21. Shiels A, Bennett T.M., Hejtmancik J.F. Cat-Map: putting cataract on the map. Mol Vis. 2010;16:2007-15.

22. The structure and diversity of hereditary pathology in Sakha Republic (Yakutia). L. Tarskaia, R. Zinchenko, G. Elchinova [et al.] // Russ J Genet. 2004;40:1264–72. https://doi.org/10.1023/B:RUGE.0000048669.22362.1c.

O.M. Zamorschikova, S.S. Sleptsova, N.R. Maksimova, A.L. Danilova, S.S. Sleptsov, L.I. Petrova

ANALYSIS OF INTERFERON GENE POLYMORPHISM IN PATIENTS WITH HDV INFECTION IN THE REPUBLIC OF SAKHA (YAKUTIA)

DOI 10.25789/YMJ.2022.80.03

УДК 616.36-002.2

The aim of the research: to analyze the frequency of occurrence of polymorphisms rs8105790 of the IFNL3 gene, rs368234815 of the IFNL4 gene, rs1831583 of the IFNA1 gene in healthy people and patients with chronic viral hepatitis D among the ethnic group of Yakuts living on the territory of the Republic of Sakha (Yakutia).

Materials and methods of the study: to study gene polymorphisms in 157 individuals with chronic HDV infection and 160 apparently healthy individuals was used polymerase chain reaction (PCR). Analysis of the results included compliance with the Hardy-Weinberg law, Pearson's chi-squared test ($\chi 2$), odds ratio and its confidence interval.

Results: the people of young working age suffer more from HDV infection, wherein the development of cirrhosis from the moment of infection with the D virus is formed on average over 6.5 years. The high replicative activity of the HDV virus in 74.1% of cases is accompanied by suppression of HBV, but with an increase in the severity of fibrosis and the formation of cirrhosis and liver cancer, there is observed simultaneous replication of hepatitis B and D viruses. According to the data obtained, the risk of developing severe fibrosis in HDV is 1.7 times higher in carriers of the ΔG-allele of the rs368234815 polymorphism of the IFNL4 gene (OR=1.784; 95% CI 0.642–4.959) and 1.8 times higher in the carriers of the C-allele of the rs1831583 polymorphism of the IFNA1 gene (OR= 1.818; 95% CI 0.340–9.713).

Conclusion: the obtained results demonstrate that the C-allele rs1831583 of the IFNA1 gene and the ΔG-allele rs368234815 of the IFNL4 gene predispose to the formation of severe fibrosis in HDV infection, Yakutia.

Keywords: chronic hepatitis, liver cirrhosis, gene polymorphism, HDV, IFNL3, IFNL4, IFNA1.

ZAMORSCHIKOVA Olga Mikhailovna – postgraduate student of the Department of Infectious Diseases, Phthisiology and Dermatovenereology of Medical Institute of M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia, olya-botty@mail.ru; **SLEPTSOVA Snezhana Spiridonovna** – M.D., Associate Professor, Head of the Department of Infectious Diseases, Phthisiology and Dermatovenereology, of Medical Institute of M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia, sssleptsova@yandex.ru; **MAKSIMOVA Nadezhda Romanovna** – M.D., chief researcher at Head of Research Laboratory "Molecular Medicine and Human Genetics" of Medical Institute of M. K. Ammosov North-Eastern Federal University, Yakutsk, Russia, nr.maksimova@s-vfu.ru; **DANILOVA Anastasia Lukinichna** – Ph.D. in Biology, senior researcher at Research Laboratory "Molecular Medicine and Human Genetics" of Medical Institute of North-Eastern Federal University named after M.K. Ammosov, Yakutsk, Russia, al.danilova@s-vfu.ru; **SLEPTSOV Spiridon Spiridonovich** – Ph.D. in Biology, associate professor, senior researcher at laboratories of clinical-population and medical-social researches of the Federal State Budgetary Scientific Institution "Yakut Scientific Center for Complex Medical Problems", Yakutsk, Russia, sachaja@yandex.ru; **PETROVA Lyubov Innokentievna** – Ph.D. in Medicine, Associate Professor of the Department of Infectious Diseases, Phthisiology and Dermatovenereology, of Medical Institute of M.K. Ammosov North-Eastern Federal University, Yakutsk, Russia, li.petrova@s-vfu.ru.

