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Research Article

First detection of cutavirus DNA in stools of patients with rheumatic diseases in Guangzhou, China

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ABSTRACT

Cutavirus (CuV) is a novel *protoparvovirus* possibly associated with diarrhea and cutaneous T-cell lymphomas. Patients with rheumatic disease are immunosuppressed and may be more vulnerable to pathogenic viruses. A descriptive study was conducted among hospitalized patients with rheumatic diseases and individuals undergoing medical health check-ups between June 2019 and June 2022 in Guangzhou, China. Stool samples of subjects were tested for CuV DNA. Demographic and fecal examination data of patients were obtained from electronic medical records. A total of 505 patients with rheumatic diseases and 244 individuals who underwent medical health check-ups were included in the study. Of the patients with rheumatic disease, 5.74% [95% confidence interval (CI): 4.03%–8.12%] were positive for CuV DNA, while no individual in the medical health check-up group was positive, indicating a close correlation between CuV and rheumatic disease. Men and patients with rheumatoid arthritis or ankylosing spondylitis, according to the disease classification, were more susceptible to being infected with CuV ($P < 0.01$). After adjustments, being male remained the only significant factor, with an adjusted odd ratio (OR) of 4.4 (95% CI: 1.7–11.4, $P = 0.002$). Phylogenetic analysis of the CuV *VP2* sequences showed three diverse clades, one of which was segregated to be a single branching independent of previously known sequences, which is possible a new genotype.

1. Introduction

Cutavirus (CuV) is an envelope-free single-stranded DNA virus that belongs to the genus *Protoparvovirus* of *Parvoviridae* (Cotmore et al., 2014; Söderlund-Venermo, 2019). The genus also includes several viruses associated with human and animal diseases, such as human bocavirus, canine parvovirus, feline parvovirus, porcine parvovirus, and minute virus of mice (Mäntylä et al., 2017; Väisänen et al., 2017). The CuV genome sequence includes two major open reading frames encoding non-structural and structural proteins. The *NS1* region, which encodes a non-structural protein (659 aa), displays resolvase activity and contains the characteristic nucleotriphosphate binding domains. The *VP1* region and *VP2* region encode structural proteins (707 aa and 569 aa), encoded as overlapping genes with different start codons and the same stop codon. There is also a smaller *ORF* located between the *NS1* and *VP1* regions, which encodes proteins of unknown function (Phan et al., 2016; Phan and Nagaro, 2020).

Cutavirus first gained attention in 2016 when it was identified in five fecal specimens using viral metagenomics: CuV DNA was found in 1.6% (4/245) and 1% (1/100) of fecal samples from children with diarrhea in Brazil and Botswana, respectively (Phan et al., 2016). The emerging virus was subsequently named after a sequence match found in a 2013 skin sample from a French patient with cutaneous T-cell lymphoma (CTCL) (Phan et al., 2016). The viral DNA was then found in patients with malignant melanoma, CTCL, and human immunodeficiency virus (HIV) infection (Mietzsch et al., 2019; Väisänen et al., 2019; Wieland et al., 2019; Phan and Nagaro, 2020). Väisänen et al. conducted a retrospective case-control study and found CuV DNA in skin biopsies of 4/25 (16.0%) of CTCL and 4/136 (2.9%) of transplant patients (Väisänen et al., 2019). However, no CuV DNA was detected in the 159 skin samples of 98

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healthy adults. In addition, CuV DNA has been detected in plasma samples in adult allogeneic hematopoietic stem cell transplantation recipients (Zanella et al., 2021). Moreover, anti-CuV serum antibodies were found in CTCL patients, immunosuppressed solid-organ transplant recipients, and leukemia patients (Väisänen et al., 2019; Mohanraj et al., 2021). The high detection rate of serum IgG antibodies in immunocompromised people suggests that they might be more susceptible to CuV than the general population.

Rheumatic diseases exhibit a broad spectrum of immune system malfunction, including inflammatory diseases such as rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, and systemic vasculitis such as giant cell arteritis (Galmiche et al., 2022). The prevalence of rheumatoid arthritis in developed countries is approximately 0.5%–1% of the adult population, but its incidence (at least among women) seems to be rising in recent years (Smolen et al., 2016; Otón and Carmona, 2019). Ankylosing spondylitis typically presents in patient's twenties, and it is more common in males, with the prevalence ranging from 0.1% to 1.4%. The high prevalence of ankylosing spondylitis in young populations will lead to substantial individual and societal cost (Braun and Sieper, 2007; Crossfield et al., 2021). Rheumatic diseases are triggered by both cell- and antibody-mediated processes and are associated with concurrent immunodeficiency.

According to previous studies, patients with rheumatic diseases can be considered as an immunosuppressed population (Goldman et al., 2021; Galmiche et al., 2022). We hypothesized that CuV infection might occur in individuals with rheumatic disease. Due to the complexity of rheumatic disease, including the uncertainties regarding its precise etiological trigger, and the lack of similar investigations in the Chinese population, we conducted this study to verify the hypothesis.

2. Materials and methods

2.1. Study participants and demographic data collection

The ethics committee of Southern Medical University approved the study protocol (2019001). We investigated patients hospitalized with rheumatic disease and individuals who presented for medical health check-up (MHC) at the Third Affiliated Hospital of Southern Medical University in Guangzhou, China, between June 2019 and June 2022 (Fig. 1). Rheumatic disease was diagnosed according to the American College of Rheumatology classification criteria (Hochberg, 1997).

