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Phylogenetic Analysis of Human Parvovirus B19 Sequences from Eleven Different Countries Confirms the Predominance of Genotype 1 and Suggests the Spread of Genotype 3b▽

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Phylogenetic analysis of 166 human parvovirus B19 sequences from 11 different countries attributed 91.57% to genotype 1, 5.42% to genotype 3b, and 3.01% to genotype 3a. Very similar viruses of genotype 1 circulated widely in Europe and Israel. Genotype 3b seems to show an increasing spread outside of Africa.

Human parvovirus B19 (B19V) infections are usually associated with mild disease, but in immunocompromised and anemic patients, as well as during pregnancy, severe complications can occur. Based on the genetic variability of 994 nucleotides (nt) of the NS1/VP1-unique region junction, three distinct genotypes of B19V have been proposed (13). A recent report presented evidence that certain complications might be preferentially associated with certain virus genotypes (6). Several studies demonstrated that previously published or commercially available assays show differences in their diagnostic performance, including the inability to detect certain genotypes, especially genotype 3, subtype 3b (1, 5). Despite these important implications for the diagnosis of B19V, little is known about the genotypes prevalent in many countries.

Serum samples collected between 2000 and 2008 mostly from rash/fever patients negative for both measles and rubella from 11 different countries were analyzed for B19V (Table 1). A nested PCR was performed with the forward primers e1855f and e1863f (13) and reverse primers B19-R1 (5′-GGGAACT TCCGGCAAACTTCCTTG-3′) and B19-R2 (5′-GTAGTCTT TTACTACTTGTGCTTG-3′), yielding fragments of 1,239 and 1,168 nt. Previously published reverse primers (13) have a maximum of three (e2953r) and four (e2960r) mismatches compared to B19V GenBank sequences, including 3′ and 5′

TABLE 1. Origin and characteristics of the 166 human parvovirus B19 sequences studied

Location	No. of patients	Age range (yr)	Yr(s) of sample collection	No. of patients with genotype:			Mean genetic distance (%)[a]
				1	3a	3b	
Bishkek, Kyrgyzstan	12	<1–21	2006–2007	12			1.07
Osh, Kyrgyzstan	12	1–29[b]	2006–2007	8		4	5.95
Bulgaria	54	3–39	2005–2007	53		1	0.54
Burkina Faso	5	20–29	2007–2008		5		0.41
Estonia	8	4–17	2008	8			0.66
Georgia	3	<1–14	2008	3			0.27
Greece	21	13–44[b]	2000–2005	20		1	1.77
Israel	14	7–66	2001–2006	14			0.52
Luxembourg	21	7–58	2000–2006	21			0.81
Nigeria	5	5–69	2005, 2007	2		3	7.3
Russia	5	<1–38	2006–2007	5			0.41
Serbia	6	3–31	2007	6			0.34
Total	166	<1–69[b]	2000–2008	152	5	9	2.48

[a] Distance between all sequences from the same location.
[b] Age not recorded for all patients.

