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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Supplementary Materials

Supplemental Methods

UCSF Center for Next-Gen Precision Diagnostics Metagenomic Next-Generation Sequencing

Briefly, total nucleic acid extract from lysed samples was enriched for microbial nucleic acid and prepared into DNA (methyl-DNA reduced) and RNA (DNase treated) libraries. Barcoded libraries were sequenced by Illumina NextSeq 550 instrument with at least 5 million reads and analyzed using the SURPI+ bioinformatics pipeline for pathogen detection.[3,4]

UCSF Generation of Gene Expression Based Classifier Models

FASTQ files from patients who had clinical CSF mNGS testing performed at UCSF were preprocessed for removal of primers and low-quality or low-complexity sequences.[13] The resulting reads were aligned using STAR.[12] After exclusion of long non-coding RNAs, read counts were log transformed. Assignment of patient samples as autoimmune / non-infectious diseases or viral infection was performed by review of the patient electronic medical record under protocols approved by the UCSF institutional review board (IRB).

Samples were partitioned into training and test sets in an approximately 80%/20% ratio. Training set samples (n=117 samples from patients with autoimmune / non-infectious disease, 117 samples from patients with viral infection) were used to generate the final model. For selection of differentially expressed genes to include in the model, feature selection using LassoCV from Scikit-learn 0.20.4 (Python software) was performed using iterative cycles of cross-validation and gene pruning (10 cycles of 5 80%/20% cross-validation splits) to identify appropriate parameters for the final model. The final highest performing model yielded in 48 genes, 33 associated with autoimmune / non-infectious disease and 13 with viral infection. Heatmaps and

dendrograms were generated using pheatmap version 1.0.12 in R 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). RNA gene expression levels were log-transformed and normalized prior to visualization.

Infectious Diseases Pathology Branch Immunohistochemistry (IHC)

For identification of yellow fever (YF) virus in tissue specimens, IHC was performed using monoclonal antibody against 17D YF vaccine strain. This antibody shows cross-reactivity with Zika and dengue viruses but no cross-reactivity with chikungunya or Japanese encephalitis viruses. Prepared 4µm tissue sections were incubated at ambient temperature for 30 minutes with 1:200 dilution of 17D anti-NS1 monoclonal antibody (clone 1D6) followed by sequential incubation with polymer-based indirect aminoalkane phosphatase detection system (MACH 4Universal AP Polymer Kit, Biocare Medical, Pacheco, California). The antibody/polymer conjugate was visualized by Permanent Red Chromogen (Cell Marque, Rocklin, California). Tissue sections were counterstained in Mayer's modified hematoxylin (Poly Scientific R&D Corp., Bay Shore, New York) for microscopy examination. Uninfected mouse serum was used as a non-specific control for primary antibody incubation with sequential tissue sections from case patients. Specifically for the heart recipient, sections of dentate, hippocampus, pons, and basal ganglia were examined for YF virus RNA using primers and probes targeting the 5' noncoding region (NCR) and 3' untranslated region.

Arboviral Diseases Branch Metagenomic Next-Generation Sequencing

Briefly, RNA was treated with DNase to remove host genome, followed by cDNA synthesis (Tecan Genomics), generation of sequencing libraries, and read assembly, as previously described.[9] Libraries were prepared for sequencing using the Ion Chef 530 sequencing kit and

sequenced on the Genestudio S5 instrument (Thermo Fisher). Specimens were sequenced on 530 chips (Thermo Fisher) resulting in an average of 8 million reads per sample. Individual sequence reads with matches to viral genes were identified using custom Python scripts that separated reads into individual FASTA files and submitted to the Basic Local Alignment Search Tool nucleotide (BLASTn) program using default parameters. Output data were formatted to include alignment quality information (alignment length, percentage of identical matches, number of mismatches, number of gap openings, E-values, the start and end of the alignment in the query, and subject and bitscores) and was filtered by reads that aligned to viral genes.

Supplemental Table. Test results for diseases[a] other than yellow fever on specimens from organ and tissue recipients

Patient	Days relative to transplant	Specimen	Pathogen	Test	Result
Right kidney recipient	31	NP swab	SARS-CoV-2, influenza A/B	PCR	Negative
	32	NP swab	SARS-CoV-2	PCR	Negative
	36	CSF	Multiple pathogens	Biofire® FilmArray® ME panel	Negative
			Human polyoma virus 2	PCR	Negative
			Treponema pallidum	VDRL	Negative
			Histoplasma capsulatum	Antibody	Negative
			Cryptococcus neoformans	Antigen	Negative
			Acid-fast bacilli (AFB)	Smear, culture	Negative
			Fungi	Culture, Fungitell®	Negative
			Toxplasma gondii	PCR	Negative
			Epstein Barr virus (EBV)	PCR (quantitative)	Negative
	36	Serum	Fungi	β-D-glucan	Negative
	40	CSF	Multiple pathogens	Biofire® FilmArray® ME panel	Negative
			WNV, SLEV, EEEV, POWV, HRTV, CVV, CAL serogroup	Real-time RT-PCR	Negative
			WNV	IgM ELISA	Negative
			SLEV, EEEV, WEEV, CAL serogroup	IgG	Negative
			WNV, POWV	E & NS1 polyvalent MIA	Negative
			LCMV	PCR	Negative
			HSV-1, HSV-2, HHV-6, VZV, CMV, EBV, Adenovirus	Real-time PCR	Negative
			Enterovirus	RT and Real-time PCR	Negative
			Alphavirus	RT-PCR	Negative

