



How can we use genomics for the diagnosis and management of patients?

Judy Breuer



PGU
Pathogen Genomics Unit

<https://www.ucl.ac.uk/infection-immunity/pathogen-genomics-unit>



Case 1

18 month old boy

Cartilage hair hypoplasia and immunodeficiency

Mutation in *RMRP*

Mitochondrial RNA-processing
endoribonuclease

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Case 1

Peripheral blood stem cell transplant from a matched unrelated donor

- Neutrophil engraftment achieved by day 13
- Persistent stool viruses (sapovirus, adenovirus)
- Adenoviraemia from day 18
treated with cidofovir and donor-derived adenovirus-specific cytotoxic T cells

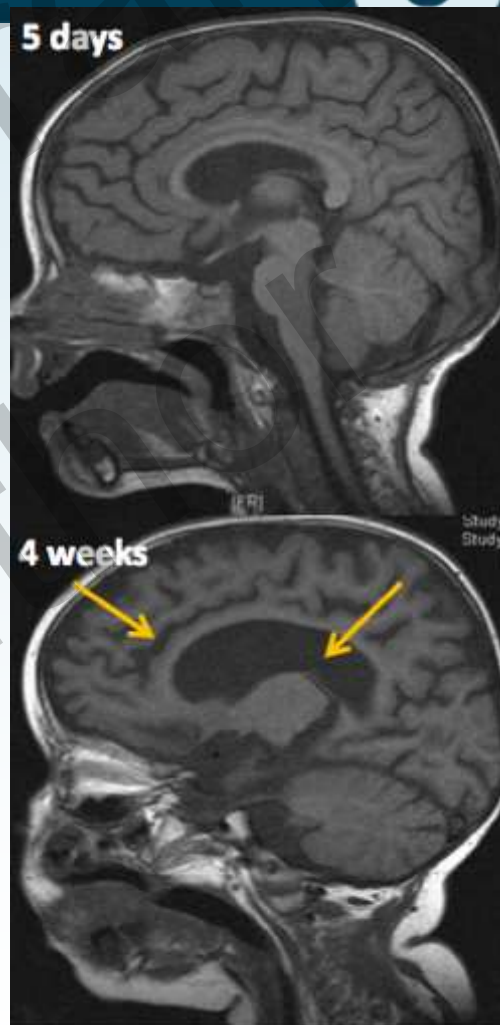


Case 1

2 week later acutely unwell

- Irritable
- Dystonia
