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Identification of the Novel KI Polyomavirus in the Respiratory Tract of an Italian Patient

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Recently, a new human polyomavirus, KIV, was detected in respiratory specimens of patients with acute respiratory tract infection. Whether this reflects a causal role of the virus in the respiratory tract is still debated. To investigate the presence of KIV in respiratory samples of Italian patients and to determine the degree of similarity with other known polyomaviruses, 222 respiratory specimens collected by general practitioners between 2006 and 2007 were screened. The entire *VP1* gene region was amplified and sequenced. Maximum Likelihood tree was generated by PAUP* software. One out of 222 samples tested was positive for KIV. Phylogenetic analysis indicated that this isolate clustered with other KIV isolates, while the WUV isolates seem to belong to a different lineage. The phylogenetic tree also showed that all other known polyomaviruses are quite distant from this isolate. This is the first report describing the presence of KIV in the respiratory tract of a 5-year-old Italian child with acute respiratory symptoms. Further investigations are needed to establish an etiological link of KIV with acute respiratory illness. ***J. Med. Virol. 80:2012–2014, 2008.***

KEY WORDS: KIV polyomavirus; respiratory tract infection; molecular epidemiology

INTRODUCTION

Polyomaviruses are small double stranded circular DNA viruses of about 5 Kb in length that can infect a variety of mammals and birds. Two human polyomavirus JCV [Padgett et al., 1971] and BKV [Gardner et al., 1971] are widespread in the human population with about 60–80% of the adults exhibiting specific antibodies against these two viral agents. Infection is thought to occur early in life and through the respiratory route, although detection of BKV and JCV in the respiratory tract has been reported rarely [Sundsfjord et al., 1994; Giuliani et al., 2007]. Once in the body, polyomaviruses establish a latent infection in the kidney [Chesters et al., 1983].

Generally, infection with BKV and JCV is asymptomatic in healthy individuals [Brown et al., 1975], while reactivation in immunocompromised hosts can have serious consequences. JCV is associated with progressive multifocal leukoencephalopathy, a demyelinating disease of the brain often seen in AIDS patients [Gordon and Khalili, 1998]. BKV can cause tubular nephritis, which can lead to allograft failure in renal transplant recipients and haemorrhagic cystitis in haematopoietic stem cell transplant recipients [Nickeleit et al., 2002; Hirsch and Steiger, 2003; Chen et al., 2004]. The designations JC and BK were derived from the initials of the patients from which the two viruses were first isolated.

Recently, a third human polyomavirus has been isolated from the respiratory tract and stool of Swedish children [Allander et al., 2007]. This virus is related only distantly to the other known primate polyomaviruses and in analogy with the nomenclature of the other human polyomaviruses the name proposed by Allander et al. [2007] is KI polyomavirus (KIV). The early region of the KIV genome, that is, the Large T antigen and Small T antigen genes showed a high degree of identity with that of BKV and JCV, while identity in the late region (VP1, VP2, and VP3 genes) was quite low. Following this finding, a fourth polyomavirus was isolated from the nasopharyngeal swab of a 3-year-old boy with pneumonia. This new virus was named WU after the initials of the infected patient [Gaynor et al., 2007].

Aim of the study was to determine the presence and the prevalence of KIV in a group of Italian patients with

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acute respiratory symptoms. A phylogenetic analysis of the KIV-*VP1* gene of the Italian isolate was carried out to determine the relationship to polyomaviruses identified previously.

MATERIALS AND METHODS

Study Group

Detection of KIV was carried out on 222 respiratory archived specimens provided by the "Istituto Superiore di Sanita", Rome, between 2006 and 2007, which were tested previously for the presence of respiratory viruses such as respiratory syncitial virus (RSV), influenza viruses A and B, parainfluenza virus types 1–3 (PIV1–PIV3) and human adenoviruses as described elsewhere [Rezza et al., 2006]. Samples were collected by nine general practitioners, including two pediatricians. Essential information such as sex, age, clinical symptoms, vaccination status were recorded on a standardized form during enrolment of the patients. Informed consent was obtained from all participants in the study.

Detection and Sequence Analysis of KIV

Total DNA was extracted from 0.2 ml of specimen using the QIAamp DNA mini kit according to the manufacturer's instruction (QIAGEN S.P.A., Milan, Italy) and then stored at −80°C until analysis. PCR conditions and primers were as originally described [Allander et al., 2007]. The PCR products were analyzed on a 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

The *VP1* gene of KI virus detected in the unique positive case was sequenced using five primer pairs which amplify overlapping fragments of the gene: FKIPyP1-3: 5′-TGCTGATACCTATAAAATTCCT-3′ (position: 1,451–1,472); RKIPyP1-4: 5′-AGGTAATTGACCAGTAATCAGG-3′ (position: 1,732–1,711); FKIPyP1-13: 5′-CCTCATTACTGGTCAATTAGCT-3′ (position: 1,711–1,732); RKIPyP1-11: 5′-AACATATTAGAAAAGTGGTTTG-3′ (position: 2,681–2,660); FKIPyP1-12: 5′-TATCATATGTGAACAACTCTA-3′ (position: 2,231–2,250). The sequencing reaction was performed using the Genome Lab DTCS Quick Start Kit (Beckman Coulter, CA) and run on a Beckman Culture CEQ2000XL sequence analyzer after column purification. The obtained sequence was submitted to the Genebank and matched against all deposited sequences (http://www.ncbi.nlm.nih.gov/BLAST).

