SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2025; 42 (2): 16324 DOI: 10.36141/svdld.v42i2.16324

Angiotensin-converting enzyme genotypes and sarcoidosis: Correlation with susceptibility, progression and treatment response

Janne Møller[1], Ole Hilberg[2], Peter Henrik Nissen[3,4], Holger Jon Møller[3,4], Elisabeth Bendstrup[1]

[1]Centre for Rare Lung Diseases, Department of Respiratory Diseases and Allergy, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Medicine, Sygehus Lillebaelt Hospital, University of Southern Denmark, Vejle, Denmark; [3]Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark; [4] Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Abstract. *Background and aim:* Previous research have yielded conflicting results regarding the association between *ACE* genotypes and both susceptibility to sarcoidosis and disease prognosis. While some studies have found no significant impact of the *ACE* genotype on sarcoidosis susceptibility, others have suggested that the DD genotype may be associated with an increased risk of developing the disease and a poor prognosis. This study aimed to evaluate the influence of *ACE* genotypes on the susceptibility to sarcoidosis, prognosis and treatment response in patients with pulmonary sarcoidosis in Denmark. *Methods*: Patients with sarcoidosis were consecutively enrolled and genotyped using allele-specific PCR and high-resolution melting methods. The distribution of ACE genotypes was then compared with that of 400 healthy Danish individuals. To assess the impact of ACE genotypes on sarcoidosis prognosis, their association with changes in pulmonary function tests, radiological staging over 24 months, the frequency of Löfgren's syndrome, and treatment response were analyzed. *Results:* Among 148 patients with sarcoidosis, the frequency of the II, ID, and DD genotypes was 25%, 52%, and 23%, respectively. No significant difference was observed in the distribution of ACE genotypes between patients and controls. Prognostic factors and treatment response also did not vary among the genotypes. The distribution of genotypes did not differ in patients with Löfgren's syndrome compared to non-Löfgren's syndrome. *Conclusions*: Our findings do not support an association between the ACE I/D genotypes and sarcoidosis susceptibility, prognosis or response to treatment in a Danish cohort.

Key words: sarcoidosis, angiotensin-converting enzyme, gene polymorphism, prognosis, treatment outcome

Introduction

Sarcoidosis is a systemic granulomatous disease of unknown origin, frequently affecting the thoracic lymph nodes and lungs. The phenotypic presentation of sarcoidosis varies significantly among ethnic groups with evidence suggesting a multifactorial origin involving genetic, environmental, and immunological factors. Among the genetic factors, the angiotensin-converting enzyme (*ACE*) gene has garnered attention due to its role in the renin-angiotensin system, which regulates blood pressure and fluid balance, and its involvement in inflammatory responses. The *ACE* gene contains an insertion (I)/deletion (D) polymorphism in intron 16, resulting in three possible genotypes: II, ID, and DD (1). This polymorphism has been linked to various cardiovascular and inflammatory diseases, including sarcoidosis (2,3). Diagnosing sarcoidosis and predicting disease course is challenging and remains a puzzle requiring the integration of pieces from clinical manifestations, imaging, blood samples and histopathology. Measurement of serum

Received: 20 August 2024
Accepted: 18 October 2024
Correspondence: Janne Møller, MD
Department of Respiratory Diseases and Allergy
Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200 Aarhus N, Denmark
E-mail: jannmoel@rm.dk
ORCID: 0000-0003-1839-3669

