

Brief Report

# Zika E Glycan Loop Region and Guillain–Barré Syndrome-Related Proteins: A Possible Molecular Mimicry to Be Taken in Account for Vaccine Development

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**Abstract:** The neurological complications of infection by the mosquito-borne Zika virus (ZIKV) include Guillain–Barré syndrome (GBS), an acute inflammatory demyelinating polyneuritis. GBS was first associated with recent ZIKV epidemics caused by the emergence of the ZIKV Asian lineage in South Pacific. Here, we hypothesize that ZIKV-associated GBS relates to a molecular mimicry between viral envelope E (E) protein and neural proteins involved in GBS. The analysis of the ZIKV epidemic strains showed that the glycan loop (GL) region of the E protein includes an IVNDT motif which is conserved in voltage-dependent L-type calcium channel subunit alpha-1C (Ca<sub>v</sub>1.2) and Heat Shock 70 kDa protein 12A (HSP70 12A). Both VSCC-alpha 1C and HSP70 12A belong to protein families which have been associated with neurological autoimmune diseases in central nervous system. The purpose of our *in silico* analysis is to point out that IVNDT motif of ZIKV E-GL region should be taken in consideration for the development of safe and effective anti-Zika vaccines by precluding the possibility of adverse neurologic events including autoimmune diseases such as GBS through a potent mimicry with Heat Shock 70 kDa protein 12A (HSP70 12A).

**Keywords:** ZIKV; Guillain–Barré syndrome; molecular mimicry; calcium channel voltage dependent; heat shock protein; vaccine

## 1. Introduction

In the last few decades, there has been an increasing number of epidemics associated with mosquito-borne RNA viruses of medical concern such as Dengue Virus (DENV), West Nile Virus (WNV) or Chikungunya Virus [1]. Like DENV and WNV, Zika Virus (ZIKV) belongs to the flavivirus genus of the *Flaviviridae* family [2]. Classically, ZIKV is transmitted by mosquito bite and the preferred vectors for ZIKV spreading are *Aedes* genus mosquitoes [2,3]. ZIKV was first reported in Uganda in 1947 in a rhesus monkey; however, the virus remained silent for years, only provoking sporadic infections until 2007 [2]. The outbreak worldwide spread urged WHO to declare ZIKV as a major public health issue, leading to a global effort to fight against the disease. ZIKV infection causes clinical manifestation in ~18% of cases ranging from mild disease with a denguelike syndrome, to more severe outcomes such as congenital microcephaly [4–6]. Of note, other neurological disorders have been associated with ZIKV infection, including encephalitis, myelitis and, finally, Guillain–Barré Syndrome (GBS) [5–8]. Moreover, uncommon modes

of transmission have been described, including sexual and vertical (mother-to-infant) transmission, increasing the threat that this virus represents [9–12].

Like other flaviviruses, ZIKV is a positive sense single-stranded RNA virus. The viral genomic RNA encodes a large polyprotein, which is processed to generate the structural proteins C, prM and E followed by the nonstructural proteins NS1 to NS5. The E protein is involved in virus binding onto the host-cell and the internalization of the viral particles. The E ectodomain is divided into three domains: I (EDI), II (EDII), and III (EDIII). The EDI comprises a glycan loop which is glycosylated on N154. The antibody-mediated virus neutralization depends on the availability of neutralizing epitopes on the E protein.

To date, there are two ZIKV lineages: the African lineage with the historical strain MR766 (1947) as prototype and the Asian lineage, the latter being responsible for the contemporary epidemics in the South Pacific and the Americas. Critically, the Asian lineage is the only one associated with GBS so far. Comparative analysis between Asian lineage ZIKV strains PF13 (French Polynesia, 2013) and BeH819015 (Brazil, 2015) with African strain MR766 showed differences in their ability to infect human host cells [13]. The epidemic ZIKV strains differ from African strain MR766-NIID by eight amino acid substitutions in ZIKV E protein at positions E-152/156/158/169/285/341/343. Interestingly, the residues E-152/156/158 are identified into the glycan loop (GL, residues E-151 to E-165) region of ZIKV. The GL region of Asian lineage ZIKV strains has a glycan linked to N154 but the African strain MR766 does not. We previously reported that the GL region influences the availability of neutralizing epitopes on ZIKV [14] and that residues E-152/156/158 play a key role in the antigenic reactivity of the GL region [14]. In the present study, we proposed that amino acids at positions E-152/156/158 of the ZIKV epidemic strains might exhibit a molecular mimicry with GBS-related proteins. The possibility that E-clusters of residues E-283/285, E-341/343, and E-437/438 could account for a molecular mimicry in relation with human proteins was also investigated.

