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Full Length Research Paper

Some genetic characteristics of sabin-like poliovirus isolated from acute flaccid paralysis cases in Nigeria

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A total of 34 sabin strains of the poliovirus isolated from 22 children with 60-day follow-up residual acute flaccid paralysis (AFP) were genetically characterized and screened for any form of recombination. Sequence analysis of the 906-nucleotide capsid showed that all the isolates were similar to their original sabin serotypes, however two of the viruses had drifted in their 3D noncapsid regions toward a sabin-sabin and sabin-nonpolio entero combination. Routine immunization in Nigeria is low and in spite of the increase in the frequency of supplemental immunizations, a lot of children are still inadequately immunized, which may be the reason for our observation in this study. Although we are not dealing with a case of circulating vaccine derived poliovirus (cVDPV) yet, if the above condition persists, the advent of cVDVP may not be too far. There is therefore the need to maintain a high quality mass immunization and sustained routine immunization.

Key words: Poliovirus, sequence, crossover, non polio enterovirus, recombination, genome, Sabin-like, vaccine, Nigeria.

INTRODUCTION

The oral polio vaccine has been proven to be effective in the control of poliomyelitis. Since its introduction in 1965, the vaccine has succeeded in reducing the number of paralytic poliomyelitis from 350,000 cases in about 125 endemic countries in 1988 to only <1000 cases in fifteen endemic countries in 2001 (WHO, 2002a, b). With about two hundred and two wild polioviruses isolated in Nigeria in the year 2002 (WHO, 2003) there is no doubt that Nigeria still constitutes a major reservoir for poliovirus. In an effort to contain the circulation of the wild poliovirus, mass and supplemental immunizations (NIDs and SNID) were conducted regularly throughout the year with an estimated target population of about nineteen million covered (WHO, 2002c). However, routine immunization especially in the northern part of the country has not matched up with the mass immunizations. The states where routine immunizations are low have therefore

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Abbreviations. AFP: acute flaccid paralysis. PCR: Polymerase chain reaction. cVDPV: circulating vaccine derived poliovirus. NID/SNID: National immunization day/supplemental national immunization day. WHO: World Health Organizations. OPV: Oral Polio vaccine. DNADIST: DNA distance.

Table 1. Sabin Recombinant Primers (Kilpatrick DR, J. Clin. Microbiol., In Press).

Primer	Sequence	Gene	Position
S-1 REC			
281S	5′- TGTAACAAAACTTAGACAAC	2C	2484-4303
231A	5′- TATGTAGTTGTTAATGGTATG	2C	4482-4462
287S	5′- TAAGGGAAATGCAAAAACTGC	3D	6423-6442
288A	5′- ATCGCACCCTACTGCTGA	3D	6648-6631
S2- REC			
283S	5′- CAAATTCATTAGTTGGTTGC	2C	4224-4243
284A	5′-TGGATAGATAGCCACCGC	2C	4412-4395
300S	5′-AGGAAATGCGGAGACTCTTA	3D	6425-6444
302A	5′-GGATCACAACCAACTGCACT	3D	6649-6630
S3-REC			
320S	5′-TGTAACCAAATTGAAACAGT	2C	4284-4303
322A	5′-TATGTAATTATTAATGGTGTG	2C	4482-4462
319S	5′-CAAAGAAATGCAAAGACTTT	3D	6423-6442
318A	5′-GGATCGCATCCAACTGCACT	3D	6650-6631

continued to harbour the wild poliovirus and have therefore continued to serve as reservoirs for the virus.

The poliovirus is among the most rapidly evolving viruses known, about 10^{-2} nt substitution/site/yr (Bellmunt et al., 1999; Kew et al., 1995; Kew et al., 1998 Martin et al., 2000). Long excretion periods and low population immunity occasioned by low coverage encourages rapid evolution and spread of the revertant virus. Oral polio vaccine (OPV) recipients usually excrete viruses for 3 to 4 weeks during which time some base substitutions are changed during the process of replication in the human intestines (Alexander et al., 1997; Minor and Dunn, 1988; Macadam et al., 1993). Some of theses changes may alter the degree of attenuation of the vaccine virus or affect the neurovirulence of the original vaccine virus, thereby leading to the disease.

In this study we have genetically characterized some of the sabin-like viruses isolated from cases of acute flaccid paralysis (AFP) with 60 days residual paralysis of some Nigerian children following vaccination with the OPV.