angiotensin-converting enzyme activity (s-ACE) is often included in diagnostic and follow-up and is thought to reflect the burden of granulomas (4). The utility of s-ACE is however debated as both sensitivity and specificity for sarcoidosis are low and the normal level of s-ACE depends on variation in the *ACE* gene. Serum ACE activity is influenced by the *ACE* gene I/D polymorphism and it has been demonstrated that genotyping increases the diagnostic value in sarcoidosis (5) though not routinely implemented in clinical practice. Existing literature presents conflicting evidence regarding the association between *ACE* genotypes and sarcoidosis. Some studies report no significant influence of the *ACE* genotype on the susceptibility to sarcoidosis, while others suggest that the DD genotype is associated with an increased risk of developing the disease. A meta-analysis of 18 studies concluded that the *ACE* DD genotype correlated with a higher risk of sarcoidosis among Caucasians, highlighting the need for further research in diverse populations (3). Controversy persists as to whether there is an association between the *ACE* genotypes and sarcoidosis prognosis and their role in response to treatment is not known. Löfgren's syndrome (LS) is a common presentation of sarcoidosis in Scandinavia (6,7) and the genetic architecture of LS patients differs from that of non-LS patients (8) therefore an association to *ACE* genotypes to LS could be hypothesised. Previously a high-resolution melting method for the detection of the I/D polymorphism of the *ACE* gene have been developed and 400 healthy Danish individuals been genotyped (9) but the relation to sarcoidosis risk and prognosis in Danish patients have not been investigated. This study aimed to evaluate the influence of *ACE* genotypes on susceptibility to sarcoidosis, disease progression and treatment response in patients with pulmonary sarcoidosis in Denmark. Additionally, it sought to investigate if *ACE* genotypes differed in patients with LS and non-LS.

Methods

Study population

The first part of the study was a case control study and the second part a prospective observational cohort study. From December 2019 to December 2021 cases were recruited consecutively from the outpatient clinic, Aarhus University Hospital, Denmark. Adult patients with a diagnosis of pulmonary sarcoidosis based on the most recent ATS diagnostic criteria (10) able to read and understand Danish, were eligible for inclusion. Participants signed written informed consent. Data on demographics, organ manifestations, pulmonary function tests, Scadding stages and treatment were retrieved from the ProSar registry (11).

Outcomes and analysis

For *ACE* genotyping, EDTA anticoagulated blood samples were obtained at inclusion and kept at -80 °C until all samples were processed in batch. DNA was extracted using the DSP mini kit on the QiaSymphony SP platform (Qiagen, Hvidovre, Denmark). Genotyping was performed using allele specific PCR and high-resolution melting on a cobas z480 real time PCR system (Roche, Copenhagen, Denmark) mainly as previously reported (9). The distribution of the *ACE* genotypes II, ID and DD in patients with sarcoidosis was compared with the distribution in 400 healthy Danish individuals (9). To evaluate prognosis in patients with pulmonary sarcoidosis, we selected three parameters: changes in pulmonary function tests over 24 months, change in chest X ray Scadding stage (0-IV) over 24 months and the frequency of patients with LS at diagnosis as this syndrome is known to predict a favorable prognosis (12). LS included patients with acute presentation of bilateral hilar lymphadenopathy, erythema nodosum and/or bilateral ankle arthritis or distinct periarticular inflammation, and sometimes fever (12). Patients whose radiological findings improved (i.e., a change from a higher to a lower Scadding stage) were classified as "improved," while those who progressed (i.e., a change from a lower to a higher stage) within 24 months were classified as "progressed." Patients who showed no change in stage were considered "stable."

Patients treated with immunosuppressive drugs for sarcoidosis were categorized as having a response to treatment if they met one or more of the following criteria:

1. Change in Scadding stage from a higher to a lower number.
2. Improvement in respiratory symptoms, defined by a change in the MRC (Medical Research Council) dyspnea scale from a higher to a lower number or cessation of cough.

3. Improvement in lung function, defined as an increase in FVC (Forced Vital Capacity) of 5% predicted or an increase in DLCO (Diffusing Capacity of the Lung for Carbon Monoxide) of 10% predicted.

Treatment response was evaluated at the first follow-up visit after treatment initiation, which took place either 12- or 24-months post-diagnosis.

Statistical analysis

Continuous data are presented as means or medians with 95% confidence intervals (95% CI). Normal distribution of data was assessed using Q-Q plots. Categorical variables are presented as percentage of the total population. To test the hypothesis of no difference between two groups, the $\chi 2$ test was used. A one-way ANOVA was conducted to determine if parameters differed between genotypes. A p value < 0.05 was considered significant. Analyses were conducted using the Stata/BE 17.0 for Mac statistical software package. The study was approved by the Central Denmark Region Data Protection Agency (case number: 1-16-02-90-19) and acknowledged by the Central Denmark Region Committee on Health Research Ethics. The study was registered on ClinicalTrials.gov (NCT04462068).

Table 1. Baseline parameters for all patients and for incident and prevalent patients individually.