Patients with rheumatic disease who had other infectious disease, such as respiratory tract infection, urinary tract infection, and tuberculosis infection, and those with incomplete clinical data were excluded. The MHC population consisted of individuals who underwent MHC at the target hospital without a history of rheumatism. The MHC population was selected by frequency matching for age and gender.

The rheumatic disease group includes 505 patients (aged 31–62 years, median age 49 years), with a female-to-male ratio of 1.5:1. The MHC group included 244 individuals (aged 31–60 years, median age 46 years), with a female-to-male ratio of 1.3:1. The demographic information of inpatients with rheumatic disease was consecutively collected, which included age, gender, and disease types.

2.2. Fecal sample collection and processing

Briefly, 2 g or 2 mL of fresh stools were collected, placed in collection tubes, and numbered sequentially. The specimens were processed into a

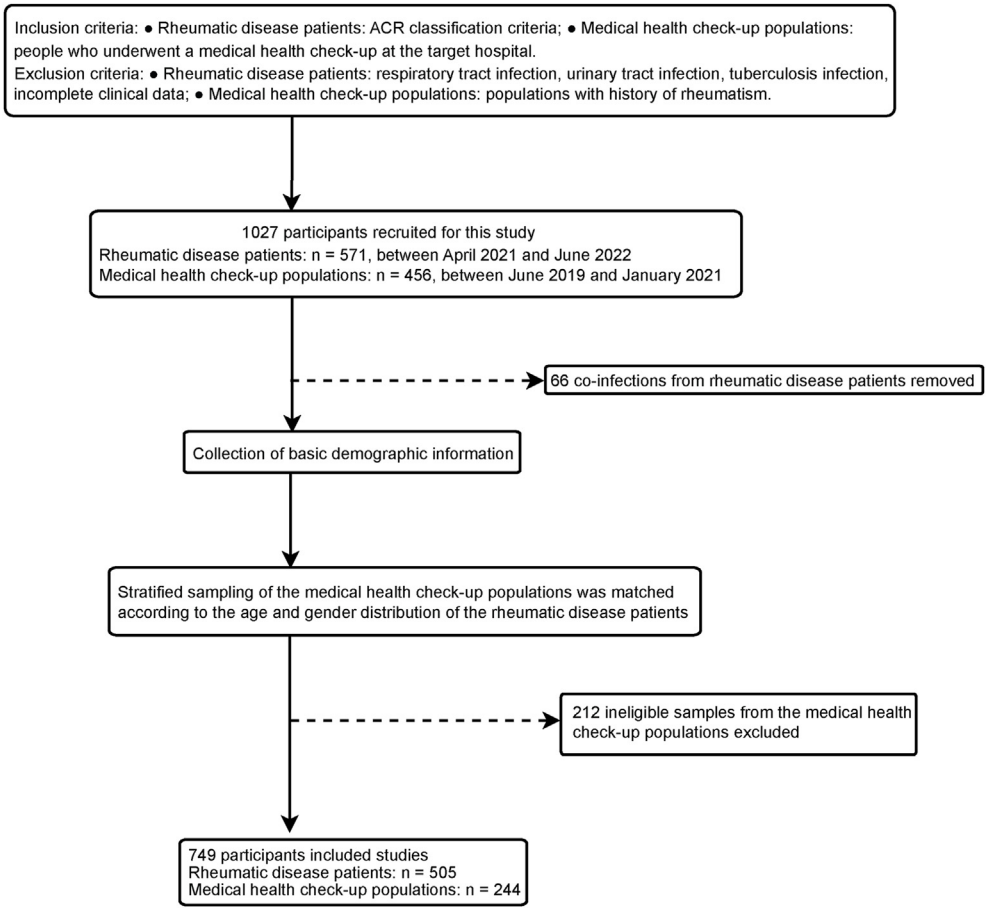

Fig. 1. Flow chart of study participant selection. Co-infections mainly refer to the diagnosed digestive system infection by enteric-associated viruses and hepatitis B virus.

20% stool suspension by adding 1 mL of phosphate buffer saline and shaking vigorously for 10 min. The collected stools were delivered to the laboratory within 12 h, stored temporarily at 4 °C, and kept in a refrigerator at −80 °C for long-term storage. Fecal analysis data were also obtained, including results of fecal features [classified using the Bristol Stool Scale (Lewis and Heaton, 1997), fecal occult blood, transferrin, microscopic fungi, and microscopic fat droplets].

2.3. Screening of the VP2 partial gene region

Nested polymerase chain reaction (nested PCR) was performed using the GoTaq Green Master Mix amplification kit (Promega, USA). Regarding the amplification reaction, the outer forward primer was 5′-$_{3361}$CAAACTACCAACTTACTGCTACCA$_{3384}$-3′, and the outer reversed primer was 5′-$_{3834}$GTTAGTCTGGTTCCTTCAGTTG$_{3858}$-3′; the inner forward primer was 5′-$_{3397}$GAATACAATAGACATAAACCAAGCAGAC$_{3424}$-3′, and the inner reversed primer was 5′-$_{3801}$TGCTTGTGAAAATGAACTGCCTG$_{3823}$-3′. The amplification conditions were as follows: denaturation at 95 °C for 5 min, 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min, 35 cycles, and extension at 72 °C for 10 min (Phan et al., 2016). PCR products were identified using 1% agarose gel electrophoresis, and the positive samples were sent for sequencing. Then, the sequencing results were compared and analyzed in the National Center for Biotechnology Information (NCBI) database.