## 2. Materials and Methods

We conducted a comparative analysis between the E sequences from epidemic Brazilian ZIKV strain BeH-819015 (Genbank access number KU365778) isolated in 2015 and laboratory-adapted African ZIKV strain MR766-NIID lineage isolated in 1947 (Genbank access number LC002520). It is of note that biological properties of viral strains BeH-819015 and MR766-NIID have been extensively studied [13–17].

We attempted to determine potential candidates for Asian lineage ZIKV E protein molecular mimicry using Blastp suite (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>, accessed on 11 November 2020) included in BLAST<sup>®</sup> NCBI tool.

For this purpose, we entered as a query the diverse peptides resulting from substitutions only observed in the epidemic strains of ZIKV, limiting ourselves to the small size peptides (5 amino acids). Then, we searched for protein with these sequences conserved among human proteins (taxid: 9605) using the reference proteins database (refseq\_protein) and protein–protein BLAST algorithm with default search parameters. Selection criteria for *in silico* determination of mimicry candidates among sequences that produced significant alignment were defined as follows: (i) primary sequence fully conserved in human candidate protein; (ii) candidate protein highly expressed in nervous system, both central and peripheral; (iii) candidate protein near or far associated with neuropathies, in the literature.

Additionally, the entire primary amino acid sequence of ZIKV (epidemic strain) envelope protein was assessed using B Cell Epitope Prediction Tools from the Immune Epitope Database (IEDB; <http://tools.iedb.org/bcell/>, accessed on 12 December 2020). Briefly, ZIKV E sequence was entered in the Antibody Epitope Prediction tool set on Bepipred Linear Epitope Prediction 2.0 in order to determine potential epitopes leading to antibody response in E protein. Subsequently, these results were cross-checked with the query sequence previously used for Blastp analysis.

Finally, prediction of peptide folding was done using PEP-FOLD3 tool [18] (available at <https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/>, accessed on 3 March

2021). To do so, we entered 30 amino acids sequence containing the pentapeptide of interest (potentially involved in molecular mimicry), running prediction with 100 independent simulations and sorting models by sOPEP. Following sequences were used for prediction (in brackets, protein name—accession number): IMLSVHGSQHSQMIVNDTGHETDEN-RAKVEV (Zika Virus polyprotein, KU365778), FSPNNRFRQLQCHRIVNDTIFTNLILFFILLS (Voltage-dependent L-type calcium channel subunit alpha-1C, Q13936) and GDTGITPLSP-SHIVNDTDSNVSEQQSFLVV (Heat shock 70 kDa protein 12A, O43301).

The illustrations in figures 1 and 3 were made with the BioRender software.

### 3. Results

One of the potential mechanisms through which ZIKV is supposed to induce GBS is molecular mimicry [19,20]. This mechanism corresponds to a similar structure between pathogens and human proteins [21]. To explore this hypothesis, we decided to develop an *in silico* approach to determine whether ZIKV E protein shares homology with human proteins (Table 1). Thus far, GBS has been associated with contemporary epidemic ZIKV strains of Asian lineage. It seems unlikely that African lineage ZIKV have the ability to mediate GBS since no case was reported to date, which could be an artefact of a poor history of infections.

**Table 1.** Host proteins obtained from query sequence alignment using Blastp. The proteins highlighted in **bold** are the only ones which fulfill the criteria of selection as candidates for molecular mimicry.

Query Sequence	Substitutions Associated	Output
IVNDT	T152I/I156T	<b>Calcium channel voltage-dependent L type <math>\alpha</math>-1C subunit</b>
		<b>Heat Shock 70 kDa protein 12A</b>
		Pecanexlike protein 2Cyclin-C
		Adhesion G-protein coupled receptor V1
		Coagulation factor VIII
		HEAT repeat-containing protein 5B
		Sodium leak channel nonselective protein
		Hexokinase-1, -2, -3, HKDC1
		Macrophage colony-stimulating factor 1
		Collagen and calcium-binding EGF domain-containing protein 1
DTGHE	I156T/Y158H	Transcription factor COE1
		Cytoplasmic FMR1-interacting protein 1
		A disintegrin and metalloproteinase with thrombospondin motifs 2
		Folliculin-interacting protein 1
		Ubiquitin-associated protein 2-like
		Prolyl endopeptidaselike
		Adapter protein CIKS
		Dermokine
		Immunity-related GTPase family M protein
		Tyrosine-protein kinase ABL2
Zinc finger protein 491		
Obscurin		
Protein prune homolog 2		
Otogelin		
eIF-2-alpha kinase activator GCN1		
BAH and coiled-coil domain-containing protein 1		
Centrosome-associated protein CEP250		
Supervillin		
Ankyrin-1		

Table 1. Cont.

