Detection of PARV4, genotypes 1 and 2, in healthy and pathological clinical specimens

Sara Botto[1], Massimiliano Bergallo[1], Francesca Sidoti[1], Maria Elena Terlizzi[1], Sara Astegiano[1], Renata Ponti[2], Cristina Costa[1], Rossana Cavallo[1]

[1]*Department of Public Health and Microbiology, Virology Unit, Turin University, Italy;*
[2]*Section of Dermatology, Department of Medical Sciences and Human Oncology, University of Turin, Italy*

SUMMARY

The molecular epidemiology and tissue distribution of Human Parvovirus 4 (PARV4) and its variant PARV5 (Parvoviridae family) are poorly known. The aim of this study was to investigate the epidemiological role and prevalence of PARV4/5 by a nested-PCR on different clinical specimens, including blood samples from healthy donors, healthy and pathological skin samples, and bronchoalveolar lavages (BAL). Among blood specimens, 2/53 were positive; 3/37 and 23/105 of healthy and pathological skin specimens resulted positive, respectively, whereas no BAL was positive. PARV4/5 may be present in different healthy and pathological samples, suggesting the need for further investigating its tissue distribution.

KEY WORDS: Human parvovirus 4, Blood donors, Bronchoalveolar lavage, Skin

Human Parvovirus 4 (PARV4) was first identified in a plasma sample from a homeless injection drug user co-infected with HBV and presenting different symptoms including fatigue, vomiting, diarrhea, sore throat and joint pains (Jones *et al.*, 2005). PARV4 belongs to the *Parvoviridae* family, along with B19 and HBoV, that is constituted by small non-enveloped viruses, with single-stranded linear DNA.

The amino acid homology between Parvovirus B19 and PARV4 is less than 30% (Longhi *et al.*, 2007), suggesting that they are just distantly related to each other and that PARV4 could be a putative member of a new parvovirus genus. In 2006 a different variant of PARV4 (genotype 1) was discovered and lately named PARV5 (also called PARV4 genotype 2) (Fryer *et al.*, 2006). The

_____
Corresponding author
Cristina Costa
Department of Public Health and Microbiology
Virology Unit, Turin University
Via Santena, 9
10126 Turin, Italy
E-mail: cristina.costa@unito.it

nucleotide divergence between the two genotypes is about 8% in the region sequenced (Fryer *et al.*, 2006, 2007a). The genome of PARV4 contains two large non-overlapping open reading frames called ORF1 and ORF2 encoding a non-structural protein and a capsid protein, respectively.

The ORF1 has a nucleotide identity that ranges from 89% to 97% with respect to non-structural proteins of the other Parvoviruses (Fryer *et al.*, 2007a).

Previous studies have detected PARV4 in lymphoid organs and bone marrow of HIV positive individuals. However, it was subsequently detected also in HIV negative samples from blood donors, thus confirming that the virus is not only detectable in subjects with an immune-compromised background (Fryer *et al.*, 2007a,b, Longhi *et al.*, 2008).

Other studies disclosed the virus in different biological samples such as the blood and plasma pool used in the manufacture of plasma-derived medicinal products (Jones *et al.*, 2005), blood samples from HCV-infected subjects (Fryer *et al.*, 2007c) and in tissue specimens such as liver from transplant recipients, donors and autopsy sam-

ples (Schneider *et al.*, 2007). A recent study detected PARV4 in serum from asymptomatic kidney transplant recipients. Previous analysis verified the absence of the virus in samples before transplantation, thus suggesting that asymptomatic infection may occur after transplantation or may be acquired through transfusion or transplantation (Vallerini *et al.*, 2008). The authors also suggested that peripheral blood mononuclear cells are not a major site of replication, similar to Parvovirus B19.

Very little is known about PARV4/5, in particular no data are available on their role in human diseases, the tissue type-specific associations or the site of persistence in infected persons, both healthy or not. Moreover, the prevalence and epidemiology of PARV4/5 remain unknown. A recent study reported Parvovirus B19 in skin biopsies from patients with clinical suspicion of primary cutaneous lymphomas, inflammatory skin disease patients, and in skin samples from healthy donors, thus confirming that skin may represent a persistent site of infection (Bergallo *et al.*, 2008).

The aim of this work was to investigate the epidemiological role and presence of PARV4, genotypes 1 and 2, using a nested PCR. We analyzed different specimens, including skin samples from healthy donors and pathological patients and blood samples from healthy donors. Moreover, we also evaluated the presence of PARV4 in bronchoalveolar lavages (BAL), as Human Bocavirus, another member of the *Parvoviridae* family, has been detected in respiratory specimens from children with lower airway infections (Sloots *et al.*, 2006, Manning *et al.*, 2006).