2.4. Genome sequencing and phylogenetic analysis

Ten fragments covering the near-full-length genome of CuV were amplified with nested PCR using specific primers (Supplementary Table S1) from the CuV isolate BR-283 (GenBank accession number KT868810). After purification, PCR fragments were sequenced using the ABI PRISM 3730XL DNA Analyzer (Applied Biosystems). Multiple sequence alignment was performed using Clustal W of Molecular Evolutionary Genetics Analysis (MEGA, version 10.0, Mega Limited, Auckland, New Zealand). All of CuV sequences in NCBI database were included in phylogenetic analysis. The phylogenetic trees for the *VP2* gene and near-complete sequences of CuV were constructed using the neighbor-joining tree method with 1000 bootstrap replicates to assess the robustness of individual nodes of each tree. Bootstrap percentages indicate the reliability of the cluster descending from that node, and the higher the number, the more reliable the estimate of the taxa that descend from that node (Hall, 2013). Generally, nodes with < 70% reliability are ignored.

2.5. Statistical analysis

The Statistical Package for Social Sciences 26.0 software (SPSS 26.0, IBM Corporation, Armonk, NY, USA) was used for data analysis to calculate odds ratios (OR), 95% confidence intervals (CI), and Cochran and Mantel-Haenszel statistics and to perform the Mann-Whitney *U* test, Fisher's exact test, and binary logistic regression analysis, as appropriate. A *P* value < 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of rheumatic diseases patients and the MHC population

A total of 749 participants were enrolled in the study (Fig. 1). Of these, 505 participants were in the rheumatic disease group, while 244 individuals were in the MHC group. No statistically significant difference in median age ($P = 0.574$) was found between the two groups (rheumatic disease: median age 49 years, range 31–62; MHC: 46 years, range 31–60, respectively) (Table 1). The female-to-male ratio in the rheumatic disease and MHC groups were 1.5:1 and 1.3:1, respectively. Similarly, there was no statistically significant difference in gender between both groups ($P = 0.560$) (Table 1).

CuV DNA was found to be more frequent in rheumatic disease patients compared to that in MHC group. The most common rheumatic disease types were rheumatoid arthritis, which accounted for 26.9% (136/505) of the cases, followed by ankylosing spondylitis (21.8%), and systemic lupus erythematosus (13.5%) (Table 1). Possible diarrhea, defined based on fecal characteristics assessed using the Bristol Stool Scale, was significantly more likely to be observed in the rheumatic disease group than in the MHC group (26.1% vs 7.4%, $P < 0.001$). The indicators of lower gastrointestinal bleeding were statistically significantly different between the rheumatic disease and MHC groups ($P < 0.01$, Table 1). Microscopic fungi were more prevalent in the rheumatic disease group compared to the MHC group ($P < 0.05$).

Table 1
Comparison of demographic and laboratory characteristics between rheumatic disease patients and the medical health check-up population.

Basic characteristic	Rheumatic disease patients (n = 505)	Medical health check-up populations (n = 244)	$P_{Z/\chi 2}$ value
Demographic data			
Age (years), median (IQR)	49 (31, 62)	46 (31, 60)	0.574
Age groups, n (%)			0.738
≤ 60	373 (73.9)	183 (75.0)	
> 60	132 (26.1)	61 (25.0)	
Sex, n (%)			0.560
Male	206 (40.8)	105 (43.0)	
Female	299 (59.2)	139 (57.0)	
Disease types, n (%)			NA
Rheumatoid arthritis	136 (26.9)	NA	
Ankylosing Spondylitis	110 (21.8)	NA	
Systemic Lupus Erythematosus	68 (13.5)	NA	
Gouty arthritis	40 (7.9)	NA	
Connective tissue diseases	39 (7.7)	NA	
Osteoarthritis	42 (8.3)	NA	
Sjogren syndrome	7 (1.4)	NA	
Juvenile idiopathic arthritis	9 (1.8)	NA	
Dermatomyositis	5 (1.0)	NA	
Scleroderma	8 (1.6)	NA	
Arthritis	5 (1.0)	NA	
Vasculitis	14 (2.8)	NA	
Psoriasis	4 (0.8)	NA	
Others	18 (3.6)	NA	
Fecal examination data			
Fecal characteristics, n (%)			< 0.001
Normal	373 (73.9)	226 (92.6)	
Possible diarrhea	132 (26.1)	18 (7.4)	
Fecal occult blood test, n (%)			< 0.001
Positive	48 (9.5)	2 (0.8)	
Negative	457 (90.5)	242 (99.2)	
Fecal transferrin test, n (%)			0.002
Positive	24 (4.8)	1 (0.4)	
Negative	481 (95.2)	243 (99.6)	
Microscopic fungi, n (%)			0.046
Yes	11 (2.2)	0	
No	494 (97.8)	244 (100.0)	
Microscopic fat droplets, n (%)			0.110
Yes	8 (1.6)	0	
No	497 (98.4)	244 (100.0)	

Statistical analysis was performed by the Mann-Whitney *U* test or Fisher's exact test. $P < 0.05$ is considered statistically significant.
IQR, interquartile range; NA, not available.

