Genomic epidemiological analysis of SARS-CoV-2 household transmission

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Abstract

Family clusters have contributed significantly to the onward spread of SARS-CoV-2. However, the dynamics of viral transmission in this setting remain incompletely understood. We describe the clinical and viral-phylogenetic characteristics of a family cluster of SARS-CoV-2 infections with a high attack rate, and explore how whole-genome sequencing (WGS) can inform outbreak investigations in this context. In this cluster, the first symptomatic case was a 22-month-old infant who developed rhinorrhea and sneezing 2 days prior to attending a family gathering. Subsequently, seven family members in attendance at this event were diagnosed with SARS-CoV-2 infections, including the infant described. WGS revealed indistinguishable SARS-CoV-2 genomes recovered from the adults at the gathering, which were closely related genetically to B.1 lineage viruses circulating in the local community. However, a divergent viral sub-lineage was recovered from the infant and another child, each harbouring a distinguishing spike substitution (N30S). This suggested that the infant was unlikely to be the primary case, despite displaying symptoms first, and additional analysis of her nasopharyngeal swab revealed a picornavirus co-infection to account for her early symptoms. Our findings demonstrate how WGS can elucidate the transmission dynamics of SARS-CoV-2 infections within household clusters and provide useful information to support outbreak investigations. Additionally, our description of SARS-CoV-2 viral lineages and notable variants circulating in Ireland to date provides an important genomic-epidemiological baseline in the context of vaccine introduction.

INTRODUCTION

Family clusters of infections have represented the majority of SARS-CoV-2 outbreaks in Ireland to date [1]. Whole-genome sequencing (WGS) has been crucial in elucidating the dynamics of virus transmission in many settings, as well as monitoring the evolution of SARS-CoV-2 [2], although few studies have examined family clusters in detail using a genomics approach. We describe the clinical, laboratory and viral phylogenetic characteristics of a family cluster of SARS-CoV-2 infections in the context of national SARS-CoV-2 genomic data, in which WGS clarified the chain of transmission and refuted the standard epidemiologically-generated hypothesis.

METHODS

Case definition and molecular diagnostics

Cases were confirmed by detection of SARS-CoV-2 RNA by real-time reverse transcriptase PCR (qRT-PCR) performed on oro- and nasopharyngeal swabs. In the context of viral genomic epidemiological surveillance, specimens in which SARS-CoV-2 RNA was detected were sent to the National Virus Reference Laboratory (NVRL), University College Dublin (UCD) for WGS. Testing for an additional 21 viral and bacterial respiratory pathogens was performed using the NxtAG Respiratory Viral Panel FAST v2 (Luminex). This included Rhinovirus/Enterovirus, Influenza A and B, Parainfluenza 1–4, Human metapneumovirus, Bocavirus,
Adenovirus, RSV A and B, seasonal coronaviruses OC43, HKU1, NL63 and 229E, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.

### Whole-genome sequencing and bioinformatics

SARS-CoV-2 genome sequences were generated from laboratory-confirmed cases referred to the NVRL, Dublin, between 25 June and 15 November 2020, with Ct values less than 30. This included the family cluster described. Viral RNA was extracted and purified using the Roche MagNa Pure 96 instrument. A tiled amplicon approach following the ARTIC v3 sequencing protocol [3] was employed, using the MinION platform (Oxford Nanopore Technologies, ONT), and assembled with the artic-nCov2019 pipeline [4]. Sequences were multiple sequence aligned against the reference genome sequence (GenBank accession number: MN908947) with MAFFT [5], and lineage identification was according to the PANGOLIN nomenclature developed by Rambaut *et al.* [6]. Maximum-likelihood phylogenetic trees were built with RAxML [7]. All virus genome sequences were deposited and are publicly available in the Global Initiative on Sharing All Influenza Data (GISAID) database, with accession IDs outlined in Table S1 (available in the online version of this article). Sequences corresponding to the family cluster include accession IDs: EPI_ISL_578327, EPI_ISL_578240, EPI_ISL_578325, EPI_ISL_578235, EPI_ISL_578324, EPI_ISL_578326 and EPI_ISL_578321.

