for graphical assistance. This work was supported in part by a research grant from Savyon Diagnostics Ltd, and by a Joint fellowship of the Hebrew University Medical School and the Hadassah Hebrew University Medical Center, both awarded to R. Nir-Paz.

REFERENCES


RESEARCH NOTE

Genetic variation of coxackie virus B5 strains associated with aseptic meningitis in Greece

A. Papa, K. Dumaidi, F. Franzidou and A. Antoniadis

A’ Department of Microbiology, School of Medicine, Aristotle University of Thessaloniki, Greece

ABSTRACT

In order to explore the genetic relationships among coxackie virus B5 strains in Greece, the nucleotide sequences of the partial VP1 gene in strains isolated from aseptic cases of meningitis were determined and compared with those of strains isolated from other countries. Phylogenetic analysis showed a high degree of divergence (25%) among Greek strains isolated in different years, which clustered with high bootstrap values in a different subgroup of viruses, suggesting that enterovirus types vary with time rather than geographical distribution. A non-synonymous mutation present in the strains of this study was not observed in other coxackie virus B5 strains.

Corresponding author and reprint requests: A. Papa, A’ Department of Microbiology, School of Medicine, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece E-mail: annap@med.auth.gr

© 2006 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, CMI, 12, 672-694
Keywords Aseptic meningitis, coxsackie virus, enterovirus, genetic relationships, Greece, molecular typing

Original Submission: 4 October 2005; Revised Submission: 10 November 2005; Accepted: 14 December 2005

Clin Microbiol Infect 2006; 12: 688–691
10.1111/j.1469-0691.2006.01476.x

Enteroviruses (EVs) comprise a large genus in the Picornaviridae family, with the 65 known human serotypes delineated into five species (A–D, plus the polioviruses) based mostly on their phylogenetic relationships [1]. Members of the enterovirus B species cause a central nervous system infection, mainly aseptic meningitis, in humans. Detection of enteroviral RNA in patient specimens is the most rapid and useful method for laboratory diagnosis and molecular characterisation of the strains involved [1–7]. In Greece, EVs are the most common cause of aseptic meningitis. Sporadic cases, as well as outbreaks, have been reported, with coxsackie virus B5 being one of the most predominant serotypes [5,6,8].

Between March 2003 and December 2004, 49 patients (32 males, 17 females; mean age 18 years, range 21 days to 73 years) with aseptic meningitis were tested for a probable enteroviral infection. All 49 patients presented with an acute onset of fever (> 38°C), headache, nausea and vomiting; a few had stiff necks and one presented with mental disturbance. Cerebrospinal fluid (CSF) showed pleiocytosis (38–1300 cells/mm3) with lymphocytosis (50–90%), moderately elevated levels of protein (18–180 mg/dL, mean 74 mg/dL) and normal levels of glucose (33–100 mg/dL, mean 57 mg/dL).

Thirty-seven CSF samples were inoculated into Hep2 (human epidermoid carcinoma) and RD (rhabdomyosarcoma) cell lines. Isolates were typed by neutralisation tests with antisera pools (RIVM, National Institute of Public Health and the Environment, Bilthoven, The Netherlands) and viral RNA was extracted directly from CSF samples using a viral RNA extraction kit (Qiagen, Hilden, Germany). Nested RT-PCR was used to amplify a 655-bp fragment of the 3’-end of the VP1 genome region [4], followed by cycle sequencing reactions with the same primers.

Nineteen of the 49 CSF samples were positive by RT-PCR, and six strains were isolated in cell culture. Sequencing and BLAST analysis revealed coxsackie viruses A9 and B5, and echoviruses 9, 11 and 14, with a predominance of coxsackie virus B5 and echovirus 11. Two clusters of cases were distinguishable, i.e., cases observed between November 2003 and June 2004 caused by coxsackie virus B5, and cases observed between June and December 2004 caused by echovirus 11.

Coxsackie virus B5 was present in four patients, GR27/03 (November 2003), GR33/03 (December 2003), GR30/04 (May 2004) and GR48/04 (July 2004), aged 62, 11, 45 and 14 years, respectively, all of whom were residents of northern Greece. The sequences obtained were submitted to GenBank with accession numbers DQ095774–DQ095777, respectively. Four isolates in Hep2 cells from the CSF samples of these patients were typed as coxsackie virus B by neutralisation tests.

The sequences obtained were aligned with other sequences retrieved from GenBank, and a consensus phylogenetic tree was constructed by the neighbour-joining method using PHYLIP software [9]. As the other available sequences were not from the same region of VP1, the phylogenetic tree was based on a common region of 279 nucleotides (positions 3004–3283 on the genome of the prototype strain Faulkner). As human coxsackie virus B5 is very similar to swine vesicular disease virus [10], a representative strain of swine vesicular disease virus HK70 (accession no. AY429470) was also included in the analysis. Human poliovirus type 1 Mahoney strain (NC_002058) was used as an outgroup.