Introduction. Chronic viral hepatitis D (CHD) is caused by the hepatitis D virus and is characterized by a predominantly progressive course with the rapid development of liver cirrhosis (LC) than other hepatitis [1,5,4,10].

The Republic of Sakha (Yakutia) is one of the regions with a high prevalence of parenteral viral hepatitis [3,8]. The ongoing annual monitoring of the incidence of chronic viral hepatitis in the Republic of Sakha (Yakutia) shows an excess of the average for the Russian Federation.

Of the 14,975 patients registered in the electronic registry, chronic hepatitis D accounts for 7.8% (1176) of the total number of all chronic viral hepatitis, while HDV infection occurs in 40.8% of people with liver cirrhosis and in 38.5% with hepatocellular carcinoma (HCC). Chronic HDV infection is more often detected in the working-age population with a predominance of indigenous people. The high index of cirrhosis of HDV infection with the development of severe complications leading to early disability and death requires an in-depth study of the causes of liver fibrosis caused by the HD virus.

The mechanisms of genetic predisposition to chronic HDV infection have not yet been elucidated. The role of nucleotide polymorphic variants of interferon I (IFNA1) and type III (IFNL3, IFNL4) genes [2] in the pathogenesis of viral hepatitis is actively studied and is due to binding to cell receptors, as well as participation in the process of viral reproduction inside the cell [14]. Many studies prove the genetic determinism of the development of the chronic course of hepatitis in HBV and HCV infections [11, 16]. Despite the relevance of genetic predictors in the study of the development of chronic HDV infection, so far, no molecular genetic studies have been conducted in the Asian ethnic group in Russia.

Purpose: to analyze the frequency of occurrence of polymorphisms rs8105790 of the IFNL3 gene, rs368234815 of the IFNL4 gene, rs1831583 of the IFNA1 gene in healthy and sick individuals with chronic viral hepatitis D among the ethnic group of Yakuts living in the Republic of Sakha (Yakutia).

Materials and methods: the study was approved by the local ethical committee of the North-Eastern Federal University named after M.K. Ammosov", complies with the ethical principles of the Declaration of Helsinki of the World Medical Association (2013). The selection of the biomaterial was carried out on the basis of the infectious diseases department of the State Budgetary Institution of the Republic of Sakha (Yakutia) "Ya-

kut Republican Clinical Hospital" (chief physician, candidate of medical sciences Vasiliev N.N.), the infectious diseases department of the NEFU Clinic (director, candidate of medical sciences Ammosov V.G.) in the period from 2020 to 2022. Molecular genetic studies were carried out in the research laboratory "Molecular Medicine and Human Genetics" of the NEFU Clinic. Whole peripheral blood samples were used as a biomaterial for studying the polymorphism of the IFNL3 (rs8105790), IFNL4 (rs368234815), IFNA1 (rs1831583) genes. The clinical group included 157 patients with established HDV infection, 71 men and 86 women, aged 22 to 77 years, mean age 48±10.5 years, of the Yakut ethnic group permanently residing in the Republic of Sakha (Yakutia). At the same time, persons of the age category up to 44 years old accounted for 47%, up to 59 years old - 41%, 60 years and older - 12%. For comparison with the frequency of occurrence of the selected polymorphisms, 160 practically healthy individuals, 75 men and 85 women, aged 20 to 75 years old, average age 49±12.7 years old, were selected.

The demographic characteristics of patients (gender, age, place of residence), the form of hepatitis D disease (hepatitis, cirrhosis, cancer) were studied. The ethnic group was determined on the basis of questionnaire data. General clinical, serological and molecular biological methods for the study of hepatitis B and D viruses were carried out. Diagnosis and assessment of the stage of liver cirrhosis was carried out on a point scale in accordance with the Child-Pugh classification and the MELD survival scale. The diagnosis was established on the basis of clinical and laboratory data and the results of ultrasound, CT and MRI of the abdominal organs and fibroelastometry of the liver. Diagnosis and stage of HCC was carried out using the TNMB system in accordance with the Barcelona classification.

Isolation of DNA from peripheral blood was carried out using a commercial kit of OOO Evrogen (Moscow, Russia). For the molecular genetic study, the TagMan SNP Genotyping Assays Applied Biosystems, Thermo Scientific (USA) genotyping kits were used. Amplification was carried out on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA) in real-time mode.

