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First detection of SARS-CoV-2 lineage A.27 in Sardinia, Italy

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Abstract

Introduction. Multiple variants of SARS-CoV-2, since the end of 2020 have emerged in many geographical areas and are currently under surveillance worldwide highlighting the continuing need for genomic monitoring to detect variants previously not yet identified.
Methods. In this study, we used whole-genome sequencing (WGS) and phylogenetic analysis to investigate A.27 lineage SARS-CoV-2 from Sardinia, Italy.
Results. The Italian A.27 lineage genomes from Sardinia appeared related in a clade with genomes from France. Among the key mutations identified in the spike protein, the N501Y and the L452R deserve attention as considered likely vaccine escape mutations. Additional mutations were also here reported.
Conclusion. A combination of features could explain our data such as SARS-CoV-2 genetic variability, viral dynamics, the human genetic diversity of Sardinian populations, the island context probably subjected to different selective pressures. Molecular and genomic investigation is essential to promptly identify variants with specific mutations with potential impact on public health and vaccine formulation.

Key words
- SARS-CoV-2
- lineage A.27
- phylogenetic analysis
- Italy

INTRODUCTION

Human coronaviruses (CoV) are enveloped positive-stranded RNA viruses belonging to the order *Nidovirales*, mostly responsible for upper respiratory and digestive tract infections [1]. An outbreak of a febrile respiratory illness due to the newly discovered Coronavirus (officially named by the World Health Organization as SARS-CoV-2) occurred in mid-December 2019, in the city of Wuhan, Hubei province (China). The virus spread across most countries in all the continents, causing a pandemic event [2-4]. Multiple variants of SARS-CoV-2, since the end of 2020 have emerged in many geographical areas and are currently under surveillance worldwide, highlighting the continuing need for genomic and epidemiological surveillance to detect variants previously not yet identified.

In particular, those viruses belonging to lineage B.1.1.7, B.1.351, P.1 and more recently to B.1.617, which contains three sub-lineages [5-10] have been considered of concern regarding a high transmissibility and/or potential immune escape [10]. During a recent survey of the diversity of SARS-CoV-2 in Mayotte, a cluster of divergent sequences within lineage A (clade 19B) was reported [11].

Specifically, SARS-CoV-2 lineage A.27, as of March 2021, was prevalent in Slovenia, France, Germany, Switzerland and the United Kingdom [12]. In this study, we report whole-genome sequencing (WGS) and phylogenetic analysis of the first three linked cases of the SARS-CoV-2 lineage A.27 in Italy.

MATERIALS AND METHODS

Patient data

Three members of the same family resulted COVID-19 positives. One patient suffers for chronic obstructive pulmonary disease (COPD), hypertensive heart disease and high BMI. He/She has been symptomatic since February 13, 2021 with a lab-confirmed COVID-19 diagnosis on February 17. At first, the patient showed symptoms characterized by osteo-muscular pain and fever (37.5 °C - 38 °C), followed by cough and dyspnea; on February 26, his/her condition worsened and intersti-

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tial pneumonia was diagnosed. The second patient has been symptomatic at February 15, confirmed two days later. Symptoms like pharyngodynia, dry cough and mild headache were reported. The clinical picture was mild with a duration of three days. The third patient, resulted positive on February 17 and asymptomatic. The younger and the third patients did not infect any other persons, despite the occurrence of not protected contacts with several colleagues for business purposes and with other family members.

Whole-genome sequencing and phylogenetic analysis

Hereby, it was reported whole-genome sequencing (WGS) and a complete molecular characterization and phylogenetic analyses of the three SARS-CoV-2 strains collected from the patients whose clinical conditions are reported above. Total nucleic acids were extracted from the naso-pharyngeal swab, collected on February 17, using QIAamp viral RNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's recommendations. SARS-CoV-2 RNA were analysed for N1, N2 and RP genes by in-house rt-Real-time PCR through the Applied Biosystems 7500 Fast System instrument using reagents and protocol from CDC (Division of Viral Diseases, Centers for Disease Control and Prevention-USA) [13].

The RNA samples were quantified using Qubit™ RNA HS Assay Kit (Q32854, Invitrogen) and 10 ng were reverse-transcribed using SuperScript™ VILO™ cDNA+ Synthesis Kit (cod 1175-4050, ThermoFisher Scientific).

Whole-genome sequencing analysis was performed using the Ion GeneStudio S5™ Series System with the Ion AmpliSeq™ SARS-Cov-2 Research Panel (Supplementary Material).

Libraries were prepared manually with the Ion Ampliseq Library Kit Plus according to the manufacturer's protocols (Pub. No. MAN0017003), quantified by Agilent 2100 Bioanalyzer (Agilent Technologies) and then pooled together in equimolar amounts.

The diluted multiplexed library was sequenced using an Ion S5™ System semiconductor-based device (Thermo Fisher scientific), according to manufacturer's protocols (Pub. No. MAN0017003; Supplementary Material).

The consensus sequences were assembled by IRMA (Iterative Refinement Meta-Assembler) Assembly method (Supplementary Material) under the Ion Torrent sequencing technology [14, 15].

