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Genetic recombination in wild-type poliovirus

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Poliovirus isolates were screened for recombinants by combined analysis of two distant polymorphic segments of the poliovirus genome (one in the capsid and the other in the polymerase-coding region). Using a restriction fragment length polymorphism (RFLP) assay, a high number of recombinant genomes was found among vaccine-derived strains excreted by poliovirus vaccine vaccinees or vaccine-associated paralytic poliomyelitis cases. Some of these subjects carried a wild-type poliovirus (non-vaccine-specific) nucleotide sequence in the 3′ part of the genome. Using a similar approach, a collection of wild-type poliovirus strains isolated in South India between 1985 and 1993 was screened for recombinants. Genotypes were defined by the parallel application of RFLP assays and genomic sequencing of the capsid protein VP1 and the 3D polymerase polypeptide. Analyses revealed several instances where the position of an isolate on the phylogenic tree for the capsid protein-coding segment did not agree with its position on the tree for the polymerase-coding region. In this way, several wild-type/wild-type and wild-type/vaccine recombinants could be identified, indicating that recombination is encountered commonly in the natural evolution of poliovirus strains.

Introduction

The genetic variability of picornaviruses is due mostly to nucleotide substitutions resulting from the high frequency of errors that occur during viral RNA replication (Holland *et al.*, 1982; King *et al.*, 1982; Kirkegaard & Baltimore, 1986). Genetic changes in poliovirus can also occur by molecular recombination (Cooper, 1977; Lai *et al.*, 1992). In several recent studies on poliovirus genotypes, a high number of recombinant genomes was found among vaccine-derived strains excreted by oral poliovirus vaccine (OPV) vaccinees or vaccine-associated paralytic poliomyelitis (VAPP) cases. Among VAPP cases, up to 50% of type 2 and 67% of type 3 Sabin strains of poliovirus have been characterized as recombinant (Furione *et al.*, 1993). Among them, a relatively high proportion [8% in Furione *et al.* (1993)] were vaccine/wild-type (V/W) recombinants carrying a non-vaccine-specific nucleotide sequence in the 3′ part of the genome (Guillot *et al.*, 2000).

Administration of trivalent OPV provides optimal conditions for multiple infections of human intestinal target cells, thus favouring the possibility of intermolecular recombination between heterotypic viral genomes. This is enhanced under endemic conditions where different genotypes (homotypic or heterotypic) of wild-type strains are in circulation. Under these conditions, and knowing the natural spreading capacity of enteroviruses (Melnick, 1990), different possibilities of recombination can occur between wild-type strains or both wild-type and vaccine-related strains.

To date, many efforts have systematically searched viral genomes for evidence of recombination among RNA viruses in animals (Lai *et al.*, 1992; Burke *et al.*, 1997) and plants (Lai *et al.*, 1992; Chenault & Melcher, 1994; Revers *et al.*, 1996). The enterovirus group is particularly well suited to such research for several reasons. First, as mentioned above, a high proportion of recombinant genomes was found among vaccine-derived strains (Minor *et al.*, 1986; Lipskaya *et al.*, 1991; Furione *et al.*, 1993; Georgescu *et al.*, 1994). Second, enteroviruses are currently endemic in most developing countries and co-circulate in humans. Finally, for vaccine safety, it has been proposed that OPV should be replaced with a recombinant virus (displaying an enterovirus genome) to lower the rate of poliomyelitis in vaccine recipients (Gromeier *et al.*, 1996). We have attempted to detect recombinant isolates in several wild-type poliovirus populations.

Using combined analysis of two distant polymorphic segments of the viral genome (one in the capsid protein and the other in the polymerase-coding region), we screened

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poliovirus field isolates for recombinants. Using a double PCR and restriction fragment length polymorphism (RFLP) assay addressing poliovirus genome segments in the regions encoding the capsid protein VP1 (RFLP1, 480 nt fragment) and the 3D polymerase polypeptide (RFLP3D, 596 nt fragment), we defined different RFLP patterns in type 1 and type 2 wild-type poliovirus strains. This was followed by sequence alignment and the detection of sequence homology in groups with similar RFLP profiles [a 150 nt fragment (nt 3296–3445) in the VP1–2A junction region and a 200 nt fragment (nt 6176–6375) of the non-structural 3D polymerase polypeptide]. Genotypes were defined by the parallel application of RFLP tests and genomic sequencing of the capsid protein VP1 and the 3D polymerase polypeptide. Our approach is based on searches of virus isolates that demonstrate different clustering properties in phylogenic trees constructed using these two short genomic regions. The results obtained may be confirmed by statistical means. We describe in this report several instances where the position of an isolate on the tree for the capsid protein-coding segment does not agree with its position on the tree for the polymerase-coding region. This demonstrates the occurrence of recombination in wild-type poliovirus and shows that this event is encountered frequently in the natural evolution of poliovirus strains.