A total of 234 samples were examined: 53 healthy blood donors from the "Blood Bank" of the Molinette Hospital of Turin (36 males, 17 females, mean age 42.3±11); 37 healthy donors skin specimens from the "Skin Bank" of the Department of Plastic Surgery and Burns Unit, Orthopaedic and Traumatology Centre of Turin (no data on age or sex, blinded samples); 105 skin biopsies from 96 patients with different dermatological diseases (12 follicle B lymphomas, 41 inflammatory dermatosis [ID, i.e. eczema, dermatitis, psoriasis], 27 Sézary syndromes, 16 mycosis fungoides), including 48 females, 48 males, mean age 63.2±13.4; 12 of these samples were fresh biopsies subsequently cryopreserved, instead the other 25

were paraffin embedded; 39 BALs specimens from 33 patients (20 solid-organ transplant recipients and 13 patients from pneumology or haematology division) subdivided into 29 males, 10 females and mean age 54.5±12.5.

DNA extraction from skin biopsies was performed using a QIAgen tissue Kit (QIAamp DNA Mini Kit; Qiagen, Hilden, Germany), and extracted samples were stored at -80°C. For BAL and blood samples the extraction was performed using the automated NucliSENS easyMAG platform system (BioMérieux, Marcy l'Etoile, France), according to the manufacturer's instructions. The starting volume of blood used for the extraction was 100 µl and the volume of eluate 50 µl, while the volume of BAL employed was 500 µl and the eluate 110 µl.

All the samples were screened for the presence of PARV4 and PARV5 by a fast nested PCR performed on a fast thermal cycler (Applied Biosystems 9800 Fast Thermal Cycler). The primer sequences were acquired from literature and were designed in a conserved region of ORF1 of PARV4 and PARV5. The oligonucleotide primers for the first-round amplification were PV4NS1F (5'-AAGACTACATACCTACCTGTG-3') and PV4NS1R (5'-TGCCTTTCATATTCAGTTCC-3'), yielding a 218 bp product, while the second-round primers were PV4NS1Fn2 (5'-GTTGATGGYCCTGTGGTTAG-3') and PV4NS1Rn2 (5'-CCTTTCATATTTCAGTTCCTGTTCAC-3'), yielding a fragment of 160 bp (Fryer *et al.*, 2007b). A nucleotide-nucleotide blast search was performed at the National Center for Biotechnology Information and The National Library of Medicine website (URL: http://ncbi.nlm.nih.gov) and confirmed that the nested PCR amplified only ORF1 of PARV4 and 5.

For the first-round amplification, 5 µl of extracted samples in a final volume of 30 µl were used, the thermal profile was as follows: 94°C 2 min for 1 cycle; 95°C for 0 s, 52°C for 15 s, 72°C for 10s for 35 cycles; then 1 cycle at 72°C for 5 min. For the second-round amplification, 1 µl of first-round PCR product into 29 µl of amplification mix was used, with the following thermal profile: 94°C 2 min for 1 cycle; 95°C for 0 s, 56°C for 15 s, 72°C for 10 s for 35 cycles; then 1 cycle at 72°C for 5 min. Subsequently, 18 µl of the second-round amplicons were analyzed by electrophoresis on a 3% (w/v) agarose gel under UV

light and the bands were visualized by ethidium bromide. As positive control, the amplification product of a positive clinical isolate (termed # 4748) was cloned into a pTopo TA cloning (Invitrogen, Milan, Italy). Plasmids were propagated into TOP10 chemically competent cells (Invitrogen). As negative control sterile water was amplified with each batch of samples. The sensitivity of the PCR was determined preparing serial 10-fold dilutions of the plasmid ranging from 10^{10} copies/µl to 1 copy/µl.

The detection limit of the nested PCR was 10 DNA copies per reaction. Precautions were taken to prevent contamination. The preparation of the amplification mix, as well as processing of samples and PCR assays were performed in separate laboratory rooms. Statistical analysis was performed by using the chi square test, utilizing a commercially available statistical software (MedCalc Software).

To check PCR inhibition, all samples were tested for the housekeeping gene glycerinaldehyde-phosphate-dehydrogenase (GAPDH) using a Fast nested PCR (first round primers: GCCAAAAGGGT-CATCATCTC forward, GGGGCCATCCACAGTC TTCT reverse; second round primers: CCAAG-GTCATCCATGACAAC forward, GTGGCAGT-GATGGCATGGAC reverse).

Results are summarized in Table 1. Considering whole blood specimens from healthy donors, 2 out of 53 resulted positive (4%); 3 out of 37

TABLE 1 - *Results of Fast Nested PCR for ORF1 detection in clinical samples. ID, inflammatory dermatosis (P not significant for all comparisons).*

Specimens (N)	ORF1 Detection Positive Samples N (%)
BALs (n=39)	0/39 (0%)
Blood donors (n=53)	2/53 (4%)
Skin Biopsies (n=142) Healthy donors (n=37) Pathological samples (n=105)	3/37 (8%)
Sézary syndrome (n=3)	1/33 (3%)
ID (n=42)	16/42 (38%)
Follicle B lymphoma (n=12)	2/12 (17%)
Mycosis fungoides (n=18)	4/18 (22%)
Total	28/234 (12%)

healthy skin specimens were positive (8%), while within the pathological skin specimens, 23 out of 105 were positive (21%), in particular 1/33 with Sézary syndrome (3%), 16/42 with ID (38%), 2/12 with follicle B lymphoma (17%), and 4/18 with mycosis fungoides (22%). No BAL sample was positive for PARV4/5 (0/39). The prevalence of PARV4/5 did not significantly differ comparing different specimen types, or healthy and pathological skin specimens.

