Check for updates

JIM Original Article

doi: 10.1111/joim.12829

Variations in maternal adenylate cyclase genes are associated with congenital Zika syndrome in a cohort from Northeast, Brazil

Á. D. Rossi[1], F. R. Faucz[2], A. Melo[3], P. Pezzuto[1], G. S. de Azevedo[3], B. L. F. Schamber-Reis[4], J. S. Tavares[3], J. J. Mattapallil[5], A. Tanuri[1], R. S. Aguiar[1], C. C. Cardoso[1,†] & C. A. Stratakis[2,†]

From the [1]Laboratório de Virologia Molecular, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [2]Section on Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA; [3]Instituto de Pesquisa Professor Joaquim Amorim Neto (IPESQ); [4]Faculdade de Ciências Médicas de Campina Grande, Núcleo de Genética Médica, Centro Universitário UniFacisa, Campina Grande, Brazil; and [5]Department of Microbiology & Immunology, F. Edward Hébert School of Medicine, Uniformed Services University, Bethesda, MD, USA

Abstract. Rossi ÁD, Faucz FR, Melo A, Pezzuto P, de Azevedo GS, Schamber-Reis BLF, Tavares JS, Mattapallil JJ, Tanuri A, Aguiar RS, Cardoso CC, Stratakis CA (Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; National Institutes of Health, Bethesda, MD, USA; Instituto de Pesquisa Professor Joaquim Amorim Neto; Centro Universitário UniFacisa, Campina Grande, Brazil; Uniformed Services University, Bethesda, MD, USA). Variations in maternal adenylate cyclase genes are associated with congenital Zika syndrome in a cohort from Northeast, Brazil. *J Intern Med* 2019; **285**: 215–222.

Background. Vertical transmission of Zika virus (ZIKV) is associated with congenital malformations but the mechanism of pathogenesis remains unclear. Although host genetics appear to play a role, no genetic association study has yet been performed to evaluate this question. In order to investigate if maternal genetic variation is associated with Congenital Zika Syndrome (CZS), we conducted a case–control study in a cohort of Brazilian women infected with ZIKV during pregnancy.

Methods. A total of 100 women who reported symptoms of zika during pregnancy were enrolled and tested for ZIKV. Among 52 women positive for ZIKV infection, 28 were classified as cases and 24 as controls based on the presence or absence of CZS in their infants. Variations in the coding region of 205 candidate genes involved in cAMP signaling or immune response were assessed by high throughput sequencing and tested for association with development of CZS.

Results. From the 817 single nucleotide variations (SNVs) included in association analyses, 22 SNVs in 17 genes were associated with CZS under an additive model (alpha = 0.05). Variations c.319T>C (rs11676272) and c.1297G>A, located at *ADCY3* and *ADCY7* genes showed the most prominent effect. The association of *ADCY3* and *ADCY7* genes was confirmed using a Sequence Kernel Association Test to assess the joint effect of common and rare variations, and results were statistically significant after adjustment for multiple comparisons ($P < 0.002$).

Conclusion. These results suggest that maternal *ADCY* genes contribute to ZIKV pathogenicity and influence the outcome of CZS, being promising candidates for further replication studies and functional analysis.

Keywords: congenital malformations, gene polymorphism, infectious disease, virology, zika.

Introduction

Zika fever is an arthropod-borne viral disease characterized by a self-limiting illness for which most common symptoms are fever, myalgia, arthralgia, rash and conjunctivitis, although most of the cases are asymptomatic [1]. Infections mainly occur by *Aedes* mosquitoes' bites but alternative transmission routes have been described such as sexual transmission [2], blood/platelets transfusion [3] and vertical

[†]C.C.C. and C.A.S. share the senior authorship of this manuscript.

© 2018 The Association for the Publication of the Journal of Internal Medicine

transmission [4]. Among them, mother-to-child transmission showed up as a major public health concern since ZIKV infection was associated with microcephaly and other developmental disorders in Brazil [5]. Nowadays, ZIKV infection during pregnancy has been clearly associated with severe neurological outcomes in foetuses beyond microcephaly, including ocular anomalies, arthrogryposis, other congenital malformations and postnatal neurodevelopmental deficits, comprising what is now known as congenital Zika syndrome (CZS) [6].