Methods

For over 15 years, the Enterovirus Laboratory, Christian Medical College Hospital, Vellore, India, has been involved in the study, surveillance and control of poliomyelitis in India (John, 1984). A bank of poliovirus isolates from children with paralytic poliomyelitis has been maintained between 1985 and 1993. Out of this, 28 type 1 and 18 type 2 poliovirus strains were selected using a VP1–2A RFLP assay and sequenced in the target genomic segments.

■ **Virus isolation and characterization.** Primary isolation of the virus was done in primary bonnet monkey (*Macaca radiata*) kidney cell cultures and initial identification and serotyping was done by neutralization with type-specific hyperimmune sera, as described previously (Melnick *et al*., 1979; John & Ratnam, 1969). For all tests, we used the Sabin (types 1 and 2) and Mahoney strains as reference viruses. For genomic analysis, the original isolates were passed once or twice in HEp2c cells.

■ **Genomic analysis.** To study the genomic variation of strains, we carried out RFLP1 (VP1–2A junction region) and RFLP3D (3D polymerase) and partial genomic sequencing of 46 strains (28 type 1 and 18 type 2 poliovirus strains) isolated in various years.

RFLP assays were carried out as described by Balanant *et al*. (1991). Two distant segments separated by 2731 nt were sampled to screen for recombinants with similar capsid (VP1) sequences but dissimilar non-capsid (3D polymerase) sequences, as described previously by Guillot *et al*. (2000).

To confirm the recombination hypothesis, 46 strains have been sequenced in these target genomic segments, as described by Guillot *et al*. (2000). Sequence alignment was done using CLUSTALW, version 1.7 (Gibson *et al*., 1994). A dendrogram of sequence relatedness was constructed using the programs included in PHYLIP, version 3.5c (Felsenstein, 1993). Distance matrices were calculated using the Kimura 2-parameter method, as implemented in the program DNADIST. The rate of transition/transversion was set as 2·0. Tree reconstruction procedures were done using the least-squares method with an evolutionary clock, as implemented in the program FITSCH (Felsenstein, 1993). Phylogenic trees were drawn using TREEVIEW (Page, 1996). The statistical significance of the branching order was estimated by performing 100 replications of bootstrap resampling of the original alignment using SEQBOOT and synthesizing the resulting set of trees using CONSENSE (Felsenstein, 1993).

■ **Statistical analysis.** The statistical test for recombination developed by Sawyer *et al*. (1989) was used as implemented in the program VTDIST2, which allows pairwise comparison of the input sequences (kindly provided by S. Sawyer, Department of Mathematics, Washington University, St Louis, Missouri, USA), to statistically validate the recombination events suspected from the clustering of the tree topologies. In this test, positions that are totally conserved are omitted (condensation) from the alignment and, for each sequence pair, a set of fragments is defined between successive sites where these two sequences differ. The following working parameters were defined: MCF (maximal size of the condensed fragments) and SSCF (sum of the squares for condensed fragments); MUF and SSUF are defined similarly but omitting the condensation step. The length of such stretches or fragments is measured in total residues (uncondensed fragment) or number of polymorphic loci (condensed fragment). We considered a fragment significant if its P value (the fraction of permuted fragment lengths greater than or equal to the observed fragment) was $\leqslant 0{\cdot}05$.

