# Supplementary Appendix

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## Methods

Rotavirus VP4, VP6, and VP7 genotyping

The stool samples obtained from the patient at 22 and 29 days after illness onset were suspended in 30% phosphate buffered saline, vortexed, and centrifuged at 1300 g for 10 min at 4 ºC. Viral RNA was extracted from 140 µL of the clarified stool supernatant using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The coding regions of the rotavirus capsid proteins VP4, VP6, and VP7 were amplified in 3 different reverse transcription (RT)-PCR reactions using previously described primers and conditions (1) and the One-Step RT-PCR kit (Qiagen, Hilden, Germany). Amplicons were detected by gel electrophoresis using a 2% agarose gel and visualized with GelRed DNA gel stain (Biotium).

Given that the dose of the Rotarix vaccine administered to the patient could not be determined and in order to compare the vaccine strain with that of the patient, a commercially lyophilized dose of the vaccine was purchased (lot number AROLC991AB) and reconstituted according to the manufacturer's instructions. The RNA was then extracted and amplified as previously described for the stool sample.

Nucleic acid sequencing and sequence analyses

Amplicons were purified using ExoProStar 1-Step (GE Healthcare - Life Sciences, UK) and sequenced in both directions by the Sanger method using the same primer pairs used for the RT-PCR and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were resolved using an automated sequencer (ABI Prism 3730XL). Sequences obtained in this study were deposited in GenBank under accession numbers OM280049-51. Nucleotide VP4 and VP7 sequences were compared with sequences available in the GenBank database using the Basic Local Alignment Search Tool. Nucleotide sequence alignment to identify mutations was performed with the ClustalW multiple alignment program within the BioEdit sequence Alignment Editor package, version 7.0.9.0. Phylogenetic trees were constructed with the Molecular Evolutionary Genetics Analysis software package, version 5.0 (http://megasoftware.net/). Evolutionary relationships were inferred by using a Neighbor Joining algorithm that applied the Kimura 2-parameter model after excluding positions containing gaps and missing data from the alignments. The reliability of the tree topologies was estimated by bootstrap analysis with 1000 replicates. Bootstrap values of >80% were used to indicate robust support for the tree topology.

**Immunologic and genetic analyses**

Flow cytometry

Peripheral blood samples were incubated with monoclonal antibodies (20–30 min at room temperature in the dark). Erythrocytes were lysed in BD FACS lysing solution (BD Biosciences, San Diego, CA, USA) and washed. Cells were stained with the conjugated anti-human antibodies (all from BD Biosciences). Stained cells were acquired in a FACSCanto II flow cytometer, using FACSDiva software for analysis (BD Biosciences).

Lymphocyte proliferation assays

Lymphocyte proliferation assays were performed with mitogens (PHA, concanavalin A, pokeweed mitogen, and OKT3) and analyzed based on tritiated thymidine (Amersham Biosciences, Piscataway, NJ, USA) uptake after 3 days.

Genetic analysis for PID genes

A next-generation sequencing (NGS) targeted panel was employed. Briefly, DNA extracted from peripheral blood was screened for mutations with a customized NGS gene panel containing 479 genes associated with immune disorders. The panel was designed with NimbleDesign software. For each sample, paired-end libraries were created with the help of the KAPA HTP Library Preparation Kit for Illumina platforms (Roche NimbleGen, Mannheim, Germany), SeqCap EZ Library SR (Roche NimbleGen), and the NEXTflex-96 PreCapture Combo Kit for indexing (Bioo Scientific, Austin, TX, USA). Sequencing was conducted on a MiSeq system (Illumina, San Diego, CA, USA), according to the standard operating protocol.

**Supplementary Table 1:** Nucleotide and amino acid differences in rotavirus genes 4, 6, and 9 between rotavirus vaccine strains and rotavirus obtained from stool specimens

| Genome segment | Protein | Geno type | Protein size | Amplicon size | Mutations | | | | | | | | GenBank accession number |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Nucleotides | | | | Amino Acid | | | | |
| | | | | | Position | Rotarix Vaccine* | Rotarix Vaccine † | Study Strain | Position | Rotarix Vaccine* | Rotarix Vaccine † | Study Strain | |
| 4 | VP4 | [8] | 2359 | 620 (nt 163 to 783) | 508 | T | T | C | 170 | F | F | L | OM280050 |
| | | | | | 590 | A | A | C | 197 | N | N | T | |
| 6 | VP6 | 1 | 1194 | 336 (nt 737 to 1073) | - | - | - | - | - | - | - | - | OM280051 |
| 9 | VP7 | 1 | 978 | 836 (nt 25 to 861) | 605 | T | T | C | 202 | M | M | T | OM280049 |