3.2. Prevalence of CuV DNA in patients with rheumatic diseases and MHC population

In total, 29 of 505 (5.74%; 95% CI, 4.03–8.12%) rheumatic disease patients and none of the MHC participants were positive for CuV DNA ($P < 0.001$). There was no significant difference in CuV DNA detection rate between participants ≤ 60 years and those > 60 years [5.90% (22/373) vs 5.30% (7/132), $P > 0.05$, Table 1). The prevalence of CuV DNA in the 21–30 years and 51–60 years age groups was 7.69% (7/91) and 7.55% (8/106), respectively (Fig. 2A). Male patients had a higher positivity (10.19%, 21/206) of CuV DNA than female patients (2.68%, 8/299) ($P < 0.001$) (Table 2, Fig. 2B). CuV was identified throughout the year, especially during spring and autumn, with two peaks in April (11.1%) and November (8.8%), respectively (Fig. 2C). Patients with rheumatoid arthritis accounted for 34.5% (10/29) of the CuV-positive patients with rheumatic diseases, which is comparable to the percentage for those with ankylosing spondylitis (Table 2). Specifically, ignoring disease classifications with small sample sizes, the highest positive rate of CuV DNA was found in patients with ankylosing spondylitis (9.1%) and rheumatoid arthritis (7.4%) (Fig. 2D).

3.3. Comparison of characteristics of CuV DNA-positive and negative patients with rheumatic diseases

CuV-positive patients did not differ significantly from CuV-negative patients by age. There were significant differences between CuV-positive and CuV-negative patients by gender ($P < 0.001$) and disease types ($P = 0.003$) (Table 2). After adjustment for age and disease types, males were significantly more likely to be CuV-positive compared to females (adjusted OR, 4.41; 95% CI, 1.71–11.39; $P = 0.002$) (Table 2). Within the age strata, difference of CuV DNA positivity by gender was only observed in the 61–70 age group ($P = 0.018$, Fig. 3A). Within the disease type strata, only the rheumatoid arthritis group showed a significant difference of CuV DNA positivity by gender ($P < 0.001$, Fig. 3B). These findings suggest that being male could be an important risk factor for CuV infection in patients with rheumatic diseases, especially in patients with rheumatoid arthritis or aged 60–71 years. In addition, there was no significant change in fecal examination findings between CuV DNA-positive and negative patients (including changes in fecal features of inflammation and microscopic pathogen) ($P > 0.05$) (Table 2).

3.4. Phylogenetic characterization of CuV

The amplified *VP2* partial region was highly variable in our study. Remarkably, phylogenetic analysis of all existing sequences of CuV and those most similar to the reference sequences showed that CuV variants consisted of three genetically distinct clusters (Fig. 4A). Three of the *VP2* partial sequences in our study (GZ-F267, GZ-F150, and GZ-F200) clustered closely together with sequence KX685945.1 from melanoma patients in Denmark, with a nucleotide similarity of up to 88.0%. Most *VP2* partial sequences in our study were genetically close to the reference sequences from feces samples collected from population with

