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Intrahost viral evolution during chronic sapovirus infections

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ABSTRACT

Background: Sapovirus is a common cause of self-limiting diarrhea. In immunocompromised individuals chronic infections occur, but are incompletely investigated.
Objectives: To investigate viral evolution in immunocompromised hosts during chronic sapovirus infection.
Study design: From January 2010 to September 2018 stool samples of 5333 in-patients were analyzed for the presence of sapovirus RNA by real-time RT-PCR. In follow-up samples of chronic diarrhea cases nucleic acid sequencing of sapovirus genomes was performed. Amino acid mutations were identified by alignments and compared to data from GenBank. Sapovirus genotypes were assessed by phylogenetic analysis of viral capsid gene (VP1).
Results: Sapovirus RNA was present in 146 stool samples of 95 patients (1.8%), most frequently in young children and infants. In patients older than 14 years, sapoviruses were exclusively detected in immunocompromised patients. Chronic diarrhea occurred in almost one third of the sapovirus positive immunocompromised individuals (n = 5) and was established by different sapovirus genotypes (GI.2, GII.1, GII.3). The maximum observed duration of sapovirus shedding was 119 days. Accumulation of amino acid mutations during chronic infection was most often detected within VP1 P2 protruding subdomain. Reduction of immunosuppression was associated with decrease of viral load and clearance of sapovirus in stool.
Conclusions: Clinicians should consider immunocompromised individuals at risk to develop chronic diarrhea due to persistence of SaV infection. The identified VP1 mutations contribute to an understanding of sapovirus-host interactions. For further conclusions regarding virus immune escape and altered viral fitness structural data on sapovirus capsid and virus/receptor complex are necessary.

1. Background

The genus Sapovirus (SaV) belongs to the family *Caliciviridae* and is associated with gastroenteritis in humans and animals. The viral single-stranded positive-sense RNA genome is 7.1–7.7 kb in size. It contains two open reading frames (ORFs). The larger one, ORF1, encodes a polyprotein which is processed by the virus-encoded protease into at least six non-structural proteins (NS1 to NS6-7) and the major outer capsid protein, VP1. The minor structural protein, VP2, is encoded by ORF2 [1]. Sapovirus VP1 consists of several domains; an N-terminal (N) arm inside the capsid shell, as well as the shell (S) and protruding (P) domains, which are connected via a flexible hinge (H). The P domain is placed at the surface of the capsid protein and contains the N-terminal (P1-1) and C-terminal (P1-2) parts of the P1 subdomain, and the hypervariable P2 subdomain positioned in between of them [2,3].

Based on VP1, SaV are phylogenetically classified into 15 genogroups (GI to GXV). Each of which can be subdivided into genotypes [4,5]. The VP1 genogrouping and genotyping corresponds with SaV antigenicity [6]. Human pathogenic strains are assigned to four genogroups and belong to at least 18 genotypes (GI.1 to GI.7, GII.1 to GII.8, GIV.1, GV.1, and GV.2) [4].

Human SaV largely fail to grow in vitro [7]. Due to lack of cell culture models and crystal structure of its proteins, the mechanisms of human SaV binding and entry into target cells are largely unknown [1]. For porcine SaV, however, an efficient cell culture system has been established [8] and binding of sialic acids has been shown [9]. Capsid subdomain P2 is thought to be the most important site of host factor interactions [10]. The site of infection and the susceptibility of cell types to SaV in vivo remain unknown [1,7].

In humans, SaV are less common than noroviruses, but increasingly diagnosed since the introduction of real-time RT-PCR detection methods [11,12]. Sapoviruses are estimated to cause 4% of acute gastroenteritis outbreaks in Europe [11]. The clinical course of SaV infections is generally milder than in noroviruses [13,14]. Asymptomatic shedding is common [15] and re-infections occur as protective immunity is transient and likely restricted to antigenically homologous

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Table 1
Nucleic acid sequencing data.