- Reduced consciousness

Progressive cerebral atrophy





Clinical differential diagnosis

Drug toxicity

Infection

Immune reconstitution

GvHD

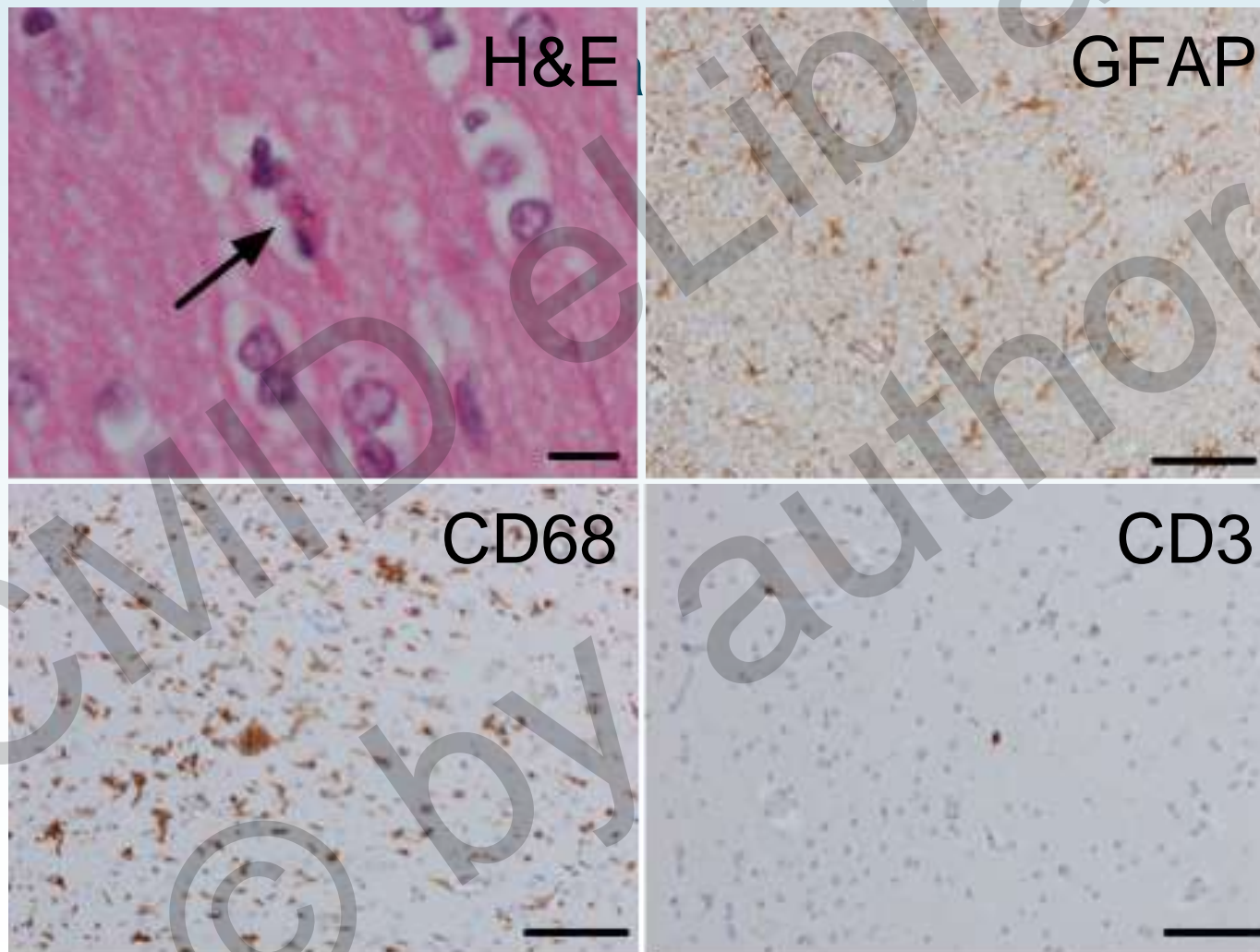
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CSF

Negative for:

- Adenovirus
- HSV 1 or 2
- VZV
- CMV
- EBV
- HHV 6 and 7
- BK & JC
- Measles
- Norovirus
- Sapovirus
- Astrovirus (HAstV 1-8)
- Rotavirus
- Enterovirus
- Parechovirus
- Toxoplasma



8 weeks after onset of neurology

4-9 cases per 100,000 per year (England)¹

2,600 – 5,850 cases per year

7.4% mortality²

Fatal cases more likely in immunocompromised patients (OR 3.44)³

71% long lasting sequelae⁴

Fatigue, problems with attention and concentration, memory loss, behavioural changes, epilepsy