Despite the strong evidence for ZIKV vertical transmission, the mechanisms by which ZIKV impairs foetal development remain to be elucidated [7, 8]. Virus evolution and the search for pathogenic mutations have been the focus in many studies [9, 10] although, no specific viral mutations responsible for severe cases have been described [11]. In addition, even in regions where more pathogenic strains are present, most cases do not result in foetal malformations, indicating that additional risk factors such as environmental and host genetics, might be involved in Zika pathogenesis [6].

The first evidence for a role of host genetics in CZS was reported in a recent study of Brazilian twins, which showed higher outcome discordance among dizygotic pairs than between monozygotic twins and suggested that recessive and multifactorial inheritance might be involved in CZS susceptibility [12]. Cyclic adenosine monophosphate (cAMP) has been shown to modulate different mechanisms of viral pathogenesis in hepatitis B, hepatitis C and HIV infections. Recently, this second messenger was also implicated in ZIKV neuropathogenesis, suggesting genes in this pathway as candidates for association studies [13–17].

To better explore the role of host's genetics in CZS, we conducted a case-control study in a cohort of Brazilian women infected with ZIKV during pregnancy. A total of 205 candidates genes involved in cAMP pathway or in immune response to ZIKV were investigated. Our results, though in a relatively small cohort, suggest an important association between maternal adenylate cyclase genes and susceptibility to CZS.

Material and Methods

Subjects and study design

A total of 100 women who attended to Instituto de Pesquisa Professor Joaquim Amorim Neto (IPESQ), Campina Grande, PB Brazil from 2015 to 2017 and reported ZIKV-related disease symptoms during pregnancy were considered eligible for this study. After careful review of medical records, all participants were classified as cases or controls based on the presence of severe outcomes from ZIKV infection in infants (Congenital Zika Syndrome). The following congenital malformations were considered severe outcomes: brain calcifications, ventriculomegaly, microcephaly, cerebellar hypoplasia, arthrogryposis, lissencephaly and/or hydrocephaly. The control group consisted of infected mothers who gave birth to healthy babies. All clinical diagnoses were performed by specialized physicians from IPESQ. Medical ultrasound exams with the Samsung WS80 ELITE ultrasound equipment were performed during pregnancy. Magnetic resonance was realized postdelivery in cases with apparent neurological damage with the Siemens MAGNETOM Espree scanner.

To confirm ZIKV infection, all samples were subjected to molecular and serological analysis. Molecular diagnosis was performed by a Real-time RT-qPCR assay on 7500 Real-Time PCR System (Thermo Fisher Scientific) using OneStep RT-PCR Kit (QIAGEN) and 10 μL of total RNA. Primers and probe (ZIKV1107-FAM) used were specific for the envelope sequence, described previously [18]. Serological tests for ZIKV were performed in addition to tests for DENV, CHIKV and for STORCH infections in order to exclude possible confounders. Different kits were used to access the serum levels of IgG/IgM from collected samples. For ZIKV, DENV and CHIKV, tests were performed with XGen kits (Biometrix). For STORCH, DIA.PRO kits (Dia.Pro Diagnostic Bioprobes) were used. Protocols were followed as specified by the manufacturer. The criteria for discrimination of IgG cross-reactivity between ZIKV and DENV were values for ZIKV at least three times higher than for DENV. Subjects with negative results for ZIKV infection or positive results for other infections (based on IgM for DENV, CHIKV or STORCH) were excluded from the study.

After molecular and serological tests, the final sample size included 52 women (28 cases and 24 controls). General characteristics of cases and controls are summarized in Table 1. The present study was approved by the Institutional Review Board at University of São Paulo and written informed consent was obtained from all individuals included in the study.

13652796, 2019, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/joim.12929 by CAPES, Wiley Online Library on [07/06/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 1 *Characteristics of the cohort from Campina Grande, Brazil*

	Controls ($N = 24$)	Cases ($N = 28$)
Gestational age		
First trimester	13 (46.42)	20 (71.4)
After	15 (53.58)	8 (28.6)
ZIKV diagnosis		
PCR+	13 (54.16)	9 (32.14)
IgM+	8 (33.33)	0 (0)
IgG+	3 (12.5)	19 (67.86)
Microcephaly[a]	–	22 (78.6)
Other	–	6 (21.4)

Results are represented as % of N.
[a]Individuals in this category may present other malformations in addition to microcephaly.