The generated sequences were submitted to GISAID (accession numbers: EPI_ISL_ 2244910, EPI_ISL_ 2244911 and EPI_ISL_ 2244912) [16].

To explore the lineages of the new sequences the "Pangolin COVID-19 Lineage Assigner" was used [17] in order to assign the lineages. The assignment of the clade was performed according to Nextstrain classification [18] (https://Nextstrain.org/).

The identification of the amino acid mutations was performed through visualization of the alignments compared to Wuhan-Hu-1 Reference SARS-CoV-2 genome (Accession Number: NC_045512.2).

For phylogenetic analysis, 181 additional complete genome foreign SARS-CoV-2 sequences lineage A.27 were retrieved from GISAID [16] database (last access 23 March 2021) to investigate the relationships among strains. All the sequences were aligned using MAFFT [19] under the Galaxy platform Galaxy Version 7.221.3 [20] (https://usegalaxy.org/) (*Supplementary Material*) and manually edited through Bioedit software [21]. The best fitting substitution model, together with the maximum likelihood (ML) phylogenetic tree, were obtained with IQ TREE [22]. Support for the tree topology and clades was estimated with the bootstrap test (1000 bootstrap replicates).

RESULTS

The lineage analysis showed that the three SARS-CoV-2 sequences belonged to lineage A.27 and the clade assignment was 19B (last access to Pangolin COVID-19 Lineage Assigner and Nextstrain: 23 March 2021).

The maximum likelihood phylogenetic tree *(Figure 1)* shows a supported cluster including four genomes from Germany, and a main supported clade.

The sequences of the three patients identified as lineage A.27, appeared located in the main clade related (bootstrap value 81%) with eight genomes from France.

The A.27 lineage genomes collected from other countries were distributed in other statistically supported clusters within the main clade.

The non-synonymous amino acid variations identified in the three A.27 lineage Italian genomes compared to the Wuhan-Hu-1 reference NC_045512.2, were reported in *Table 1*.

In particular, the variations identified inside the spike protein were: L18F, T95I, L452R, N501Y, T572S, A653V, H655Y, D796Y, S939F, H1083Y, G1219V.

DISCUSSION

We described whole-genome sequencing (WGS) and phylogenetic analysis of the first three linked cases of the SARS-CoV-2 lineage A.27.

The three genomes here investigated belonged to lineage A.27 and represent, to the best of our knowledge, the first identification of this lineage in Italy at that time (February 17, 2021). In fact, other A.27 genomes deposited in Gisaid in Italy with older collection dates were related to 18 March 2021.

Phylogenetic analysis consistently placed the three Italian genomes related among them and showed the Italian patient's strain in a supported cluster mainly related with genomes from France. Among the amino acids mutations found in the three A.27 genomes if compared to other A.27 genomes present on Gisaid from other countries, the following: T572S, H1083Y (*spike*), L146F (*nsp12*), E261D (*nsp13*) were identified only in Italian A.27 genomes from Sardinia region.

Our data could be the result of a combination of events, such as the SARS-CoV-2 genetic variability, the viral dynamics (within and between individual hosts), and the island context probably promoting a different selective pressure [23, 24].

These observations reinforce the need for a continuous genomic surveillance. The rise in mutational variants of SARS-CoV-2, especially with changes in the Spike