	Patients *n*=148
Age	47.3 (45.0; 49.6)
Male gender	88 (59%)
Ethnicity, Caucasian	100%
Never smokers **Former smokers** **Current smokers** **N/A**	95 (64%) 42 (28%) 8 (5%) 3 (2%)
Time since diagnosis, days	300 (245; 354)
FEV1 % predicted **FVC % predicted** **DLco % predicted (*n*=125)**	92 (89; 95) 101 (98;103) 84 (81; 87)
Scadding stage, Chest X-ray (*n*=143) **0** **I** **II** **III** **IV**	 27 (19%) 74 (52%) 32 (22%) 7 (5%) 3 (2%)
Löfgren's Syndrome	35 (24%)
Extrapulmonary manifestations	42 (28%)
Treatment ongoing or initiated **Glucocorticoid (n = 47)/** **Methotrexate: (n = 4)**	48 (32%)

Numeric variables are listed as number of patients and percentage (). Continuous variables are reported as means with 95% CI (), otherwise specified. Abbreviations: N/A: no answer, BMI: Body mass index. FEV1: Forced expiratory volume. FVC Functional vital capacity. TLC: Total lung capacity. DLco: Diffusion capacity of the lungs for carbon monoxid.

Results

A total of 163 patients met the inclusion criteria and 152 patients accepted participation. Two patients were excluded as their diagnosis was later changed, and two were excluded as ACE genotyping was not performed. *ACE* genotype was measured in 148 eligible patients.

The demographics of the patients at diagnosis are shown in Table 1. Extensive baseline characteristics of the cohort have previously been published (7).

ACE genotype frequency

The distribution of *ACE* genotypes and allele frequencies in patients with sarcoidosis (n=148) are shown in Table 2. The ID genotype was the most prevalent in both groups. For patients with sarcoidosis, the second most frequent genotype was II (ID > II > DD), whereas in the control group, it was DD (ID > DD > II). No significant difference in the distribution of *ACE* genotypes was observed between patients and controls. The frequency of the I allele was higher than that of the D allele in patients with sarcoidosis while in control subjects, the D allele was slightly more prevalent. This difference was minimal and not statistically significant (p = 0.5412). Overall, there was no significant difference in allele frequencies between patients with sarcoidosis and the control group.

ACE genotype and prognosis

The frequencies of prognostic factors in the different genotypes are listed in Table 3. The frequencies of Löfgren's syndrome in genotypes were (DD: ID: II) 0.26: 0.27: 0.14 and did not differ significantly. The frequency of radiological progression was higher in the DD and II groups than in ID though no significant difference was found. Overall, PFT

Table 2. ACE genotype and allele frequencies in sarcoidosis patients and controls.

	Patients (n=148)		Controls (n=400)			
	n	Frequency	n	Frequency	Odds ratio (95% CI)	p-value
Genotypes						
II	37	0.25	90	0.23	1.15 (0.72; 1.81)	0.5380
ID	77	0.52	205	0.51	1.03 (0.69; 1.53)	0.8716
DD	34	0.23	105	0.26	0.84 (0.52; 1.33)	0.4337
Alleles						
I	151	0.51	385	0.48		0.3957
D	145	0.49	415	0.52		

Table 3. *ACE* genotypes prognosis and treatment response.

	Total	DD	ID	II	Anova	P val
Frequency of Löfgren's syndrome	0.24 (n=148)	0.26 (n=34)	0.27 (n=77)	0.14 (n=37)	F(2,145) = 1.41	0.2485
Frequency of RP	0.12 (n=127)	0.16 (n=31)	0.09 (n=70)	0.15 (n=26)	F(2,124) = 0.78	0.4604
Frequency of TR	0.56 (n=55)	0.75 (n=12)	0.50 (n=32)	0.56 (n=11)	F(2,52) = 1.10	0.3399
ΔFEV1 %	2.01 (-0.17; 4.19) (n=134)	1.19 (-3.00; 5.37) (n=32)	2.14 (-0.16; 4.44) (n=72)	2.57 (-4.57; 9.70) (n=30)	F(2,131) = 0.10	0.9071
ΔFVC%	3.63 (1.78; 5.49) (n=134)	3.19 (1.13; 7.50) (n=32)	3.14 (0.59; 5.68) (n=134)	5.30 (1.60; 9.00) (n=30)	F(2,131) = 0.45	0.6387
ΔDlco%	4.41 (2.72; 6.11) (n=107)	6.36 (2.55; 10.16) (n=28)	2.43 (0.23; 4.64) (n=51)	6.07 (2.56; 9.59) (n=28)	F(2,104) = 2.52	0.0851