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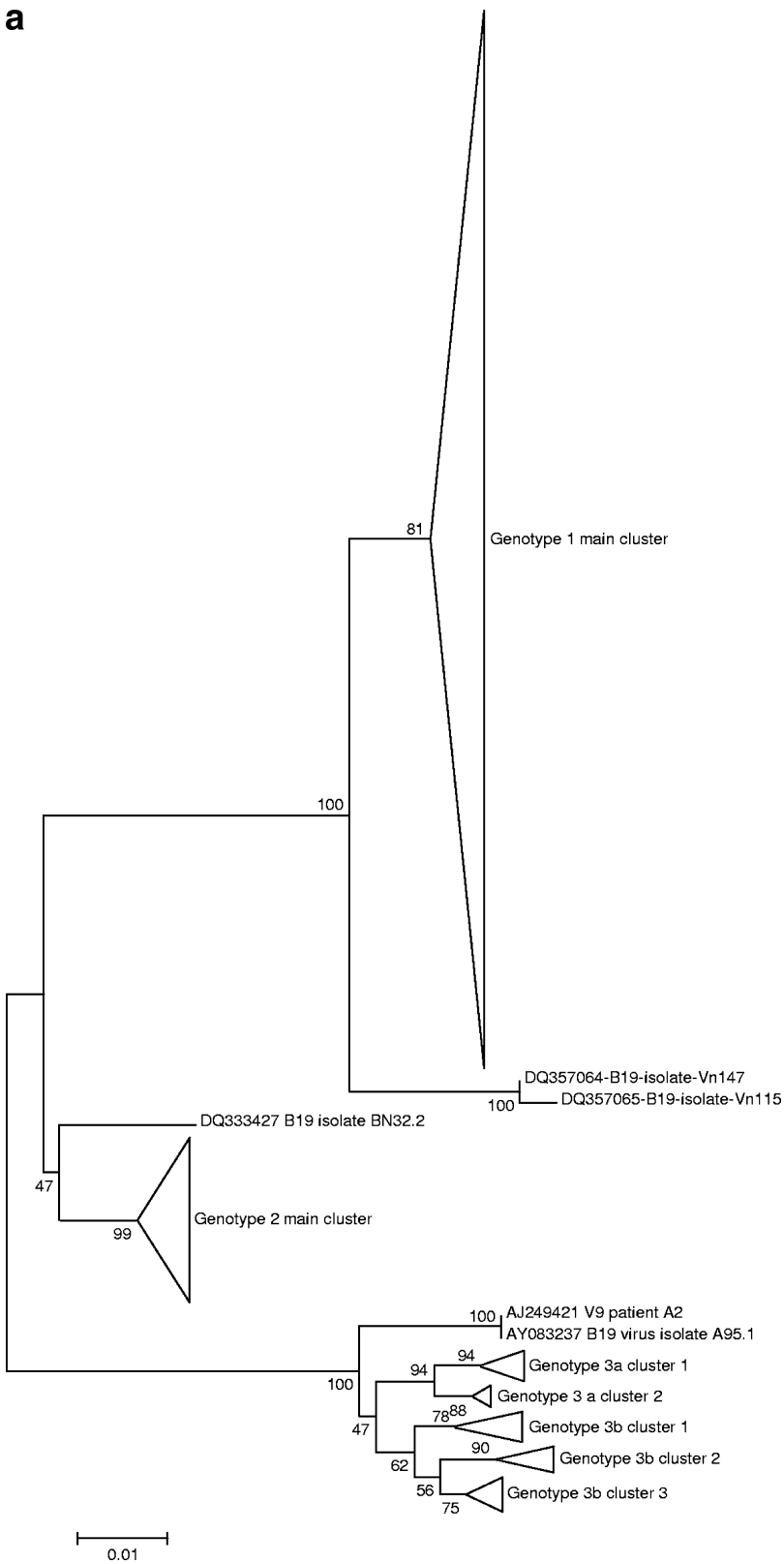

FIG. 1. (a) Phylogenetic tree based on 319 sequences (166 obtained in this study and 153 downloaded from GenBank). The basis of each isosceles triangle is proportional to the number of sequences. (b) Phylogenetic tree based on 92 nonredundant sequences from this study and a set of sequences downloaded from GenBank used as references for the different genotypes. The reference sequences are shown without symbols and are labeled with their GenBank accession number, name, and genotype designation. Phylogenetic analyses are based on a region of 994 nt and the neighbor-joining algorithm using the Kimura two-parameter model. Sequences are from 12 different locations in 11 countries: Bishkek, Kyrgyzstan (black circles); Osh, Kyrgyzstan (dark gray circles); Bulgaria (light gray circles); Burkina Faso (black squares); Estonia (dark gray squares); Georgia (light gray squares); Greece (black triangles); Israel (dark gray triangles); Luxembourg (inverted black triangles); Nigeria (inverted dark gray triangles); the Russian Federation (black diamonds); and Serbia (dark gray diamonds).

FIG. 1—*Continued.*

mismatches. Considering the impact of mismatches in the primer binding sites (7), our new reverse primers with no more than two mismatches would be more sensitive to amplify the NS1/VP1-unique region junction proposed for genotyping by sequencing and phylogenetic analysis (13).