DNA extraction and storage

DNA extraction was performed using QIAamp DNA Blood Mini Kit following manufacturer's recommendation (QIAGEN®). After extraction, the DNA concentration was determined with a Qubit HS DNA Assay kit (Invitrogen®) and stored at −80 °C until sequencing.

Exon sequencing and genotyping

The exomes from the 205 targeted genes were queried using probes designed in Illumina's DesignStudio using TruSeq Custom Amplicon v1.5 with the following options: CDS only; nominal amplicon size 250-bp; 10-bp padding. This resulted in 3603 amplicons covering 504 099-bp. Targeted Amplicon-Seq libraries were constructed from 100 to 250 ng of genomic DNA using Illumina's TruSeq Custom Amplicon kit. The libraries were barcoded, pooled together and sequenced on an Illumina MiSeq using v2 chemistry to generate 2 × 250 bp reads. This yielded an average of 200 000 paired-end reads per sample. Sequenced data was aligned to the human genome build hg19 using BWA. Mutation calls were generated in the capture design regions using Sentieon Genomics and annotated using SnpEff and dbNSFP.

In silico prediction of variant functional impact

Functional impact of each variant was predicted using the algorithms CADD (v1.4), DANN, FATHMM (v2.3), MutationTaster2, PolyPhen-2 and SIFT. Scores were interpreted as recommended by the developers [19–24].

Statistical analyses

All statistical analyses were performed using R software (version 3.4) and the packages "SNPassoc", "qqman", "multtest" and "SKAT". Deviations from Hardy-Weinberg equilibrium (HWE) were assessed by χ^2 tests. The association between each SNP and development of CZS was assessed by unconditional logistic regression models with adjustment for gestational age at the moment of infection (1st trimester or other). We primarily considered additive genetic models and secondarily considered dominant and codominant models. Possible effects of population stratification were assessed using the inflation factor λ. Values below 1.05 were considered benign [25].

Genes with at least one variation with statistically significant association under an alpha of 0.05 were included in gene-level analysis using a sequence kernel association test (SKAT). Associations between each gene and the phenotype were assessed using a SKAT version which determines the joint effect of common and rare (minor allele frequency <0.01) variations [26]. Equal weights were applied for all variants. A Bonferroni adjustment for multiple comparisons was performed to minimize type I error.

Results

Quality control and single SNP analysis

A total of 1591 single nucleotide variations (SNV) were found in our sample ($N = 52$ individuals). From these, 653 were removed from further analysis because they could not reach a 98% call rate ($N = 412$) or because they were monomorphic in control group ($N = 241$). In addition, 121 variants that showed deviation from Hardy-Weinberg equilibrium among controls were also removed. The remaining 817 SNVs were included in the case-control analyses (Figure 1).

Twenty-two variations in 17 genes were associated with the development of CZS under an additive model considering a 5% significance level (Fig. 1; Table S1). From these, 14 were single nucleotide polymorphisms (SNPs) registered in dbSNP (Build 151) with minor allele frequencies (MAF) varying from 3.8 (rs61731968, *AKAP1* gene) to 48.1% (rs11676272; *ADCY3* gene). The remaining 8

© 2018 The Association for the Publication of the Journal of Internal Medicine

13652796, 2019, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/joim.12829 by CAPES, Wiley Online Library on [07/06/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