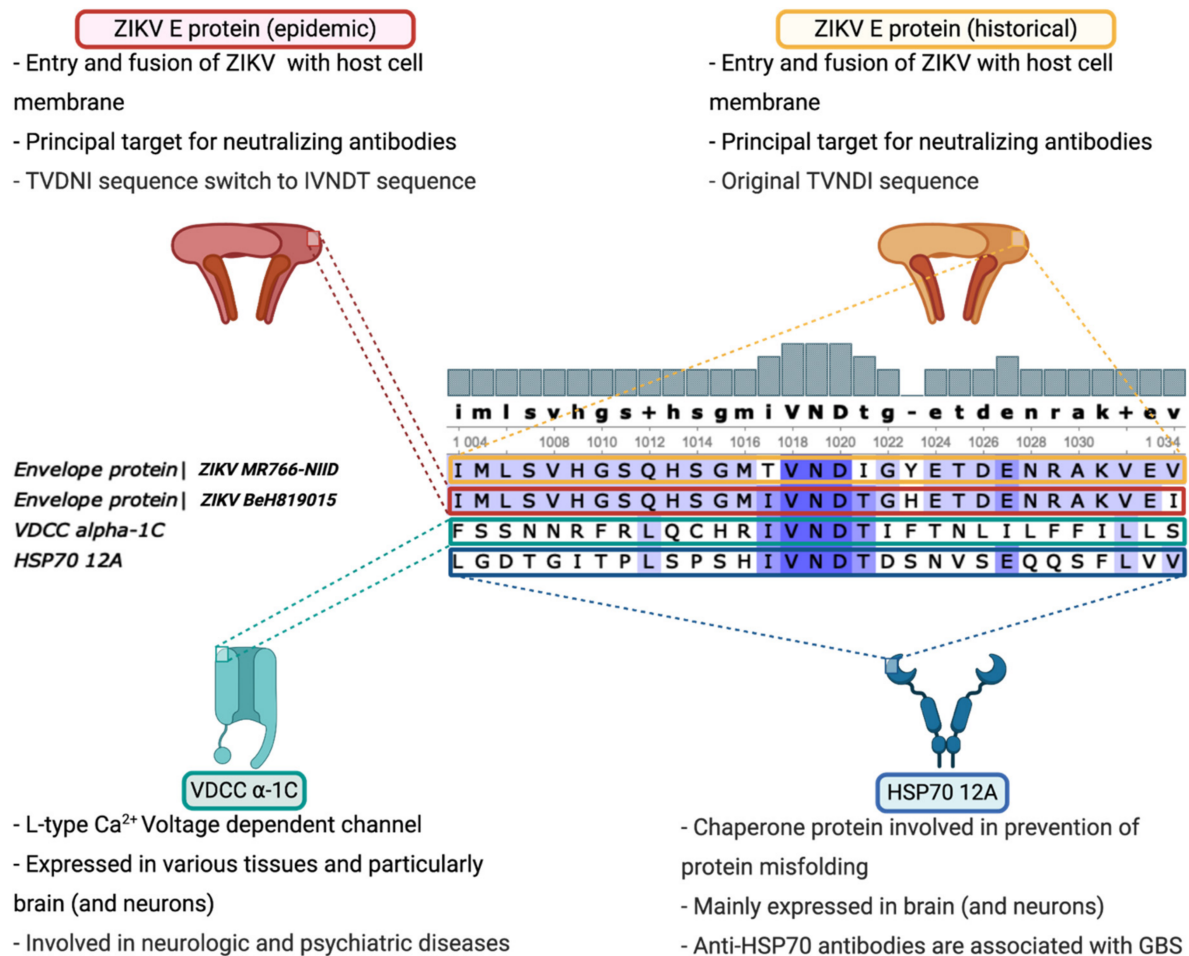
Query Sequence	Substitutions Associated	Output
GRLSS	K283R/F285S	Pikachurin precursor
		Hamartin
		Ankyrin repeat and LEM domain-containing protein 2
		Testis-expressed protein 10
		GRB2-associated and regulator of MAPK protein 2
		Low-density lipoprotein receptor-related protein 3
		Ligand of Numb protein X 2
		Synaptotagminlike protein 4
		Chromodomain Y-like protein 2
		Oxygen-dependent coproporphyrinogen-III oxidase, mitochondrial precursor
		Sodium- and chloride-dependent GABA transporter 2
		Acetyl-CoA acetyltransferase, cytosolic
		ER membrane protein complex subunit 10
		Histamine H4 receptor
		Protein GOLM2
		Protein FAM83A
		Uncharacterized protein C9orf163
		Uncharacterized protein C3orf18
		Mucin-16
		Obscurin
Nesprin-1		
VPAQM	I341V/V343A	1-acyl-sn-glycerol-3-phosphate acyltransferase gamma
		E3 ubiquitin-protein ligase HERC2
		Histone-lysine N-methyltransferase 2A
		Serine/threonine-protein kinase mTOR
		Myosin light chain kinase, smooth muscle
		Zinc finger protein 646, 292, 831, GLI1
		Galactoside alpha-(1,2)-fucosyltransferase 2
		Tensin-2
		Neurolysin, mitochondrial
		Filamin-A
		Spectrin beta chain, nonerythrocytic 5
		Structure-specific endonuclease subunit SLX4
		Adhesion G protein-coupled receptor L3
		Tau-tubulin kinase 1
		IQ domain-containing protein N
		N-acetylglucosamine-1-phosphotransferase subunits alpha/beta
		FERM domain-containing protein 4A
		RE1-silencing transcription factor
		Protocadherin-8
		Protein transport protein Sec24D
Adhesion G-protein coupled receptor G2		
Protein FAN		
Kinesinlike protein KIF18B		

