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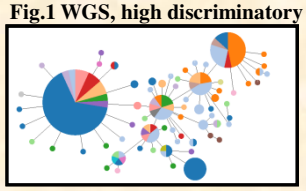
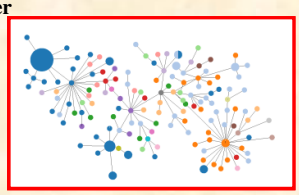
**Fig.1 WGS, high discriminatory power**



HA 67/160 (42%)



WGS 144/160 (90%)

**Fig. 2. Phylogenetic tree of representative viral whole-genome sequences**
**black**– the 2016/2017 vaccine strain, **red** – vaccinated cases, **grey** – unvaccinated, **yellow**- clade 3C.2a1, **brown** – clade 3C.2.

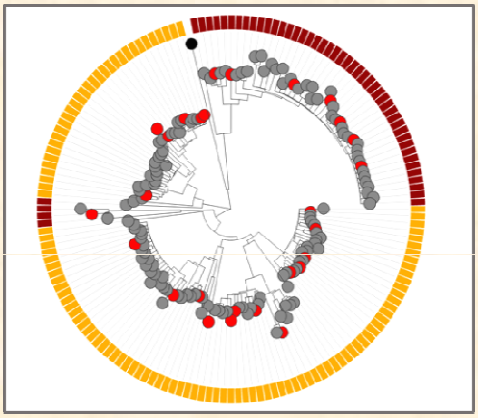


**Table I. Amino acid substitutions found only in viruses detected in vaccinated cases**

| Viruses | PB2 | PB1 | PB1-F2 | N40 | PA | PA-X | HA | NP | NA | M1 | M2 | NS1 | NS2 | AA Substitutions Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EVA079 | R251G | | | | | | | | V116A | | | | | 2 |
| EVA080 | | | | | | | G5R | | V240I | | | | | 2 |
| EVA088 | | | | | | | | | | | | | | 0 |
| EVA089 | | | | | | | L531S | | | | | | | 1 |
| EVA091 | | | | | | | | | | | | P212H | L55I | 2 |
| EVA094 | | | | | L469M | | S124I | | | | | T49A | | 3 |
| EVA102 | | | | | | | | | | | | | | 0 |
| EVA107 | | | | | | | I230L | | I307L | | | | | 2 |
| EVA119 | | | | | | | | | | | | Q63K | | 1 |
| EVA124 | | | | | | | | | | | | | | 0 |
| EVA126 | | | | | | | | | | | | | | 0 |
| EVA133 | | | | | | | | | | | | | | 0 |
| EVA157 | | | | | | | | | | | | | | 0 |
| EVA162 | | | | | | | F193S | M163L | | | | | | 2 |
| EVA163 | | | | | | | | | | | | | | 0 |
| EVA164 | | | | | | | | | V165I | | | | | 1 |
| EVA169 | | | | | N350K | | S46F N126D | | E344K | V15I | | | | 5 |
| EVA182 | | | | | N359T | | | K452R | | | | | | 2 |
| EVA186 | | | | | | | | | | N236K | | | | 1 |
| EVA200 | | | | | | | | | | | | | | 0 |
| EVA209 | | | | | | | | | | | | | | 0 |
| EVA221 | | | | | I407M | | | | S332P | | | | | 2 |
| EVA239 | V731M | | | | | | | | | | | | | 1 |
| EVA250 | | | | | | | | | | | | | | 0 |
| EVA263 | | | | | | | | | | | | | | 0 |
| EVA268 | | | | | | V192G | | | I20T | | | | | 2 |
| EVA311 | | | | | | | D408N | | | V138I | | | | 2 |
| AA Substitutions Total | 2 | 0 | 0 | 0 | 4 | 1 | 8 | 2 | 7 | 3 | 0 | 3 | 1 | 31 |