	Diarrhea	Time interval (d)[a]	Sapovirus	Sequencing data	GenBank
Patient#1	Chronic	–	Hu/DE/2017/GII.1/LeipzigP1-A	Near CG	MH541021
		8	Hu/DE/2017/GII.1/LeipzigP1-B	VP1, VP2	MH541022
		22	Hu/DE/2017/GII.1/LeipzigP1-C	VP1, VP2	MH541023
		39	Hu/DE/2017/GII.1/LeipzigP1-D	VP1, VP2	MH541024
		39	Hu/DE/2017/GII.1/LeipzigP1-Ddel[b]	VP1, VP2	MH541025
		49	Hu/DE/2017/GII.1/LeipzigP1-E	VP1, VP2	MH541026
		49	Hu/DE/2017/GII.1/LeipzigP1-Edel[b]	VP1, VP2	MH541027
		58	Hu/DE/2017/GII.1/LeipzigP1-F	VP1, VP2	MH541028
		64	Hu/DE/2017/GII.1/LeipzigP1-G	Near CG	MH541029
Patient#2	Chronic	–	Hu/DE/2017/GII.1/LeipzigP2-A	Near CG	MH541030
		7	Hu/DE/2017/GII.1/LeipzigP2-B	VP1, VP2	MH541031
		32	Hu/DE/2017/GII.1/LeipzigP2-C	Near CG	MH541032
Patient#3	Chronic	–	Hu/DE/2018/GII.1/LeipzigP3-A	Near CG	MH541033
		14	Hu/DE/2018/GII.1/LeipzigP3-B	VP1, VP2	MH541034
		25	Hu/DE/2018/GII.1/LeipzigP3-C	VP1, VP2	MH541035
		38	Hu/DE/2018/GII.1/LeipzigP3-D	VP1, VP2	MH541036
		58	Hu/DE/2018/GII.1/LeipzigP3-E	VP1, VP2	MH541037
		69	Hu/DE/2018/GII.1/LeipzigP3-F	Near CG	MH541038
Patient#4	Chronic	–	Hu/DE/2015/GI.2/LeipzigP4-A	Near CG	MH541039
		10	Hu/DE/2015/GI.2/LeipzigP4-B	VP1, VP2	MH541040
		15	Hu/DE/2015/GI.2/LeipzigP4-C	VP1, VP2	MH541041
		36	Hu/DE/2015/GI.2/LeipzigP4-D	Near CG	MH541042
Patient#5	Chronic	–	Hu/DE/2010/GII.3/LeipzigP5-A	Near CG	MH541043
		16	Hu/DE/2010/GII.3/LeipzigP5-B	VP1, VP2	MH541044
		32	Hu/DE/2010/GII.3/LeipzigP5-C	VP1, VP2	MH541045
		59	Hu/DE/2010/GII.3/LeipzigP5-D	VP1, VP2	MH541046
		91	Hu/DE/2010/GII.3/LeipzigP5-E	VP1, VP2	MH541047
		119	Hu/DE/2010/GII.3/LeipzigP5-F	Near CG	MH541048
PatientAD1	Acute	–	Hu/DE/2009/GII.3/LeipzigAD1	VP1, VP2	MH763826
PatientAD2	Acute	–	Hu/DE/2010/GII.3/LeipzigAD2	VP1, VP2	MH763827

Near complete genomes (CG) did not cover 5′NTR, three codons of NS1, and 22–28 nucleotides of 3′NTR. Sequencing data of VP1 and VP2 is framed by short partial sequences of NS6-7 and 3′NTR.
NS, non-structural protein; VP, viral structural protein.
[a] The number of days that have elapsed since the first sapovirus positive stool was sampled.
[b] Nucleic acid sequencing showed deletion of VP1 codon 343 in a viral subpopulation.

SaV [16–18]. Shedding of SaV may continue after symptoms have subsided but mostly decreases to undetectable levels within two weeks [14,19]. Persistent SaV diarrhea (> 14 days) is uncommon [20]. Chronic SaV infection (> 1 months) is reported less frequently than chronic norovirus infections [21,22].

2. Objectives

To investigate viral evolution in immunocompromised hosts during chronic sapovirus infection.

3. Study design

3.1. Sample collection, RNA extraction and virus detection

From January 2010 to September 2018 a total of 7722 stool samples were obtained from 5333 in-patients at Leipzig University Hospital, Germany. Thereof, 1798, 676 and 2859 patients aged less than five, 5–14 and 15 years or older, respectively. Total RNA was extracted from 10% stool suspensions in PBS (MagNA Pure system, Roche Applied Science, Mannheim, Germany) and tested for presence of SaV RNA by a real-time RT-PCR that target the polymerase-capsid junction [23]. The immune status of all SaV positive patients was assessed.