Table 1. Cont.

Query Sequence	Substitutions Associated	Output
GALNS	V437A/F438L	A-kinase anchor protein 12
		EMILIN-1 precursor
		DENN domain-containing protein 1C
		Gelsolin
		Tudor domain-containing protein 10
		CXXC-type zinc finger protein 4
		Obscurin
		Microtubule-actin cross-linking factor 1
		Usherin
		Ryanodine receptor 2
		Dynein heavy chain 8, heavy chain 14, heavy chain 7
		Sacsin
		Basement membrane-specific heparan sulfate proteoglycan core protein
		DNA-dependent protein kinase catalytic subunit
		Protein bassoon
		Transformation/transcription domain-associated protein
Xin actin-binding repeat-containing protein 2		
Protocadherin-16 precursor		

Based on the amino acid changes observed between the E proteins of viral strains BeH-819015 and MR766-NIID, we identified four clusters of amino acid substitutions making good candidates to further explore molecular mimicry hypothesis (Table 1). Into the EDI domain, residues at positions E-152/156/158 may play a key role in antigenic reactivity of ZIKV GL region [14]. The E-GL region of Asian lineage ZIKV encompasses the sequence IVNDT (residues E-152 to E-156) which includes the sequon NDT where a glycan is linked to N154. African ZIKV strain MR766-NIID includes the pentapeptide TVNDI in place of IVNDT due to the (T, I) permutation at positions E-152/156 leading to a nonglycosylated ZIKV [14]. The amino acid substitution E-Y158H has been identified between MR766-NIID and epidemic Brazilian strain BeH819015 of Asian lineage. It is of note that two substitutions at positions E-156/158 of the GL region change the pentapeptide DIGYE into DTGHE. Other amino acid residues in the E protein are candidates for searching for a sequence homology with GBS-related proteins. There are the EDII residues E-283/285 with a pentapeptide GKFLS for BeH819015 but GRLSS in MR766-NIID. Additionally, the EDIII residues E-341/343 compose the pentapeptide VPAQM in BeH819015 but IPVQM in MR766-NIID. Finally, the V437A and F438L permutations lead to a GALNS peptide for BeH819015 compared to GVFNS for MR766-NIID.

With the aim to understand the underlying mechanism for ZIKV-mediated GBS, *in silico* methods were employed to identify potential protein candidates for molecular mimicry. Consequently, protein sequence alignment based on the E proteins of BeH819015 and MR766-NIID was assessed with human proteins, following the criteria described in the Materials and Methods section. From all the candidates found using this method (Table 1), calcium channel voltage-dependent L type, alpha 1C subunit and Heat Shock 70 kDa protein 12A were the only ones to fulfill all the criteria (Figure 1). Indeed, the IVNDT sequence is totally conserved and these proteins are highly expressed in the nervous system, both central and peripheral [22–24]. Likewise, as described below, a correlation has been reported between development of neuropathies and autoantibodies directed against both the voltage-dependent Ca<sup>2+</sup> channel and HSP70. Thus, these proteins could be exposed to the immune system and therefore to an autoantibody response.