Causes of encephalitis

Up to 63% of encephalitis cases no causative agent identified¹

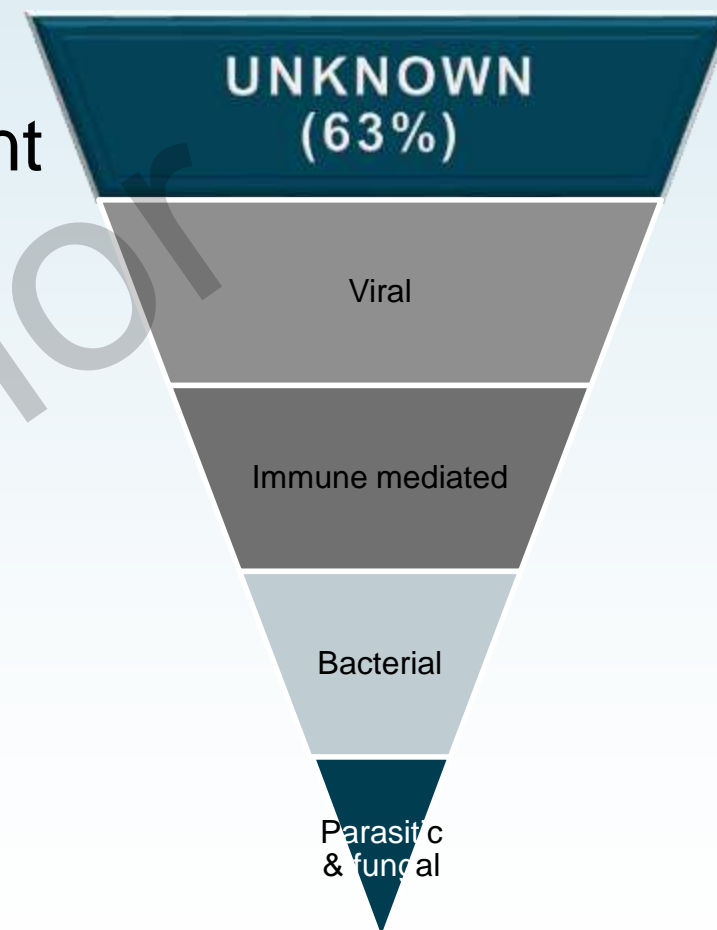
Non-infectious
(autoimmune)

Infectious
(microorganisms)

↓
Immunosuppression

Treatment

↓
**Avoid
immunosuppression**



¹Glaser *et al.* (2006).

Standard virological approach to diagnosis



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Immunocompetent

HSV-1 and -2
VZV
Enterovirus
HHV6

Immunocompromised

CMV
Adenovirus
EBV
JC

Mumps
Measles
Flu A and B

Gastro viruses (rotavirus, norovirus, astrovirus, sapovirus)
Respiratory viruses (Coronavirus, RSV, Human metapneumovirus, Parainfluenza)
Parvovirus
Rubella
Toxoplasma
Parechovirus

- Specific qPCR for each target
— 25 targets
- Need to know what you're looking for
- Specimen may be insufficient

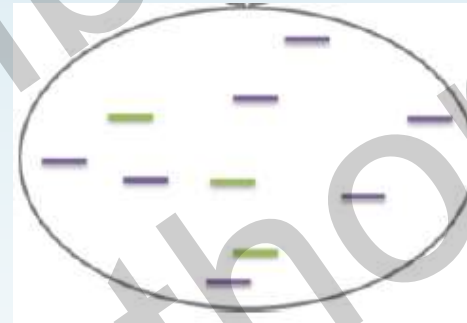


Why deep sequencing?



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- PCR:
 - Multiple targets (insufficient specimen)
 - Need to know what you're looking for
- Deep sequencing:
 - Sequence total RNA in a sample, including host and any pathogens present
 - No prior knowledge or assumptions about the type of pathogen (don't need to know what you're looking for)
 - One test: sufficient specimen
 - RNASeq: RNA genomes and transcripts



— Host RNA transcripts
— Viral RNA genomes or transcripts

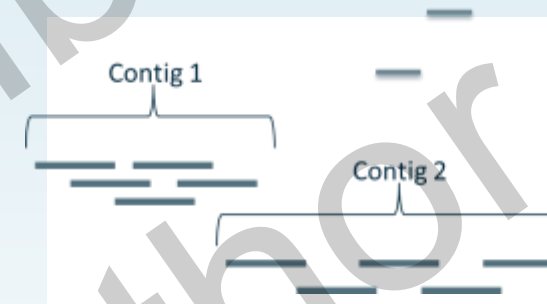
How: RNASeq



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Brain biopsy total RNA
(ribodepletion or polyA enrichment)

— Host RNA transcripts
— Viral RNA genomes or transcripts



Deep sequencing of cDNA
(NextSeq)
60 million reads



Discard Human sequences (~97%)

Assemble non-human sequences into contigs

Compare to database of known sequences

- Mixture model (MetaMix)
- Infer species and genome
 - Relative abundance
 - Assign probability