Numeric variables are listed as frequencies and number of patients (n=). Continuous variables are reported as means with 95% CI (). *Abbreviations:* TR: Treatment response. RP Radiologic progression.LS: Löfgren's syndrome. Changes (Δ) in FEV1: Forced expiratory volume. FVC: Functional vital capacity. DLco: Diffusion capacity of the lungs for carbon monoxide. Val=value. F= F-value

parameters increased over 24 months. Mean changes in PFT were small and did not differ between *ACE* genotypes. A total of 55 patients were treated with immunosuppression during the 2 years of follow up. Treatment response did not differ among the genotypes.

The distribution of genotypes in patients with LS did not differ from that in patients with non-LS, (Table 4).

Table 4. The distribution of *ACE* genotypes in patients with Löfgren's syndrome (LS) and non-LS

	LS (n=35)	Non-LS (n=113)	Difference	P value
DD	0.26	0.22	0.04 (-0.13; 0.20)	0.6591
ID	0.60	0.50	-0.10 (-0.08; 0.29)	0.2799
II	0.14	0.28	-0.14 (-0.28; 0.00)	0.0939

Discussion

This study aimed to investigate the relationship between the *ACE* I/D polymorphism and the susceptibility of sarcoidosis in Denmark. Additionally, we sought to evaluate if factors of prognosis were associated with the genotypes. This study is to our knowledge the first to evaluate the association of the *ACE* I/D polymorphism with treatment response. We found no association between genotype- or allele frequencies, prognosis or treatment response in this population. We found the ID genotype to be the most frequent in Denmark. This aligns with several other European populations (13–15) as well

as Afro-Americans (16), whereas the DD genotype is more prevalent in Turkish and Iranian sarcoidosis populations (17,18). The *ACE* I/D polymorphism and risk of sarcoidosis have been studied in different populations, with I/D frequencies varying among them with different results (13): British and Czech populations were studied by McGrath et al. They found no association and neither did a Swedish study (14). However, a meta-analysis from 2015 including 18 studies concluded that the *ACE* DD genotype correlated with an increased risk of sarcoidosis in Caucasians (3). In studies published since 2015, a Finnish study from 2017 did not find an increased risk related to genotype (15), whereas an Iranian study found the DD genotype to be more prevalent in patients with sarcoidosis than in controls (17). These differing results suggest that the role of the I/D polymorphism depends on the population being studied, adding to the variability of the disease. In sarcoidosis, the heterogeneity of the clinical presentation and prognosis warrants biomarkers to help predict disease trajectory and inform treatment strategies. Our study did not show any association to prognosis or treatment response. Other studies have investigated the *ACE* I/D polymorphism and its clinical implications in sarcoidosis, with variable results. No association was found in British and Czech populations with respect to pulmonary disease severity, fibrosis, and progression (13). Similarly, no association with Löfgren's syndrome or HLA-DR alleles known to be linked to Löfgren's was found in Spanish and Swedish populations (14,19), mirroring the results of the present study, which showed no difference in *ACE* genotype distribution between patients with Löfgren's syndrome and those without. Serum-ACE activity is reported to be significantly higher in individuals with the DD genotype compared to those with the II and ID genotypes (5,17). In line with these findings, a Finnish study from 2001 demonstrated that statistically significantly more patients with the DD genotype had a poor prognosis compared to those with II and ID genotypes (20) and Papadopoulos et al. reported an increased frequency of the DD genotype in patients with autoimmune manifestations and major granuloma mass (Scadding stage III) (21). Contrary to this, a US study found a moderate association between the II genotype and radiographic progression (16). In contrast, we did not find any associations between *ACE* polymorphisms and prognostic outcomes. No studies have previously explored if *ACE* genotypes is related to treatment response. Perhaps not surprisingly, we could not prove such a relationship.