3.2. Sanger sequencing

In five chronic SaV diarrhea cases near complete SaV genomes were amplified from the earliest and most recent SaV positive samples by overlapping RT-PCRs (OneStep RT-PCR Kit, Qiagen, Hilden, Germany) (Table S1). In case of failure of sequencing the next-to-last positive sample was used.

For several stool samples obtained at time points between the first and the last sapovirus positive sample, viral cDNA of SaV positive samples was synthesized using genotype specific 3′NTR anti-sense primers (SuperScript III First-Strand Synthesis System, Invitrogen, Carlsbad, USA). Complete VP1 and VP2 sequences were amplified (Phusion High Fidelity DNA polymerase, Thermo Scientific, USA) using genotype specific primer pairs (Table S1). Amplicons were purified (Wizard SV Gel and PCR Clean-Up System, Promega, Mannheim, Germany) and sequenced (BigDye Terminator v1.1 Cycle Sequencing kit and ABI 3500 Genetic Analyzer, PE Applied Biosystems, Foster City, CA). The obtained sequences have been deposited in the GenBank database (Table 1).

3.3. Sequence analysis

Overlapping electropherograms were aligned and analyzed (Geneious v10.2.2, Biomatters Ltd, New Zealand). Putative ORFs, cleavage sites and functional domains were identified by comparison to available GenBank data, and substitutions between different time points by alignments.

3.4. Phylogenetic analysis

Complete VP1 nucleic acid sequence alignments of the present SaV and available reference data from GenBank were phylogenetically analyzed using Maximum Likelihood algorithm in MEGA5 [24]. Statistical support was assessed by bootstrapping with 1000 replicates.

4. Results

Sapovirus RT-PCR yielded positive results in 146 samples of 95

Table 2
Patients' characteristics.

	Age	Sex	Health information	Immunosuppressive drugs (adjustments after infection)
Patient#1	2 yrs	Male	Intestinal lymphangiectasia, thymic hypoplasia, panlymphopenia, hypogammaglobulinaemia; substitution of immunoglobulins	–
Patient#2	13 mo	Female	Severe refractory epilepsy	Prednisolone (tapered)
Patient#3	2 yrs	Male	Embryonal brain tumor	Vincristine, ifosfamide, adriamycine, actinomycine D
Patient#4	65 yrs	Male	Chronic glomerulonephritis, kidney transplantat (early post-operative phase)	Tacrolimus, mycophenolate mofetil (suspended), prednisolone (reduced)
Patient#5	57 yrs	Male	B-cell chronic lymphocytic leukemia (Binet-C), bone-marrow transplant	Fludarabine, bendamustine, rituximab, dexamethasone, cytarabine, cisplatin

patients (1.8%). Sapovirus positivity rate was 4.2%, 1.6% and 0.3% in patients aged less than five, 5–14 and 15 years or older, respectively. In the oldest age group, SaV was exclusively detected in immunocompromised individuals. In total, SaV occurred in 16 immunocompromised patients of whom three children and two adults developed chronic diarrhea (31%) (Table 2). Patient #1, an immunodeficient child, experienced repeated episodes of viral infections. Since SaV diarrhea persisted, the administration of polyspecific immunoglobulins was intensified. Patients #2 to #5 received immunosuppressive drugs. In case of non-malignant disease, i.e., patients #2 and #4, immunosuppression was reduced after SaV detection (Table 2). The day of diarrhea onset was documented in patients #1-4, but remained unidentified in patient #5 (Fig. 1). Short diarrhea was reported four days prior to conditioning therapy of patient #5, but first enteric symptoms may have occurred earlier than this. Diarrhea reappeared when salvage therapy of progressive B-cell chronic lymphocytic leukemia was initiated.

The time interval between the first and the last SaV positive sample of individual patients ranged from 32 to 119 days and included episodes without diarrhea. Sapovirus testing of the most recent samples was negative in patients #1 and #2, but positive in patients #3-5; albeit low viral load in patient #4 (Fig. 1). Almost complete SaV genomes were assessed in the earliest sample of all patients, in the last positive sample of patients #1-3 and #5, and in the next-to-last sample of patient #4 (Table 1).