Data processing was carried out using statistical criteria using the IBM SPSS Statistics 26.0 software package. To compare the means, we used T-test of independent samples (CI=95%; p> 0.05). The distribution of genotypes for the studied polymorphisms was checked for compliance with the Hardy-Weinberg equilibrium using Fisher's exact test. To compare allele frequencies between both sick and healthy groups, there was used Pearson's $\chi 2$ test with Yates' correction for continuity. Results were considered significant at $p<0.05$.

Results and discuss: distribution according to nosological forms of the study group revealed that in 36.9% (58) patients chronic hepatitis D was without severe fibrosis, in 63.1% (99) patients hepatitis D was in the stage of cirrhosis, including those with hepatocellular carcinoma 15 people.

When studying the epidemiological anamnesis of the clinical group, it was found that 46.5% of patients had a fact of surgical intervention, 44.6% of patients mentioned endoscopic and other invasive medical interventions. 33.1% of the surveyed do not exclude infection with HDV infection through blood transfusion, cases of intra-family contacts were observed in 32.1% of cases. A history of donation was noted in 2.3%, a possible sexual transmission in 5.3% of patients. Past acute viral hepatitis in history was registered in 39.5%. The duration of the disease was 19.2±11.7 years, the time from the moment of infection with the hepatitis D virus to the formation of liver cirrhosis is 6.5 years on average.

Clinical symptoms in most patients with HDV infection were manifested by asthenic syndrome in 100% of cases, skin manifestations in the form of telangiectasia and palmar erythema in 72.6%, and heaviness in the right hypochondrium in 68.8% of cases. Bleeding gums, nose, including episodes of bleeding from varicose veins of the esophagus were observed in 54.1% of patients, jaundice in 46.5%, edematous syndrome, including ascites, was observed in 40.8% of patients, splenomegaly was observed in 44 % and 41.4% of patients complained of pain in the joints. Among the most common comorbidities in patients were diseases of the gastrointestinal tract in 45.2%, diseases of the cardiovascular system in 21%, endocrine system in 11.5% and genitourinary system in 18.5% of cases.

In a laboratory study of peripheral blood of patients with CHD (n=157), pronounced cell cytolysis was observed in patients at the stage of liver cirrhosis. The level of serum aminotransferases revealed a 2.5-fold excess of ALT ($p<0.000$) in the initial manifestations of cirrhosis, also a 3-fold increase in AST activity ($p<0.000$) and a 4-fold increase in the level of bilirubin fractions ($p<0.000$) were detected in decompensated forms liver cirrhosis. A significant increase in the level of alpha-fetoprotein ($p<0.000$) in this group became a prognostically unfavorable factor in the course of severe liver fibrosis.

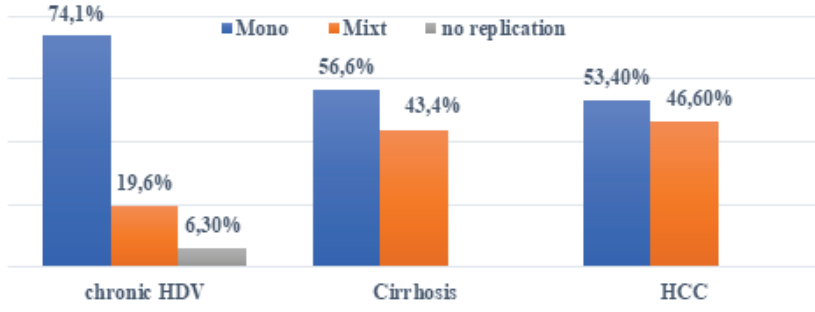
Previous studies on the incidence of chronic viral hepatitis B, C and D in the region has revealed a direct relationship between the incidence rate and the incidence of HCC in this category of patients [9]. The most aggressive course is detected in patients with chronic HDV infection, which is characterized by mixed replication of HBV and HDV viruses [6]. In the study of replicative activity by PCR (n=157), mono-replication of HDV was detected in 101 people, mixed replication of HDV in 51 people, 5 of the studied patients had no replication. In the CGD group without liver cirrhosis, mono-replication prevailed 74.1%, mixed replication was 19.6%, and 6.3% of patients were without replication. In the CGD group with liver cirrhosis, mixed replication was observed in 43.4% of cases, and mono-replication in 56.6%. In patients with HCC, the activity of the virus showed mixed replication in 46.6%, and mono-replication in 53.4% of cases (Fig. 1).