**Figure 1.** Calcium channel voltage-dependent L type  $\alpha$ -1C subunit and Heat Shock 70 kDa protein 12A are potential candidates for molecular mimicry following ZIKV infection. Comparative analysis of Brazilian strain of ZIKV (BeH819015) and laboratory-adapted historical strain of ZIKV (MR766-NIID) revealed that an IVNDT polypeptide, only found in epidemic strain, might be related to ZIKV-related GBS due to calcium channel voltage-dependent L type  $\alpha$ -1C subunit or Heat Shock 70 kDa protein 12A molecular mimicry.

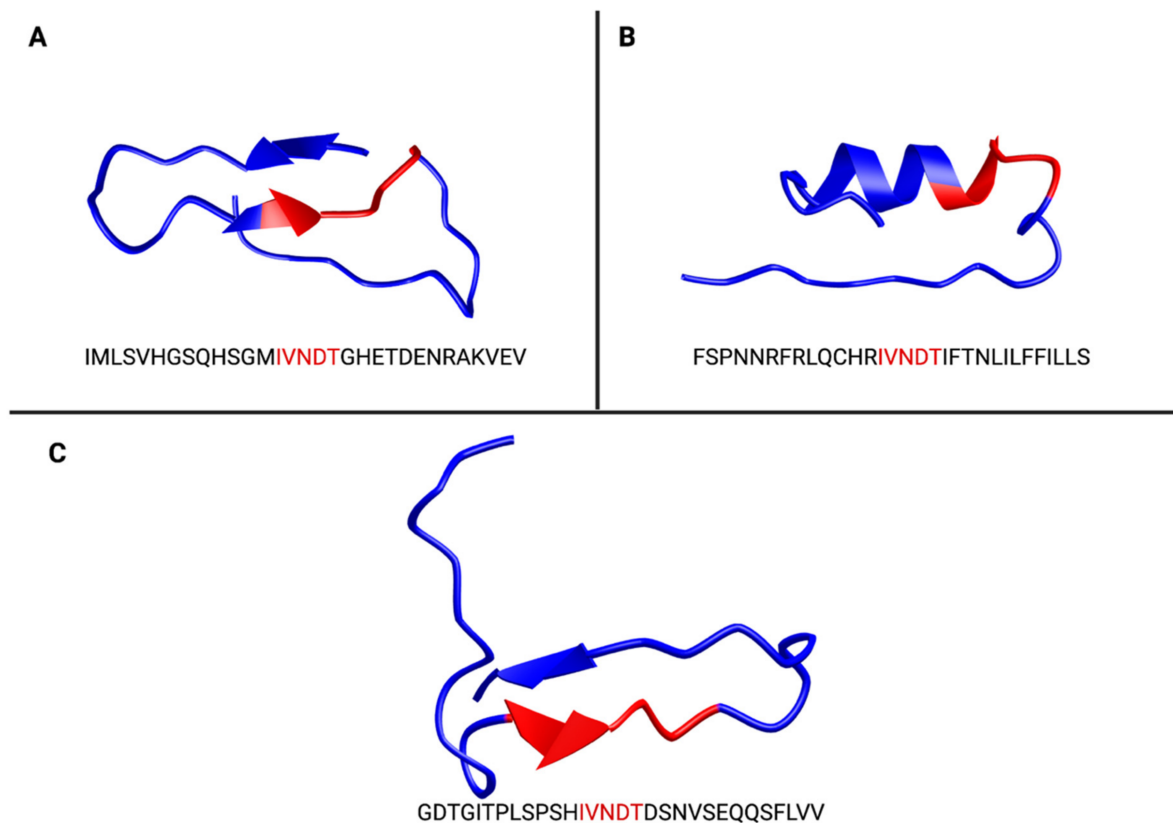
Moreover, according to antibody epitope prediction using IEDB tools, we determined that among all the predicted epitopes potentially leading to antibody response in ZIKV E sequence, only two peptides relate to regions where amino acid changes were observed in the epidemic ZIKV strain and that served in the previously cited alignment (Table 2). Strikingly, one of them contained the IVNDT sequence. Considering that other predicted antibody epitopes do not include any known amino acid substitutions between African- and Asian lineage ZIKV, it is highly unlikely that such E-associated epitopes may play a role in the development of ZIKV-mediated GBS. However, in order to rule out any possibility, we tested all the pentapeptides derived from each of these epitopes, using the methodology described previously. Conversely to IVDNT previously mentioned, no matching candidate was found with any of the tested pentapeptides here (Tables S1–S3).