Analysis of VP1 showed GII.1 genotypes in patients #1-3, GI.2 in patient #4, and GII.3 in patient #5 (Fig. 2). Nucleic acid identity between GII.1 strains Hu/DE/2017/GII.1/LeipzigP1-A and Hu/DE/2017/GII.1/LeipzigP2-A was high (99.3%), but lower in comparison to strain Hu/DE/2018/GII.1/LeipzigP3-A (91.1%). At amino acid level identity was high between all three GII.1 strains (99.1–99.8%). In VP1 analysis strain Hu/DE/2010/GII.3/LeipzigP5-A showed relatedness and high nucleotide identity (97.8% and 97.2%) with GII.3 acute diarrhea strains Hu/DE/2009/GII.3/LeipzigAD1 and Hu/DE/2010/GII.3/LeipzigAD2 (Fig. 2).

The number of heterogeneous sites in the earliest assessed SaV genome was low in patients #1-4 ($n \leq 2$) but high in patient #5 ($n = 29$). Comparative analysis with SaV in follow-up samples showed non-synonymous (dN) to synonymous (dS) substitution ratios above 1.0 in patients #1-4 (1.5, 1.7, 1.2 and 2.5, respectively). In contrast, dN/dS ratio was below 1.0 in patient #5 (0.4). Distributional analysis identified dN accumulation in VP1 in patients #1, #2 and #4. In contrast, results were non-significant in patient #3 and patient #5 (Table 3).

In alignments of all complete VP1 sequences of GII.1, GII.3 and GI.2 SaV from GenBank (Fig. S1) the entire VP1 was highly conserved in GII.1. In GII.3, variability was marginally higher and showed polymorphism of N. In contrast, GI.2 variability was high, including strong P polymorphism (Fig. 3a).

Longitudinal analysis of VP1 residues identified 7, 4, 3, 4 and 6 fixed mutations in patients #1, #2, #3, #4, and #5, respectively, and several unfixed mutations (Fig. 1). Mutations were mainly located within P2 and P1-1 subdomains. Putative mutation hotspots in GII SaV were identified in P2 codons 341–349 and 404–411 (Fig. 3b). Notably, three P2 mutations occurred at identical sites of GII.1 strains Hu/DE/2017/GII.1/LeipzigP1-A and Hu/DE/2017/GII.1/LeipzigP2-A (Fig. 1).

As VP1 variability of GII.3 SaV was low (Fig. 3a), an attempt to identify putative VP1 mutations which occurred prior to the observation period was conducted in patient #5. For this purpose, sequences of Hu/DE/2010/GII.3/LeipzigP5-A and –F were compared to all complete GII.3 VP1 sequences from GenBank and to local acute diarrhea strains Hu/DE/2009/GII.3/LeipzigAD1 and Hu/DE/2010/GII.3/LeipzigAD2 (Fig. S1). By this approach, seven putative preceding mutations across the VP1 were identified (Fig. 3b).