### RESULTS

#### Outbreak description

**Cases 1 and 2**

On 13 August 2020, a 22-month-old girl (case 1) who lived with her parents in the south-east of Ireland, developed purulent rhinorrhoea and sneezing, followed by diarrhoea shortly thereafter. Two days later, she was brought to a family gathering which lasted two-and-a-half hours, where she had close contact with nine family members. This included her mother, both maternal grandparents, two uncles and their partners, and two cousins aged 1 and 3 years. Upon leaving the gathering, the mother of case 1 (case 2) experienced pronounced fatigue, headache and mild diarrhoea, with a low-grade fever of 37.8 degrees Celsius. Case 2 sought medical attention from her General Practitioner and was referred for testing for suspected SARS-CoV-2 infection on 17 August,
with viral RNA detected by qRT-PCR (Cycle threshold, Ct 15.7).

As close contacts of case 2, all who attended the family gathering were referred for SARS-CoV-2 RNA testing, which took place on 20 August. This included case 1, who also had SARS-CoV-2 RNA detected (Ct 25). Case 1 experienced persistent nasal congestion for a total of 14 days, whereas case 2 developed mild shortness of breath and anosmia between 22 August and 24 August; this improved without the need for specific medical treatment.

**Cases 3 to 7**

Five of eight other family members who were present at the gathering on 15 August yielded 'SARS-CoV-2 RNA detected' results (Ct range 16–27) when tested on 20 August. All had close contact with case 1 at the event and had developed mild symptoms in the days that followed. The timeline of symptoms for each case is depicted in Fig. 1, with the range of symptoms observed in Fig. 2 and qRT-PCR results with Ct values shown in Table 1.

Case 3, a 65-year-old female with type 2 diabetes mellitus and grandmother of case 1, developed a non-productive cough on 17 August; this was followed by nasal congestion and fatigue for 7 days. She had ten hours of close contact with case 1 between 14 and 16 August, which included the family gathering.

Case 4, a 35-year-old uncle of case 1, developed myalgia, fatigue and subjective fevers between 18 August and 20 August; this was followed by mild shortness of breath and

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**Table 1.** Real-time qRT-PCR results, including cycle threshold (Ct) levels for SARS-CoV-2 targets, corresponding to the household outbreak

| Case | Sample date | SARS-CoV-2 PCR | E gene | S gene | N gene | NxTAG respiratory viral Panel |
|------|-------------|----------------|--------|--------|--------|-------------------------------|
| Case 1 | 20/08/2020 | RNA DETECTED* | n/a | n/a | 25 | RHINOVIRUS/ENTEROVIRUS RNA DETECTED |
| Case 2 | 17/08/2020 | RNA DETECTED† | 15.7 | 15.8 | n/a | VIRAL NUCLEIC ACID NOT DETECTED |
| Case 3 | 20/08/2020 | RNA DETECTED* | n/a | n/a | 18.8 | VIRAL NUCLEIC ACID NOT DETECTED |
| Case 4 | 20/08/2020 | RNA DETECTED* | n/a | n/a | 21 | VIRAL NUCLEIC ACID NOT DETECTED |
| Case 5 | 20/08/2020 | RNA DETECTED* | n/a | n/a | 19 | VIRAL NUCLEIC ACID NOT DETECTED |
| Case 6 | 20/08/2020 | RNA DETECTED* | n/a | n/a | 16 | VIRAL NUCLEIC ACID NOT DETECTED |
| Case 7 | 20/08/2020 | RNA NOT DETECTED‡ | n/a | n/a | n/a | VIRAL NUCLEIC ACID NOT DETECTED |
|       | 27/08/2020 | RNA DETECTED‡ | n/a | n/a | n/a | VIRAL NUCLEIC ACID NOT DETECTED |

*IDEXX SARS-CoV-2 (COVID-19) RealPCR Test.
†Altona RealStar SARS-CoV-2 RT-PCR Kit.
‡GSD NovaPrime SARS-CoV-2.
E, Envelope; S, Spike; N, Nucleoprotein.
Since its recognition as a novel pathogen in late 2019, SARS-CoV-2 has been shown to transmit readily within households [8], and SARS-CoV-2 clusters within private homes have represented the majority of community outbreaks recorded in Ireland to date [1]. Although meta-analyses have estimated secondary attack rates within families to be 18.1–18.8% [9, 10], a recent prospective study found transmission rates to be as high as 53% [11]; considerably higher than outside of this environment.