The genotype frequency of the examined patients corresponded to the Hardy-Weinberg equilibrium, which made it possible to carry out the distribution of alleles and genotypes of the polymorphic variant of the IFNL4 (rs368234815), IFNL3 (rs8105790) and IFNA1 (rs1831583) genes. The studied samples showed differences between the patients of the main group (n=157) and the control group (n=160) (Table 1). The sample of healthy patients was characterized by the largest number of carriers of homozygous AA - genotype rs1831583 of the IFNA1 gene, TT / TT - genotype rs368234815 of the IFNL4 gene and TT genotype rs8105790 of the IFNL3 gene without the presence of mutant allele, carriers of the homozygous AA genotype - genotype of the rs1831583 gene of the IFNA1 gene, TT/TT - genotype of the rs368234815 gene of the IFNL4 gene, TT genotype rs8105790 of the IFNL3 gene, but in the observed distribution, the proportion of heterozygous genotypes (carriers of the mutant allele) was higher than in the healthy group. Similar work was carried out by Karataylı S.C. and co-authors on the study of the rs8105790 polymorphism of the IFNL3 gene in patients with chronic HBV infection. Patients with the CC/TC genotype for rs8105790 ($p<0.0001$) were found to be more likely to be inactive HBsAg car-

riers [12].

In the studied groups, no significant differences were found in the frequency of genotype distribution (p>0.05), which indicates a fairly frequent occurrence of these markers among a healthy, uninfected population. The distribution of alleles showed significant differences for the C-allele of the rs1831583 locus of the IFNA1 gene (p=0.035) and the ΔG-allele of the rs368234815 locus of the IFNL4 gene (p=0.009). No significant differences were found in the rs8105790 polymorphism of the IFNL3 gene, which is probably due to the protective function of this gene, and it does not play a role in the development of chronic hepatitis D. According to the data, the probability of developing severe fibrosis in HDV is 1.7 times higher in carriers of the mutant allele of the polymorphism ΔG-allele of the rs368234815 polymorphism of the IFNL4 gene and 1.8 times higher in carriers of the C-allele of the rs1831583 polymorphism of the IFNA1 gene (Table 2). The research of the polymorphism of the genes we study is being carried out by many scientists [7,10,13]. A group of Chinese scientists examined 3128 people for the possibility of HBV infection with a number of polymorphisms of interferon genes in 14 loci, where the formation of HCC was associated with polymorphisms of the IFNA1 genes. In the studied groups of patients with liver fibrosis, the development of HCC was indicated in polymorphic variants of the IFNA1-rs1831583 and IFNA2-rs649053 genes [15]. There are indications that polymorphic variants of the IFNL3, IFNA1, and IFNL4 genes are associated with the development of liver cirrhosis in patients with chronic hepatitis B; polymorphic variants rs1831583 of the IFNA1 gene and rs649053 of the IFNA2 gene indicated the development of liver cancer [13]. There are studies that note the relationship between the development of liver cirrhosis and HCC in patients with HCV with the rs368234815 polymorphism of the IFNL4 gene [11], at the same time, unlike the Caucasians there is data that indicates the frequency of spontaneous clearance of HCV in carriers of the TT genotype of the rs368234815 polymorphism of the IFNL4 gene in representatives of the mongoloid race [7].

Conclusion: The Republic of Sakha (Yakutia) is one of the "endemic" regions of the Russian Federation in terms of the prevalence of viral hepatitis D. A feature of the course of HDV infection is the susceptibility to this pathology of people of indigenous nationality and of working age. The course of chronic hepatitis D



Share of HDV replication activity in patients with and without cirrhosis (n=157)

Table 1

The frequency of occurrence of polymorphisms of the studied genes in patients with CHD and healthy people of indigenous nationality