Results

Sequence divergence in the VP1–2A and 3D regions

Type 1 and 2 poliovirus field isolates from the south of India were genotyped by RFLP screening and nucleotide sequencing of the VP1–2A and the 3D polymerase-coding regions. RFLP screening results have been published elsewhere. A sequence divergence of more than 15% defines a genotype (Rico-Hesse *et al*., 1987). Genomic sequence divergence in the VP1–2A-coding region groups type 1 strains into three major genotypes. The first type 1 genotype, named Sab-1/Mah, had an average of 99% cluster strains very close to prototype Sabin 1 and Mahoney strains. Wild-type strains are clustered in two genotypes, namely W1-1 and W1-2. Strains grouped in genotype W1-1 were isolated in various years, from 1986 to 1993. Within this genotype, the isolates were classified into two subclusters separated from each other by a sequence divergence of 2–10%. Genotype W1-2 group strains were isolated in 1985.

Sequence analysis showed the presence of three genotypes among type 2 strains. Except for strain 92/285, which clustered in genotype W2-2, all other wild-type isolates were of the same genotype, W2-1. Within this genotype (W2-1), the isolates could be classified further into three clusters, differing from each other by 8–10%. Cluster 1 (W2-1 cluster 1) included strains isolated in 1986 (86/375, 86/624, 86/1193 and 86/1683). In cluster 2 (W2-1 cluster 2) are strains isolated in 1987, 1988 and 1990 that are closely related in their VP1–2A genomic region (87/122, 87/297, 87/1305, 87/1808, 88/669, 90/787, 90/960 and 90/2439). Cluster 3 (W2-1 cluster 3)