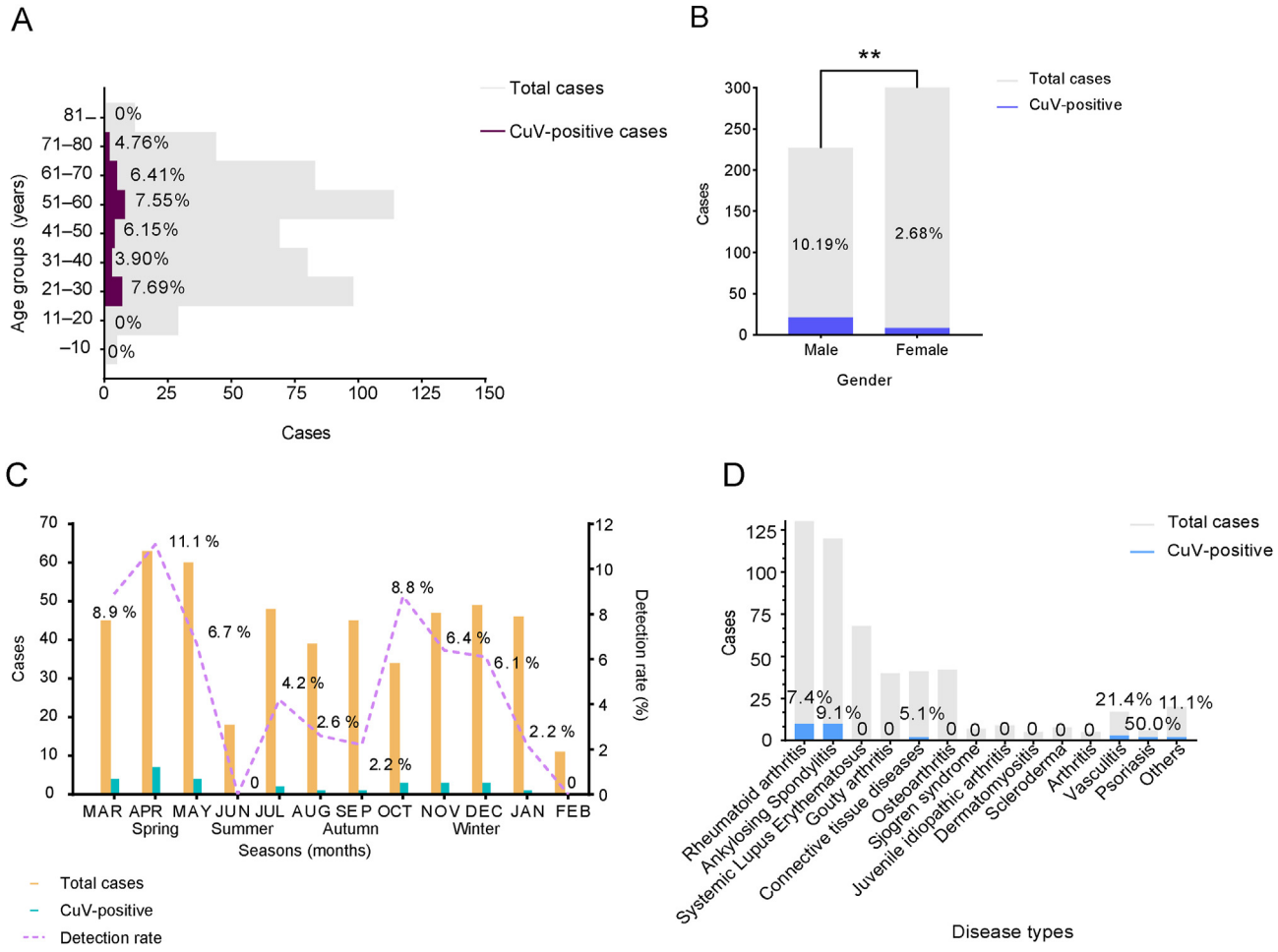

Fig. 2. Distribution of cutavirus (CuV) DNA by age (**A**), gender (**B**), season (**C**) and disease type groups (**D**). Statistical analysis was performed by Fisher's exact test. $^{**}P < 0.01$.

Table 2
Comparisons of the demographic and laboratory characteristics of cutavirus-positive and negative patients.

Basic Characteristic	Rheumatic disease patients (n = 505)	CuV-positive (n = 29)	CuV-negative (n = 476)	$P_{Z/\chi 2}$ value	OR (95% CI)	P_{OR} value
Demographic data						
Age (years), median (IQR)	49 (31, 62)	53 (30, 60)	49 (31, 62)	0.905	0.998 (0.978–1.019)	0.852
Age groups, n (%)				0.801	1.119 (0.467–2.684)	0.801
≤ 60	373 (73.9)	22 (75.9)	351 (73.7)			
> 60	132 (26.1)	7 (24.1)	125 (26.3)			
Sex, n (%)				< 0.001	4.413 (1.709–11.393)[a]	0.002[a]
Male	206 (40.8)	21 (72.4)	185 (38.9)			
Female	299 (59.2)	8 (27.6)	291 (61.1)			
Disease types, n (%)				0.003	NA	0.784
Rheumatoid arthritis	136 (26.9)	10 (34.5)	126 (26.5)			
Ankylosing spondylitis	110 (21.8)	10 (34.5)	100 (21.0)			
Systemic lupus erythematosus	68 (13.5)	0	68 (14.3)			
Gouty arthritis	40 (7.9)	0	40 (8.4)			
Connective tissue diseases	39 (7.7)	2 (6.9)	37 (7.8)			
Osteoarthritis	42 (8.3)	0	42 (8.8)			
Sjogren syndrome	7 (1.4)	0	7 (1.5)			
Juvenile idiopathic arthritis	9 (1.8)	0	9 (1.9)			
Dermatomyositis	5 (1.0)	0	5 (1.1)			
Scleroderma	8 (1.6)	0	8 (1.7)			
Arthritis	5 (1.0)	0	5 (1.1)			
Vasculitis	14 (2.8)	3 (10.3)	11 (2.3)			
Psoriasis	4 (0.8)	2 (6.9)	2 (0.4)			
Others	18 (3.6)	2 (6.9)	16 (3.4)			
Fecal examination data						
Fecal characteristics, n (%)				0.855	0.925 (0.399–2.141)	0.855
Normal	373 (73.9)	21 (72.4)	352 (73.9)			
Possible diarrhea	132 (26.1)	8 (27.6)	124 (26.1)			
Fecal occult blood test, n (%)				0.256	2.098 (0.762–5.778)	0.152
Positive	48 (9.5)	5 (17.2)	43 (9.0)			
Negative	457 (90.5)	24 (82.8)	433 (91.0)			
Fecal transferrin test, n (%)				1.000	0.703 (0.092–5.400)	0.735
Positive	24 (4.8)	1 (3.4)	23 (4.8)			
Negative	481 (95.2)	28 (96.6)	453 (95.2)			
Microscopic fungi, n (%)				0.482	1.664 (0.206–13.465)	0.633
Yes	11 (2.2)	1 (3.4)	10 (2.1)			
No	494 (97.8)	28 (96.6)	466 (97.9)			
Microscopic fat droplets, n (%)				0.379	2.393 (0.284–20.130)	0.422
Yes	8 (1.6)	1 (3.4)	7 (1.5)			
No	497 (98.4)	28 (96.6)	469 (98.5)			

Statistical analysis was performed by binary logistic regression analysis.
$P < 0.05$ is considered statistically significant.
IQR, interquartile range; NA, not available.
[a] The adjusted OR for sex with adjusting for age and disease types.

unexplained diarrhea (Phan et al., 2016). Specifically, one sequence (GZ-F157) identified in this study was segregated into a single clade, which could not be clustered with any of the reference strains.