Our study focused on the screening of different specimen types to investigate the molecular epidemiology of PARV4, genotypes 1 and 2. PARV4 was searched for in skin specimens considering the skin tropism of Parvovirus B19 (Bergallo *et al.,* 2008). Like B19 genotype 2 (K71), PARV4 positive samples were found in both pathological and healthy skin biopsies, thus suggesting the lack of a pathological role in this context. PARV4 was also detected in healthy blood samples, as previously reported (Vallerini *et al.,* 2008, Fryer *et al.,* 2006). The contamination of blood and haemoderivatives suggests that further studies are necessary to establish whether PARV4 could be dangerous to recipients. On the other hand, BAL samples were negative in all the cases, suggesting the absence of PARV4 in the lower respiratory tract.

In conclusion, based on data detected by us and by other groups, PARV4 may be present in different specimens, such as liver, blood from healthy donors or intravenous drug users, both HIV-positive or -negative (Fryer *et al.,* 2007a,b,c and 2006; Schneider *et al.,* 2007, Longhi *et al.,* 2007), and skin from healthy donors and pathological patients.

Instead, we found that this Parvovirus was not detected in BAL, suggesting a different tissue distribution from HBoV which was identified in respiratory specimens from children with lower respiratory tract diseases (Sloots *et al.,* 2006, Manning *et al.,* 2006). We can also suggest that respiratory routes are not the transmission route of these viruses, unlike the other genera of Parvovirus like B19 and HBoV, whereas it is transmitted through parenteral routes (Simmonds *et al,* 2008). These data call for further studies to investigate the tissue-type distribution of PARV4 and to develop quantitative assays to determine its epidemiological and pathological role in different clinical contexts.

REFERENCES

Bergallo M., Costa C., Sidoti F., Novelli M., Ponti R., Castagnoli C., Merlino C., Bernengo M.G., Cavallo R. (2008). Variant of Parvovirus B19: Bioinformatical evaluation of nested PCR assays. *Intervirology.* **51**, 75-80.

Fryer J.F., Delwart E., Bernardin F., Tuke P.W., Lukashov V.V., Baylis S.A. (2007a). Analysis of two human parvovirus PARV4 genotypes identified in human plasma for fractionation. *J. Gen. Virol.* **88**, 2162-2167.

Fryer J.F., Delwart E., Hecht F.M., Bernardin F., Jones M.S., Shah N., Baylis S.A. (2007b). Frequent detection of parvoviruses, PARV4 and PARV5, in plasma from donors and symptomatic individuals. *Transfusion.* **47**, 1054-1061.

Fryer J.F., Kapoor A., Minor P.D., Delwart E., Baylis S.A. (2006). Novel parvovirus and related variant in human plasma. *Emerg. Infect. Dis.* **12**, 151-154.

Fryer J.F., Lucas S.B., Padley D., Baylis S.A. (2007c). Parvoviruses PARV4/5 in hepatitis C virus infected patients. *Emerg. Infect. Dis.* **13**, 175-176.

Jones M.S., Kapoor A., Lukashov V.V., Simmonds P., Hecht F., Delwart E. (2005). New DNA viruses identified in patients with acute infection syndrome. *J. Virol.* **79**, 8230-8236.

Longhi E., Bestetti G., Acquaviva V., Foschi A., Piolini R., Meroni L., Magni C., Antinori S., Parravicini C., Corbellino M. (2007). Human Parvovirus 4 in the bone marrow of Italian patients. *AIDS.* **21**, 1479-1490.

Manning A., Willey S.J., Bell J.E., Simmonds P. (2007). Comparison of tissue distribution, persistence, and molecular epidemiology of Parvovirus B19 and novel parvoviruses PARV4 and human bocavirus. *J. Infect. Dis.* **195**, 1345-1352.

Schneider B., Fryer J.F., Reber U., Fischer H.P., Tolba R.H., Baylis S.A., Eis-Hubinger A.M. (2008). Persistence of novel human parvovirus PARV4 in liver tissue of adults. *J. Med. Virol.* **80**, 345-351.

Simmonds P., Manning A., Kennell R., Carnie F.W., Bell J.E. (2008). Parenteral transmission of the Novel Human Parvovirus PARV4. *Emerging. Inf. Dis.* **13**, 1386-1388.

Sloots T.P., McErlean P., Speicher D.J., Arden K.E., Nissen M.D., Mackay I.M. (2006). Evidence of human coronavirus HKU1 and human bocavirus in Australian children. *J. Clin. Virol.* **35**, 99-102.

Vallerini D, Barozzi P, Quadrelli C, Bosco R, Potenza L, Riva G, Gregorini G, Sandrini S, Tironi A, Montagnani G, De Palma M, Torelli G, Delwart E, Luppi M. (2008) Parvoviruses in blood donors and transplant patients, Italy. *Emerging. Inf. Dis.* **14**, 185-186.