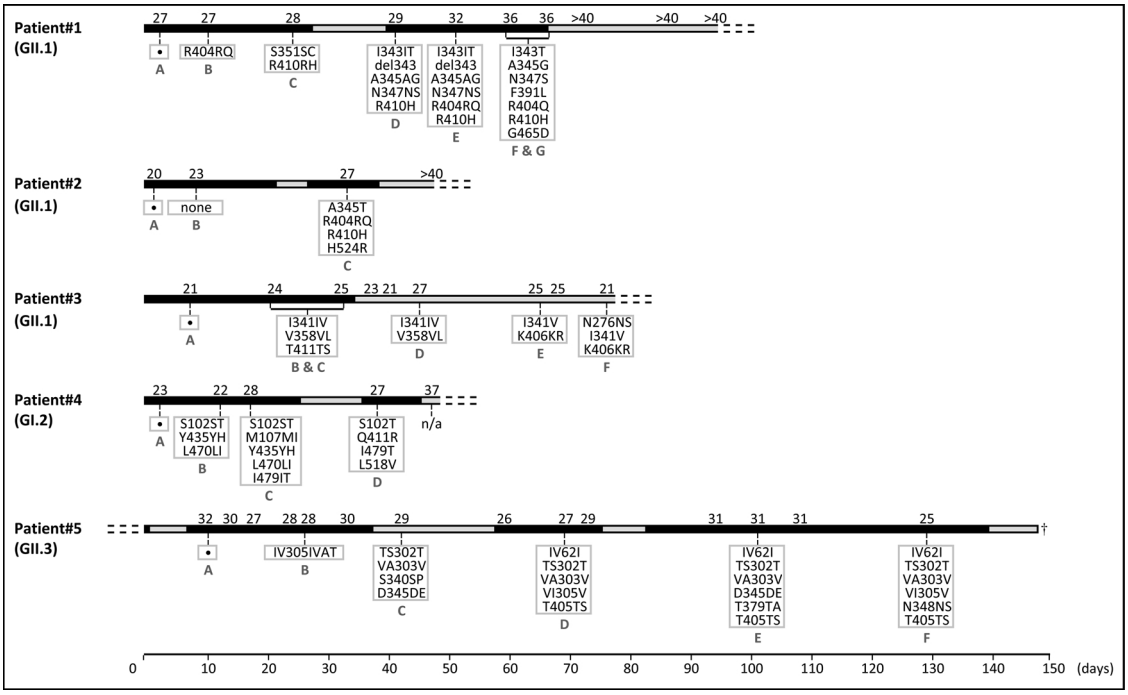

Fig. 1. Clinical and virological findings during chronic sapovirus (SaV) infection. The patient number and SaV genotype are shown at the left. The horizontal bars indicate episodes with (black) and without (grey) diarrhea. In patients #1-4 the day of SaV diarrhea onset is equal to day zero and follow-up ended after SaV remained negative or diarrhea subsided. In patient #5 diarrhea was reported at day zero, but prior enteric symptoms may have remained unreported. Patient #5 died from bacterial pneumonia and neutropenic sepsis. Above the bars cycle threshold (Ct) values of SaV real-time RT-PCR in stool samples are shown. Samples which show Ct values above 40 were considered negative. At different time points, amino acid changes of VP1 in comparison to the earliest SaV positive sample (•) are shown below the bars in small boxes. The numbering of residues refers to the deduced amino acids of the subgenomic VP1 transcript of the respective SaV strain. The capital letters below the boxes (A to G) indicate the analyzed sequences of the patients' samples as listed in Table 1 (GenBank accessions MH541021-48). Amplification of VP1 was not successful in the most recent positive sample of patient #4 (n/a). Notably, residues 345, 404, and 410 of the two GII.1 SaV of independent patients #1 and 2 were altered in both strains.

5. Discussion

This paper adds five cases of chronic SaV infections in immunocompromised individuals. In line with recent reports, overall SaV positivity rate was low in stool samples, and sapovirus infection was more common in infants and young children [25,26]. In adults, SaV infections were rarely detected and restricted to samples from immunocompromised individuals. In the present paper, chronic infection occurred in nearly one third of the SaV affected immunocompromised patients, which was clearly more frequent than in a previous report (12/80, 15%). However, in the prior study information about patients' immune status remained incomplete and chronic infection might have been more frequent in the immunocompromised subgroup [25]. Chronic SaV infections are rare and published cases are incompletely studied. In addition to the above mentioned chronic SaV infection in 12 children, two more cases in adults were reported. These two renal transplant recipients suffered from long-lasting diarrhea. Association with SaV positivity in single stool samples is described, and chronic SaV shedding in stool was not demonstrated [21,27]. Intrahost viral evolution was investigated in none of the reported 14 cases and the viral genotype was only assessed in one adult as GII.1, without providing sequence data [21].

This investigation showed chronic SaV shedding in five independent immunocompromised individuals. Sequencing of follow-up samples identified different viral genotypes, excluded SaV re-infections, and allowed for analysis of viral intrahost evolution based on sequence data of all viral genes. The earliest investigated samples of patients #1-4 were obtained shortly after diarrhea onset when heterogeneity of the viral population was low. In agreement with this observation, viral populations of related noroviruses are known to be homogeneous shortly after infection due to the genetic bottleneck effect, where a significant number of variants found in the donor are lost at transmission [28]. In contrast, a high number of heterogeneous sites and alterations of otherwise conserved VP1 sites were shown in the earliest investigated sample of patient #5. Accordingly, recent acquisition of SaV infection by patient #5 is unlikely. In fact, the heterogeneous viral population shown in the earliest sample of patient #5 rather points to viral intrahost evolution and accumulation of mutations prior to first sampling, probably during several weeks to months [28,29]. In agreement with this assumption, phylogenetically related GII.3 strains with conserved VP1 sites circulated in acute diarrhea patients in the same area. It can be therefore assumed, that the actual duration of sapovirus shedding in stool of patient #5 was longer than the one observed (> 119 days).

