

Genetic analysis of West Nile New York 1999 encephalitis virus

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Analysis of the genome of the flavivirus responsible for the 1999 New York City encephalitis epidemic cloned from human brain by reverse-transcription polymerase chain reaction indicates its identity as a lineage I West Nile virus (WNV; WNV-NY1999) closely related to WNVs previously isolated in the Middle East.

The *Flaviviridae* include human pathogens such as yellow fever, dengue, Japanese encephalitis, St Louis encephalitis, Kunjin (KUNV), and West Nile (WNV) viruses. WNV infections are often mild, but may result in encephalitis. WNV is transmitted by mosquitoes. Birds are maintenance hosts and may carry WNV. Epidemics have been reported in Israel, France, South Africa, and Romania where an outbreak near Bucharest in 1996-97 had an up to 5% mortality.¹ WNV infection was not recognised in the Americas until August, 1999, when there was an outbreak of encephalitis in New York City.² WNV-specific immunological and molecular reagents confirmed infection in 59 human beings; 103 birds; 12 horses; and in *Culex pipiens*, other *Culex* species, and *Aedes vexans* mosquitoes. The distribution of isolates indicated that the zone of infection extended beyond New York City to surrounding counties, as well as to Connecticut and Maryland. We cloned the genome of the New York WNV (WNV-NY1999) from necropsy human brain samples by RT-PCR. Due to the autolytic

interval, RNA integrity was reduced: 17 overlapping amplification products were required to assemble a WNV-NY1999 virus genome sequence comprising 10 945 nucleotides.

Initial database analysis of WNV-NY1999 revealed sequence similarity to WNV-Wengler (Nigeria) and KUNV-MRM61c (Australia).³ After cloning the WNV-NY1999 genome, sequences representing the E, NS3, NS5, and 3'-UTR regions were compared with published and unpublished flavivirus sequences. Subtypes of WNV are distinguished by antigenic variations in the E (envelope) protein and the presence of an N-glycosylation site (Asn-Tyr-Ser) at aminoacids 154-156.^{3,4} Two lineages of WNVs are proposed based on signature aminoacid motifs: lineage I includes KUNV as well as WNVs from Europe; the Middle East; and North, Central and West Africa; lineage II includes WNVs from West, Central, and East Africa, and from Madagascar. Deduced WNV-NY1999 E aminoacid sequence showed an intact N-glycosylation site and the presence of lineage I signature motifs (Ala₁₇₂, Asn₁₉₉, Thr₂₀₅, Thr₂₀₈, and Thr₂₁₀).⁴ Phylogenetic analysis of E nucleotide sequence also indicated membership in lineage I (figure 1A). Alignment of available NS3 and NS5 sequences confirmed assignment of WNV-NY1999 to the WNV and KUNV genotype lineage I defined by Berthet et al⁴ based on E gene sequence (figure 1B, C). Alignment of 3'-UTR sequences, which are divergent immediately downstream of the polyprotein stop codon,⁵ showed conservation between WNV-NY1999 and WNV-EGY-Eg101 (table). High levels of E nucleotide sequence conservation were also found between WN-NY1999 and WNV-EGY-Eg101,