We amplified the near-full-length sequence from a CuV-positive sample. With a length of 4423 bp, GZ-F264 (GenBank: OQ101998) was the longest near-full-length fragment that could be amplified in our sample. The GZ-F264 obtained from fecal samples of patients with rheumatoid arthritis showed 93.8%–97.4% identity at the nucleotide level and 88.5%–94.6% amino acid similarity with all CuV reference sequences (Supplementary Table S2). The sequence of GZ-264 clustered closely together with NC_039050.1 and KT868811.1 from children with diarrhea (Phan et al., 2016), with similarity up to 100% (Fig. 4B). The results showed that the near-full-length sequence of GZ-F264 was classified in the CuV branch and did not cluster with human bufavirus (BuV) and human tusavirus (TuV) of the same genus.

4. Discussion

In this study, we initially found a relatively high detection rate (5.74%; 95% CI, 4.03%–8.12%) of CuV DNA in stool samples from Chinese patients with rheumatic disease. Although CuV has not been previously detected in feces of rheumatic patients, the positive rate in our research was remarkably higher than that in several populations (Phan et al., 2016; Wieland et al., 2019). The CuV DNA prevalence among patients with diarrhea was 1.6% in Brazil and 1.0% in Botswana (Phan et al., 2016). CuV DNA has also been demonstrated in skin swabs or tumor biopsy tissues from patients with skin malignancies. Patients with melanoma have a 4%–10% detection rate of CuV DNA (Mollerup et al., 2017; Väisänen et al., 2019). CuV DNA was found in 6 of 130 (4.6%) paraffin-embedded skin biopsy specimens of patients with CTCL, all of whom had mycosis fungoides (Kreuter et al., 2018). Additionally, CuV DNA was detected in skin biopsies of 4/25 (16.0%) CTCL patients (Väisänen et al., 2019). CuV DNA was also detected in swab specimens from healthy skin of HIV-positive individuals, with a prevalence of 17.1% (Wieland et al., 2019). Notably, the prevalence of CuV DNA identified using mNGS in highly immunocompromised plasma samples (25 allogeneic hematopoietic stem cell transplantation recipients) was 4% (Zanella et al., 2021). In contrast, skin biopsies from 4/136 (2.9%) transplant patients were found to contain CuV DNA (Väisänen et al., 2019). Moreover, the positive detection rate of serum samples for CuV IgG in transplant recipients was 6.5% (Väisänen et al., 2019). In this study, however, none (0/244) of the MHC population had CuV DNA in their stool. Similarly, CuV DNA was not detected in any of the 159 skin samples of 98 healthy adults (Väisänen et al., 2019) and was absent in all patients with other types of lymphoma (Kreuter et al., 2018). In addition, the IgG seroprevalence of CuV was 9.5% among CTCL patients and 3.8% in healthy adults (Väisänen et al., 2019). Generally, CuV is undetectable in the general population, suggesting that healthy people are less