Strengths of this study included genotyping of a relatively large number of sarcoidosis patients and controls. We investigated the association of genotypes to treatment response which has not previously been investigated. Our study has some limitations. We included patients from only one center raising the question about generalizability to the broader sarcoidosis population in Denmark. However, the age and gender distribution in our cohort was very similar to register-based incidence data (22). The variable results in the present and other studies further reduce generalizability among populations, instead underscoring the heterogeneity of the disease. The follow-up of 2 years is shorter than some of the comparing studies which may influence the results regarding prognosis as sarcoidosis is often described resolving over 2-5 years and we cannot exclude that longer follow up would alter the results with regards to Scadding stage and pulmonary function tests. However, with the relatively discrete changes in PFTs and RP we do not consider this a major issue. Also, our study included relatively few patients with advanced sarcoidosis and need for anti-inflammatory treatment which may have added to the lack of an association between *ACE* genotypes and treatment responses.

Conclusion

Our study found no evidence to support an association between the *ACE* I/D genotypes and sarcoidosis on susceptibility, prognosis or treatment response. The distribution of genotypes did not differ in patients with LS compared to non-LS patients. The variability in findings across different studies underscores the complexity of sarcoidosis and the influence of genetic and environmental factors. Further research is necessary to fully understand the role of *ACE* I/D polymorphism in sarcoidosis and to identify reliable biomarkers for disease prediction and management.

Acknowledgements: None.

Conflict of Interest: JM: Received support for the manuscript from Spydspidspuljen and The Frølich Foundation. Honoraria for lectures from Boehringer Ingelheim and Novartis. Support

for attending meetings and for travel by Boehringer Ingelheim. OH, PHN and HJM have no conflicts of interest related to this article. EB received honoraria for lectures from AstraZeneca, Daichii Sankyo and Boehringer Ingelheim. Participation on Advisory Board for Simbec-Orion.

Authors' Contribution: JM, OH, and EB all contributed to the planning and designing of the study. JM collected the data and performed the initial data analyses. PHN and HJM did the analyses of the *ACE* genotypes. The first draft of the manuscript was written by JM, with input from EB. All authors participated in the discussion and interpretation of the results and made substantive suggestions for revision. All authors accepted the final version and take responsibility for the integrity of the work as a whole.