MATERIALS AND METHODS

Virus isolation, identification and typing

Stools for the virus isolation were those taken from suspected cases of AFP patients during surveillance and sent to the National WHO Polio laboratory, Ibadan and Maiduguri, Nigeria. Two stool samples taken within 48 h of each other not later than 14 days after the onset of such paralysis were sent to the two laboratories accompanied by case investigation forms giving information on the vaccination status, clinical presentation and time of collection of stool samples. Virus isolation, identification and typing in the laboratory were done according to the recommended WHO standard (WHO 2002d). Intratypic typing of the polio isolates was done by ELISA using highly specific cross-absorbed antisera (van der Avoort et al., 1995) and a molecular method involving the use of genotype specific nucleic acid probes (De et al., 1997) and genotype specific PCR primers (Yang et al., 1997).

Sequence Analysis of Isolates

RNA extraction, PCR amplification, and analysis were prepared as already described (Yang et al., 1991; De et al., 1997). VPI region of the polioviruses were sequenced using the fluorescent dye-labeled dideoxynucleotide chain terminator (Applied Biosystem, Forster City, CA, USA).

Sabin recombinant PCR

Isolates were further screened for recombinant noncapsid sequences using the PCR primers targeting the P2 and P3 region characteristic for each Sabin strain (Kilpatrick, J.Clin.Microbiol, In Press). Briefly described, 2 μl of the RNAs were inoculated into the PCR tube containing PCR buffers and primers. The following S1, S2 and S3 Sabin recombinant (REC) primers were used (Table 1).

S1- REC 281S, 287S, 288A (10 pm each) and 321A (80 pm/ul)
S2- REC 283S, 284A, 300S, 302A (10 pm/ul)
S3- REC 318A, 319S, 320S (10 pm/ul each 80 ul) and 322A in S3-REC mix.

The mixtures were heated at 95ºC for 5 min to denature. This was placed on ice and spun in micro centrifuge to concentrate. 14.5 ul of the enzyme mix was added per reaction. The enzyme mix was made up of 14.5 ul of water, 0.06 ul of RNAse Inhibitor, 0.06 of reverse transcriptase and 1.2 ul of Taq. The reaction was then subjected to PCR conditions of 94ºC to denature, 50ºC for annealing and 65ºC for extension. Each temperature regime lasted