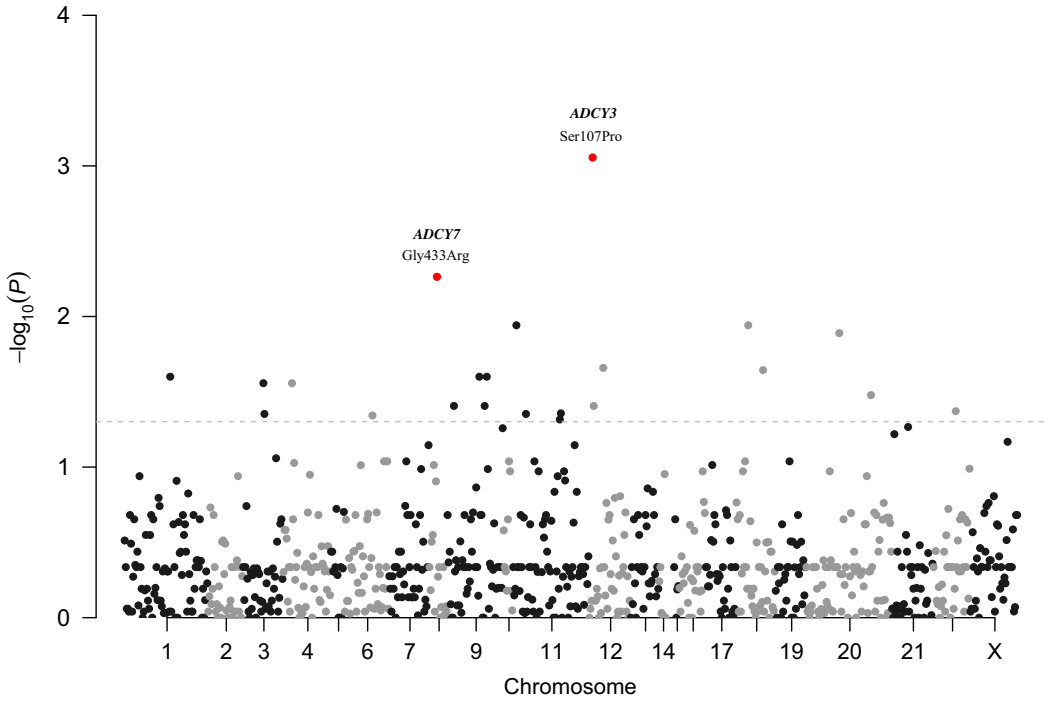

Fig. 1 *Manhattan plot showing the 817 single nucleotide variations (SNVs) included in the association analysis. SNVs were plotted according to chromosomal location (x axis), with −log10 P values (y axis) derived from logistic regression analysis under an additive model. The horizontal dashed line indicates P = 0.05. Variations highlighted in red showed the most prominent associations among the 22 SNVs above the threshold.*

variations were all *missense* variations with frequencies between 3.8 and 23% in our cohort (Table S1). The calculated inflation factor (λ = 1.03) did not suggest an impact of population stratification.

Variations in genes encoding A-kinase anchor proteins and adenylate cyclases were the most frequent among the statistically significant associations (16 SNVs in 9 genes and 7 SNVs in 4 genes, respectively, including results obtained after adjustment for gestational age - Table S1). The strongest effect was observed for the missense variants c.319T>C (rs11676272) at *ADCY3* (OR = 3.69; $_{95\%}$CI = 1.58–8.65; P = 0.00087) and c.1297G>A, at *ADCY7* (OR = 0.2; $_{95\%}$CI = 0.06–0.65; P = 0.00545). After adjustment for gestational age at the moment of infection (first trimester or not), rs11676272 and c.1297G>A remained significant and rs11676272 had even a stronger association (OR = 5.04; $_{95\%}$CI = 1.76–14.42; P = 0.0005004) (Table S1). The total number of statistically significant associations (including both models) increased to 55 variations at 39 genes with this change (Table S1). However, due to the small sample size, they were not statistically significant after Bonferroni adjustment for multiple comparisons (data not shown).

Variant functional impact

Possible functional impacts of the nonsynonymous variations rs11676272 and c.1297G>A were investigated *in silico* using six different algorithms (Table 2). Results obtained from all algorithms classified c.1297G>A as a functional and potentially pathogenic variation. The data suggest a direct functional impact for c.1297G>A since PolyPhen2 and SIFT results indicated that this variant affects protein activity and/or structure. No evidence of a functional impact was observed for rs11676272 (Table 2).

Sequence kernel association test (SKAT)

Sequence kernel association test analyses were performed to investigate the joint effect of

13652796, 2019, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/joim.12829 by CAPES, Wiley Online Library on [07/06/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 2 In silico *prediction of functional effects of rs11676272 and c.1297G>A according to six different algorithms*

Gene	Variant	Amino acid change	CADD[a]	DANN[a]	FATHMM[a]	Mutation Taster	PolyPhen2	SIFT[a]
ADCY3	rs11676272	Ser107Pro	0.001	0.662	0.79	Polymorphism	Benign	0.888
ADCY7	c.1297G>A	Gly433Arg	26.9	0.999	0.83	Disease Causing	Damaging	0.001

[a]Scores higher than 20 (CADD), 0.91 (DANN), 0.8 (FATHMM) and lower than 0.05 (SIFT), were considered pathogenic.