Our sequence was then aligned and compared with a set of reference sequences (Table S1) using ClustalX software [Thompson et al., 1997] followed by manual editing using the Bioedit software [Hall, 1999]; gaps were removed from the final alignment. The best fitting nucleotide substitution model was tested with a hierarchical likelihood ratio test, using a neighbor-joining (NJ) tree with LogDet corrected distances as base tree [Swofford and Sullivan, 2003]. A maximum likelihood (ML) tree was then inferred with the selected model and ML-estimated substitution parameters. The heuristic search for the best tree was performed using an NJ tree as starting tree and the TBR branch-swapping algorithm. NJ trees were also obtained with ML estimated pair-wise distances using the best fitting nucleotide substitution model. Calculations were performed with PAUP* software [version 4.0; Swofford, 2002]. Statistical support for specific clades was obtained with the ML-based zero branch length test for the ML tree [Swofford, 2002], by bootstrapping (1,000 replicates) for the NJ tree. Tree were rooted by ML rooting by outgroup rooting. The nucleotide sequence obtained in this study has been submitted to Gen-Bank under accession number EU807841.

RESULTS

Of the 222 respiratory specimens screened, one from a 5-year-old child was positive for the novel polyomavirus KIV. The virus was detected in January when there is usually a peak for respiratory diseases and the patient presented acute respiratory symptoms (fever, cough, headache, nasal congestion, croup).

Phylogenetic analysis of the *VP1* gene of the KIV-Rome isolate showed a high degree of identity (>99%) to the Stockholm and Brisbane isolates and all clustered together (Fig. 1, white triangle), while the WUV strains clustered in a distinct group as supported by the bootstrap value (Fig. 1, gray triangle). The others polyomaviruses grouped in two different clusters genetically distant from the KIV-Rome isolate (Fig. 1).

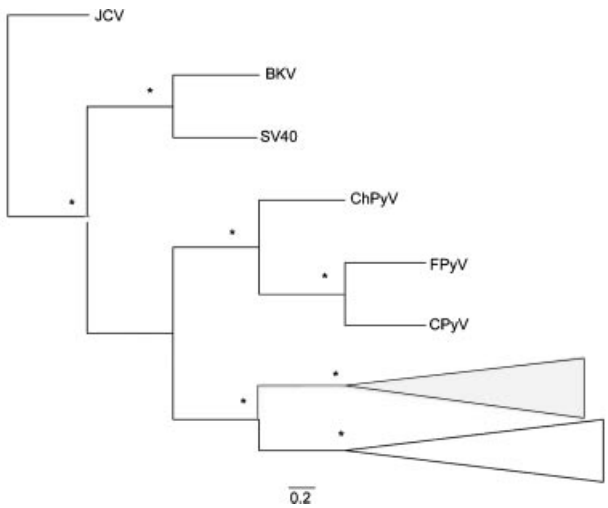

Fig. 1. The data set included the KIV-Rome sequence (Accession number: EU807841) and 23 reference sequences of polyomaviruses downloaded from the NCBI Sequence Database, see Table S1. The tree was rooted using JCV strain as outgroup. Branch lengths were estimated with the best fitting nucleotide substitution model according to a hierarchical likelihood ratio test [Swofford and Sullivan, 2003], and were drawn in scale with the bar at the bottom indicating 0.2 nucleotide substitutions per site. One asterisk (*) along a branch represents significant statistical support for the clade subtending that branch ($P < 0.001$ in the zero-branch-length test, and bootstrap support (>75%). The KIV-Rome strain clustered within the white triangle with KIV strains used as reference sequences, see Table S1.The gray triangle represent the WUV strains (Table S1).

DISCUSSION

KIV is a novel human polyomavirus identified for the first time in the respiratory tract of Swedish children [Allander et al., 2007] and then in Australian patients [Bialasiewicz et al., 2007], suggesting a worldwide distribution of the virus. This study describes the presence of KIV in the respiratory secretions of an Italian patient who presented to the general practitioner with acute respiratory symptoms in 2007. The virus was detected in 1 out of 81 children (1.2%) with respiratory disease studied during the winter season. A similar prevalence was reported in the Swedish cohort [Allander et al., 2007]. The present and previous studies [Allander et al., 2007; Bialasiewicz et al., 2007] suggest that children are more prone to KIV infection or its reactivation as described for BKV and JCV [White and Khalili, 2004].

In order to determine the genetic diversity between the strain identified in this study and the other known polyomaviruses we sequenced the *VP1* gene. Phylogenetic analysis showed that the new isolate clustered with other KIV isolates (Fig. 1, white triangle), while the WUV isolates seem to belong to a different lineage (Fig. 1, gray triangle). The phylogenetic tree also indicated that all other known polyomaviruses are quite distant from the new isolate, Figure 1.

The results of this study do not allow conclusions on the role of KIV as respiratory pathogen because of limited sample size and very similar symptoms with other respiratory diseases. Further investigations and careful epidemiological surveys are needed to establish a firm link to human disease, considering the oncogenic potential of the known human polyomaviruses (JCV and BKV) and their association with several human tumors [White and Khalili, 2004; Giuliani et al., 2007; Zheng et al., 2007], it will be interesting to study the role of KIV in human tumors.

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