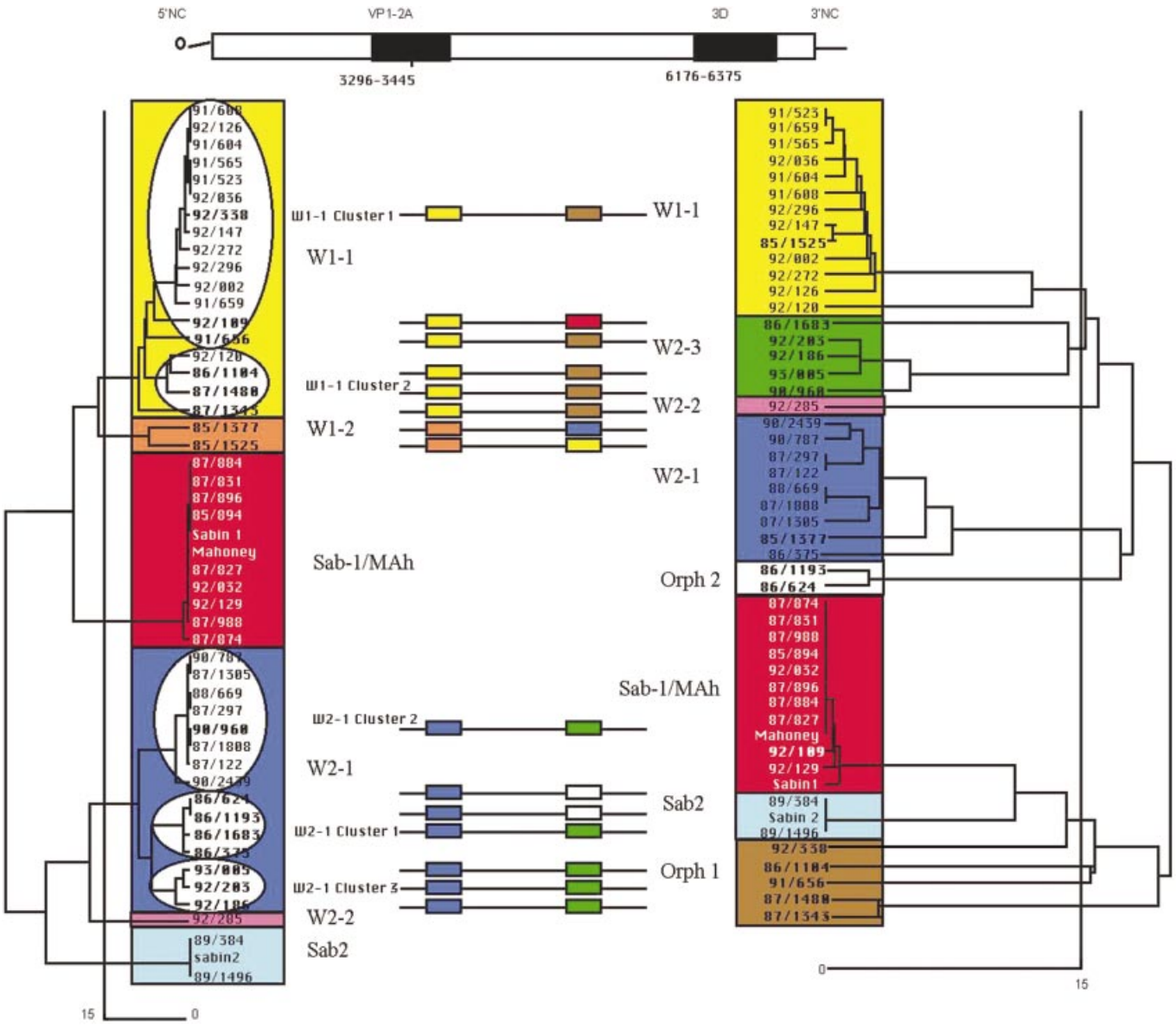

Fig. 1. Phylogenic analysis of poliovirus type 1 and 2 strains from India. The dendrogram is based on relatedness between nucleotide sequences in the VP1–2A (left) and 3D polymerase (right) encoding regions. VP1–2A: type 1 polioviruses, W1-1 (yellow), W1-2 (orange) and Sab-1/Mah (red); type 2 polioviruses, W2-1 (blue), W2-2 (pink) and Sab-2 (cyan). 3D polymerase: type 1 polioviruses, W1-1 (yellow), W1-2 (orange), Orph1 (white) and Sab-1/Mah (red); type 2 polioviruses, W2-1 (blue), W2-2 (pink), W2-3 (green), Orph2 (white) and Sab-2 (cyan). EMBL nucleotide sequence accession numbers are as follows: type 1 VP1–2A sequences, AJ248493–AJ248523; type 2 VP1–2A sequences, AJ248475–AJ248492; type 1 3D sequences, AJ248989–AJ249018; type 2 3D sequences, AJ249019–AJ249036.

groups strains isolated in 1992 (92/203 and 92/186) and 1993 (93/005). Genotype 1, named Sab-2, clustered strains closely related to the prototype Sabin 2 strains (Fig. 1).

A similar approach was adopted for genotyping the same strains in the polymerase 3D-coding region. Grouping into distinct genotypes was done according to the same criterion – more than 85% sequence homology. The name of each genotype was conserved for strains with frequent associations with the VP1–2A genotypes. The same clustering is observed for both type 1 and 2 strains. However, for type 2 strains, a new genotype, W2-3, was identified. For both type 1 and type 2 strains, another genotype appears. Named Orphan (Orph1 and Orph2), these strains could not be related to the genotypes defined above and could be wild-type poliovirus or non-polio/enterovirus (Fig. 1).

Identification of recombinant genotypes

We defined genotypes by the parallel application of RFLP assays and genomic sequencing of the capsid protein VP1 and