Phylogenetic analysis (MEGA version 3.1) of 166 sequences obtained in this study (Table 1) and 153 sequences downloaded from GenBank (February 2009) showed that the majority of the new samples (n = 152 [91.57%]) and 245 sequences in total clustered together to form the genotype 1 group, including two outliers (DQ357064 and DQ357065) (Fig. 1a). Genotype 1 dominated in 9 of the 11 countries investigated here. Thus, our results further confirm the predominance of genotype 1 in most parts of the world (15). The mean genetic distance among all genotype 1 sequences within the 994-nt region was 0.91%, and the maximal genetic distance was 4.70% (DQ357065 and DQ293995).

Recent studies suggest that genotype 2 viruses circulated widely in Europe half a century ago, before they were largely replaced by genotype 1 (6, 9). Not a single genotype 2 virus was found in this study, further indicating that this genotype has essentially disappeared from circulation (9). Thirty-eight of the 39 sequences from GenBank attributed to genotype 2 clustered together, while one sequence (DQ333427) seems to represent an outlier of genotype 2 (Fig. 1a). The genotype 2 sequences exhibited an overall mean genetic distance of 1.17% and a maximal genetic distance of 4.71% (DQ333427 and AY064476).

The 35 sequences belonging to genotype 3, including the 14 new sequences (5 subtype 3a and 9 subtype 3b) described here, grouped together with a bootstrap support of 100%. All sequences except two identical outliers (AJ249421 and AY083237) fell into the two groups previously designated as genotypes 3a (n = 12) and 3b (n = 21) (10). Within genotype 3a, two clusters supported by bootstrap values of 94 and 88 could be distinguished, and within 3b, three main clusters (bootstrap values 78, 90, and 75) were observed (Fig. 1a). The genotype 3 sequences showed by far the largest mean genetic distance (2.38%), with a maximum distance of 4.49% (DQ408305 and NC_004295). Genotype 3 has been found in France (13), Brazil (11), the United Kingdom (3), the United States (FJ265736 [unpublished data]), and foreigners in Germany (12), and it was the most prevalent genotype in Ghana (2). Interestingly, three of our five sequences from Nigeria and all five sequences from Burkina Faso also belonged to this genotype (Fig. 1b). Despite the limited number of sequences, this may suggest a wider distribution and a predominance of this genotype in West Africa. Besides the two single sequences from Bulgaria and Greece, four sequences from Osh in Kyrgyzstan also belonged to genotype 3 (Fig. 1b). These sequences were associated with each of the three clusters of genotype 3b, covering the full range of genetic variability currently observed within this subtype. Subtype 3b viruses were all collected and/or reported during the last decade. They were found in three different non-African countries in our study as well as recently in Brazil (4) and in foreigners from North Africa and Turkey living in Germany (12). This indicates either that 3b viruses are spreading outside of Africa, or they were previously missed due to the diagnostic problems mentioned before (1, 5). In contrast, subtype 3a viruses were only found in Burkina Faso, and they all belonged to cluster 1 of this subtype.

Mutation rates of B19V are high for a DNA virus and comparable to those of RNA viruses (8, 14). Within the 994-nt region, genotype 3 is by far the most diverse, with a mean intragenotype distance of 2.38%, followed by genotypes 2

(1.17%) and 1 (0.91%). Although these percentages may be biased by many identical or very similar genotype 1 sequences, they indicate that genotype 3 has a longer evolutionary history than the other two genotypes and that genotype 1 may be of a more recent origin (8). This hypothesis is also supported by our finding that very similar genotype 1 viruses circulate widely throughout Europe and Israel (topmost cluster in Fig. 1b).

In conclusion, this study provides genotype information for 11 countries in Europe, Asia, and West Africa and doubles the number of B19V sequences spanning the NS1/VP1-unique region junction available in GenBank. While the worldwide predominance of genotype 1 and the disappearance of genotype 2 have been confirmed, genotype 3 seems to be more widely distributed than previously thought. The latter genotype seems to be predominant in parts of West Africa and has now also been found in three additional non-African countries. Whether the recent reports of genotype 3b viruses are due to improved diagnostics or whether this subtype shows an increasing spread also outside of Africa deserves further investigation. The high genetic diversity of genotype 3 viruses with several clusters within subtypes 3a and 3b compared to genotype 1 and 2 viruses is indicative of a longer evolutionary history, probably in Africa.