Table 3 *Gene-based association analysis with Congenital Zika Syndrome in the cohort from Campina Grande, Brazil*

	Variants analyzed (*N*)		SKAT results (*P*-value)	
Gene	Rare	Common	Crude	Adjusted*
ADCY3	2	9	**0.00191**	**0.00115**
ADCY7	2	3	**0.00146**	**0.00180**
ADCY10	6	15	0.07424	0.01397
AKAP1	7	17	0.06081	0.04884
AKAP3	2	10	0.06084	0.03914
AKAP8	2	5	0.04259	0.06712
COL1A2	2	10	0.02810	0.02797

Results were obtained from a SKAT model allowing for the joint effect of common and rare variants.
*Results adjusted for gestational age at the moment of infection (first trimester or other). *P*-values that remained statistically significant after Bonferroni adjustment for multiple comparisons ($\alpha = 0.00294$) are shown in bold.

common and rare variations from each of the 17 genes with SNVs associated to CZS in univariate models. All analyses were performed with and without adjustment for gestational age at the moment of ZIKV infection, resulting in a total of 7 genes with statistically significant associations (Table 3).

The most prominent results were again obtained for *ADCY3* and *ADCY7* genes, which showed a very low *P*-value compared to others, remaining statistically significant even after Bonferroni adjustment for multiple comparisons ($\alpha = 0.00294$ considering 17 genes included in SKAT analyses) (Table 3). This finding strongly supports the conclusion that variations observed in these two maternal *ADCY* genes are in fact associated with development of CZS in this cohort.

Discussion

The cAMP pathway is one of the most ubiquitous signalling pathways in cellular physiology, being operational in both prokaryotes and eukaryotes [27]. In mammals, this pathway is involved in many biological processes such as immune regulation, stress responses, hormone signalling and development [28–31]. During pregnancy, cAMP has been associated with decidualization of the endometrium, invasion of the trophoblast, syncytialization and placental function/physiology [32–35]. Recently, cAMP was also implicated in ZIKV neuropathogenesis (Olmo *et al.*, 2017), which makes genes related to the cAMP pathway promising candidates for genetic association studies in the context of ZIKV infection.

In the present study, SNVs in three genes encoding adenylate cyclases (*ADCY3*, *ADCY7* and *ADCY10*) were associated with CZS. The association of those genes with susceptibility to CZS was confirmed after gene-based analysis, and results for *ADCY3* and *ADCY7* remained statistically significant after Bonferroni adjustment for multiple comparisons. Moreover, *in silico* analyses showed that c.1297G>A variation had a high potential for damaging ADCY7 protein structure and/or function. Although rs11676272 was considered benign by the algorithms, this variation has already been reported as an expression quantitative trait locus (eQTL) for *ADCY3* gene, with the G allele associated with higher transcription levels in whole blood samples [36]. Altogether, these data show that genetic variation in cAMP-related genes in pregnant women of this cohort influence the risk for CZS in their newborns and support a role for this pathway on ZIKV replication, vertical transmission and/or pathogenesis.

Adenylate cyclase genes encode proteins that catalyze the synthesis of cAMP from ATP, being crucial for its generation. cAMP levels act as a potent regulator of innate and adaptive immune responses and manipulation of its cellular concentration is a well-known pathogen strategy to avoid host immune system [37]. By increasing cAMP production, pathogens inhibit NF-kB activation and, consequently, impair downstream

13652796, 2019, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/joim.12829 by CAPES, Wiley Online Library on [07/06/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

proinflammatory events, sometimes making use of adenylate cyclase to do so [38–40]. Moreover, in the context of ZIKV infection, higher levels of cAMP could not only stimulate virus replication but also affect placental innate immunity as previously shown, facilitating mother-to-child transmission [41]. Recent data also show that inhibition of cAMP downstream effectors suppress ZIKV replication, supporting the relevance of cAMP levels on ZIKV pathogenesis [42].

In addition, our data may also suggest that higher levels of cAMP could influence ZIKV severe outcomes. The rs11676272 G allele was associated with a risk effect and it could induce higher levels of ADCY3 [36], which would consequently lead to higher levels of cAMP. On the other hand, c.1297G>A was associated with a protective effect and considering that this variation could damage ADCY7 function and impair cAMP production, it could lead to lower levels of cAMP. This makes cAMP a very interesting molecule for future *in vitro* studies regarding its ability to influence ZIKV replication.