**Fig. 3 Intra-host minor SNVs syn/non-syn targeting the HA gene**
In **red**-non-syn SNVs in vaccinated cases

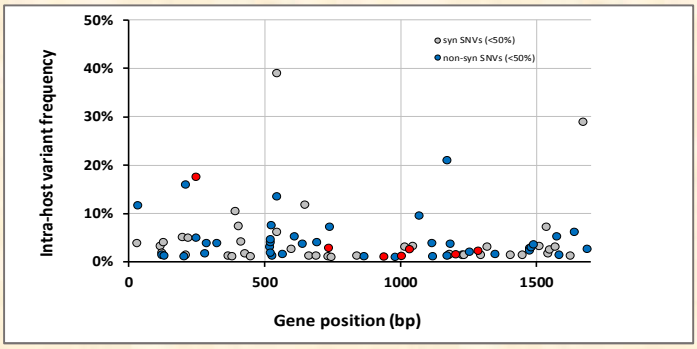
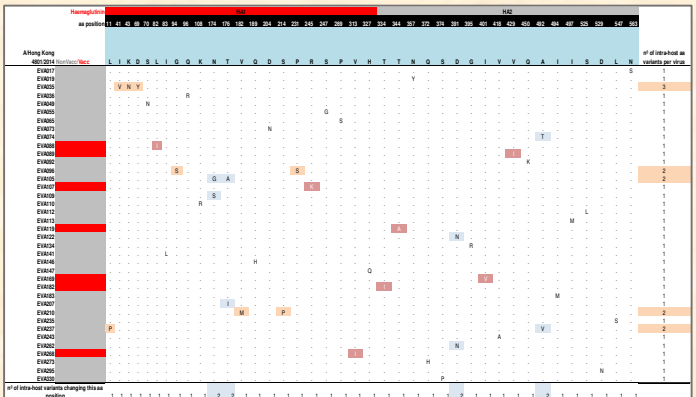



**Table II. Amino acid substitutions changed by intra-host variants**



## SUMMARY


- WGS enabled a higher discriminatory power compared to Hemaglutinin Sanger sequencing;
- All viruses clustered in 2 genetic groups: 3C.2a and the predominant 3C.2a1. Viruses from vaccinated were detected in both clades. Three recombinant viruses were detected, 1 from a vaccinated;
- AA substitutions exclusively detected in viruses from vaccinated cases showed no redundancy;
- Non-synonymous intra-host minor SNVs targeting the HA gene were detected in 7 viruses from vaccinated cases (26%). Still, the aa substitutions also showed no redundancy.


## GOAL

**To search for influenza genetic traits underlying vaccine failure**

## METHODS

In the scope of the EuroEVA/I-MOVE 2016/2017, nasopharyngeal swabs were collected from patients with influenza like illness selected in primary care settings. Viral RNA was extracted directly from biological samples and after multiplex PCR amplification, the whole genome was sequenced for 159 influenza A(H3) viruses by deep sequencing on a MiSeq platform. The influenza gene sequences were assembled using a in-house multi-software pipeline with a mean depth of coverage of 1075x. Multiple gene alignments and mutational analysis was performed on MEGA software 6.0. The bioinformatics tool (www.INSaFLU.insa.pt) will soon be released.

## RESULTS

The whole-genome sequence was determined for 159 influenza A(H3), all clustered in 2 genetic groups: 3C.2a and the predominant 3C.2a1 (Fig.2).

Twenty seven viruses (17%) were detected in vaccinated individuals (16.3% in 3C.2a and 17.3% in 3C.2a1 groups) (Fig.2).

Substitutions shared by the 10 viruses from vaccinated cases were also found among viruses from unvaccinated cases. Although 17 viruses from vaccinated cases presented substitutions not found in viruses from unvaccinated cases (Table I), no redundancy was observed.

86 positions of the HA gene showed intra-host minor SNVs, 46 non syn SNVs. 7 viruses from vaccinated cases showed intra-host minor non syn SNVs. Still, the AA substitutions were different from each other and were scattered along the protein (Fig. 3, Table II).

## CONCLUSIONS

The proportion of vaccinated cases was similar in both 3C.2a and the new 3C.2a1 groups. The limited number of amino acid substitutions exclusive of vaccinated cases occurred sporadically. Inspection of intra-host SNVs affecting HA did not provide clues about vaccine failure but warrants further investigation both at genome scale and sample size.