During intrahost evolution heterogeneity of viral populations increased in all patients and successive amino acid mutations appeared. Similar to data from noroviruses, mutations mainly accumulated within the P domain of VP1and remained rare in non-structural proteins and VP2 [30]. Immune-driven selection of escape mutants may explain this observation [31], as neutralizing epitopes are suggested within the P domain of calicivirus' capsids [32,33].

The present findings suggest positive selection and highest non-synonymous substitution rates in patients #1, #2 and #4, and support the concept of enhanced viral adaptation rates in hosts under moderate immunosuppression, as weak selective pressure still drives evolution but is unable to completely control viral replication [34]. Notably, reduction of immunosuppression in three of the five patients of this paper was associated with decreasing viral loads, and (near) clearance of virus in stool. In contrast, severe immunosuppression persisted in cancer patients #3 and #5, who showed weak positive selection (patient #3) or negative selection (patient #5) and failed to control the virus. Conceivably, fitness costs of mutations have limited viral immune

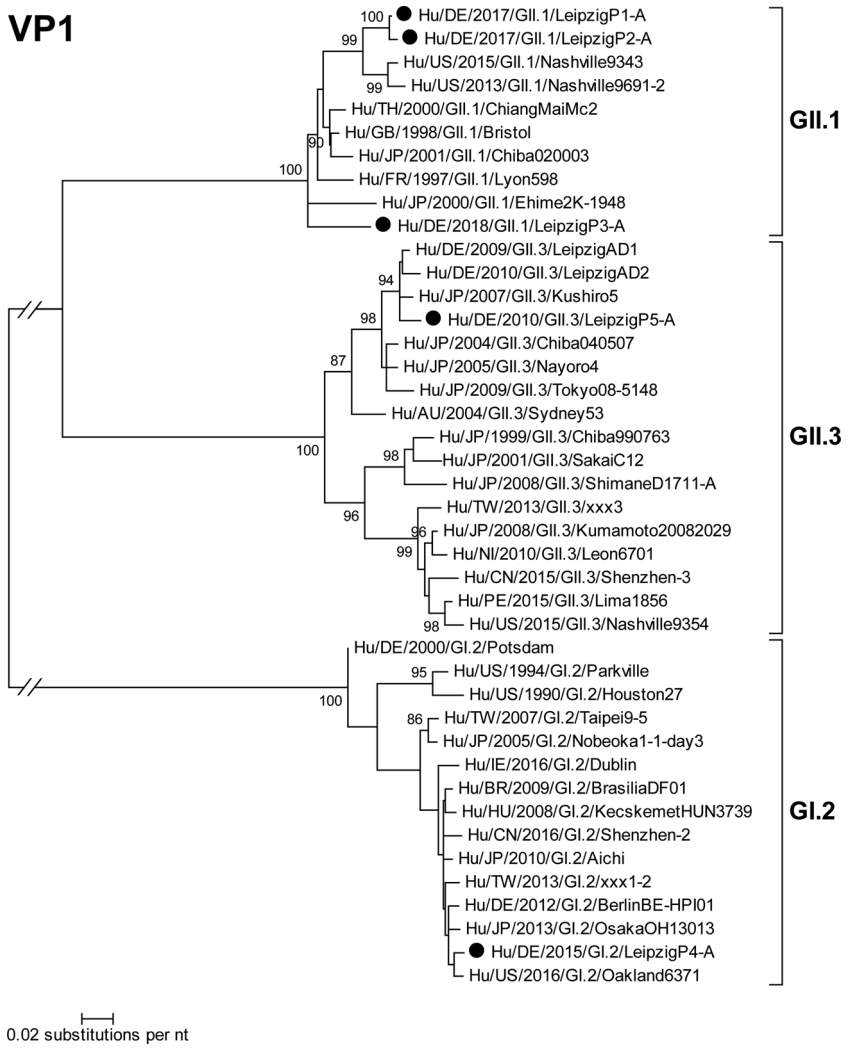

Fig. 2. Phylogenetic analysis of complete sapovirus (SaV) viral protein 1 (VP1). Complete VP1 nucleic acid sequences of the present stool sample set and SaV GI.2, GII.1 and GII.3 reference data from GenBank were aligned. Phylogenetic analysis was performed using Maximum Likelihood algorithm in MEGA5. Statistical support was assessed by bootstrapping with 1000 replicates. Only bootstraps values above 80% are shown. Strains of chronically infected patients of the present investigation are labelled by a filled circle.