We also would like to address that among our results, SNVs not yet described appear with high frequency in the present cohort, including c.1297G>A, with a frequency of 23.1% in our study. This might be explained by some inbreeding observed in Brazilian Northeast region [43–45] and the lack of genetic studies in this region of Brazil. On the other hand, frequency of the SNP rs11676272 in the present study (48.1%) was the same as described in ExAC Browser for the general population (48%) and similar to that observed among individuals with European ancestry (46%). According to data from the 1000 Genomes project retrieved from Ensembl genome browser, Africans showed the highest frequency of the G allele (85%) while the lowest was observed among Native Americans (AMR, 39%). The population from the Brazilian Northeast region is quite admixtured, with a high contribution of European and African ancestries, followed by Native American ancestry [46, 47].

Another mechanism that might be causing an enrichment of new SNVs in this study is a possible association with susceptibility to ZIKV infection *per se*, since all subjects included in our study presented with symptomatic ZIKV infection. Finally, the high frequency of such variants may also be a consequence of the sample size of our cohort, reinforcing the importance of replication studies with independent cohorts to validate the associations found here.

Taken together, our data suggest a role for *ADCY* genes in ZIKV infection and/or related disease, not only highlighting them as promising candidates for further population and functional studies but also providing the first evidence of maternal genetics influencing the risk to CZS.

Conflict of interest

The authors have nothing to disclose.

Acknowledgements

This work was supported by the following funds: Intramural Research Program (IRP) of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) to CAS (Project# Z1A HD008920), Uniformed Services University (USU) to JJM (grant# R0739273), National Council for Scientific and Technological Development (CNPq – grant# 440900/2016-6) and National Council for the Improvement of Higher Education (CAPES – grant# 88881.130757/2016-01). The authors are indebted to the NICHD's IRP Molecular Genomics Core (Dr. Steven Coon, Dr. James Iben, and Dr. Li Tianwei). We also thank Biometrix Diagnostica for donating kits for serology tests.

References

1 Cao-Lormeau V-M, Blake A, Mons S *et al.* Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet* 2016; **387:** 1531–9.
2 Musso D, Roche C, Robin E, Nhan T, Teissier A. Potential sexual transmission of Zika. *Virus* 2015; **21:** 2013–5.
3 Motta IJF, Spencer BR, da Silva SGC *et al.* Evidence for transmission of Zika virus by platelet transfusion. *N Engl J Med* 2016; **375:** 1099–101.
4 Besnard M, Lastère S, Teissier A, Cao-Lormeau VM, Musso D. Evidence of perinatal transmission of zika virus, French Polynesia, December 2013 and February 2014. *Eurosurveillance* 2014; **19:** 8–11.
5 De Oliveira Melo AS, Aguiar RS, Amorim MMR *et al.* Congenital Zika virus infection: Beyond neonatal microcephaly. *JAMA Neurol* 2016; **73:** 1407–16.
6 Brasil P, Pereira JP, Moreira ME *et al.* Zika virus infection in pregnant women in Rio de Janeiro. *N Engl J Med* 2016; **15:** 2321–34.
7 Tabata T, Petitt M, Puerta-Guardo H *et al.* Zika virus targets different primary human placental cells, suggesting two

 © 2018 The Association for the Publication of the Journal of Internal Medicine

13652796, 2019, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/joim.12829 by CAPES, Wiley Online Library on [07/06/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