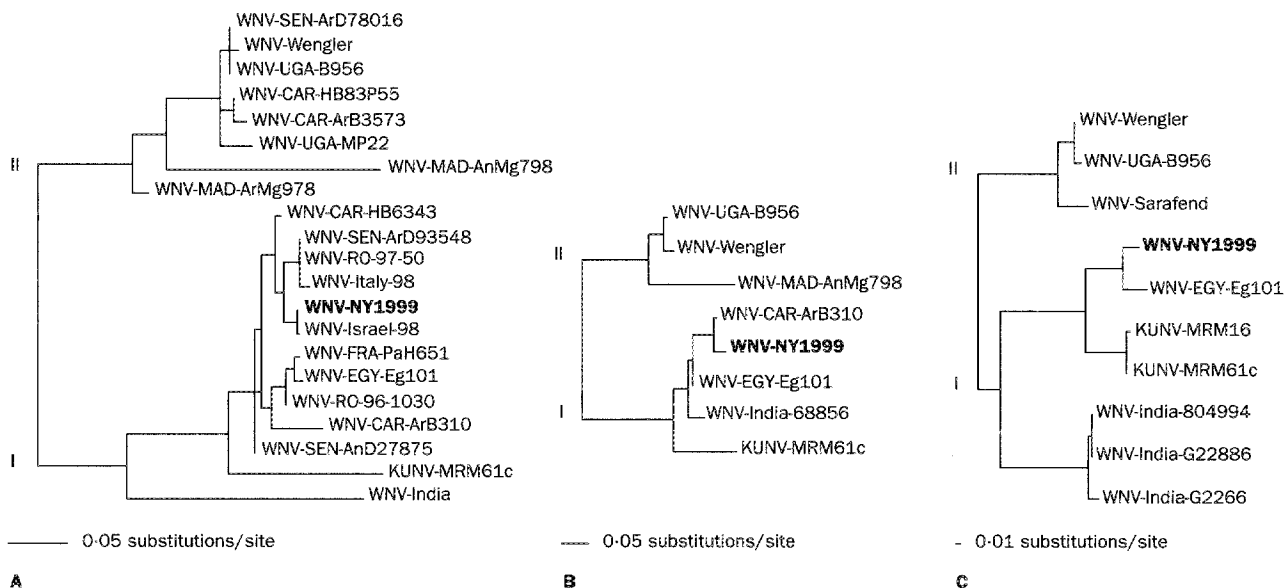


Figure 1: Analysis of WNV-NY1999 E, NS3, NS5, and 3'-UTR sequences

A, phylogenetic tree based on 227 nucleotides of E region sequence indicating membership of WNV-NY1999 in lineage I. B, phylogenetic tree based on 182 nucleotides of NS3 sequence indicating the relationship of WNV-NY1999 to lineage I viruses WNV-CAR-ArB310 and WNV-EGY-Eg101. C, phylogenetic tree based on 236 nucleotides of NS5 sequence indicating the relationship of WNV-NY1999 to lineage I viruses WNV-EGY-Eg101 and KUNV MRM61c.

Viral sequences used in figures 1 and 2: WNV-Wengler, GenBank #M12294, KUNV-MRM61c, #D00246. Envelope: WNV-UGA-B956, this study; WNV-UGA-MP22, #AF001562; WNV-MAD-ArMg978, #AF001574; WNV-MAD-AnMg798, #AF001559; WNV-SEN-ArD78016, #AF001556; WNV-SEN-AnD27875, #AF001569; WNV-SEN-ArD93548, #AF001570; WNV-CAR-HB83P55, #AF001557; WNV-CAR-ArB3573, #AF001565; WNV-CAR-HB6343, #AF001558; WNV-CAR-ArB310, #AF001566; WNV-EGY-Eg101, #AF001568; WNV-FRA-PaH651, #AF001560; WNV-RO-96-1030, #AF130363; WNV-RO-97-50, #AF130362; WNV-Romania-96, WNV-Italy-98, and WNV-Israel-98, WNV-India: V Deubel, unpublished. NS3: WNV-UGA-B956; WNV-MAD-AnMg798 (Dak-MG798), WNV-CAR-ArB310 (Dak-B310), WNV-EGY-Eg101 (E101), WNV-India-68856: Porter KR, et al. Am J Trop Med Hyg 1993; **48**: 440-46. NS5 and 3'-UTR: KUNV-MRM16, #L48979; WNV-UGA-B956, this study (#AF208017); WNV-EGY-Eg101, #AF017254; WNV-Sarafend, #L48977; WNV-India-804994, JSM, RAH, and JS, unpublished (#AF196540); WNV-India-G2266, JSM, RAH, and JS, unpublished (#AF196537); WNV-India-G22886, JSM, RAH, and JS, unpublished (#AF196538).

	WNV-NY1999										
WNV-NY1999		WNV-Israel-98									
WNV-Israel-98	100.0		WNV-CAR-HB6343								
WNV-CAR-HB6343	97.8	97.8		WNV-RO-97-50							
WNV-RO-97-50	96.9	96.9	97.4		WNV-SEN-ArD93548						
WNV-SEN-ArD93548	96.9	96.9	97.4	100.0		WNV-Italy-98					
WNV-Italy-98	96.5	96.5	96.9	99.1	99.1		WNV-SEN-AnD27875				
WNV-SEN-AnD27875	96.0	96.0	97.4	96.5	96.5	96.0		WNV-Romania-96			
WNV-Romania-96	95.2	95.2	95.6	95.6	95.6	95.2	96.5		WNV-RO-96-1030		
WNV-RO-96-1030	95.2	95.2	95.6	95.6	95.6	95.2	97.4	99.1		WNV-FRA-PaH651	
WNV-FRA-PaH651	95.2	95.2	95.6	95.6	95.6	95.2	97.4	98.7	98.7		WNV-EGY-Eg101
WNV-EGY-Eg101	94.7	94.7	95.2	95.2	95.2	94.7	96.9	97.8	97.8	98.7	WNV-CAR-ArB310
WNV-CAR-ArB310	93.0	93.0	93.8	93.0	93.0	92.5	94.7	93.0	93.8	93.0	92.5