Table 3
Non-synonymous substitutions in the viral genome during chronic sapovirus infection.

	Time interval (days)	Non-synonymous substitutions n (rate)			
		NS	VP1	VP2	total
Patient#1	64	2 (0.18)	7 (1.95)*	0	9 (0.58)
Patient#2	32	1 (0.13)	4 (1.55)*	0	5 (0.44)
Patient#3	69	6 (0.51)	3 (0.78)	2 (1.75)	11 (0.65)
Patient#4	36	1 (0.16)	4 (1.95)*	0	5 (0.57)
Patient#5	119	7 (0.34)	6 (0.90)	2 (1.01)	15 (0.52)

The near complete viral coding sequence of the earliest sapovirus positive sample was compared with the most recent available viral sequence of the same patient. The number of non-synonymous substitutions (dN) is shown for the genetic regions of the non-structural proteins (NS) and the structural proteins, VP1 and VP2. To allow comparison of the occurrence of dN between the patients the rate of dN (dN/amino acid site/100 days*10^{-2}) is indicated in brackets. Significance ($p < 0.05$) of the dN rate in a genetic region was assessed by Fisher's Exact Test and is indicated by *.

escape and enabled patients #1, #2 and #4 to finally clear the virus. Viral fitness can be reduced by escape mutations due to overlap of VP1 antigenic sites and receptor binding sites, as shown for noroviruses [35]. Furthermore, capsid plasticity is thought to be crucial for fitness of escape mutants. For instance, it has been suggested for noroviruses that prevalent GII.4 genotypes may evolve rapidly as they tolerate high capsid variation without losing viral function [36,37]. In agreement with this observation, the present analysis identified highest VP1 variation within SaV GI.2 genotype. As described in GII.4 noroviruses, rapidly evolving SaV GI.2 drift variants have emerged in recent years and GI.2 became the most prevalent SaV genotype in humans [11]. In contrast, the antigenic diversity is thought to be limited in less prevalent non-GII.4 noroviruses because of lower capsid plasticity [36,37]. Infrequent GII.1 and GII.3 SaV showed low VP1 variation in the present analysis of published sequence data, which may point to restricted capsid plasticity in these genotypes.

Comparison of intrahost mutations showed similar distribution across VP1 in the present GII SaV but a distinct pattern in the GI.2 SaV. Interestingly, VP1 mutations of the GI.2 strain were distinct from reported intrahost mutations of a persistently shed and finally cleared GI.2 outbreak strain too [19]. Late mutations of GI.2 SaV in patient #4, however, may have been missed as VP1 sequencing of the last, low-positive, sample failed.

Within the VP1 mutation hotspots of the present GII SaV, identical residues were altered in GII.1 strains of two independent patients. Interestingly, the altered sites were otherwise conserved in all available