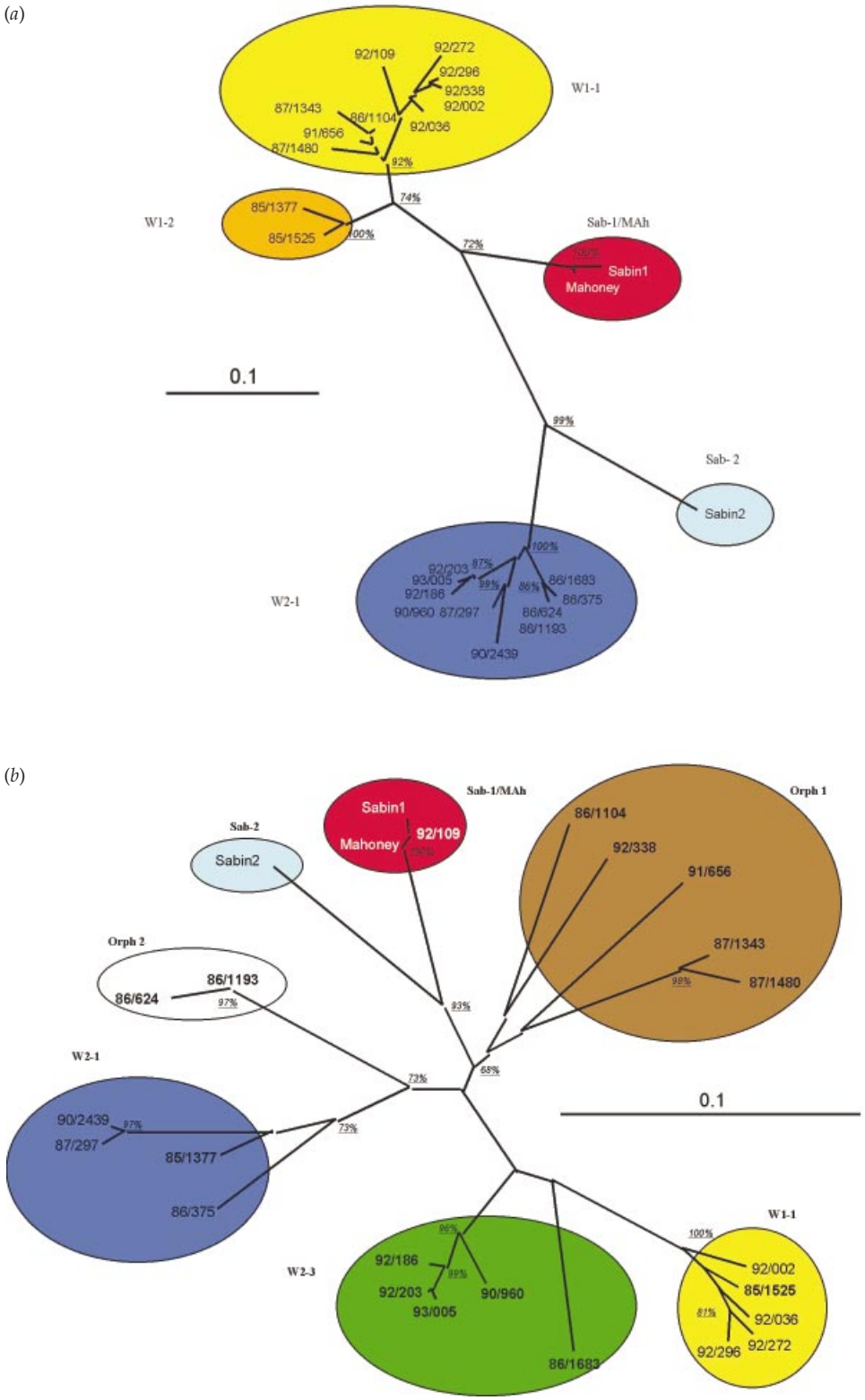

Fig. 2. Phylogenic analysis of poliovirus type 1 and 2 strains from India. Dendograms are based on relatedness between nucleotide sequences in the VP1–2A (a) and the 3D polymerase (b) encoding regions. VP1–2A: type 1 polioviruses, W1-1 (yellow), W1-2 (orange) and Sab-1/Mah (red); type 2 polioviruses, W2-1 (blue) and Sab-2 (cyan). 3D polymerase: type 1 polioviruses, W1-1 (yellow), W1-2 (orange), Orph1 (brown) and Sab-1/Mah (red); type 2 polioviruses, W2-1 (blue), W2-3 (green), Orph2 (white) and Sab-2 (cyan).

Table 1. VTDIST analysis of the regions studied

P values were calculated on the proportion of permuted datasets out of 10000 yielding higher scores than the actual data. We considered a fragment significant if $P \leqslant 0{\cdot}05$ (the fraction of permuted fragment lengths greater than or equal to the observed fragment).

Region	Parameter	Score	*P* value	SD above simulation mean	SD of simulations
VP1–2A	SSCF	184415	0·03	4·71	8004,3166
	MCF	83	100·00	0·00	0
	SSUF	572789	47·25	−0·05	36875,0036
	MUF	150	100·00	0·00	0
3D polymerase	SSCF	74266	4·01	1·89	2975,2942
	MCF	69	5·94	1·40	7,1169
	SSUF	847000	0·46	2·88	30533,6035
	MUF	197	5·94	1·35	19,6372
Chimera	SSCF	266999	0·00	14·33	6871,2931
	MCF	100	46·19	0·03	19,097
	SSUF	1484389	0·03	4·34	57577,9769
	MUF	209	80·38	−0·93	42,7017