routes for vertical transmission. *Cell Host Microbe* 2016; **20:** 155–66.
8 Nguyen SM, Antony KM, Dudley DM *et al.* Highly efficient maternal-fetal Zika virus transmission in pregnant rhesus macaques. *PLoS Pathog* 2017; **13:** 1–22.
9 Wang L, Valderramos SG, Wu A *et al.* From mosquitos to humans: genetic evolution of Zika virus. *Cell Host Microbe* 2016; **19:** 561–5.
10 Ye Q, Liu Z-Y, Han J-F, Jiang T, Li X-F, Qin C-F. Genomic characterization and phylogenetic analysis of Zika virus circulating in the Americas. *Infect Genet Evol* 2016; **43:** 43–9.
11 Wang A, Thurmond S, Islas L, Hui K, Hai R. Zika virus genome biology and molecular pathogenesis. *Emerg Microbes Infect* 2017; **6:** e13.
12 Caires-Júnior LC, Goulart E, Melo US *et al.* Discordant congenital Zika syndrome twins show differential *in vitro* viral susceptibility of neural progenitor cells. *Nat Commun* 2018; **9:** 1–11.
13 Zhang X, Liu H, Xie Z *et al.* Epigenetically regulated miR-449a enhances hepatitis B virus replication by targeting cAMP-responsive element binding protein 5 and modulating hepatocytes phenotype. *Sci Rep* 2016; **6:** 1–13.
14 Farquhar MJ, Harris HJ, Diskar M *et al.* Protein kinase A-dependent step (s) in Hepatitis C Virus Entry. *J Virol* 2008; **82:** 8797–811.
15 Moreno-fernandez ME, Rueda CM, Rusie LK, Chougnet CA. Regulatory T cells control HIV replication in activated T cells through a cAMP-dependent mechanism. *Blood* 2017; **117:** 5372–81.
16 Olmo IG, Carvalho TG, Costa VV *et al.* Zika virus promotes neuronal cell death in a non-cell autonomous manner by triggering the release of neurotoxic factors. *Front Immunol* 2017; **8:** 1–14.
17 Riva L, Song O, Prentoe J *et al.* Identification of Piperazinyl-benzenesulfonamides As New Inhibitors of Claudin-1 Trafficking and Hepatitis C Virus Entry. *J Virol* 2018; **92:** e01982–17. Available from: http://jvi.asm.org/lookup/doi/10.1128/JVI.01982-17
18 Campos G, Bandeira A, Sardi S. Zika virus outbreak, Bahia Brazil. *Emerg Infect Dis* 2015; **21:** 1881.
19 Vaser R, Adusumalli S, Leng SN, Sikic M, Ng PC. PROTOCOL UPDATE SIFT missense predictions for genomes. *Nat Protoc* 2009; **4:** 1073–82.
20 Adzhubei IA, Schmidt S, Peshkin L *et al.* A method and server for predicting damaging missense mutations. *Nat Methods* 2010; **7:** 248–9.
21 Schwarz JM, Cooper DN, Schuelke M, Seelow D. Mutationtaster2: mutation prediction for the deep-sequencing age. *Nat Methods* 2014; **11:** 361–2.
22 Shihab HA, Gough J, Cooper DN *et al.* Predicting the functional, molecular, and phenotypic consequences of amino acid substitutions using hidden markov models. *Hum Mutat* 2013; **34:** 57–65.
23 Kircher M, Witten DM, Jain P, O'roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet* 2014; **46:** 310–5.
24 Quang D, Chen Y, Xie X. DANN: a deep learning approach for annotating the pathogenicity of genetic variants. *Bioinformatics* 2015; **31:** 761–3.
25 Price AL, Zaitlen NA, Reich D, Patterson N. New approaches to population stratification in genome-wide association studies. *Nature Reviews Genetics* 2012; **11:** 1–20.
26 Lee S, Emond MJ, Bamshad MJ *et al.* Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. *Am J Hum Genet* 2012; **91:** 224–37.
27 Buck J, Sinclair ML, Schapal L, Cann MJ, Levin LR. Cytosolic adenylyl cyclase defines a unique signaling molecule in mammals. *Proc Natl Acad Sci* 1999; **96:** 79–84.
28 Klein M, Bopp T. Cyclic AMP represents a crucial component of treg cell-mediated immune regulation. *Front Immunol* 2016; **7:** 1–5.
29 Truttmann MC, Ploegh HL. rAMPing Up Stress Signaling: protein AMPylation in Metazoans. *Trends Cell Biol* 2017; **27:** 608–20.
30 Moretti C, Mencacci C, Frajese GV, Cerilli M, Frajese G. Growth hormone-releasing hormone and pituitary adenylate cyclase-activating polypeptide in the reproductive system. *Trends Endocrinol Metab* 2002; **13:** 428–35.
31 Nicol X, Gaspar P, Curie M. Routes to cAMP : shaping neuronal connectivity with distinct adenylate cyclases. *Eur J Neurosci* 2014; **39:** 1742–51.
32 Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. *Endocr Rev* 2014; **35:** 851–905.
33 Gupta SK, Malhotra SS, Malik A, Verma S, Chaudhary P. Cell signaling pathways involved during invasion and syncytialization of trophoblast cells. *Am J Reprod Immunol* 2016; **75:** 361–71.
34 Weedon-Fekjær MS, Taskén K. Review: spatiotemporal dynamics of hCG/cAMP signaling and regulation of placental function. *Placenta* 2012; **33(SUPPL.):** S87–91.
35 Schanton M, Maymó JL, Pérez Pérez A, Sánchez- V, Varone CL. Involvement of leptin in the molecular physiology of the placenta 1. *Reproduction* 2015; **155:** R1–R12.
36 Consortium GTex. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* 2015; **348:** 648–60.
37 Mosenden R, Taskén K. Cyclic AMP-mediated immune regulation - Overview of mechanisms of action in T cells. *Cell Signal* 2011; **23:** 1009–16.
38 Wall EA, Zavzavadjian JR, Chang MS *et al.* Suppression of LPS induced TNF-a production in marcophages by cAMP is mediated by PKA-AKAP95-p105. *Sci Signal* 2009; **2:** 1–17.
39 McDonough KA, Rodriguez A. The myriad roles of cyclic AMP in microbial pathogens: from signal to sword. *Nat Rev Microbiol* 2012; **10:** 27–38.
40 Pulliainen AT, Pieles K, Brand CS *et al.* Bacterial effector binds host cell adenylyl cyclase to potentiate G s-dependent cAMP production. *Proc Natl Acad Sci* 2012; **109:** 9581–6.
41 Olmos-Ortiz A, García-Quiroz J, Avila E *et al.* Lipopolysaccharide and cAMP modify placental calcitriol biosynthesis reducing antimicrobial peptides gene expression. *Am J Reprod Immunol* 2018; **79:** e12841. Available from: https://doi.org/doi.wiley.com/10.1111/aji.12841
42 Cheng F, Ramos da Silva S, Huang I-C, Jung JU, Gao S-J. Suppression of Zika virus infection and replication in endothelial cells and astrocytes by PKA inhibitor PKI 14-22. *J Virol* 2017; **92:** e02019–17. Available from: http://jvi.asm.org/lookup/doi/10.1128/JVI.02019-17
43 Freire-Maia N. Inbreeding in Brazil. *Am J Hum Genet* 1957; **9:** 284–98.