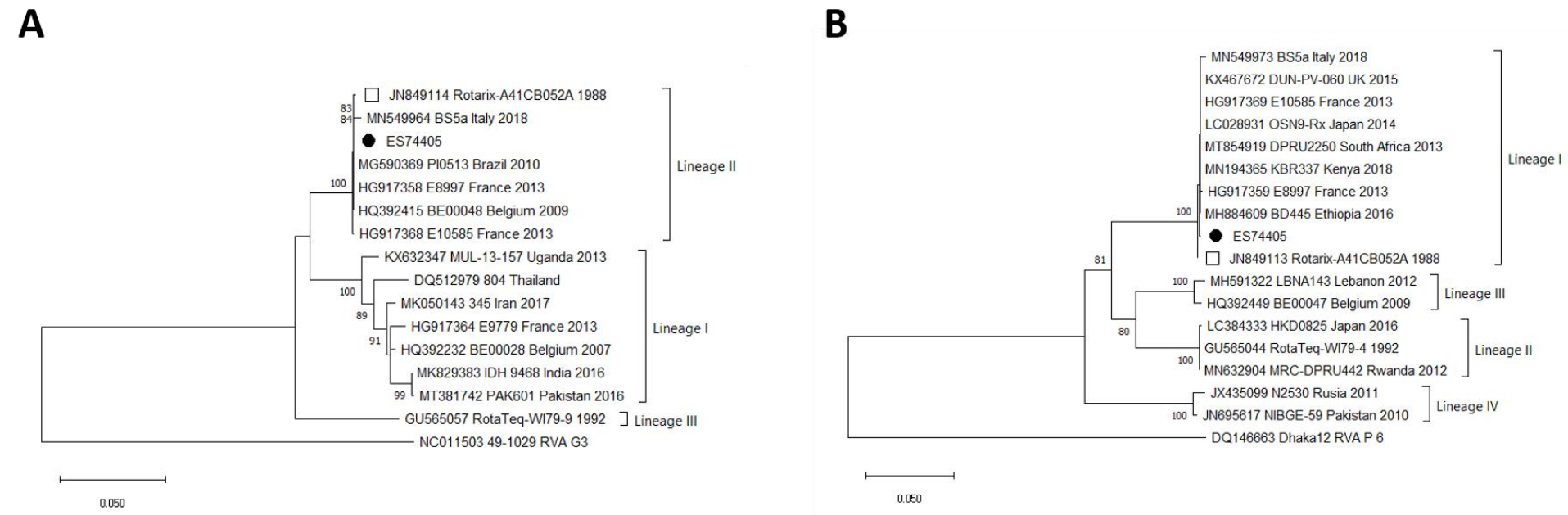
*Referral sequences: For VP4 JN849113; for VP6 KX954619; for VP7 JN849114. All from RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A[8]. Nt, nucleotide

†Lot number AROLC991AB

**Supplementary Table 2**: Cases of Rotarix vaccine-acquired rotavirus infections in infants with reported underlying conditions for which VP4 and VP7 sequences have been published in GenBank

| Strain name | Country | Date of sampling (m/y) | Age at presentation (in months) | AGE episode ? | Vaccination history | Underlying conditions | Rotavirus strain detected | Mutation L170F in VP4 | Mutation T197N in VP4 | Mutation T202M in VP7 | Accession number for VP4 | Accession number for VP7 | Ref |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ES74405 | Spain | 10/2020 | 2 | Yes | 1st dose RV1 22 days prior to sample collection | Intestinal lymphangiectasia | RV1 vaccine strain | Yes | Yes | Yes | OM280050 | OM280049 | This study |
| E10585 | France | 11/2013 | 3 | Yes | 2nd dose RV1 prior to sample collection | SCID | RV1 vaccine strain | Yes | No | Yes | HG917369 | HG917368 | (2) |
| E8997 | France | 1/2013 | 3 | Yes | 2nd dose RV1 prior to sample collection | SCID | RV1 vaccine strain | Yes | No | Yes | HG917359 | HG917358 | (2) |
| BS5a-g | Italy | 6/2018 | 4 | Yes | 2nd dose RV1 prior to sample collection | SCID | RV1 vaccine strain | Yes | No | Yes | MN549973-79 | MN549964-70 | (3) |

**Supplementary Figure**. Phylogenetic analysis based on the partial VP7 nucleotide sequences of the G1 genotype (A) and partial VP4 nucleotide sequences of the P[8] genotype (B) of the Rotarix vaccine-derived G1[P8] study strain (ES74405) and other rotavirus reference strains from GenBank. The study strain is marked with a black circle and the reference Rotarix strain is marked with a white square. Trees were built with the Neighbor Joining algorithm that applied the Kimura 2-parameter model and were bootstrapped with 1000 repetitions. Bootstrap values below 80 are not shown.



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