	40	Serum	WNV, SLEV, EEEV, POWV, HRTV, CVV, CAL serogroup	Real time RT-PCR	Negative
			WNV	IgM ELISA	Negative
			SLEV, EEEV, WEEV, CAL serogroup	IgG	Negative
			WNV, POWV	E & NS1 polyvalent MIA	Negative
			LCMV	PCR, sequencing	Negative
			Alphavirus	RT-PCR	Negative
	41	Serum	*Ehrlichia chafeensis*, *E. ewingii/caris, E. muris-like, Anaplasma phagocytophilum*	PCR	Negative
			Rickettsia rickettsii	IgM/IgG ELISA	Negative
			Aspergillus spp	Galactomannan	Negative
			HTLV	ELISA	Negative
	42	Serum	Coxsackie B (1–6)	Antibody	Negative
	43	Serum	HBV, HCV	PCR	Negative
	43	Urine	Microsporidia	PCR	Negative
	44	Stool	Microsporidia	PCR	Negative
	49	Stool	*Clostridioides difficile*	Toxin NAAT	Negative
Heart recipient	9, 17, 23	Heart tissue	Microsporidia, human polyoma virus	IHC	Negative
	31	BAL cell block	*Encephalitozoon cuniculi*, human polyoma virus	IHC	Negative
	29	CSF	Multiple pathogens	Biofire® FilmArray® ME panel	Negative
			C. neoformans	Antigen	Negative

			AFB	Smear, culture	Negative
			Fungi	Culture	Negative
			WNV	IgM, IgG ELISA	Negative
			CMV, EBV, human polyoma virus 2	PCR	Negative
	36	CSF	Multiple pathogens	Biofire® FilmArray® ME panel	Negative
			C. neoformans	Antigen	Negative
			Fungi	Culture	Negative
			WNV	IgM, IgG ELISA	Negative
			CMV, EBV	PCR	Negative
			Human polyoma virus 2	PCR	Positive
			Autoimmune disease	Autoimmune panel	Negative
Liver recipient	0	Liver tissue	Microsporidia, HSV-1, HSV-2	IHC	Negative
	21	Serum	CMV, EBV	PCR	Negative
	23	Stool	*C. difficile*	Toxin NAAT	Positive
			C. difficile	GDH Antigen	Negative
			Multiple pathogens	Biofire® FilmArray® GI panel	Negative
	24	Serum	WNV	IgM, IgG ELISA	Negative
			C. neoformans	Antigen	Negative
			Parvovirus B19, HHV-8, HHV-6, CMV	PCR	Negative
			HSV-1, HSV-2	PCR	Negative
			Borrelia burgdorferi	PCR, EIA	Negative
			T. pallidum	Antibody cascade/EIA	Negative
	24	CSF	Multiple pathogens	Biofire® FilmArray® ME panel	Negative
			AFB	Smear, culture	Negative
			Fungi	Culture	Negative
			Viral	Culture	Negative

			T. gondii	IgG ELISA	Negative
	26	Serum	*R. rickettsii*	IgM, IgG ELISA	Negative
			T. gondii	PCR (qualitative)	Negative
			Babesia microti	PCR	Negative
			Parasites	Smear (thick & thin)	Negative
			Plasmodium spp	Antigen	Negative
	28	Serum	BK virus	PCR (quantitative)	Negative
			EEEV, WEEV	IgM IFA, IgG IFA	Negative
			WNV, SLEV, EEEV, POWV, LACV, JCV	IgM ELISA	Negative
			JCV, CVV, HRTV	RT-PCR	Negative
			A. phagocytophilum, E. chaffeensis, B. microti	IgM ELISA, IgG ELISA	Negative
			B. duncani	IgG ELISA	Negative
			B. burgdorferi	Antibody screen	Negative
			Blastomyces dermatitidis	Antibody immunodiffusion	Negative
			C. immitis	IgM, IgG ELISA	Negative
			Paraneoplastic syndrome	Paraneoplastic antibody panel	Negative
	28	Urine	*Histoplasma capsulatum*	Antigen	Negative
	29	CSF	EEEV, WEEV	IgM IFA, IgG IFA	Negative
			Multiple pathogens	Biofire® FilmArray® ME panel	Negative
			AFB	Smear, culture	Negative
			Fungi	Culture	Negative
			LCMV	IgM ELISA, IgG ELISA	Negative
			Human polyoma virus 2	PCR	Negative
			C. immitis, H. capsulatum	Antigen quantitative EIA	Negative
	30	NP/OP swab	SARS-CoV-2	NAT	Negative