The relative contribution of young children in the onward transmission of SARS-CoV-2 in the context of household clusters is debated. In the outbreak described in the present study, the chronology of symptoms suggested that a 22-month-old infant was likely to be the primary case, which was notable as children under 4 years of age represented less than 1% of
Fig. 3. (a) Phylogeny of Irish community SARS-CoV-2 sequences (n=674), including the assigned viral lineages and notable spike variants observed. SARS-CoV-2 whole-genome sequences were generated from laboratory-confirmed cases between 25 June and 15 November 2020. This family cluster is highlighted in the red dashed box. (b) Maximum-likelihood phylogenetic tree representing the family cluster, rooted to the reference strain Wuhan-Hu-1 (GenBank accession number: MN908947). Distinctive mutations are shown next to the branches, non-synonymous mutations and the mutated ORFs are detailed. A combination of two synonymous mutations separate the cluster from other B.1 lineage genomes from the region, with the acquisition of a further non-synonymous A-to-G mutation in the ORF encoding the spike protein (S:N30S) identified in Cases 1 and 7.
identified Irish SARS-CoV-2 infections at that time [12]. In a detailed analysis of 58 household clusters, Laws et al. observed secondary infection rates that were similar for both adults (30%) and children (28%), although children experienced symptoms less frequently than adults [13]. This contrasts with an analysis from Switzerland of familial clusters involving 39 paediatric SARS-CoV-2 infections, which showed that in only three cases (8%) did a child develop symptoms before any other household contact [14] and several other reports which have also described an absence of transmission of SARS-CoV-2 from infants to their caregiver despite prolonged close contact [15–17]. However, it is unknown whether these findings represent a genuine difference in susceptibility to infection due to age-related factors, or if infants are being excluded from testing programmes due to their tendency to display milder symptoms [18, 19].

SARS-CoV-2 cases co-infected with other respiratory viruses

As noted in this cluster, common respiratory viruses such as rhinovirus are not easily distinguished from SARS-CoV-2 infections on the basis of clinical symptoms alone. In fact, the presence of rhinorrhea and sneezing caused by other viral pathogens could mask COVID-19 diagnosis, yet conceivably increase the propensity to spread concomitant SARS-CoV-2 infection by droplet and aerosol routes. Moreover, there is evidence emerging to suggest an upregulation of factors mediating SARS-CoV-2 entry across the human upper-airway epithelium, including the ACE2 receptor, by human rhinovirus A16 [20, 21]. Studies from early 2020 have estimated rates of SARS-CoV-2 co-infection with rhinovirus/enterovirus at 6.9% [22]: as such, ongoing surveillance through the (northern hemisphere) winter season will be important to establish both the burden of these co-infections, and the impact they may have on SARS-CoV-2 attack rates.

Role of WGS in SARS-CoV-2 outbreak investigations

Throughout the pandemic, WGS has played an important role in efforts to characterize the dynamics of transmission of SARS-CoV-2 within clusters and outbreaks. In the initial phase, genomic data generated in the context of a household outbreak provided important evidence for human-to-human transmission of the virus in China [8] and subsequently played a key role in the investigation of early cryptic transmission in the United States [23]. As the virus has spread and evolved, hundreds of thousands of SARS-CoV-2 genomes have been made available publicly on platforms such as GISAID [24], and such data has been key to informing public health policy development across many jurisdictions [25, 26]. It is noteworthy that a high proportion of mutations in the SARS-CoV-2 genomes correspond to C-to-U and A-to-G mutations, which are attributed to the effects of host-dependent RNA-editing proteins of the APOBEC and ADAR families, respectively [27]; the three mutations distinguishing the genome sequences derived from the family cluster from other sequences circulating in the region exemplify such effects. In this cluster, WGS supplemented the epidemiological investigation with high-resolution data about the source and directionality of transmission of infection, as well as providing important background genomic data about locally circulating viruses. Firstly, it supported the entry, from a single point source, of infection into this family from genetically similar B.1 lineage viruses circulating in the south-east of the country; secondly, even in a setting whereby the history of events and chronology of symptoms pointed to case 1 as the primary case, WGS identified a genetically distinct viral sub-lineage from her sample that suggested she was infected later than other family members.