**Table 2.** Antibody epitope prediction for epidemical ZIKV (BEH819015) envelope protein. Summary of all antibody predicted epitope using IEDB tools with ZIKV (BEH819015) envelope protein as query. In **bold** are highlighted the sequences with substitutions in BEH819015 strain compared with MR766-NIID strain.

Start-End	Peptide
5–9	GVSNR
66–103	SDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDGRGWGN
126–133	TGKSIQPE
146–163	SQHSGMIVNDTGHETDEN
193–197	RTGLD
218–240	FHDIPLPWHAGADTGTPHWNNKE
274–279	EAEMDG
312–322	TFTKIPAETL
349–352	MQTL
368–371	STEN
395–408	KITHHWHRSGSTIG
428–440	AWDFGSVGGALNS

In addition, a previous work has shown that susceptibility to Zika virus infection is increased in differentiated neural cells compared to their undifferentiated counterparts [25]. Additionally, this work suggests that this may be related to the several neurological outcomes of ZIKV infection [25]. Thus, we wondered if one or several GBS-related proteins could be differentially expressed in differentiated neural cells compared to undifferentiated neural cells, maybe explaining the development of GBS following ZIKV infection. To answer this, we performed a differential expression analysis of the transcriptome dataset GSE121534 using DEBrowser [26] as described in Supplemental Materials. We compared a list of genes with a fold value >5 (Table S1) to a set of GBS-related proteins summarized in Table S2, but no match was observed between the transcriptome dataset GSE121534 and the list of GBS-related proteins. Remarkably, calcium channel voltage-dependent L type, alpha 1C subunit and Heat Shock 70 kDa protein 12A were expressed by differentiated and undifferentiated SH-SY5Y in the transcriptome dataset GSE121534 analysis. This led us, again, to privilege the hypothesis of molecular mimicry with these two proteins.

Finally, based on the predicted secondary structure data (Figure 2) obtained using PEP-FOLD3 [18], as described in the Materials and Methods section, it seems that the folding of the ZIKV E glycan loop from the Asian lineage is close to Heat Shock 70 kDa protein 12A. Indeed, in both cases, IVNDT sequence belongs to a  $\beta$ -sheet structure, strengthening the theory that this pentapeptide could elicit an autoimmune response. Taken altogether, these data form a growing body of evidence for antibody-directed against IVNDT peptide and either calcium channel voltage-dependent L type, alpha 1C subunit or Heat Shock 70 kDa protein 12A.