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REFERENCES

1. **Baylis, S. A., J. F. Fryer, and P. Grabarczyk.** 2007. Effects of probe binding mutations in an assay designed to detect parvovirus B19: implications for the quantitation of different virus genotypes. J. Virol. Methods **139:**97–99.
2. **Candotti, D., N. Etiz, A. Parsyan, and J.-P. Allain.** 2004. Identification and characterization of persistent human erythrovirus infection in blood donor samples. J. Virol. **78:**12169–12178.
3. **Cohen, B. J., J. Gandhi, and J. P. Clewley.** 2006. Genetic variants of parvovirus B19 identified in the United Kingdom: implications for diagnostic testing. J. Clin. Virol. **36:**152–155.
4. **Freitas, R. B., F. L. Melo, D. S. Oliveira, C. M. Romano, M. R. Freitas, O. Macedo, A. C. Linhares, P. M. de A. Zanotto, and E. L. Durigon.** 2008. Molecular characterization of human erythrovirus B19 strains obtained from patients with several clinical presentations in the Amazon region of Brazil. J. Clin. Virol. **43:**60–65.
5. **Hokynar, K., P. Norja, H. Laitinen, P. Palomäki, A. Garbarg-Chenon, A. Ranki, K. Hedman, and M. Söderlund-Venermo.** 2004. Detection and differentiation of human parvovirus variants by commercial quantitative real-time PCR tests. J. Clin. Microbiol. **42:**2013–2019.
6. **Kuhl, U., D. Lassner, M. Pauschinger, U. M. Gross, B. Seeberg, M. Noutsias, W. Poller, and H. P. Schultheiss.** 2008. Prevalence of erythrovirus genotypes in the myocardium of patients with dilated cardiomyopathy. J. Med. Virol. **80:**1243–1251.
7. **Kwok, S., D. E. Kellogg, N. McKinney, D. Spasic, L. Goda, C. Levenson, and J. J. Sninsky.** 1990. Effects of primer-template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies. Nucleic Acids Res. **18:**999–1005.
8. **Norja, P., A. M. Eis-Hübinger, M. Söderlund-Venermo, K. Hedman, and P. Simmonds.** 2008. Rapid sequence change and geographical spread of human parvovirus B19: comparison of B19 virus evolution in acute and persistent infections. J. Virol. **82:**6427–6433.
9. **Norja, P., K. Hokynar, L. M. Aaltonen, R. Chen, A. Ranki, E. K. Partio, O. Kiviluoto, I. Davidkin, T. Leivo, A. M. Eis-Hubinger, B. Schneider, H. P. Fischer, R. Tolba, O. Vapalahti, A. Vaheri, M. Soderlund-Venermo, and K. Hedman.** 2006. Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. Proc. Natl. Acad. Sci. USA **103:**7450–7453.
10. **Parsyan, A., C. Szmaragd, J. P. Allain, and D. Candotti.** 2007. Identification and genetic diversity of two human parvovirus B19 genotype 3 subtypes. J. Gen. Virol. **88:**428–431.
11. **Sanabani, S., W. K. Neto, J. Pereira, and E. C. Sabino.** 2006. Sequence variability of human erythroviruses present in bone marrow of Brazilian patients with various parvovirus B19-related hematological symptoms. J. Clin. Microbiol. **44:**604–606.
12. **Schneider, B., A. Hone, R. H. Tolba, H. P. Fischer, J. Blumel, and A. M. Eis-Hubinger.** 2008. Simultaneous persistence of multiple genome variants of human parvovirus B19. J. Gen. Virol. **89:**164–176.
13. **Servant, A., S. Laperche, F. Lallemand, V. Marinho, G. De Saint Maur, J. F. Meritet, and A. Garbarg-Chenon.** 2002. Genetic diversity within human erythroviruses: identification of three genotypes. J. Virol. **76:**9124–9134.
14. **Shackelton, L. A., and E. C. Holmes.** 2006. Phylogenetic evidence for the rapid evolution of human B19 erythrovirus. J. Virol. **80:**3666–3669.
15. **Toan, N. L., A. Duechting, P. G. Kremsner, L. H. Song, M. Ebinger, S. Aberle, V. Q. Binh, D. N. Duy, J. Torresi, R. Kandolf, and C. T. Bock.** 2006. Phylogenetic analysis of human parvovirus B19, indicating two subgroups of genotype 1 in Vietnamese patients. J. Gen. Virol. **87:**2941–2949.