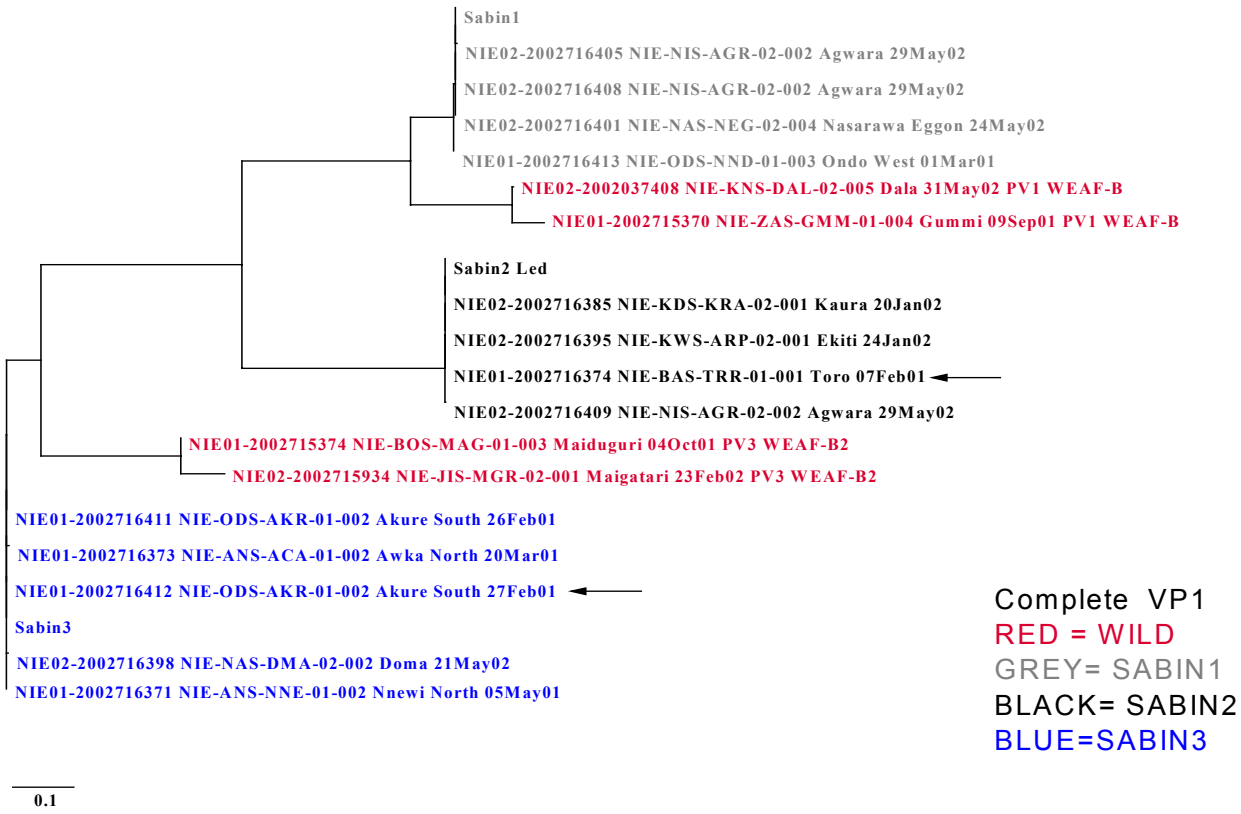

Figure 1. Phylogenetic tree of the VP1 region showing the relationship of viruses 6374 and 6412 (arrowed) with some Nigerian Sabin and wild strains. Colour codes differentiate the serotypes.

for 45 s. A total of 32 cycles of the reaction were run. Conditions for polyacrylamide gel electrophoresis and detection of amplified products by ethidium bromide staining have been as described elsewhere (Kilpartrick et al., 1998).

Sequence Analysis of 3D region

Three of the Poliovirus isolates which did not show any band in the electrophoretic gel, and the S2 225 bp 3D band and the S3 228 bp 3D band were further sequenced (Liu et al., 2000). The following primers were used: 187A, 947A, 4073A, 153A, 4121S, 188S, 239S, 190A, 5761A, 230S, 186S, 600A, 455S, 7450A, 7024A, 235A, 5918A, 2365A and 7024S (Yang et al., 1997).

Whole genome sequence

Two of the viruses- 374 and 412 which showed some degree of mutation from the prototype strain were subjected to whole genome sequencing to determine the area of crossover in the nucleotide sequence. This whole genome sequence was done as described earlier (Yang et al., 1991). Relationship among the diverse type polio genotypes were estimated from the VP1 and the 3D region sequences by the method of Felsentein (1981) using the the Wisconsin Package. Genetic distances were calculated using the program DNADIST and summarized in a tree constructed by neighbor-joining method (Saitou and Mei, 1987) using the NEIGHBOR program of the PHYLIP 3.752c program package (Felsentein, 1993).

RESULTS

A total of 34 Sabin isolates representing 22 suspected AFP cases were collected. All the cases were from children vaccinated with the OPV but with varying degree of OPV status. All the children had residual paralysis after 60 days. Six of the isolates were PV1, 9 PV2, and 19 PV3.

Sequence analysis of the VP1 capsid region showed that all the isolates were similar to their original Sabin genotype with between 0-3 nucleotide substitutions (Figure 1). Extracted RNA from the viruses were further amplified with the S1 S2, S3- REC primers. Our result showed that two of the viruses-6374 and 6412 yielded negative PCR results; the 3D products were not amplified, indicating that recombination may have taken place. This prompted us to do a partial sequence of the 3D non-capsid region. Sequence analysis showed that virus 6374 had only three nucleotide changes in the VP1 region but had recombined in the 3D region with a non-polio enterovirus, closest similarity being to CA 20 (85%). Likewise virus 6412 which was originally a Sabin 3 had recombined in the 3D region with Sabin 1 with a similarity of 99.8% (Figure 2).

Sequence analysis of the whole genome of virus 6374 showed one nucleotide change in the VP1 region plus the

