (ML) method using MEGAX and 1000 bootstrap repeat methods for statistical robustness. The best substitution model was also obtained from MEGA, based on the Bayesian information criterion (BIC). (**A) Nucleotide tree.** The Tamura-Nei model with gamma and invariable sites correction (TN93+G + I) method was the model of nucleotide substitution that best fitted the data. Nucleotide (1485 bp) sequences of the DENV2-E gene from 151 reference strains, including all five genotypes – American/Asian, American, Asian I, Asian II and cosmopolitan – were retrieved from GenBank. The tree was rooted on a sylvatic genotype outgroup. Bootstrap supporting values greater than 70 are shown at the nodes. The American/Asian genotype clade containing lineages I and II is expanded. Taxa color tip markers indicate geographic location. The Peruvian DENV-2 samples sequenced in this study are in bold and highlighted in a grey box. Peruvian viruses from previous epidemics are represented with red markers. The green and blue bars in the branch of the Peruvian samples represents the amino acid changes at position I379 T and V484I, respectively. The taxon label is presented in the following format: GenBank accession number|strain name|country|year. (B) **Map of the Loreto region, showing the provinces Alto Amazonas (yellow) and Maynas (green).** Yurimaguas and Iquitos are the capitals of each province, respectively.

most potent (Renner et al. 2018). Analysis of the DENV-E amino acid (AA) sequences of AM/AS strains (Fig. 2B) revealed that, aside from the single AA polymorphisms at positions 91, 129, 131, 170, 203, 340, and 380 relevant for phylogenetic classification (Bennett et al. 2006; Oliveira et al. 2010; Barcelos Figueiredo et al. 2014), viruses from the recent outbreak had a non-conservative and a conservative substitution at positions 379 and 484, respectively.

Mutation I379T is located in the β-strand F of the E-DIII region, against which the DENV 3H5, Zika ZV-67 and Zika Z006 NAbs have been reported (Dussupt et al. 2020), while mutation V484I is located in the transmembrane region of the E-protein, a highly conserved region among flaviviruses, playing an important role in the assembly of the protein (Hsieh et al. 2010).

Given that specific DENV genotypes have been associated with mild or severe disease (Bell et al. 2019), the introduction of the new genetic variant reported here might be associated with an increase in the number of severe DENV cases currently reported in HSGY.

Unfortunately, our study was limited by the scarcity of Peruvian sequences publicly available from 2011 onwards, as well as by our focus on only the envelope gene. Future functional studies and long-term follow-up are therefore needed to determine if these specific mutations correspond with functional antigenic changes, and to assess their possible implications for viral fitness, as well as for the pathogenicity and dynamics of dengue disease in Yurimaguas.



B

Serotype	Genotype	Lineage	Country	Strain name	Accession number	Year	City	Amino acid position respect to the envelope protein of DENV-2										
								II	II	II	I	I	II	III	III	III	TM	TM
								91	129	131	160	170	203	340	379	380	484	485
DENV-2		1	Jamaica	N.1409	M20558	1983		L	V	L	K	I	D	M	I	I	V	V
DENV-2	DENV-2 American/Asian	1	Peru	OBT1012/PER/00	JX051767	2000	Piura	I	.	.	.	.	E	.	.	.	.	.
DENV-2	DENV-2 American/Asian	1	Peru	IQD2979	JX051776	2002	Iquitos	I	.	.	E	.	E	.	.	.	.	.
DENV-2	DENV-2 American/Asian	1	Peru	FMD1337	JX051779	2007	Madre de Dios	I	.	.	.	.	E	.	.	.	.	.
DENV-2	DENV-2 American/Asian	1	Peru	FMD2210	JX051780	2008	Madre de Dios	I	.	.	.	.	E	.	.	.	.	.
DENV-2	DENV-2 American/Asian	2	Peru	MIS1313	JX051798	2011	Tumbes	I	.	Q	.	T	.	T	.	.	.	.
DENV-2	DENV-2 American/Asian	2	Peru	FMD2311	JX051783	2009	Madre de Dios	I	I	Q	.	T	.	T	.	V	.	.
DENV-2	DENV-2 American/Asian	2	Peru	FMD2285	JX051784	2009	Madre de Dios	I	I	Q	.	T	.	T	.	V	.	.
DENV-2	DENV-2 American/Asian	2	Peru	IQA 2080/10	KC294221	2010	Iquitos	I	I	Q	.	T	.	T	.	V	.	.
DENV-2	DENV-2 American/Asian	2	Peru	FPI01399	KC294209	2011	Iquitos	I	I	Q	.	T	.	T	.	V	.	.
DENV-2	DENV-2 American/Asian	2	Peru	FPY019	JX051791	2010	Yurimaguas	I	I	Q	.	T	.	T	.	V	.	.
DENV-2	DENV-2 American/Asian	2	Peru	FPM149	JX051795	2011	Puerto Maldonado	I	I	Q	.	T	.	T	.	V	.	.
DENV-2	DENV-2 American/Asian	2	Peru	FPY00016	KC294215	2010	Yurimaguas	I	I	Q	.	T	.	T	.	V	.	.
DENV-2	DENV-2 American/Asian	2	Peru	FPI2645	JX051793	2011	Iquitos	I	I	Q	.	T	.	T	.	V	I	.
			Peru	FA4IQ|2018	--	2018	Iquitos	I	I	Q	.	T	.	T	.	V	I	.
			Peru	FA4IQ084	--	2018	Yurimaguas	I	I	Q	.	T	.	T	T	V	I	.
			Peru	FA4YU|2018	--	2018/2019	Yurimaguas	I	I	Q	.	T	.	T	T	V	I	.

Figure 2. (**A**) Percentage of DENV cases registered in Hospital Santa Gema Yurimaguas (HSGY). Percentages of cases are depicted in different colors according to the World Health Organization (WHO) classifications as follows: dengue without warning signs (D–W) in green, dengue with warning signs (D + W) in yellow, and severe dengue fever (SDF) in red. (**B**) **Amino acid sequence polymorphism of the American/Asian genotype DENV-2 envelope protein.** Partial alignment of the envelope protein shows the sites of amino acid polymorphism within the American/Asian genotype. Alignment of sequences is based on the Jamaica M20558 strain. Dots indicate residues identical to the reference strain. Isolates are arranged according to their genotype. Nucleotide alignment was carried out using ClustalX and the amino acid sequence was inferred from the nucleotide sequences using AliView v 1.26, following the standard genetic code.

Funding source

This work was supported by the 4th Framework Agreement of the Belgian Development Cooperation program (DGD FA4) of the Belgian Federal Government.

Conflicts of interest

The authors report no conflicts of interest.

Ethical approval

Ethical approval was obtained from the Ethical Committee of the Universidad Peruana Cayetano Heredia (UPCH), the Research Ethics Committee (CEI) of the Hospital Regional de Lambayeque, the Institutional Review Board of the Institute of Tropical Medicine Antwerp and the Ethical Committee of the University Hospital Antwerp.

Acknowledgements

We would like to thank the staff at Hospital Santa Gema in Yurimaguas and the Laboratorio Satelite in Iquitos for their valuable help and dedication in patient recruitment.

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