

genotypes, it was slightly overrepresented in AFLP cluster IVb (CC 121) compared with major clusters I and III. Whether the prevalence of PVL in carriage- and blood-culture isolates is higher and differs among distinct genetic clusters of *S. aureus* in countries with endemic CA-MRSA has to be investigated further.

In conclusion, we have shown that the PVL-encoding phage has entered distinct staphylococcal lineages, although its prevalence differs per clonal group. PVL is associated with skin and soft tissue infections but not with bacteremia, which suggests that PVL is not likely to be involved in the pathogenesis of bacteremia. Infections caused by PVL-positive *S. aureus* strains have been documented since the 1930s. Expansion and increased incidence of such infections, however, are more recent, and further epidemiologic studies for tracking this phenomenon are still warranted.

**Damian C. Melles,\*  
Willem B. van Leeuwen,\*  
Hélène A.M. Boelens,\*  
Justine K. Peeters,\*  
Henri A. Verbrugh,\*  
and Alex van Belkum\***

\*University Medical Center Rotterdam, Rotterdam, the Netherlands

## References

- Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M, et al. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet*. 2002;359:753–9.
- Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis*. 1999;29:1128–32.
- Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. *Staphylococcus aureus* isolates carrying Panton-Valentine leukocidin genes in England and Wales: frequency, characterization, and association with clinical disease. *J Clin Microbiol*. 2005;43:2384–90.
- Panton PN, Valentine FCO. Staphylococcal toxin. *Lancet*. 1932;1:506–8.
- Prevost G, Cribier B, Couppie P, Petiau P, Supersac G, Finck-Barbançon V, et al. Panton-Valentine leukocidin and gamma-hemolysin from *Staphylococcus aureus* ATCC 49775 are encoded by distinct genetic loci and have different biological activities. *Infect Immun*. 1995;63:4121–9.
- Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, et al. Necrotizing fasciitis caused by community-associated methicillin-resistant *Staphylococcus aureus* in Los Angeles. *N Engl J Med*. 2005;352:1445–53.
- Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis*. 2003;9:978–84.
- Melles DC, Gorkink RF, Boelens HA, Snijders SV, Peeters JK, Moorhouse MJ, et al. Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. *J Clin Invest*. 2004;114:1732–40.
- Prevost G, Couppie P, Prevost P, Gayet S, Petiau P, Cribier B, et al. Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. *J Med Microbiol*. 1995;42:237–45.
- von Eiff C, Friedrich AW, Peters G, Becker K. Prevalence of genes encoding for members of the staphylococcal leukotoxin family among clinical isolates of *Staphylococcus aureus*. *Diagn Microbiol Infect Dis*. 2004;49:157–62.

Address for correspondence: Damian C. Melles, Erasmus MC, University Medical Center Rotterdam, Department of Medical Microbiology & Infectious Diseases, Rm L-313, Dr Molewaterplein 40, 3015 GD Rotterdam, the Netherlands; email: d.melles@erasmusmc.nl



## Small Anellovirus in Hepatitis C Patients and Healthy Controls

**To the Editor:** Torquetenovirus (TTV) and torquetenominivirus (TTMV) are characterized by a small, negative-sense, circular, single-stranded DNA genome and by an extraordinary ability to produce chronic plasma viremia. Indeed, >80% of humans harbor variably high viral loads of TTV, TTMV, or both, in plasma, regardless of geographic provenance, age, sex, and health conditions (1). Currently, TTV and TTMV are classified as distinct species in the floating (although closely linked to the family *Circoviridae*) genus *Anellovirus*, but their extreme genetic heterogeneity and some distinctive features in genomic organization have led some to suggest that they should be classified as an independent family (2,3). Most recently, after examining serum specimens from patients with symptoms of an acute viral infection by using DNase sequence-independent single-primer amplification, Jones et al. (4) identified, among other viruses, 2 novel TTV- and TTMV-like agents. Because of their even smaller genomes ( $\approx 2.4$  and 2.6 kb vs. 3.6–3.8 kb for TTV and 2.8–2.9 kb for TTMV), these agents were named small anelloviruses (SAVs).

Because tissue culture and serologic methods are not yet available, diagnosis of anellovirus infection relies exclusively on viral DNA detection. We tested 55 Italian hepatitis C patients (mean age  $56 \pm 14$  years, male/female ratio 30/25, 53 TTV positive) and, for comparison, 35 healthy donors (mean age  $36 \pm 12$  years, male/female ratio 17/18, 33 TTV positive) for SAV in plasma by using the polymerase chain reaction (PCR) primers described by Jones et al. (4), followed by direct amplicon

sequencing. To increase assay sensitivity, a heminested PCR format was adopted that used a sense primer designed in a segment of the untranslated region that is highly conserved among all anelloviruses (5'-TCAAGGGCA ATTCGGGCT-3'). We found 5 positive results among the hepatitis C patients (9.1%, all of whom were TTV positive) and 3 positive results among healthy controls (8.6%); and all were confirmed by sequence data.