References

1. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest. 1990;86(4):1343–6. doi: 10.1172/JCI114844.
2. Sayed-Tabatabaei FA, Oostra BA, Isaacs A, Van Duijn CM, Witteman JCM. ACE polymorphisms. Circ Res. 2006 May;98(9): 1123–33. doi: 10.1161/01.RES.0000223145.74217.e7.
3. Yang H, Mo T, Nie W, Li B. Angiotensin converting enzyme I/D polymorphism and sarcoidosis risk. Sarcoidosis, Vasculitis and Diffuse Lung Diseases. 2015;32(4):284–8. PMID: 26847094.
4. Gilbert S, Steinbrech DS, Landas SK, Hunninghake GW. Amounts of angiotensin-converting enzyme mRNA reflect the burden of granulomas in granulomatous lung disease. Am Rev Respir Dis. 1993;148(2):483–6. doi: 10.1164/ajrccm/148.2.483.
5. Fløe A, Hoffmann HJ, Nissen PH, Møller HJ, Hilberg O. Genotyping increases the yield of angiotensinconverting enzyme in sarcoidosis - A systematic review. Dan Med J. 2014;61(5):1–6. PMID: 24814734.
6. Grunewald J, Brynedal B, Darlington P, et al. Different HLA-DRB1 allele distributions in distinct clinical subgroups of sarcoidosis patients. Respir Res. 2010 Feb 26;11(1). PMID: 20187937.
7. Møller J, Hilberg O, Bendstrup E. Fatigue in Patients with Sarcoidosis in Denmark. Lung. 2023 Feb 1;201(1):103–10. doi: 10.1007 /s00408-023-00602-0.
8. Grunewald J. HLA associations and Löfgren's syndrome. Vol. 8, Expert Review of Clinical Immunology. 2012. p. 55–62. doi: 10.1586 /eci.11.76.
9. Nissen PH, Campbell NB, Højskov CS, et al. Development of a high-resolution melting genotyping assay for the angiotensin I converting enzyme insertion/deletion polymorphism and establishment of genotype-specific reference intervals in a Danish population. Ann Clin Biochem. 2015;52(1):105–12. doi: 10.1177/0004563214529261.
10. Crouser ED, Maier LA, Baughman RP, et al. Diagnosis and Detection of Sarcoidosis An Official American Thoracic Society Clinical Practice Guideline. Vol. 201, American Journal of Respiratory and Critical Care Medicine. American Thoracic Society; 2020. p. E26–51. doi: 10.1164/rccm.202002-0251ST.
11. Møller J, Hilberg O, Bendstrup E. Design and rationale of ProSar, the first Danish sarcoidosis registry. Sarcoidosis Vasc Diffuse Lung Dis. 2022;38,4. doi: 10.36141/svdld.v38i4.11605.
12. Grunewald J, Brynedal B, Darlington P, et al. Different HLA-DRB1 allele distributions in distinct clinical subgroups of sarcoidosis patients. Respir Res. 2010 Feb 26;11(1). doi: 10.1186/1465-9921-11-25.
13. McGrath DS, Foley PJ, Petrek M, et al. ACE Gene I/D Polymorphism and Sarcoidosis Pulmonary Disease Severity. 2012 Dec 14; 164(2):197–201. doi: 10.1164/ajrccm.164.2.2011009.
14. Planck A, Eklund A, Yamaguchi E, Grunewald J. Angiotensin-converting enzyme gene polymorphism in relation to HLA-DR in sarcoidosis. J Intern Med. 2002 Mar 3 ;251(3):217–22. doi: 10.1046 /j.1365-2796.2002.00946.x.
15. Lahtela E, Wennerström A, Pietinalho A, et al. ACE gene variants and sarcoidosis in a Finnish population. Sarcoidosis, Vasculitis, and Diffuse Lung Diseases. 2017;34(2):104. PMID: 32476831
16. Maliarik MJ, Rybicki BA, Malvitz E, et al. Angiotensin-converting enzyme gene polymorphism and risk of sarcoidosis. Am J Respir Crit Care Med. 1998;158(5 Pt 1):1566–70. doi: 10.1164/ajrccm .158.5.9804123.
17. Javadi A, Shamaei M, Zarei M, Rezaeian L, Kiani A, Abedini A. Insertion/Deletion Polymorphisms and Serum Angiotensin-converting Enzyme Levels in Iranian Patients with Sarcoidosis. Iran J Public Health. 2016 Nov 26 ;45(11):1473. PMID: 28032065.
18. Yilmaz D, Karkucak M, Coşkun F, Yakut T, Kunt Uzaslan E. ACE gene I/D polymorphism and risk of sarcoidosis development in Turkish patients. Tuberk Toraks. 2012;60(3):201–6. doi: 10.5578/tt.3935.
19. Alía P, Mañá J, Capdevila O, Álvarez A, Navarro MÁ. Association between ACE gene I/D polymorphism and clinical presentation and prognosis of sarcoidosis. Scand J Clin Lab Invest. 2005; 65(8):691–8. doi: 10.1080/00365510500354128.
20. Pietinalho A, Furuya K, Yamaguchi E, Kawakami Y, Selroos O. The angiotensin-converting enzyme DD gene is associated with poor prognosis in Finnish sarcoidosis patients. European Respiratory Journal. 1999 Apr 1;13(4):723–6. doi: 10.1034/j.1399-3003.1999.13d04.x.
21. Papadopoulos KI, Melander O, Orho-Melander M, Groop LC, Carlsson M, Hallengren B. Angiotensin converting enzyme (ACE) gene polymorphism in sarcoidosis in relation to associated autoimmune diseases. J Intern Med. 2000 Jan 1;247(1):71–7. doi: 10.1046 /j.1365-2796.2000.00575.x.
22. Sikjµr MG, Hilberg O, Ibsen R, Løkke A. Sarcoidosis: A nationwide registry-based study of incidence, prevalence and diagnostic work-up. Respir Med. 2021 Oct 1;187. doi: 10.1016/j.rmed.2021.106548.