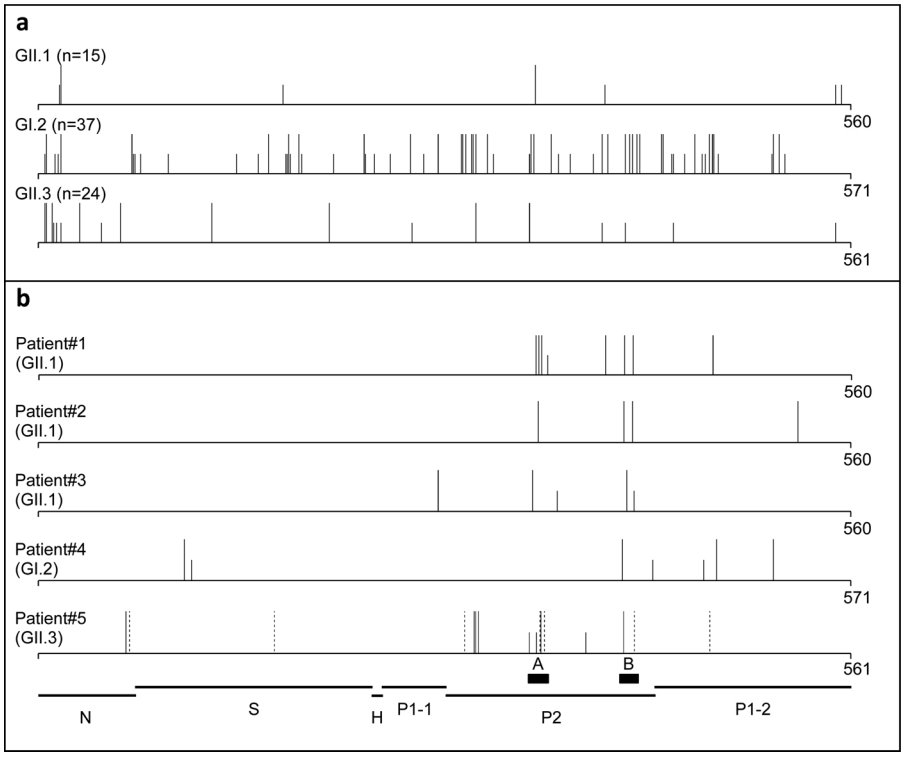

Fig. 3. Heterogeneity and intrahost amino acid changes within sapovirus (SaV) viral protein 1 (VP1). The eight charts depict the complete SaV VP1. The VP1 subdomains (N, N-terminal arm; S, shell; P, protruding domain; P1-1, N-terminal P1; P1-2, C-terminal P1) are indicated at the bottom. The total number of VP1 residues is indicated at the right. Fig. 3a shows VP1 amino acid heterogeneity assessed by comparison of all complete VP1 sequences of GII.1, GI.2 and GII.3 SaV from GenBank. The number of compared strains per chart is indicated at the left. Each vertical bar indicates amino acid polymorphism at the respective VP1 site. Short vertical bars indicate that the residue was conserved in all but one strain. In Fig. 3b vertical bars indicate intrahost amino acid changes during chronic SaV infection. Amino acid changes were assessed by comparison of the earliest and most recent complete SaV VP1 sequence of the patients (GenBank accessions MH541021 and -29, MH541030 and -32, MH541033 and -38, MH541039 and -24, MH541043 and -48). Short vertical bars refer non-fixed amino acid changes during the observed intrahost evolution. In patient #5 dashed lines indicate seven putative preceding amino acid changes, which may have occurred during chronic infection but prior to the observation period: The seven residues (Val64, Val164, Val295, Asp347, Gly350, Val419, Tyr464) of Hu/DE/2010/GII.3/LeipzigP5-A and -F differ from all complete GII.3 VP1 sequences available at GenBank as well as from closely related SaV acute diarrhea strains Hu/DE/2009/GII.3/LeipzigAD1 and Hu/DE/2010/GII.3/LeipzigAD2 (GenBank accessions MH763826 and MH763827) (Fig. S1). Two putative hotspots of P2 amino acid changes in GII strains, A and B, are marked by black cuboids.

GII.1 sequence data and both mutated SaV were subsequently cleared by the patients. We therefore hypothesize that the altered sites may be under immune-driven selective pressure and associated with viral fitness. Mapping of varied sites onto a VP1 three-dimensional crystal structure should provide more insight into the potential role of the mutations in capsid stability, viral antigenicity and receptor usage [38]. Yet, although an intermediate resolution structure of SaV VP1 has been assessed, it is not available in the database [3]. Thus, efforts to predict the structure of SaV VP1 by homology modelling based on feline calicivirus or vesivirus VP1 were made in the past [10,39]. Amino acid identities between different caliciviruses are however low within the most antigenic and most host interacting P2 subdomain [33]. Thus, homology modelling of SaV to other caliciviruses would be considerably erroneous in P2 and was not performed in this paper.

Most of the present assumptions about the impact of mutations are based on data of related noroviruses, and may well be inaccurate for SaV. Another important limitation is the restricted number and heterogeneity of analyzed chronic diarrhea cases. Amino acid mutations were observed and described, but statistical analysis of their significance was not possible. Future studies should be able to identify and investigate more cases, if a targeted SaV screening of immunocompromised individuals with chronic diarrhea is performed.

Taken together, clinicians should consider immunocompromised individuals at risk to develop chronic diarrhea due to persistence of SaV infection. The identified intrahost VP1 mutations may contribute to an understanding of sapovirus-host interactions. For further conclusions regarding virus immune escape and altered viral fitness structural data on SaV capsid structure and virus/receptor complex are necessary.

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Competing interests

The authors declare that they have no competing interests.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Leipzig University (AZ 298/16-ek).

Author contributions

CP and UGL designed the study. CP carried out the laboratory experiments and performed the data analysis. CP drafted the manuscript, UGL revised it. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.02.001.

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