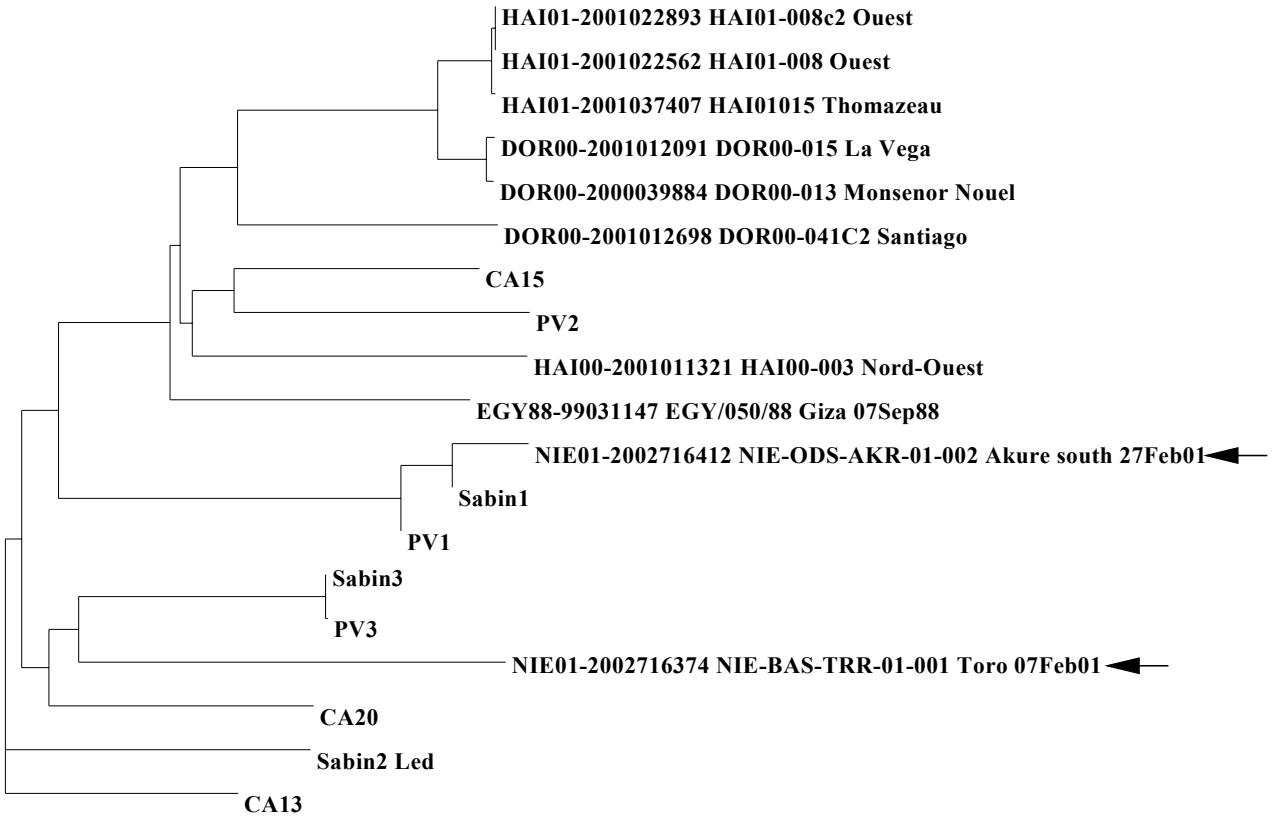

Figure 2. Phylogenetic tree of the 3D non-capsid region showing the relationship of viruses 6373 and 6412 (arrowed) with some other cVDPVs (Kew et al., 2002) and non polio enterovirus.

following nucleotide changes prior to the recombination point (bp positions in bold) of **G→A$_{481}$**, **C→T$_{2909}$** and **T→G$_{4290}$**. The area of cross over was actually in the 3D noncapsid region from position 5208 where most of the nucleotide substitutions took place.

DISCUSSION

In this study we have attempted to characterize some sabin-like polio viruses isolated from cases of acute flaccid paralysis among some Nigerian children vaccinated with the oral polio vaccine. Data on immunization status of non-polio AFP cases in Nigeria during the time of this study suggests that in spite of increasing frequency of supplemental immunizations particularly in the high risk transmission states, many children were inadequately vaccinated against poliovirus. This is coupled with the general routine immunization coverage which is associated with low population immunity. All these factors seem to must have contributed to our observation in this study. Areas at high risk for emergence of revertant polio vaccine are those where polio vaccine coverage is low and epidemiological conditions favored the transmission of the virus (Kew et al., 1995).

Two types of recombination were observed; a sabin-to-sabin recombination and a sabin to other species, C-enterovirus. This is consistent with the general behavior of the virus (Kew et al., 1998; Liu et al., 2000). Many poliovirus clinical isolates are recombinants. Heterotypic recombinants are frequently isolated from patients given OPV vaccine. Whole genome sequence of one of the viruses showed that the area of the crossover was in position 5802 of the noncapsid region. Again this is consistent with earlier findings of other workers who observed that crossovers are most common in the noncapsid region than in the untranslated and capsid regions (Kew et al., 2002, Martin et al., 2000).

The fact that none of the sabin viruses showed a significant divergence in the capsid nucleotide sequence (>1% from the parental OPV strain) underscores the fact that we are not dealing with a circulating virus derived polio virus (cVDPV) but if the condition that predisposes to our observation in this study persists, it may not be long before we see the advent of cVDPV in this region as this may actually be a predictor of a future possible cVDPV. This will not be good for the program in Nigeria.

The implication of this study is to maintain high quality mass immunization with a tested routine immunization all over the country, while concentrating more attention on those reservoir states in the Northern Nigeria where the virus is still very much in circulation.

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