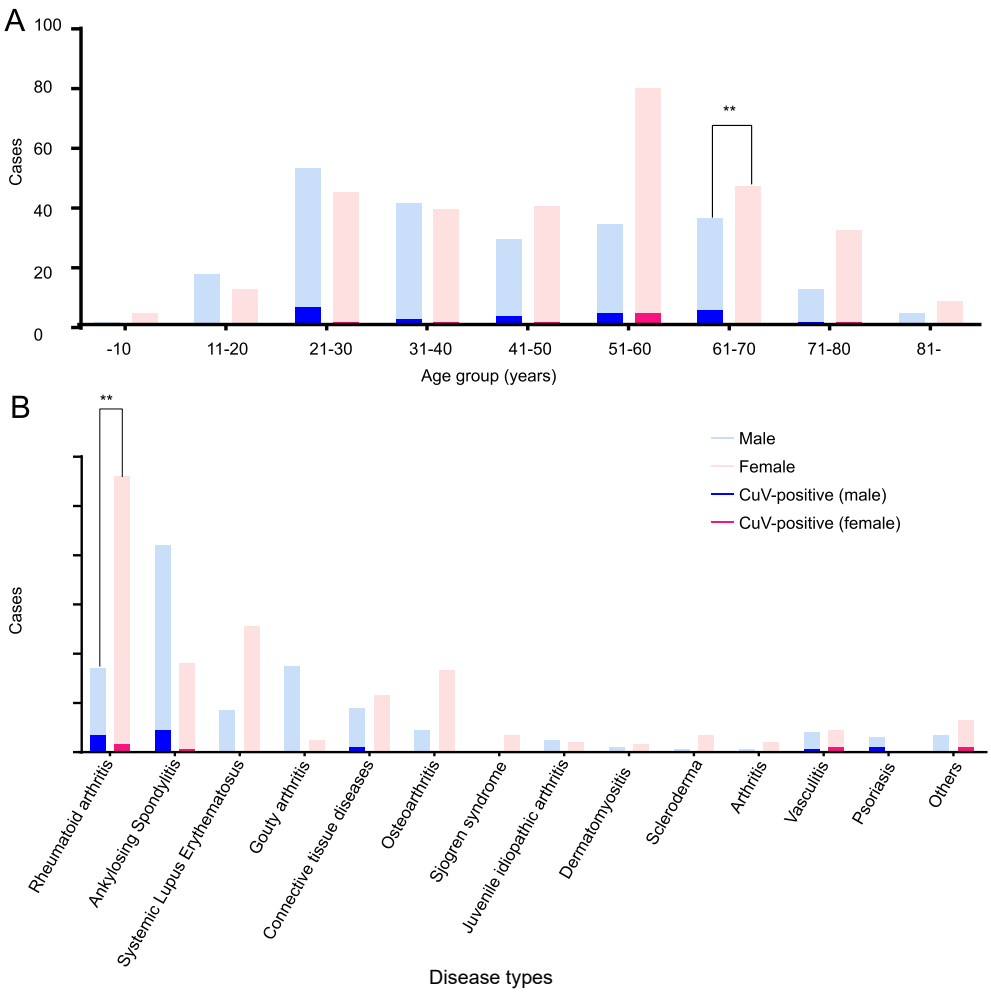

Fig. 3. Differences of CuV DNA positivity between male and female in different age (**A**) and disease type (**B**) groups. Statistical analysis was performed by Fisher's exact test. $^{**}P < 0.01$.

vulnerable to the virus. CuV might be a potentially pathogenic virus, and those with immunosuppressive disorders may be susceptible to it.

A clinical review showed that autoimmune diseases are more prevalent in females than males (3:1) (Favalli et al., 2019). However, we found significant differences between CuV DNA-positive and negative patients in terms of male sex [adjusted OR (95% CI) = 4.41 (1.71–11.39), $P = 0.002$) even after adjustment for age and disease types. This finding is consistent with the previous gender distribution of CuV infection in patients with CTCL (mycosis fungoides) (Kreuter et al., 2018). Likewise, 87.5% of CuV DNA-positive patients were male (Väisänen et al., 2019), revealing that being male might be one of the risk factors for CuV infection. This result is similar to the findings in most epidemiological studies indicating that men are more susceptible to infectious diseases than women (Gay et al., 2021). Most of the patients with rheumatic diseases [73.9% (373/505), Table 1) were younger than 60 years in this study. CuV DNA detection rates were comparable in groups of participants below and above 60 years old [$\leq$ 60 years groups vs $>$ 60 years groups: 5.90% (22/373) vs 5.30% (7/132); $P > 0.05$] (Table 1). In contrast, CuV DNA was significantly less prevalent in gastroenteritis patients below 60 years compared to those older than 60 years (0.2% vs 5.1%, $P < 0.001$) in Finland (Mohanraj et al., 2021). This can be as a result of the samples coming from various populations.

There is no relevant literature report on the seasonal distribution of CuV. In this study, CuV DNA was identified throughout the year, with two peaks in spring and autumn. Despite the fact that the seasonal distribution of CuV infection is not yet understood, findings in this study may offer some insight into virus epidemiology.

The prevalence of CuV DNA was predominant in patients with rheumatoid arthritis (7.4%) and ankylosing spondylitis (9.1%). Significant differences in CuV DNA positivity between disease types were observed in univariate analysis ($P = 0.003$) but not in multivariate analysis, suggesting that patients with rheumatoid arthritis and ankylosing spondylitis, like other immunocompromised (Goldman et al., 2021) populations, were more vulnerable to weakly pathogenic or commensal viruses.

Concerning the trends in CuV DNA positivity in different age groups for both rheumatoid arthritis and ankylosing spondylitis disease types, the prevalence of viral DNA did not increase with age and remained prevalent in the 21–30 years and the 51–70 years age groups (Supplementary Figs. S1A and S1B). There was no correlation between CuV DNA detection rate and age in rheumatic patients during our observation period. Although the CuV DNA positivity did not increase with age in this study, the prevalence of CuV infection in older patients still requires further study. Meanwhile, whether CuV affects acute attacks of rheumatic diseases such as group A streptococcus remains to be further investigated (Chakravarty et al., 2014). In addition, CuV was found in patients with severe plaque psoriasis (2/4) and vasculitis (3/14). By contrast, a study showed that skin samples from parapsoriasis lesions tested negative for CuV DNA (Phan et al., 2016). This discrepancy might be due to the small size of patients with these diseases. Wolfe et al. (Wolfe