Although the identification of a spike substitution from the two children in this family outbreak cluster provided useful information about the chain of transmission between family members, the functional significance of this variant has yet to be established. This highlights the important role that WGS plays in tracking the genomic evolution of this virus and in informing public health responses, particularly in the context of widespread vaccine use. In recent months, a variety of important amino acid substitutions and deletions within the SARS-CoV-2 spike protein have been identified by genomic surveillance efforts in many countries with the potential to impact viral transmissibility, pathogenesis and/or facilitate immune-escape from neutralizing antibodies. For example, the B.1.1.7 'Variant of Concern', which is defined by a set of 23 genomic variations including an S:N501Y substitution and deletions at positions 69–70, spread rapidly throughout the United Kingdom from late November 2020 [28] and received significant attention due to evidence supporting increased viral transmissibility and its association with the failure of S gene qPCR targets [28, 29]. Notable variants observed in Irish SARS-CoV-2 sequenced genomes between 25 June and 15 November 2020, are represented in Fig. 3 (a). These include D614G, associated with the B.1 lineage observed in this family cluster, which is now ubiquitous and appears to have a moderate effect on SARS-CoV-2 transmissibility [30]; A222V, present in the 20A.EU1 (B.1.177) cluster which has spread quickly across Europe from Spain and become dominant in Irish sequences [31]; and N439K, a variant for which there is evidence to suggest increased ACE2 receptor binding affinity and immune-escape from neutralising antibodies [32].

Limitations of WGS

In this cluster, most family members were tested for SARS-CoV-2 by qRT-PCR on the same day, including case 1, and the majority had genetically indistinguishable viruses. Additionally, the moderate substitution rate in SARS-CoV-2 due to the replication with proofreading activity [33] limits the accumulation of mutations among samples. This meant that although a putative direction of transmission between the adult family members and case 1 could be hypothesized, the identity of the primary case could not be definitely proven. Although the possibility that more than one introduction of SARS-CoV-2 into the family cannot be out RULED, the high nucleotide sequence similarity among the viral sequences recovered from the family members.
and the genetic distance to other sequences generated from contemporaneous community cases, indicated a single introduction into the family with spread between those in attendance at the gathering was the most parsimonious series of events. However, these points serve to highlight the potential limitations of WGS in this context; although it can add weight to hypotheses generated by outbreak investigations, directionality of transmission is not always possible to ascertain, and phylogenetic information must be taken in the context of metadata derived from a complete epidemiological evaluation.

**CONCLUSION**

SARS-CoV-2 transmits readily within households and may be clinically indistinguishable from seasonal respiratory viruses. Genomic data from WGS provided high-resolution phylogenetic characterization of this household cluster, elucidating the chain of transmission between family members, and affording an insight into the source of infection from SARS-CoV-2 viral lineages in community circulation in Ireland. This underlines the important role that WGS can play as an aid to epidemiological investigations and efforts to better understand the transmission dynamics and genomic evolution of SARS-CoV-2.

**Funding information**

The authors received no specific grant from any funding agency.

**Author contributions**

D.H. investigation, data curation, project administration, writing – original draft; G.G. formal analysis, data curation, investigation, software, visualization, writing – review and editing; J.D. supervision, writing – review and editing; K.M.D. investigation, methodology, supervision, writing – review and editing; C.D.G. conceptualization, resources, supervision, writing – review and editing; K.M.D. investigation, writing – review and editing; J.D. supervision, writing – introduction into the family with spread between those in attendance at the gathering was the most parsimonious series of events. However, these points serve to highlight the potential limitations of WGS in this context; although it can add weight to hypotheses generated by outbreak investigations, directionality of transmission is not always possible to ascertain, and phylogenetic information must be taken in the context of metadata derived from a complete epidemiological evaluation.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethical statement**

Written informed consent was obtained from all patients described in this cluster. Whole-genome sequencing of SARS-CoV-2 viral genomes was performed in the context of national genomic epidemiological surveillance in support of public health outbreak investigations.

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