Genotypes. alleles	Chronic HDV n=157	healthy group n=160	$\chi 2$	*P*
Полиморфизм *rs1831583* гена *IFNA1*				
AA – genotype	95.5 (150/157)	96.8 (155/160)	p=0.565*	-
AC – genotype	3.8 (6/157)	3.1 (5/160)		-
CC – allele	0.6 (1/157)	0		-
A – allele (%)	97.0 (306/316)	98.0 (315/322)	-	p=0.035**
C – allele (%)	3.0 (8/267)	2.0 (5/250)	-	
Полиморфизм *rs8105790* гена *IFNL3*				
TT – genotype	85.3 (134/157)	79.4 (127/160)	p=0.078**	-
TC - genotype	14.6 (23/157)	20.6 (33/160)		-
CC – genotype	0	0		-
T – allele (%)	93.0 (291/313)	90.0 (287/319)	-	P=0.053**
C – allele (%)	7.0 (23/329)	10.0 (33/330)	-	
Полиморфизм *rs368234815* гена *IFNL4*				
TT/TT – genotype	87.2 (137/157)	87.5 (140/160)	p=0.598*	-
TT/ΔG – genotype	12.1 (19/157)	12.5 (20/160)		-
ΔG/ΔG – genotype	0.64 (1/157)	0		-
TT (%)	93.0 (293/316)	94.0 (300/320)	-	p= 0.009**
ΔG (%)	7.0 (21/300)	6.0 (20/334)	-	

* Analysis of arbitrary cross tables using the chi-square test;
**Analysis of four-field contingency tables. Pearson contingency coefficient (C)

Table 2

The incidence of liver cirrhosis in persons with HDV infection depending on the carriage of the mutant allele of the studied genes

Polymorphisms genes	Groups Research	Chances in groups	95%CI	OR
rs1831583 гена *IFNA1*	Carrier M	0.065	95%	1.818
	Not carrier M	0.036		
	Not carrier M	0.137		
rs368234815 гена *IFNL4*	Carrier M	0.206	95%	1.784
	Not carrier M	0.115		

Note: Carrier M - carrier of the mutant allele (XY, YY); Non-carrier M - homozygous for the normal allele (XX); OR- odds ratio (odds ratio), 95% CI - 95% confidence interval OR

is characterized by an aggressive nature and rapid development of complications. Decompensated forms of the disease are more often recorded in persons with HDV infection in the stage of cirrhosis and hepatocellular liver cancer. A prognostically unfavorable marker for the formation of liver fibrosis is the mixed replication of HBV and HDV viruses.

The obtained results demonstrate that the risk of developing severe liver fibrosis in HDV is 1.8 times higher in carriers of the C-allele rs1831583 of the IFNA1 gene and 1.7 times higher in carriers of the ΔG-allele rs368234815 of the IFNL4 gene. These groups of genes can become candidate genes for the formation of cirrhosis and liver cancer, which requires further study of other variants of the loci of the studied genes.

Information about the funding of the study and about the conflict of interest: the work was prepared with the financial support of the Russian Foundation for Basic Research. Grant No. 20-315-90046.

Reference

1. Abdurakhmanov D.T. Khronicheskij gepatit V i D [Chronic hepatitis B and D]. Educational-method. Manual. M.: GEOTAR-Media, 2010; 288 (In Russ.).]

2. Arsentieva N.A., Semenov A.V., Totolyan A.A. Rol' Polimorfizma genov citokinov pri virusnom gepatite S [The role of polymorphism of cytokine genes in viral hepatitis C / // Infection and immunity]. Infekciya i immunitet [Infection and immunity. 2012; 4 (2): 687-698 (In Russ.).] DOI: 10.15789/2220-7619-2012-4-687-698.

3. Zamorshchikova O.M., Slepcova S.S., Slepcov S.S., Malov S.I. HDV-infekciya: prediktor tyazhelogo fibroza pecheni [HDV infection: a predictor of severe liver fibrosis]. Yakut medical journal. 2021; 2(74): 113-115 (In Russ.).] DOI 10.25789/YMJ.2021.74.29.

4. Yushchuk N.D., Vengerov Yu.Ya. Infekcionnye bolezni: nacional'noe rukovodstvo [Infectious diseases: national guidelines. 3rd edition. M: GEOTAR-Media, 2021: 1104 (In Russ.).]