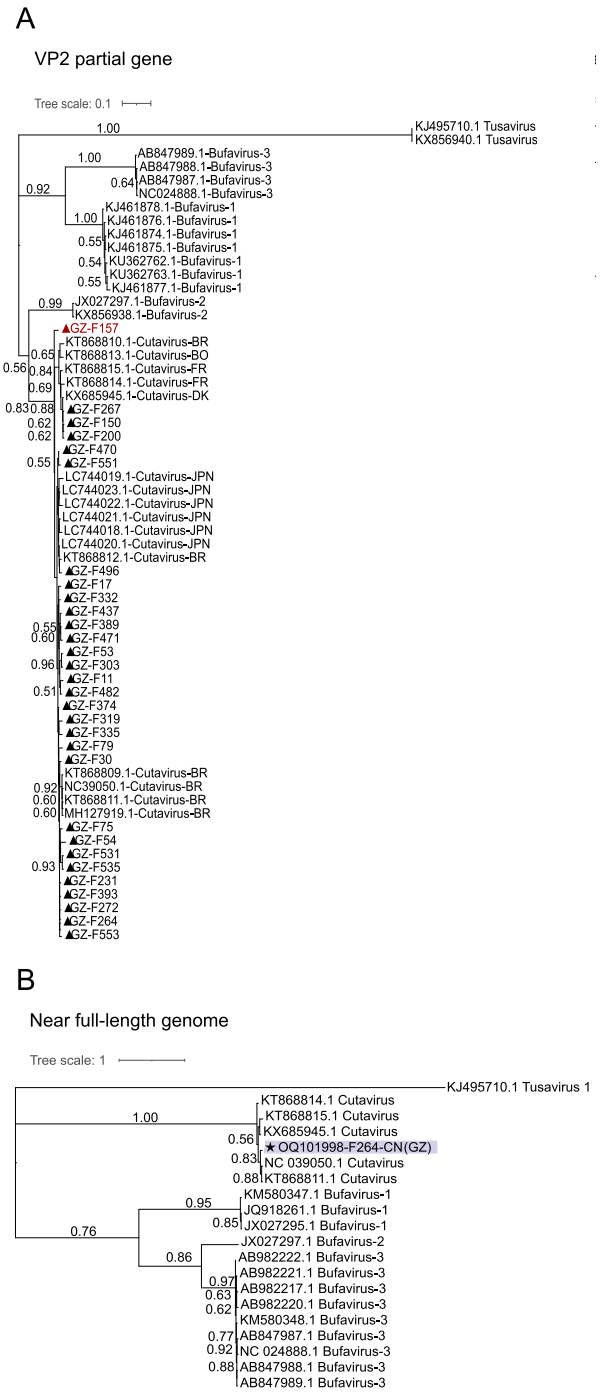

Fig. 4. Phylogenetic analyses of CuV VP2 partial gene regions (427bp) (**A**) and near full-length genome (**B**) from rheumatic diseases patients. **A** GenBank no., species, and sample types (only in CuV clusters) are indicated. The solid triangles are the CuV sequences detected in this study; the sequence in red (GZ-F157) is the independent phylogenetic sequence found in this study. The neighbor-joining phylogenetic tree was generated with bootstrap values determined by 1000 replicates in the MEGA (Version 10.0). Bootstrap values are shown if > 50%. VP2, viral capsid protein 2. **B** GenBank no., species and country are indicated. The neighbor-joining phylogenetic tree was generated with bootstrap values determined by 1000 replicates. Bootstrap values are shown if > 50%.

and Michaud, 2007) identified an association between rheumatoid arthritis biologic treatment and an increased risk of non-melanoma skin cancer and melanoma. Moreover, vasculitis was more commonly reported in patients with melanoma (Salem et al., 2018). Biologic therapies for common inflammatory diseases, such as psoriasis and rheumatoid arthritis, can lead to significant increases in melanoma risk (Esse et al., 2020). Further studies on molecular mechanisms are required to determine whether a high detection rate of CuV DNA in rheumatic disease patients denotes a causative pathway from rheumatic disease to cancer.

All CuV-related sequences in the NCBI library were included in this analysis. However, the partial sequences in the *VP2* region were split into three major clusters in this study, possibly related to their highly variable and short fragments. The viral sequences in this study were mainly clustered into the skin melanoma reference strain (Mollerup et al., 2017) and the Brazilian diarrhea reference strain (Phan et al., 2016). Melanocytes are also present in small numbers in the enteric epithelium, where melanomas can occur, although rarely (Li et al., 2014). Therefore, the cell tropism and potential chain of dissemination of CuV remain to be investigated.

There were several limitations in this study. First, based on the results, we could not determine whether these patients suffered from an acute or persistent CuV infection. Second, we only tested for CuV nucleic acids in stools. However, the presence of viral nucleic acids in the stools does not indicate acute infection. Whether CuV, like norovirus or rotavirus, can be detected in stool as an indication of infection is unclear. Additionally, we could not completely collect specific clinical examination results of autoimmune patients, such as the levels of C-reactive protein and TNF-alpha. Prospective studies with large sample sizes are needed in future.

5. Conclusions

This study provides evidence of prevalence of CuV DNA in stool samples of patients with rheumatic disease in Chinese. Men may be more susceptible to CuV infection than women. Phylogenetic analysis showed that the VP2 partial region of CuV variants consisted of three genetically distinct clusters, one of which clustered independently and is potentially a new genotype. The significance of CuV infection in rheumatic disease patients is worth studying further.

Data availability

The original data presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding authors. The full-length sequences appearing in the paper are available in the NCBI database with GenBank number OQ101998, and the Genbank number of *VP2* partial region sequences is from OR536283 to OR536310.

Ethics statement

The study is approved by the Ethics Committee of Southern Medical University (2019001). Informed consent has been obtained from each participant. Our research data is completely anonymous and does not involve patient privacy.

Author contributions

Yongzhi Li: methodology, investigation and writing-original draft. Liting Zheng: investigation, validation and formal analysis. Huan He: investigation and data curation. Hushen Xiong: investigation and data curation. Jiaqi Chen: writing reviewing. Hengbiao Sun: sample collection and editing. Juxian Xian: sample collection. Caiyun Chen: sample collection. Minyi Liang: sample collection. Qiushuang Li: investigation. Jiaqi Fu: investigation. Fei Wu: methodology and supervision. Yuhan Gao: reviewing and editing. Gang Xiao: project administration and conceptualization. Qing Chen: funding acquisition, supervision, reviewing, and editing.

Conflict of interest

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2023.10.006.

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