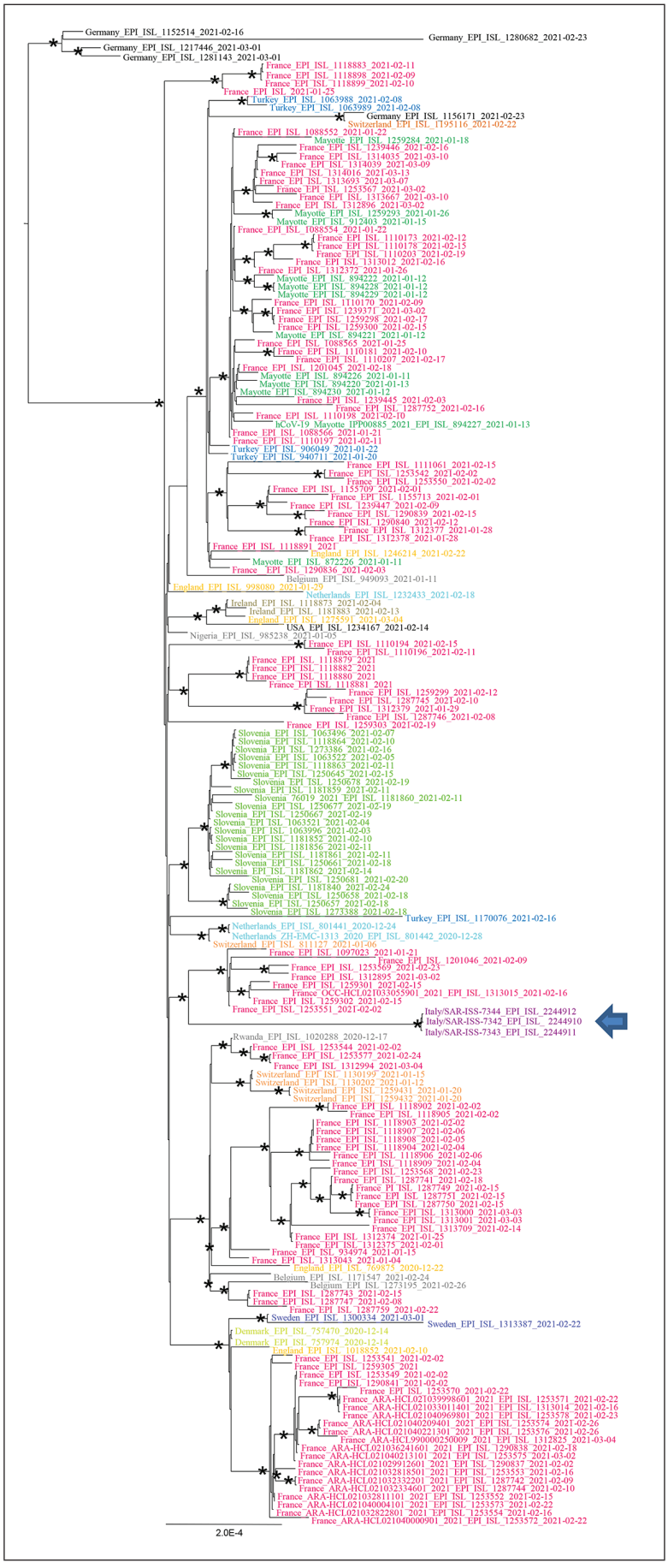

Figure 1

Maximum Likelihood Phylogenetic analysis of three SARS-CoV-2 lineage A.27 Italian complete genomes from Sardinia, plus 181 lineage A.27 downloaded from GISAID (collected from other countries). The tree was rooted by using the midpoint rooting method. Branch lengths were estimated with the best-fitting nucleotide substitution model according to a hierarchical likelihood ratio test. The scale bar at the bottom represents nucleotide substitutions per site. An asterisk along a branch represents significant statistical support for the clusters subtending that branch (bootstrap support and aLRT >80%). The colors of the tips represent genomes from different countries (Germany, black; France, red; Turkey, blue; Switzerland, dark orange; Mayotte, dark green; England, ocra yellow; Netherlands, celestial blue; Belgium, grey; Ireland, brown; Nigeria and Rwanda, dark grey; Slovenia, light green; Italy, violet; Sweden, intermediate blue; Denmark, super light green).

Table 1
The non-synonymous amino acid mutations harbored by the three Italian SARS-CoV-2 genomes lineage A.27 here investigated

Mutation	Target
L18F	*spike*
T95I	*spike*
L452R	*spike*
N501Y	*spike*
T572S	*spike*
A653V	*spike*
H655Y	*spike*
D796Y	*spike*
S939F	*spike*
H1083Y	*spike*
G1219V	*spike*
S202N	*nucleocapsid*
P106L	*nsp2*
D217G	*nsp4*
N82S	*nsp6*
L146F	*nsp12*
P77L	*nsp13*
E261D	nsp13
V50A	*ORF3a*
L84S	*ORF8*

protein, is of significant concern due to the potential ability for these mutations to increase viral infectivity, virulence and/or ability to escape immune response.

In particular, the lineage A.27 was interesting because comprises a combination of different amino acid changes and in particular the N501Y and L452R amino acid substitutions. The N501Y in the spike also found in the alfa, in the beta and in the omicron variant (https://outbreak.info/situation-reports/omicron) is associated with an increased viral transmission [25]. The mutation L452R first got attention at that time, as part of the epsilon variant, but additional evidences have shown that several lineages carried L452R mutations [26], also including the delta variant [27]. The findings seem to suggest that the L452R may provide a competitive advantage, and that the replacement with the arginine may create a much stronger attachment of the virus to the human cells, and might allow it to avoid the neutralizing antibodies trying to interfere with this attachment [26, 27]. Ongoing molecular surveillance is essential to promptly identify variants with specific mutations that may act as trigger to an increase of COVID-19 cases.

CONCLUSION

Molecular and genomic investigation is essential to promptly identify variants with specific mutations with potential impact on public health and vaccine efficacy.

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Authors' contributions

ALP contributed to the conception, design of the study, to investigation, to phylogenetic analysis, and writing original draft. FC contributed to patient data curation, clinical picture, resourses, writing, review and editing. ADM contributed to investigation, data curation, writing, review and editing. SF and RC contributed to data curation, writing, review and editing. MM and MC contributed to whole-genome sequencing, consensus assembly, with the supervision of MC. GO contributed to resources, data curation, writing, review and editing. GR contributed to supervision, writing, review and editing. PS contributed to project administration, supervision, writing, review and editing. The first draft of the manuscript was written by ALP. All Authors commented on earlier version of the manuscript. All Authors have read and approved the final manuscript.

Ethical considerations

This study is a part of a larger research approved by the Ethics Committee of the ISS (Prot. PRE BIO CE n. 0026259).

Conflict of interest statement

The Authors declare that they have no conflict of interest.

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