Virus Genes (2013) 47:556–558
DOI 10.1007/s11262-013-0968-1

Rosavirus: the prototype of a proposed new genus of the *Picornaviridae* family

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Received: 20 May 2013 / Accepted: 31 July 2013 / Published online: 13 August 2013

Abstract We describe a 8,724-nucleotide-long picornavirus genome encoding a single 2,470-aa polyprotein obtained from the feces of a wild mouse. Rosavirus is genetically closest to the double ORF *Dicipivirus* found in canine feces that is currently the only picornavirus with a second internal ribosome entry site (IRES). Of note, a section of rosavirus' 5′UTR showed strong sequence and structural conservation with the type II IRES from the *Parechovirus* and *Hungarovirus* genera possibly reflecting exchange of genetic modules between genera. Based on genetic distance criteria rosavirus qualifies as prototype of a new genus of the *Picornaviridae* family.

Keywords Picornavirus · Feces · Mice · Genus

The study

We previously reported a partial (~3,956 bases) genome of rosavirus (*ro*dent *s*tool *a*ssociated picorna*virus*) sequenced together with the genome of another picornavirus named mosavirus (GenBank JF973687) from the stool sample of a single wild canyon mouse (*Peromyscus crinitus*) collected in California in May 2010 [1]. Using the Illumina MiSeq platform (http://www.illumina.com/systems/miseq.ilmn) and 5′RACE [2] we generated and describe here a near-complete 8,724-nucleotide-long genome of rosavirus A (GenBank JF973686).

Alignment and RNA structure prediction of the 5′UTR revealed a type II internal ribosomal entry sites (IRES) structure (Fig. 1) similar to that of parechoviruses [3] and hungaroviruses of cattle and sheep [4]. This structural homology and extended regions of substantial nucleotide sequence similarity to picornaviruses in other genera (e.g., 84 and 71 % identity from positions 155–221 and 332–503 between rosavirus and Ljungan virus sequences) reflect a possible example of modular exchange of type II IRESs comparable to that observed for type IV IRES elements in other picornavirus genera [5]. Since the available rosavirus 5′UTR sequence only starts at a position homologous to the G loop of parechovirus [6] it is likely that ~250 bases are missing from the 5′UTR.

A Kozak sequence, RNNAUGG (AACAUGG), was found at the beginning of the rosavirus ORF [7]. The P1 polypeptide was 869 aa in length sharing the closest aa identity of 35 % with the P1 of canine picodicistrovirus (CPDV) (Table 1) also known as Cadicivirus A, prototype and currently single member of the *Dicipivirus* genus. CPDV was found in canine feces and contains an unusual 2nd IRES between P1 and P2 [8]. P1 amino acids identities of rosavirus to the next closest picornaviruses were 18 and 14 % to Aichi virus and turdivirus 2 (also known as oscivirus A1), respectively (Table 1). Rosavirus P1 contained the conserved motif GXXXT/S (^{3}GRKDS^{7}) for myristoylation [9]. Similar to CPDV, rosavirus did not have a putative L protein preceding the capsid