the 3D polymerase polypeptide. RFLP characterization demonstrated that type 1 strains 85/1377, 85/1525, 86/1104, 92/109, 92/120 and 92/338 and type 2 strains 86/375 and 86/1683 had different restriction patterns, clustering in the polymerase 3D-coding region compared to the capsid region. Sequence analysis for 15 isolates revealed that the position on the tree for the capsid protein-coding segment did not agree with the position of these isolates on the tree for the polymerase-coding region. The segregation of these strains in the 3D polymerase region differed from that in the VP1–2A region (Figs 1 and 2). This is indicative of discontinuities in the evolutionary lineage of the two different genomic segments. This can also be illustrated by the fact that although pairs of strains, 86/624 and 86/1193, 87/297 and 88/669, 87/1305 and 87/787, and 87/1808 and 90/960, were similar in the VP1–2A region, they showed significant sequence divergence in the 3D polymerase-coding region. The most likely mechanism for the production of such discontinuities is recombination. To evaluate the significance of such potential recombination events, we applied two different statistical tests: bootstrap values and Sawyer's test to detect recombination event.

Statistical determination of recombinants

We first evaluated the statistical value of each node of the trees obtained for the VP1–2A and 3D-coding regions. We applied the following programs of the PHYLIP package (Felsenstein, 1993): SEQBOOT was used to create 100 different alignments, from which DNADIST deduced 100 distance matrices. From these matrices, NEIGHBOR was used to calculate 100 trees and infer the 'best tree'. Then, CONSENSE was used to validate each node of this best tree and draw it with TREEVIEW (Page, 1996). Only bootstrap values greater than 70% were considered significant. All groups in the VP1–2A tree were stable with bootstrapping values greater than 70%, whereas in the 3D tree, only the type 1 Orph group received a bootstrap value of less than 70%. So, in the VP1–2A tree, strain 92/109 clusters in W1-1, with a bootstrap value of 92%, but this isolate shifts in the Sab-1/Mah cluster (bootstrap value of 100%) in the 3D tree. Isolates 85/1377 and 85/1525 are strongly grouped in cluster W1-2 in the VP1–2A tree (bootstrap value of 100%). But in the 3D polymerase region, isolate 85/1377 is closely related to genotype W2-1, with a bootstrap value of 73%, and isolate 85/1525 is closely related to cluster W1-1 (bootstrap value of 100%). In the VP1–2A tree, cluster W2-1 is a single strong group (bootstrap value of 100%), whereas in the polymerase region, this cluster is broken into three different subclusters: cluster W2-3, including isolates 86/1683, 90/960, 92/186, 92/203 and 93/005, cluster W2-1, including isolates 85/1377, 86/375, 87/297 and 90/2439, and cluster Orph2 with isolates 86/624 and 86/1193 (Fig. 2).

In the second step of our analysis, we applied Sawyer's statistical tests for genetic rearrangement based on imbalances in size distribution of regions where sequence pairs within a data set were identical (Sawyer *et al.*, 1989). Both the global analysis and the SSCF parameters indicated that the probability of recombination in the VP1–2A region is high in the inner group (0·03% – no recombination occurred) (Table 1). This probability is lower in the polymerase-coding region. VTDIST offers the possibility of comparing the sequence pairs and, for each sequence, calculated the mean *P* values (a *P* value less than 0·05 was considered significant) obtained when these sequences were compared with others isolates (Table 2). This

Table 2. Statistical determination of recombinant isolates: SSCF-associated *P* values (as percentages) determined using VTDIST

We considered a fragment significant if its *P* value was $\leqslant 0{\cdot}05$ (the fraction of permuted fragment lengths greater than or equal to the observed fragment). First, all sequences were analysed together, then, in the second step of the analysis, pairs of sequences were compared. VP, 150 nt of VP1–2A; 3D, 200 nt of polymerase 3D; Ch, chimera of the two region sequences; Ancre, the representative strain of the genotype.