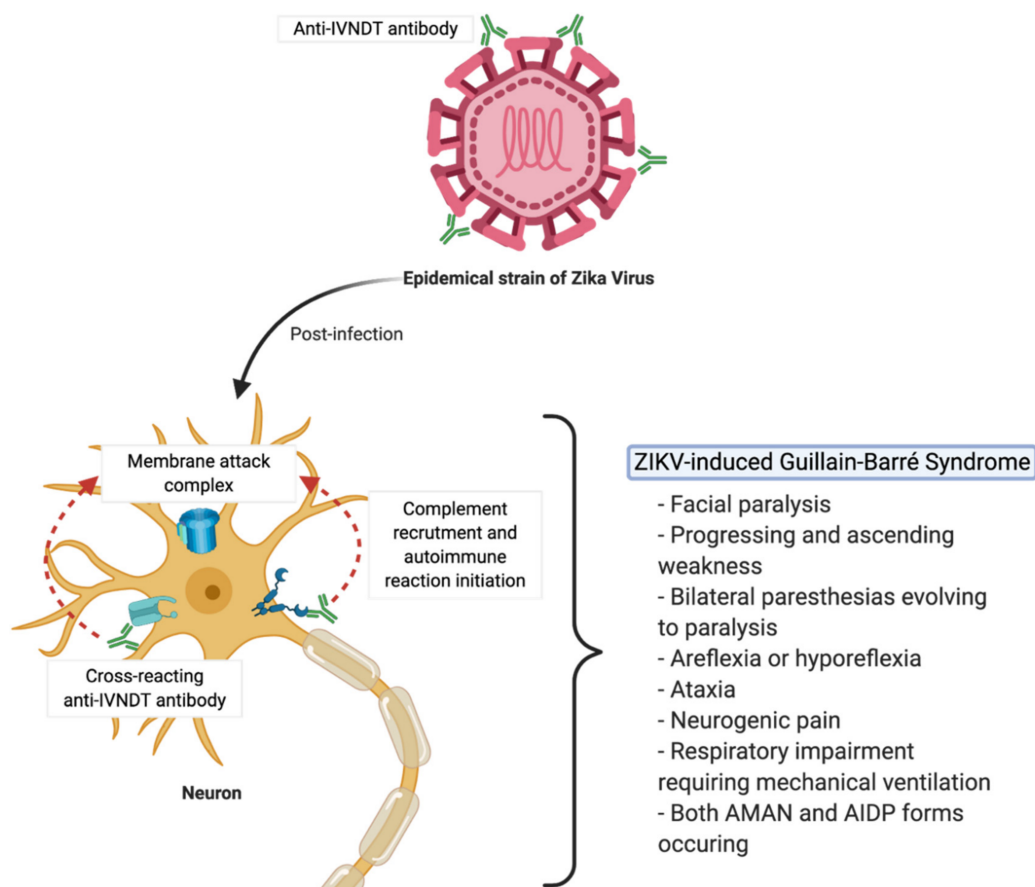


**Figure 2.** Predicted folding of epitope containing IVNDT (in red). ZIKV Asian lineage (A), VDDC alpha 1C (B) and HSP70 12 A (C). Prediction of peptide folding was done using PEP-FOLD3. HSP70 12A folding seems to be close to ZIKV E glycan loop folding, conversely to VDDC alpha 1C.

#### 4. Discussion

Voltage-dependent calcium channels (VDCCs) are key proteins modulating  $\text{Ca}^{2+}$  entry into electrically excitable cells, following depolarization. Calcium channel voltage-dependent L type  $\alpha$ -1C subunit, also called  $\text{Ca}_v1.2$ , is part of L-type VDCCs family [22]. This  $\text{Ca}^{2+}$  channel participates in hippocampal long-term potentiation, hippocampus-dependent forms of memory, peripheral vascular resistance, cardiac inotropy or insulin secretion [22,27]. The impairment of similar channels by anti-GM1 antibodies was implicated in development of GBS [28,29]. Since anti-GM1 antibodies are found at low levels in ZIKV-induced GBS patients, it is likely that voltage-dependent  $\text{Ca}^{2+}$  channel is targeted by different cross-reacting antibodies. Here, we showed the existence of an IVNDT conserved sequence between  $\text{Ca}_v1.2$  and ZIKV (BEH819015) envelope protein. Even if  $\text{Ca}_v1.2$  has never been directly implicated into development of GBS, nor any other neuropathy, we suggest that the recognition of  $\text{Ca}_v1.2$  on a neuron by cross-reacting anti-IVNDT antibodies following ZIKV infection might lead to the initiation of the immune response and development of the GBS pathophysiology (Figure 3). However, this hypothesis remains to be tested. Since  $\text{Ca}_v1.2$  is mainly involved in cardiac and memory functions, the development of the symptoms associated with these functions should be expected (e.g., cardiac and psychiatric forms), but to date none was reported. Moreover,  $\text{Ca}_v1.2$  folding in the region of the IVNDT pentapeptide seems to be quite far from ZIKV E glycan loop folding (Figure 2A,B). Of note, predicting  $\text{Ca}_v1.2$  topology using PredictProtein [30], we noticed that IVNDT region is likely to be cytoplasmic, decreasing the probability of molecular mimicry with this candidate.





**Figure 3.** ZIKV-induced Guillain–Barré Syndrome might be promoted by autoantibodies directed against calcium channel voltage-dependent L type  $\alpha$ -1C subunit or Heat Shock 70 kDa protein 12A.

Heat Shock Proteins (HSP) are divided into subfamilies according to their theoretical molecular weights (27-kDa, 60-kDa, 70-kDa and 90-kDa). They are known for their crucial role in preventing protein misfolding and aggregation, as for their induction in case of cellular stress such as increased temperature radiation, exposure to chemicals, oxidative stress and various physiological and pathological stimuli [31]. An additional role of HSP is the participation in antigen presentation/cross-presentation [32] and inflammatory signaling [33]. Among this large family, HSP70s (to which belongs Heat Shock 70-kDa protein 12A—HSP70 12A) have been associated with autoimmune neurological disorders and more specifically immune-induced neuropathies like GBS [34]. Indeed, an increased prevalence of anti-HSP70 antibodies in GBS patients has been reported [31,35]. Here, we showed the existence of an IVNDT conserved sequence between HSP70 12A and ZIKV (BeH819015) envelope protein. As HSP70 12A is mainly expressed in neural cells of the central nervous system (CNS), such a finding might be meaningful for ZIKV-associated GBS pathophysiology insights. Additionally, in contrast to Cav1.2, HSP70 12A folding obtained via PEP-FOLD3 [18] fits well with the ZIKV E glycan loop from the Asian lineage (Figure 2A,C). Accordingly, the body of evidence would be more directed towards molecular mimicry against HSP70 12A. Based on our molecular mimicry hypothesis, it is proposed that anti-IVNDT antibodies produced during ZIKV infection cross-react with HSP70 expressed by neurons promoting complement recruitment, membrane attack complex formation and autoimmune reaction initiation (Figure 3).