The amino acid sequences inferred from the coding segment of the amplicon of SAV in this study and the corresponding sequences of the 10 SAV in GenBank at the time of this writing were then aligned with representative TTV and TTMV sequences (online Appendix Figure 1, available from <http://www.cdc.gov/ncidod/EID/vol12no07/06-0234-G1.htm>). This method allowed us to identify the motif WX<sub>7</sub>HX<sub>3</sub>CXCX<sub>5</sub>H, which is highly characteristic of the open reading frame 2 (ORF2) of anelloviruses (5), in all SAVs. SAV sequences, as well as a large number of TTVs and all TTMVs, were then used to construct a phylogenetic tree and to calculate the extent of genetic divergence within SAV, TTV, and TTMV. Although a precise phylogenetic description will require the analysis of full-length ORF2, the SAV sequences clustered quite separately from those of TTV and TTMV, and the extent of divergence observed among SAV was huge and in the same range as among TTV or TTMV. Furthermore, SAVs obtained from hepatitis C patients and healthy participants were intermingled (online Appendix Figure 2, available from <http://www.cdc.gov/ncidod/EID/vol12no07/06-0234-G2.htm>).

While this study was under way, Biagini et al. reported a 12% prevalence of SAV viremia in French blood donors (6). Our results confirm the high prevalence of SAV viremia in healthy persons and extend the finding to hepatitis C patients. Our data,

combined with those of Biagini et al., indicate that, since SAV clusters separately from previously identified anelloviruses, it should be considered a distinct species (or possibly genus). This would increase the already high genetic diversity of anelloviruses, further arguing for the appropriateness of creating a separate viral family.

Because the clinical and viral parameters of hepatitis C in SAV-positive patients were not significantly different from those in the SAV-negative patients (data not shown), our results suggest that, similar to TTV (7), SAV has little or no effect on the course of hepatitis C. Although anelloviruses have not yet been definitely linked to any specific disease, evidence is growing that they might be involved in acute respiratory diseases in children (8,9). Furthermore, a florid TTV replication in the respiratory tract correlated with severity of lung impairment in children with asthma (10). A precise appreciation of the wide range of viruses classified within the anelloviruses is a prerequisite to understanding such disease associations and the disease-inducing potential of these viruses in general.

**Elisabetta Andreoli,\*  
Fabrizio Maggi,\* Mauro Pistello,\*  
Silvia Meschi,\*  
Marialinda Vatteroni,\*  
Luca Ceccherini Nelli,\*  
and Mauro Bendinelli\***

\*University of Pisa, Pisa, Italy

#### References

1. Bendinelli M, Pistello M, Maggi F, Fornai C, Freer G, Vatteroni ML. Molecular properties, biology and clinical implications of TT virus, a recently identified widespread infectious agent of man. *Clin Microbiol Rev.* 2001;14:98–113.
2. Hino S. TTV, a new human virus with single stranded circular DNA genome. *Rev Med Virol.* 2002;12:151–8.
3. Biagini P, Todd D, Bendinelli M, Hino S, Mankertz A, Mishiro S, et al. Anellovirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. *Virus taxonomy*, 8th report of the International

Committee for the Taxonomy of Viruses. New York: Elsevier/Academic Press; 2004. p. 335–41.

4. Jones MS, Kapoor A, Lukashov VV, Simmonds P, Hecht F, Delwart E. New DNA viruses identified in patients with acute viral infection syndrome. *J Virol.* 2005;79:8230–6.
5. Takahashi K, Hijikata M, Samokhvalov EI, Mishiro S. Full or near full length nucleotide sequences of TT virus variants (types SANBAN and YONBAN) and the TT virus-like mini virus. *Intervirology.* 2000;43:119–23.
6. Biagini P, de Micco P, de Lamballerie X. Identification of a third member of the *Anellovirus* genus ("small anellovirus") in French blood donors. *Arch Virol.* 2006;151:405–8.
7. Nishizawa Y, Tanaka E, Orr K, Rokuhara A, Ichijo T, Yoshizawa K, et al. Clinical impact of genotype 1 TT virus infection in patients with chronic hepatitis C and response of TT virus to alpha-interferon. *J Gastroenterol Hepatol.* 2000;15:1292–7.
8. Biagini P, Charrel RN, de Micco P, de Lamballerie X. Association of TT virus primary infection with rhinitis in a newborn. *Clin Infect Dis.* 2003;36:128–9.
9. Maggi F, Pifferi M, Fornai C, Andreoli E, Tempestini E, Vatteroni ML, et al. TT virus in the nasal secretions of children with acute respiratory diseases: relations to viremia and disease severity. *J Virol.* 2003;77:2418–25.
10. Pifferi M, Maggi F, Andreoli E, Lanini L, De Marco E, Fornai C, et al. Associations between nasal torquetenovirus load and spirometric indices in children with asthma. *J Infect Dis.* 2005;192:1141–8.

Address for correspondence: Mauro Bendinelli, Virology Section and Retrovirus Center, Department of Experimental Pathology, University of Pisa, via San Zeno 35–37, I-56127 Pisa, Italy; email: bendinelli@biomed.unipi.it