	SSCF-associated *P* values (%)				SSCF-associated *P* values (%)			
Strains	VP1	3D	Ch	VP ancre	VP1	3D	Ch	3D ancre
92/109	2	72	0	92/272	17	28	0	Sabin
87/1343	0·24	89	0	92/272	6	43	0	86/1104
87/1480	1·25	82	0	92/272	46	17	0	86/1104
91/656	0·34	54	0	92/272	69	9	0	86/1104
86/1104	1	12	0	92/272				
92/338	49	67	0	92/272	1·3	30	0	86/1104
85/1525					0·59	12	1	92/272
85/1377					38	5	0	87/2439
86/624	68	14	5	87/2439	1·3	9	0·5	
86/1193	68	11	6	87/2439	1·3	14	0·7	
86/375	46	44	55	87/2439	0·44	8	0·68	85/1377
87/297	53	35	52	87/2439	37	23	0	85/1377
90/2439				87/2439	37	5	0	85/1377

method allowed us to determine not only which isolates were recombinants but also from which cluster they had shifted. This was done by calculating *P* values between the discrete VP1–2A and 3D sequences and 'virtual' chimeras of the two sequences, produced by joining VP1–2A and 3D into a single discontinuous sequence. Here, again, phylogenic analyses and bootstrapping were concordant with those of the Sawyer test for isolates 85/1525, 85/1377 and 92/109. We also obtained good correlation between isolates grouped into the Orph1 cluster (86/1104, 87/1343, 87/1480, 91/656 and 92/338) and both type 1 strains and type 2 putative recombinants. This suggests that these 15 isolates were the result of RNA recombination. These 15 strains are 92/338, 92/109, 91/656, 86/1104, 87/1480, 87/1343, 85/1377 and 85/1525 for type 1 polioviruses and 86/624, 86/1193, 86/1683, 93/005, 92/203, 92/186 and 90/960 for type 2 polioviruses.

Thus, three categories of recombinants can be defined: (i) W/W intratypic recombinants (85/1525 and 92/109 in type 1 strains and 86/1683, 92/186, 86/203, 90/960 and 93/005 in type 2 strains); (ii) W/W intertypic recombinants (85/1377); (iii) W/Orph recombinants (86/1104, 87/1343, 87/1480, 91/656 and 92/338 in type 1 strains and 86/624 and 86/1193 among type 2 strains). Of the W/W recombinants, 28% of the genome was documented in type 1 (8 of 28) and type 2 (7 of 18) strains, respectively. In 46% (8 of 15) of the recombinant strains, a wild-type poliovirus was identified as parent of the 3D-coding genomic sequences analysed. In the other seven strains, non-polio/enterovirus origin could not be excluded. All these isolates show markedly different affinities depending on the genomic segment considered.

Discussion

So far, only one possible or probable instance of recombination in wild-type poliovirus has been described (Rico-Hesse *et al.*, 1987). In earlier studies using a double RFLP assay, a high number of recombinant genomes was found among vaccine-derived strains excreted by OPV vaccinees or from VAPP cases (Furione *et al.*, 1993; Georgescu *et al.*, 1994). Some of these carried a wild-type (non-vaccine-specific) nucleotide sequence in the 3′ part of the genome. In this work we have systematically searched for the presence of RNA recombination in wild-type poliovirus. First, we defined genotypes by a parallel application of RFLP assays and genomic sequencing focused on the capsid protein VP1 and the 3D polymerase polypeptide. Frequent association between a group identified in the capsid region of the genome to another in the polymerase segment define a genotype (related strains). Then, we used a simple approach for rapid identification of putative recombinant isolates, similar to the one that allowed Chenault & Melcher (1994) to demonstrate recombination in a plant DNA virus and Revers *et al.* (1996) to do the same in potyviruses, plant RNA viruses with a genetic organization reminiscent of that of picornaviruses (Goldbach, 1986). Two different domains encoding the capsid protein were analysed separately and the trees obtained were compared. In the absence of recombination, the general topologies of the trees should be similar, whereas if recombination had occurred in some isolates, the corresponding branches would be expected to differ in their location in the two trees, being more closely related to different parental clusters in each tree. A statistical

analysis was then performed to calculate probabilities for the occurrence of no recombination within and between the regions VP1–2A and 3D so as to confirm the occurrence of recombination events suspected from the tree topology.

