Retrospective Study on Poliomyelitis Cases in Northern Greece

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Here we report the results of a retrospective study on the epidemiological characteristics and genetic relationships of the virus isolates responsible for the last poliomyelitis cases in Greece. The last wild poliomyelitis case in Greece was detected

in 1996, and the last vaccine-related strain was isolated in 1998. The whole of Europe, including Greece, is now considered to be polio-free. *J. Clin. Lab. Anal.* 18:296–298, 2004. © 2004 Wiley-Liss, Inc.

Key words: poliomyelitis; poliovirus; Greece; vaccine; PCR

INTRODUCTION

From 1950 to 1963, poliomyelitis was endemic in Greece. The Salk inactivated polio vaccine (IPV) was first administered in Greece in 1962, and in 1964 this was replaced by the Sabin live attenuated oral polio vaccine (OPV) (1). Vaccine uptake within the Greek population is high, with the exception of a small proportion of the gypsy community, which has lower uptake because many of these individuals refuse, for unknown reasons, to have their children vaccinated. The introduction of IPV/OPV resulted in a decline in mortality from 1962 onward; however, polio eradication strategies were enhanced in Greece in 1996 after a large outbreak occurred in Albania.

MATERIALS AND METHODS

Since 1976 a total of 20 poliovirus isolates have been detected in our laboratory (where samples from the whole of northern Greece are tested) from stool specimens or rectal swabs from 19 patients with acute flaccid paralysis (AFP). Fourteen of these isolates were wild strains, and six were Sabin-like ones (Table 1).

In 1976 an outbreak of paralytic poliomyelitis occurred in the gypsy communities of Greece. There were seven confirmed cases, including two fatal ones, and one additional case was observed in April of 1977 (2). In 1981–1982, four further cases were observed in unvaccinated children. A large outbreak of paralytic poliomyelitis occurred in Albania in 1996, with 145 cases (mostly in young adults) causing persisting paralysis in 87 individuals and 16 deaths (mortality rate = 12%) (3). A wild-type 1 strain of poliovirus was spread from Albania to the neighboring countries of Yugoslavia and Greece (4). In total, five of the Greek polio cases were

related in this outbreak. Three of these cases were found in southern Greece (5). The other two cases (isolates 7/96 and 10/96) were observed in northern Greece and referred to our laboratory.

In addition to the wild polio isolates, six vaccine-related strains were isolated from five children with paralytic disease.

Isolation procedures in Hep-2 (human epidermoid carcinoma) cell lines and identification of isolates by microneutralization assays were performed according to standard protocols in a biosafety level (BSL) 2 cabinet (6). Viral RNA was extracted from culture supernatant samples, and a 305-nucleotide genome fragment from the junction region between VP1 (the gene encoding the major structural protein) and 2A (the gene encoding the viral protease) was amplified by RT-PCR using primers JunS (bp 3209–3228) and JunA (bp 3493–3514) (4). Nucleotide sequencing was performed on five wild poliovirus isolates (8/76, 40/76, 44/77, 15/82 and 10/96) and one vaccine-related isolate (79/77). The sequences determined in this study were aligned with their respective corresponding poliovirus sequences obtained from the GenBank database. The aligned sequences were analyzed phylogenetically with the use of PHYLIP software (7).

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Table 1. Epidemiological characteristics of polioviruses (wild and vaccine-related) isolated in northern Greece during 1976–1998