5. Isaeva O.V., Kyuregyan K.K. Virusnyj gepatit del'ta: nedoocenennaya ugroza [Delta viral hepatitis: an underestimated threat]. Infekcionnye bolezni: novosti, mneniya, obuchenie [Infectious disease: news, opinions, training. 2019; 2 (8): 72-79 (In Russ.).] DOI: 10.24411/2305-3496-2019-12010.

6. Zamorshchikova O.M., Sleptsova S.S., Sleptsov S.S., Ohlopkov V.S. Kliniko-laboratornye osobennosti formirovaniya cirroza pecheni pri HDV-infekcii v Respublike Sakha (Yakutiya) [Clinical and laboratory features of the formation of liver cirrhosis in HDV infection in the Republic of Sakha (Yakutia)]. Zabajkal'skij medicinskij vestnik [Transbaikalian Medical Bulletin. 2022; 3: 44-49 (In Russ.).]

7. Malov I.V., Baaratkhuu O., Ogarkov O.B., Stepanenko L.A., Nyamdava P., Oyunbilehg ZH., Malov S.I., Savilov E.D., Amarsanaa ZH., Gantulga D. Osobennosti polimorfizma genov interferona III tipa u bol'nykh virusnym gepatitom C na sopredel'nykh territoriyakh Rossii i Mongolii [Features of polymorphism of type III interferon genes in patients with viral hepatitis C in the adjacent territories of Russia and Mongolia]. Zhurnal infektologii [Journal Infectology. 2017; 3 (9): 46-53 (In Russ.).]

8. Semenov S.I., Pisareva M.M., Fadeev A.V. [et al.] Struktura virusa gepatita del'ta v Respublike Sakha (Yakutiya) [The structure of the delta hepatitis virus in the Republic of Sakha (Yakutia)]. Zhurnal infektologii [Journal of Infectology]. 2020; 2 (12): 112-113 (In Russ.).]

9. Sleptsova S.S., Sleptsov S.S., Andreev M.N. [et al.] Khronicheskie virusnye gepatity i pervichnyj rak pecheni v respublike Sakha (Yakutiya) [Chronic viral hepatitis and primary liver cancer in the Republic of Sakha (Yakutia)]. Zhurnal infektologii [Journal Infectology. 2019; 11 (4): 79-84 (In Russ.).]

10. Chen H., Shen D., Ji D. [et al.] Prevalence and burden of hepatitis D virus infection in the global population: a systematic review and meta-analysis. Gut. 2018. 68. P. 381–382.

11. Da Silva A.M.V., Alvarado-Arnez L.E., Azamor T. [et al.] Interferon-lambda 3 and 4 Polymorphisms Increase Sustained Virological Responses and Regulate Innate Immunity in Antiviral Therapy with Pegylated Interferon-Alpha. Frontiers in Cellular and Infection Microbiology. Front. Cell. Infect. Microbiol. 2021; 11 (656393): 1-12. DOI: 10.3389/fcimb.2021.656393.

12. Karataylı S.C., Bozdayı M., Karataylı E. [et al.] Interleukin-28 gene polymorphisms may contribute to HBsAg persistence and the development of HBeAg-negative chronic hepatitis B. Liver Int. 2015; 35(3): 846-853. doi:10.1111/liv.12595

13. Lee M.H., Yang H.I., Lu S.N., [et al.] Polymorphisms near the IFNL3 Gene Associated with HCV RNA Spontaneous Clearance and Hepatocellular Carcinoma Risk. Sci.Rep. 2015; 5(17030): 1-12. doi:10.1038/srep17030

14. Lopusna K., Rezuchova I., Betakova T. [et al.] Interferons lambda, new cytokines with antiviral activity. Acta virologica. 2013; 57 (2): 171-179.

15. Ma N., Zhang X., Yang L., Zhou J, Liu W., Gao X, Yu F., Zheng W., Ding S., Gao P., Yuan M., Liu D.J. Role of Functional IFNL4, IFNLR1, IFNA, IFNAR2 Polymorphisms in Hepatitis B virus-related liver disease in Han Chinese population. Viral. Hepat. 2018; 25 (3): 306-313. DOI: 10.1111/jvh.12817.

16. Sakaue S., Kanai M., Tanigawa Y. [et al.] A cross-population atlas of genetic associations for 220 human phenotypes. Nat Genet. 2021; 53: 1415–1424. DOI:10.1038/s41588-021-00931-x