The approach we have used has several limitations that can lead to the underestimation of the extent of recombination. First, sequence alignment and phylogenic analysis of strains according to two different genomic regions (the VP1–2A junction and the 3D region) suggest that these regions show different rates of evolution. This can be explained by different selection pressures acting on different genomic regions. The evolution rate of the poliovirus genome is about 10^4 per cycle (Kew *et al.*, 1995). Capsid proteins are the target of host immune pressure and represent the leading front of evolution, while the 5′ non-coding region is the region with the slowest rate of evolution (Poyry *et al.*, 1992). In the absence of recombination and if we consider similar evolution rates in the VP1–2A junction region and in the polymerase 3D-coding region, the general topologies of the trees should be similar. However, if recombination had occurred in some isolates, the corresponding branches could be expected to differ in their location in the two trees, being more closely related to a different parental cluster in each tree. In this way, it is possible to explain all the putative recombinants we have found. But, if we consider that the polymerase-coding region evolves more rapidly than the VP1–2A region, we can easily imagine that no recombination occurred among type 2 strains and consider the different clustering obtained in the polymerase-coding region as a result of the normal evolution of these strains. Genotype W2-1 evolves into three separate clusters: W2-1, W2-3 and Orph2. This can also explain the bursting of the main genotype W1-1 in the VP1–2A tree into two different clusters in the 3D tree, W1-1 and Orph1. But this phenomenon is not robust enough to explain the shift of isolate 85/1377 from W1-2 (VP1–2A tree) to W2-1 (3D tree), of isolate 85/1525 from W1-2 (VP1–2A tree) to W1-1 (3D tree) and of isolate 92/109 from W1-1 (VP1–2A) tree to Sab-1/Mah in the 3D tree. This shift can be explained only by the occurrence of RNA recombination events. Another limitation to our approach is that multiple recombination events might escape detection, since these types of isolates will cluster similarly in regions located on the two sides of two recombination breakpoints.

Considering the Orph clusters, a non-polio/enterovirus cannot be excluded as the parent of the 3D sequence. We are limited by the fact that there are not enough available sequences for this region of non-polio/enteroviruses field isolates in the EMBL database to identify the possible parent. Several recent studies on poliovirus genotypes in our laboratory showed that a high number of recombinant genomes found among vaccine-derived strains excreted by OPV vaccinees or isolated from VAPP cases. Up to 50% of type 2 and 67% of type 3 Sabin strains among VAPP cases have been characterized as recombinant (Furione *et al.*, 1993). Among them, a relatively high proportion (8% in above study) were V/W recombinants carrying a non-vaccine-specific nucleotide sequence in the 3′ part of the genome and, for some of them, a non-polio/enterovirus could not be excluded as the parent (Guillot *et al.*, 2000).

Physical evidence for recombination is most easily demonstrated when the genomic sequences of both parental viruses are known, as is generally the case for vaccine-related strains but not for wild-type poliovirus isolates. RFLP provides valuable information on the circulation and prevalence of different poliovirus variants in the environment. This double test can be used to rapidly screen for recombinants and to obtain an overview of a target group of patterns with similar RFLP profiles to be sequenced. In this study, we describe recombination in wild-type poliovirus and clearly demonstrate that this event is encountered frequently in the natural evolution of poliovirus strains and that different genomic regions may have different rates of evolution (VP1–2A region and 3D).

Not all combinations theoretically possible between wild-type polioviruses, vaccine-derived strains and non-polio/enteroviruses may give rise to recombinants in nature because of epidemiological and biological barriers. Enteroviruses can superinfect persons who are currently infected by other enteroviruses (type, serotype or genotype). Different enteroviruses can concurrently infect single cells in cell cultures *in vitro*, as can poliovirus in free cells (Tang *et al.*, 1997). Wild-type poliovirus genotypes are not uniformly distributed worldwide. Except for the Indian subcontinent and sub Saharan Africa, only a unique serotype or a single genotype is usually encountered. Efforts to stop wild-type poliovirus transmission leads to the spread of vaccine-derived strains all over the world, especially in endemic areas. This allows different types of enteroviruses to co-circulate, leading to opportunities for recombination events to occur.

The double RFLP test can be used to rapidly screen for recombinants and to obtain an overview of target groups of homologous patterns to be sequenced. Using a parallel application of RFLP and genomic sequencing addressing the capsid protein VP1 and the 3D polymerase polypeptide, we identified several W/W and W/V recombinants. Our results suggest that recombination is commonly encountered in the natural evolution of poliovirus.

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