Isolated number/year	Month of onset	OPV			Location	Type
		Gender	Age	Doses		
8/76	February	F	10 m	0	Thrace	Wild polio 1
24/76	April	F	11 m	0	Thrace	Wild polio 1
25/76	April	F	18 m	0	Central Macedonia	Wild polio 1
26/76	May	F	9 m	0	Thrace	Wild polio 1
32/76	May	M	13 m	0	Thrace	Wild polio 1
40/76	June	M	10 m	0	Central Macedonia	Wild polio 1
49/76	July	M	2 y	0	Central Macedonia	Wild polio 1
44/77	April	F	10 m	0	Thrace	Wild polio 1
79/77	November	M	7 m	2	Central Macedonia	1 + 3 polio, SL
43/81	November	F	17 m	0	Thrace	Wild polio 1
9/82	March	F	15 m	0	Thrace	Wild polio 1
14/82	May	M	3 y	0	Thrace	Wild polio 1
15/82	May	M	3.5 y	0	Thrace	Wild polio 1
42/85	May	F	3.5 y	1	Thrace	Polio 3, SL
4/96	May	M	4 m	1	Central Macedonia	Typed only by polyvalent sera
7/96	September	M	5 m	0	Central Macedonia	
10/96	September	M	2.5 y	0	Central Macedonia	Wild polio 1
7/98	June	M	12 m	0	Thessalia	Polio 3, SL
19/98	December	F	5 m	1	Central Macedonia	Polio 3, SL

m, months; y, years; SL, sabin-like.

RESULTS

A phylogenetic tree including strains from this study and their respective corresponding poliovirus sequences obtained from the GenBank database was constructed (Fig. 1). The Greek wild-type 1 poliovirus isolates from 1976–1977 differed from the 1982 and 1996 isolates by 17% and 33%, respectively, while the 1982 isolate differed from the 1996 isolates by 29%. A genetic similarity of 98–99% was observed among the Greek polio isolates from 1976–1977, which comprise an independent clade. The closest strains to the 1982 Greek polio isolate were those isolated in 1978 in The Netherlands, and in 1977 in Turkey. The 1996 Greek isolate showed a genetic diversity of only 2.2% from the genotypes that were circulating during the outbreak in Albania. Isolate 79/77, which was vaccine-associated, showed a genetic difference of 2.2% from the Sabin strain at the nucleotide level.

DISCUSSION

By default, a sequence divergence of >15% distinguishes genotypes, while a sequence similarity of at least 98% implies a direct epidemiological link (8). The low similarity observed between isolates in Greece from different years indicates a multisource origin. The genetic similarity of 98–99% among the Greek 1976–1977 polio isolates suggests that there was an indigenous strain circulating at that time in Greece. The high similarity (97.8%) between the 1996 Greek isolate and

the genotypes that circulated during the 1996 outbreak in Albania suggests that the genotype was imported directly from Albania. By immunological and molecular methods, the agent responsible for the 1996 outbreak was identified as a single strain of wild poliovirus type 1, which showed a 95% similarity with a wild-type 1 poliovirus strain isolated in Pakistan in 1995 (4). Sequence analyses of different genomic regions of a number of outbreak-related Albanian polio strains and poliovirus strains isolated in Pakistan in 1995 confirmed that the poliovirus involved in the outbreak was imported directly from Pakistan, and that there was no evidence of inter- and intratypic recombinations among the strains (9).

The best tool for eradicating poliomyelitis is OPV; however, in rare cases it has been implicated in vaccine-associated paralytic poliomyelitis because of the genetic evolution of the OPV strains (10). Over the last 25 years, four such cases have been observed in northern Greece. A phylogenetic analysis of isolate 79/77 showed a genetic difference of 2.2% from the Sabin strain at the nucleotide level. Five additional vaccine-related strains were isolated in southern Greece (11).

Since 1988, when the World Health Assembly resolved to eradicate poliomyelitis worldwide, the number of countries in which polio is endemic has decreased from 127 to seven (12). Greece is now considered to be a polio-free country. However, the Albanian outbreak in 1996, as well as two poliomyelitis cases in Bulgaria in 2001 (due to the introduction of wild

Fig. 1. Phylogenetic tree constructed using the Kimura 2-parameter method based on nucleotide sequence data from the VP1-2A region. Numbers indicate the percentage bootstrap confidence values. Isolates in bold are the sequences obtained in this study.

poliovirus from India (13)), illustrate that the risk of wild-type virus being reintroduced from countries in which the virus remains endemic will persist until poliomyelitis is eradicated globally. Since a large number of refugees pass through Greek borders every year, coming from countries where pockets of the

disease are still present, public health authorities have to be aware in order to maintain effective immunization programs to prevent polio from becoming a reemerging disease.

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