Our *in silico* data allowed us to propose a molecular mimicry hypothesis between the Zika E sequence IVNDT and human neuronal proteins (e.g.,  $Ca_v1.2$  and HSP70 12A) within the CNS. The pentapeptide IVNDT which composes the sequon NDT into the EDI domain of Asian lineage ZIKV is highly conserved among contemporary epidemic viral strains. Different substrains of the historical African strain MR766 as a ZIKV prototype

have been identified as nonglycosylated ZIKV. Thus, MR766 substrain NIID (Genbank access number LC002520.1) contains a TVNDI sequence in the glycan loop region, whereas M.mulatta-tc/UGA/1947/MR-766 substrain (Genbank access number KU955594) bears a 4-aa deletion leading to a lack of VNDI sequence. To our knowledge, nonglycosylated ZIKV strains have never been involved in ZIKV-mediated GBS. It is therefore tempting to propose a link between the pentapeptide IVNDDT found in Asian lineage ZIKV in relation with human proteins  $Ca_v1.2$  and HSP70 12A and the development of GBS in Zika patients. Our molecular mimicry hypothesis will need to be proven with the detection of anti-IVNDDT cross-reacting antibodies in GBS patients diagnosed for ZIKV infection as well as the development of an animal model for understanding the mechanisms by which IVNDDT peptide-related antibodies could trigger GBS.

In conclusion, we propose that Zika IVNDDT peptide should be taken in consideration to preclude the risks of adverse neurologic autoimmune diseases such as GBS during the course of ZIKV infection. Our molecular mimicry hypothesis raises the question of vaccine development against ZIKV. Indeed, a possible causal relationship between molecular mimicry and the development of autoimmunity in response to ZIKV infection alerts us to the need for vaccine candidates exempt from the viral antibody epitopes identified as potential GBS triggers. We recently reported the development of a chimeric viral clone called ZIKALIVax, which was designed with viral strain MR766-NIID as backbone and structural protein region of epidemic strain BeH918015 [14]. The replacement of pentapeptide IVNDDT into the BeH918015 glycan loop region by the TVNDI sequence from MR766-NIID resulted in a nonglycosylated envelope protein [14]. ZIKALIVax was proposed as a live-attenuated vaccine candidate against ZIKV-related disease [14]. Based on our molecular mimicry hypothesis between ZIKV sequence IVNDDT and human proteins  $Ca_v1.2$  and HSP70 12A, it is presumed that ZIKALIVax cannot trigger GBS upon vaccination, reinforcing the attractiveness of a such chimeric viral clone as a vaccine candidate against Zika.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2076-393X/9/3/283/s1>, Supplemental methods, Table S1: Guillain Barré-related proteins; Table S2: Gene differentially expressed by differentiated versus undifferentiated SH-SY5Y; Table S3: Screening of pentapeptides derived from Table 2 epitope.

**Author Contributions:** Conceptualization: G.L., E.F., P.D., W.V.; methodology: G.L., E.F., F.B., P.K.-T., P.D., W.V.; validation: G.L., E.F., J.T.; F.B., J.-J.H., G.G., P.K.-T., P.D., W.V.; investigation: G.L., E.F., F.B., W.V.; writing—original draft preparation: G.L., E.F., F.B., P.K.-T., P.D., W.V.; writing—review and editing: G.L., E.F., J.T.; F.B., J.-J.H., G.G., P.K.-T., P.D., W.V.; supervision: P.D. and W.V.; Funding: P.D., G.L., E.F., J.T. All authors have read and agreed to the published version of the manuscript.

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**Patent:** Part of this work has been described in the patent entitled “Vaccine compositions comprising an attenuated mutant zika virus” under the number WO2017220748A1 (priority date 2016-06-23).

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

BeH819015	Clinical isolate of ZIKV collected in Brazil in 2015
$Ca_v1.2$	Calcium channel voltage-dependent L-type $\alpha$ -1C subunit
GBS	Guillain–Barré Syndrome
HSP70 12A	Heat Shock 70 kDa protein 12A
MR766-NIID	Laboratory-adapted ZIKV strain isolated in Uganda in 1947
VDCC	Voltage-dependent calcium channel
ZIKV	Zika Virus

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