Figure 2: Nucleotide conservation among indicated WNV E region sequences

Percent nucleotide identity was calculated based on 227 nucleotides of E region sequence. Note 100% sequence identity between WN-NY1999 and WNV-Israel-98.

as well as other strains for which no 3'-UTR sequence is available, including WNV-Israel-98 (isolated 1998). Indeed, sequences of WNV-NY1999 and WNV-Israel-98 were identical for the 227 nucleotides available for analysis (figure 2).

Recent outbreaks of WNV infections in Italy, Czechland, Romania, Russia, and New York City suggest maintenance hosts and arthropod vectors for WNVs are widely distributed.¹ It is possible that WNVs were present in the Americas before 1999; however, the high mortality associated with infection of native corvids is more consistent with recent introduction. Similarities in sequence between WNV-NY1999 and WNV-EGY-Eg101 and WNV-Israel-98 may suggest a Middle East origin of WNV-NY1999. Potential routes for introduction of this virus to the Eastern USA include importation of infected birds, mosquitoes, or viraemic human beings. The area within New York City where WNV was prevalent includes two international airports.

Recent outbreaks of WNVs in Europe, Asia, and North America confirm their significance as emerging infectious agents and underscores the importance of global surveillance.

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The assembled WNV-NY1999 sequence is submitted to GenBank under accession number AF202541. Protocol details may be found at:

<http://www.ucihs.uci.edu/departments/neurovirology/>

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KUNV	MRM16	ACAGTATTGT	AAATACITTG	TTAATGTAA	ATAAATAT--	-----TG	TTATTATGTG	TAGAAGTTTA
KUNV	MRM61c	ACAGTATTGT	AAATACITTG	TTAATGTAA	ATAAATAT--	-----TG	TTATTATGTG	TAGAAGTTTA
WNV	EGY-Eg101	ACAGTACTGT	AAATACITTA	TTAATGTAA	ATAGACAA--	-----TG	TAAGCATGTG	TAAAGTATA
WNV	NY1999	ACAGTACTGT	AGATAATTAA	TCAATTGTA	ATAGACAA--	-----TA	TAAGTATGCA	TAAAGTETA
WNV	India-804994	TCAGTGTGGT	AAATAGTAAC	AGTTAA----	-----GG	-----GG	TATGTGTATA	GATTAGTGT
WNV	Wengler	ACTGTTTTGT	AA-----	-----	-----	-----	-----	-----
WNV	UGA-B956	ACTGTTTTGT	AAAAAATAAA	GCTGTATTGA	GTAGTTGTAT	AGTTGTAGTG	TTCATAGCAA	TTTGAATTAT
KUNV	MRM16	GCTTTGTAAT	AGTGTTTGT	G-----TG	TTTAGAGTTA	GGAAAAATTTT	AGT-GAGGAA	GTCAGGCCGG
KUNV	MRM61c	GCTTTATAAT	AGTGTTTGT	G-----TG	TTTAGAGTTA	GAAAAATTTT	AGT-GAGGAA	GTCAGGCCGG
WNV	EGY-Eg101	GTTTTATAGT	AGCATTTAGT	GATGTTAGTG	TAAATGGTTA	AGAAAAATTTT	AAG-GAGGAA	GTCAGGCCGG
WNV	NY1999	GTTTTATAGT	AGTATTTAGT	GGTGTTAGTG	TAAATAGTTA	AGAAAAATTTT	GAG-GAGAAA	GTCAGGCCGG
WNV	India-804994	GT-AAATAGG	ATTAGCTAAA	GTATGCATGT	AGGTTAGTGT	TGAGAAATTTT	GTTAGAGGAA	GTCAGGCCGG
WNV	Wengler	-----AA	GATAGTATTA	TAGTTAGTTT	AGTGTAATA	GGA-TTATT	GAGAAATGAA	GTCAGGCCAG
WNV	UGA-B956	TTAGGCCTAA	GATAGTACTA	TAGTTAGTTT	AGTGTAATA	GGA-TTATT	GAGAAATGAA	GTCAGGCCAG

A maximum of 148 nt following the polyprotein stop codon (bold, WN-UGA-B956) were aligned. Dashes indicate gaps; points indicate nucleotides conserved between WNV-NY1999 and WNV-EGY-Eg101.

Alignment of 3'-UTR sequences