© 2018 The Association for the Publication of the Journal of Internal Medicine

13652796, 2019, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/joim.12829 by CAPES, Wiley Online Library on [07/06/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

44 Santos S, Da Silva Pequeno AA, Pessoa A *et al.* Increased prevalence of inherited neuromuscular disorders due to endogamy in Northeast Brazil: the need of community genetics services. *J Community Genet* 2014; **5:** 199–203.
45 Weller M, Tanieri M, Pereira JC, Almeida EDS, Kok F, Santos S. Consanguineous unions and the burden of disability: a population-based study in communities of Northeastern Brazil. *Am J Hum Biol* 2012; **24:** 835–40.
46 de Neves Saloum, Manta F, Pereira R *et al.* Revisiting the genetic ancestry of brazilians using autosomal AIM-Indels. *PLoS ONE* 2013; **8:** 1–11.
47 Fernanda Lima-Costa M, Rodrigues LC, Barreto ML *et al.* Genomic ancestry and ethnoracial self-classification based on 5,871 community-dwelling Brazilians (The Epigen Initiative). *Sci Rep* 2015; **5(9812).**

Correspondence: Constantine A. Stratakis, Section on Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA.
(fax: +1 301 402 0574; e-mail: stratakc@cc1.nichd.nih.gov).
and
Cynthia Chester Cardoso, Laboratório de Virologia Molecular, Instituto de Biologia, UFRJ, Av Carlos Chagas Filho 373, CCS, Bloco A, Sala 121, Ilha do Fundão, RJ 21941-902, Brazil.
(fax: +55 21 3938 6396; e-mail: cynthiac@biologia.ufrj.br).

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Single nucleotide variations associated with congenital Zika syndrome in the cohort from Campina Grande, Brazil.

13652796, 2019, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/joim.12829 by CAPES, Wiley Online Library on [07/06/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License