Phylogenetic analysis showed that the sequence divergence among the coxsackie virus B5 sequences from this study ranged from 0% to 2.5% at the nucleotide level. GR27/03 and GR33/03 were identical; however, there was no evidence of an outbreak, as the cases were observed in different months, the patients resided in different cities > 300 km apart, and no other similar strains were detected during the same period. All genomic differences were at synonymous sites (no amino-acid changes). However, the divergence from coxsackie virus B5 strains from southern Greece, isolated in 2001, was much higher (25%), with clustering with high bootstrap values in two different subgroups (Fig. 1).

Higher variability was observed in genome region 3248–3274. Fig. 2 shows the alignment of amino-acids from representative subgroups in...
this variable region. All strains from the present study have a lysine (K) in the 82nd amino-acid position of the prototype strain, which has not been detected in other coxsackie virus B5 strains; all other coxsackie virus B5 strains have glutamic acid (E) at this position, except two Japanese strains (03-158FCC2 and 8100), which have glutamine. These three amino-acid variations are hydrophilic, and it is known that this region is exposed on the virion surface [11]. However, lysine is a strongly basic, positively-charged amino-acid, while the free carboxyl group of glutamic acid makes it acidic and negatively-charged; glutamine is a polar, amide amino-acid.

The impact of this mutation on viral attachment to the cell surface is not known.

The genetic distance between the Japanese strains and the strains of this study was 9%, which was the closest relationship observed among the strains analysed. Strain 158FCC2 was isolated in 2003 in Fukuoka City, on the island of Kyushu in southern Japan, and strain 8100 was isolated in 2001 during an epidemic of aseptic meningitis in the central areas of Nara prefecture, Japan [12]. Strains clustering in the same subgroup are a Chinese strain isolated in 2002 during an outbreak of aseptic meningitis that lasted from 2002 to 2004 (11% difference), and a Finnish strain isolated in 1982 (12% difference). Sequences from the meningitis outbreak in Greece during 2001 [6] cluster in another subgroup, differing from the strains of this study by 25% (12% difference). Sequences from different coxsackie virus B5 strains can also be detected in the same area during the same year, e.g., Spanish strains isolated in 1997, and Japanese strains isolated in 2003.

The sequence of the 3’-third of the VP1-coding sequence can be used for EV taxonomy, identification of new EV types, and molecular epidemiology of enteroviral disease outbreaks [2]. It could also be used to identify recombination breakpoints or specific attributes of pathogenicity [13]. The present study yielded information concerning genetic variability among enteroviral strains in Greece, and suggests that there is not a geograph-
ical relationship among strains. Thus, Greek strains from 2003 to 2004 cluster with strains from Asia of the same period, but differ from strains isolated in Greece in 2001. Further genetic studies are needed in order to gain a better insight into the genetic variability of EV strains and any relationship with pathogenicity, and to investigate any patterns of recombination, which is a frequent event during EV evolution.

ACKNOWLEDGEMENTS

We thank F. Kamaria, physician at the Infectious Diseases Hospital of Thessaloniki, for supplying most of the CSF samples used in this study. K. Dumaidi was supported by a grant from the State Scholarships Foundation (IKY), Greece.

REFERENCES


RESEARCH NOTE

Emergence of Proteus mirabilis carrying the blaVIM-1 metallo-β-lactamase gene

S. Vourli1, H. Tsorlini2, H. Katsifa2, M. Polemis1, L. S. Tzouvelekis3, A. Kontodimou2 and A. C. Vatopoulos1

1Department of Microbiology, National School of Public Health, Athens, 2Laboratory of Microbiology, General Hospital G. Papanikolaou, Thessaloniki, 3Department of Microbiology, Medical School, University of Athens, Athens, Greece

ABSTRACT

Seven genetically related Proteus mirabilis clinical isolates from a hospital in Thessaloniki, Greece, exhibited decreased susceptibility to imipenem and carried a blaVIM-1 metallo-β-lactamase gene. PCR mapping revealed that blaVIM-1 was part of a class 1 integron that was probably located in the chromosome and also included the aacA7, dhfr and aadA genes. This is the first description of the blaVIM-1 metallo-β-lactamase gene in P. mirabilis.

Keywords blaVIM-1 gene, carbapenem resistance, integron, metallo-β-lactamase, PCR mapping, Proteus mirabilis

Original Submission: 12 September 2005; Revised Submission: 10 November 2005; Accepted: 11 December 2005

Clin Microbiol Infect 2006; 12: 691–694
10.1111/j.1469-0691.2006.01489.x

Corresponding author and reprint requests: A. C. Vatopoulos, Department of Microbiology, National School of Public Health, 196 Alexandras Ave., Athens 11521, Greece E-mail: avatopou@nsph.gr

© 2006 Copyright by the European Society of Clinical Microbiology and Infectious Diseases, CMI, 12, 672–694