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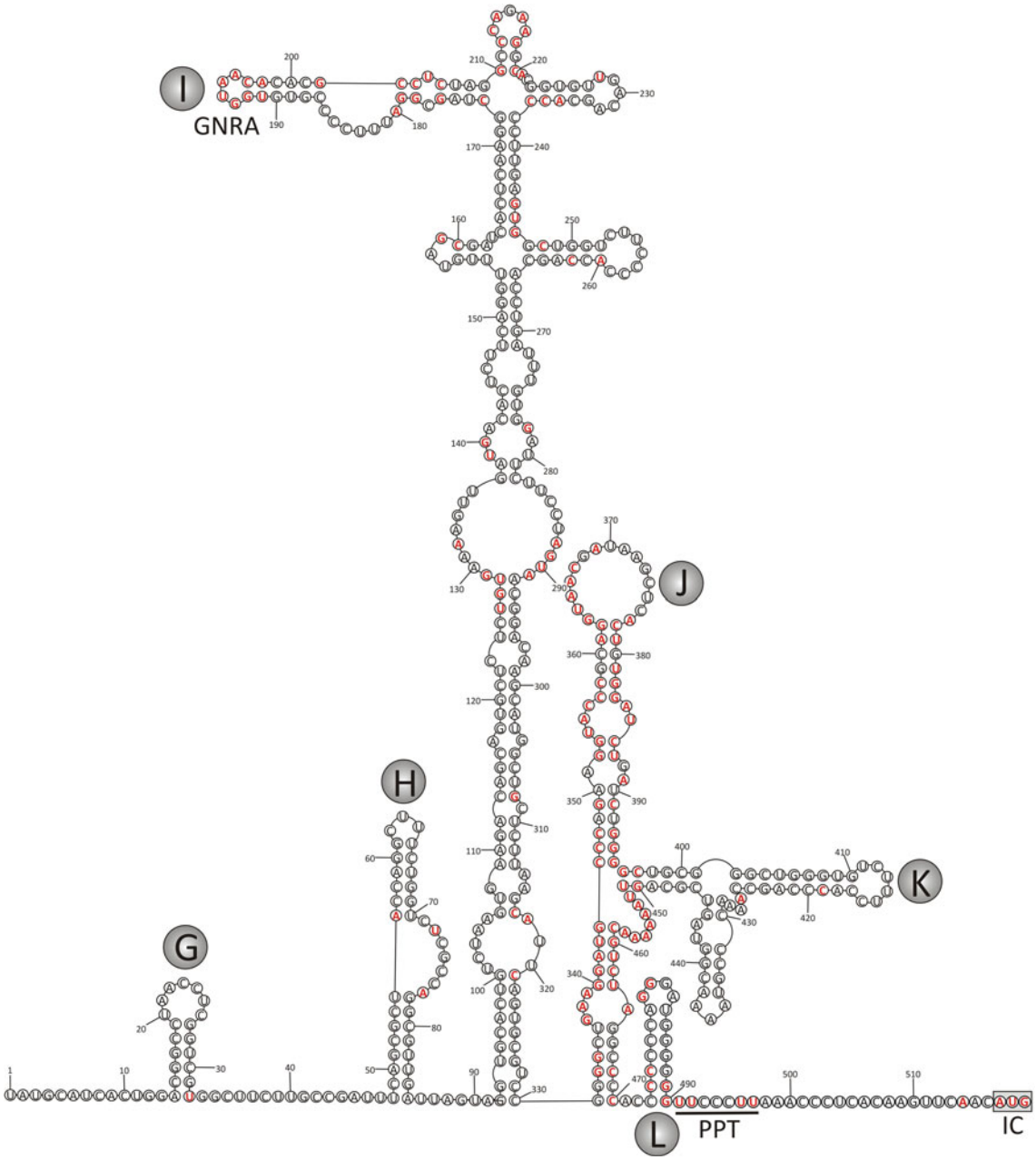

Fig. 1 Proposed RNA secondary structure of the 5′UTR of rosavirus based on minimum free energy folding predictions (MFOLD) and sequence/structural alignment with parechovirus and hungarovirus sequences. Bases conserved with parechoviruses and hungaroviruses are highlighted in red. Stem-loops G-L have been labeled using letters assigned to homologous structures in HPeV [6]. Defined IRES elements GNRA loop, polypyrimidine tract [PPT], and initiation codon [IC] have been labeled

Table 1 Comparison of amino acid sequence identity of rosavirus P1-P3 regions with those of the most closely related picornaviruses

Rosavirus			(%) aa Sequence identity with		
Region	Position	Length (aa)	CDPV (NC_021178)	Aichi virus (NC_001918)	Turdivirus 2 (GU182409)
P1	A^{1}–Q^{869}	869	35	18	14
P2	G^{870}–Q^{1649}	780	17	21	18
P3	T^{1650}–Q^{2470}	821	40	30	31

region [8]. The hypothetical cleavage map of the rosavirus polyprotein was derived from alignments with other picornaviruses and NetPicoRNA prediction [10]. The P1 was hypothetically cleaved at 1A/1B (^{58}D↓S^{59}), 1B/1C (^{314}E↓S^{315}), and 1C/1D (^{588}K↓E^{589}). The 1D protein of rosavirus and CPDV did not have motif [PS]ALXAXETG [8]. The 780-aa P2 polypeptide was hypothetically cleaved at 2A/2B (^{1102}Q↓P^{1103}) and 2B/2C (^{1312}E↓A^{1313}) and shared aa sequence identities of 17, 21, and 18 %—with corresponding polypeptides of CPDV, Aichi virus, and turdivirus 2, respectively. The 2A protein of rosavirus contained an HBox/NC domain [11]. Overall, the rosavirus P3 showed the closest identity (40 %) to CPDV followed by 30 % identity to the P3 of Aichi virus and 31 % to the avian turdivirus 2 (Table 1). Species within a picornavirus genus share >40, >40, and >50 % amino acid identities in P1, P2, and P3 regions, respectively [12], a classification largely supported by discontinuity in pair-wise evolutionary distances distribution among picornaviruses [13, 14]. Since the % aa identities of rosavirus with cogent regions of other picornaviruses are below the within-genus-distance criteria of ICTV, rosavirus is proposed as the prototype genome of the new genus *Rosavirus* in the *Picornaviridae* family, pending ICTV approval (http://www.picornaviridae.com/).

Acknowledgments We acknowledge NHLBI R01HL105770 and support from BSRI to E.D. We also thank staff at the Division of Communicable Disease Control, California Department of Public Health for sample collection.

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