Left kidney recipient	43	Stool	Multiple pathogens	Biofire® FilmArray® GI panel	Negative
		Serum	Amoebae	Serology	Negative
	44	CSF	Multiple pathogens	Biofire® FilmArray® ME panel	Negative
			AFB	Smear, culture	Negative
			Fungi	Culture	Negative
			C. neoformans	Antigen	Negative
			Treponema pallidum	VDRL	Negative
			WNV	IgM ELISA	Negative
			Human polyoma virus 2	PCR	Negative
			Multiple pathogens	16S ribosomal RNA[b]	Negative
	44	Serum	CMV	PCR (quantitative)	Negative
			C. neoformans	Antigen	Negative
			Fungi	Galactomannan, β-D-glucan	Negative
	45	Stool/urine	Microsporidia	PCR	Negative
	46	Plasma	EBV, HHV-6	PCR	Negative
Right cornea recipient	43	Urine	Microsporidia	PCR	Negative
	45	CSF	Multiple pathogens	Biofire® FilmArray® ME panel	Negative
			Human polyoma virus 2, EBV	PCR	Negative
			C. neoformans	Antigen	Negative
			Treponema pallidum	VDRL	Negative
			AFB	Smear, culture	Negative
			Fungi	Fungitell®, culture	Negative
			WNV, SLEV, EEEV, POWV, HRTV, CVV, CAL serogroup virus	Real time RT-PCR	Negative
			IgG	IgG index	Normal
			NMDA	Antibody	Negative
			Immunoglobulins	Oligoclonal banding	Negative

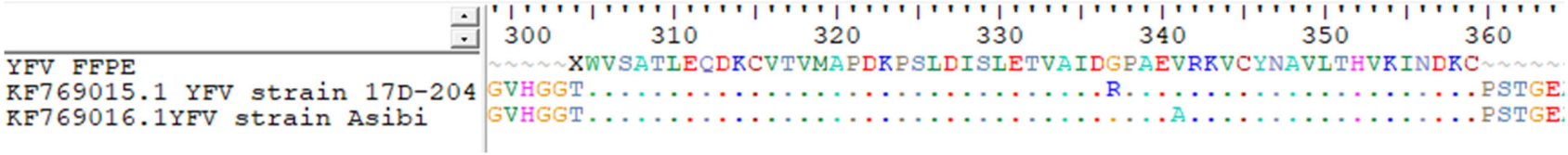
			Paraneoplastic syndrome Autoimmune disease	Paraneoplastic antibody panel Autoimmune panel	Negative Negative

Abbreviations: IHC, immunohistochemistry; Ig, immunoglobulin; ELISA, enzyme-linked immunosorbent assay; TESA, *Trypanosoma cruzi* excreted-secreted antigens; EIA, enzyme immunoassay; NP, nasopharyngeal; PCR, polymerase chain reaction; ME, meningoencephalitis; VDRL, venereal disease research laboratory; WNV, West Nile virus; SLEV, St. Louis encephalitis virus; EEEV, eastern equine encephalitis virus; POWV, Powassan virus; HRTV, Heartland virus; CVV, Cache Valley virus; CAL, California; WEEV, western equine encephalitis virus; E, envelope; NS, non-structural; MIA, microsphere immunoassay; LCMV, lymphocytic choriomeningitis virus; HSV, herpes simplex virus; HHV, human herpesvirus; VZV, varicella zoster virus; RT-PCR, reverse transcription-polymerase chain reaction; HTLV, human T-lymphotropic virus; HBV, hepatitis B virus; HCV, hepatitis C virus; NAT, nucleic acid test; GDH, glutamate dehydrogenase; GI, gastrointestinal; LACV, La Crosse virus; JCV, Jamestown Canyon virus; IFA, immunofluorescence assay; RNA, ribonucleic acid; NMDA, N-methyl-D-aspartate

a Not including routine bacterial cultures or HIV testing

b Performed at University of Washington

Supplemental Figure. Amino acid alignment of metagenomic next-generation sequencing results of RNA from brain autopsy tissue demonstrating reversion mutation in the envelope protein



A single yellow fever virus read was recovered from formalin-fixed, paraffin-embedded (FFPE) brain autopsy tissue of the heart transplant recipient covering part of the envelope (E) protein sequence. The translated sequencing read (YFV FFPE) is compared to yellow fever 17D-204 and Asibi strains of yellow fever virus. The FFPE-recovered sequence shows a mutation reverting E amino acid 52 to a glycine (Asibi strain) from an arginine (17D-204 vaccine strain). Dots in the alignment represent amino acids shared with the YFV FFPE sequence.