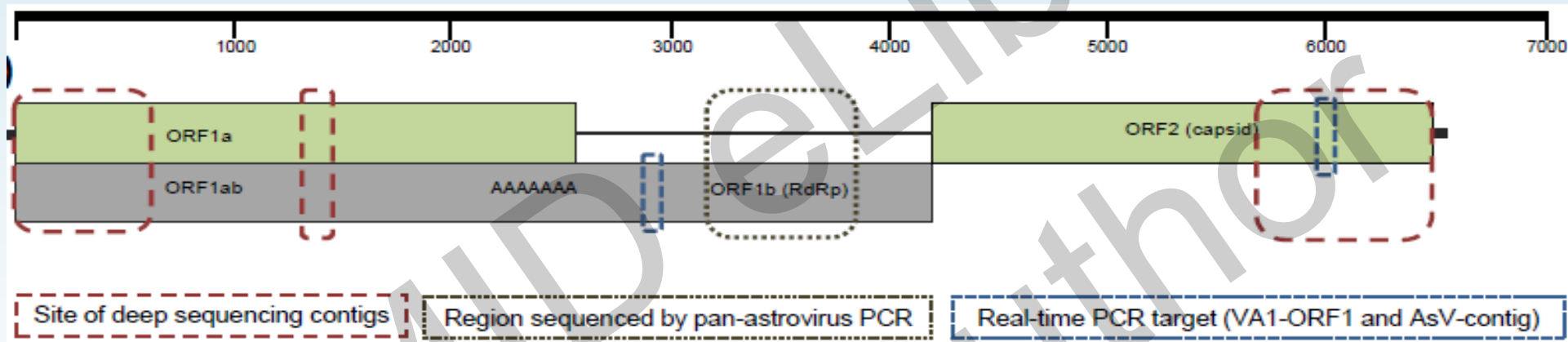
MetaMix Output

13M0217_summary_presentSpecies [Read-Only] - Microsoft Excel

taxonID	scientName	finalAssignments	poster.prob		scientName	assigned Reads	poster.prob
unknown	unknown	44848	1	human	Homo sapiens	247027	1
9606	Homo sapiens	30063	1		unknown	66759	1
10090	Mus musculus	15405	1	Environmental bacteria	Mus musculus	3650	1
1747	Propionibacterium acnes	65	1		Astrovirus VA1	579	1
525284	Gardnerella vaginalis ATCC 14019	52	1		Propionibacterium acnes	26	1
82135	Atopobium vaginae	50	1		Rothia mucilaginosa	26	1
47229	Massilia timonae	49	1		Corynebacterium pseudogenitalium	25	1
1833	Rhodococcus erythropolis	45	1		Staphylococcus capitis	19	1
69218	Enterobacter cancerogenus	44	1		Enterobacteria phage phiX174 sensu lato	19	1
13690	Sphingobium yanoikuyae	26	0.95		Neisseria mucosa	19	1
60133	Prevotella pallens	26	0.95		Alloicoccus otitis	16	1
28127	Prevotella buccalis	23	0.98		Escherichia coli O157:H7 str. EDL933	7	1
38303	Corynebacterium pseudogenitalium	20	0.9				
569	Hafnia alvei	14	0.92				

of 100 million reads

Astrovirus confirmation



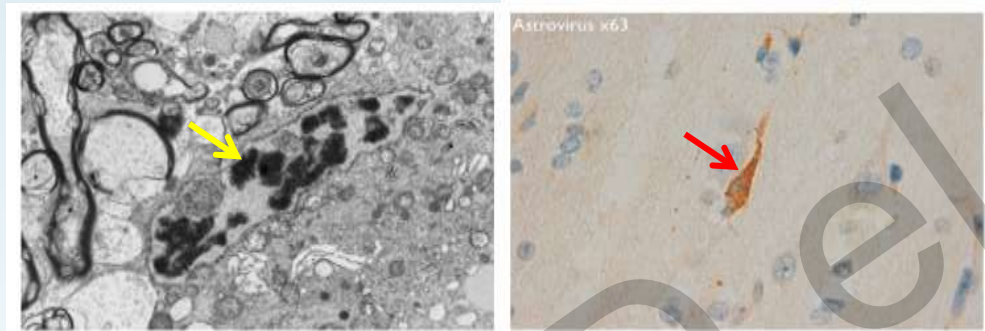
Pan-astrovirus PCR and sequencing on brain biopsy

Full genome sequencing

Histopathology

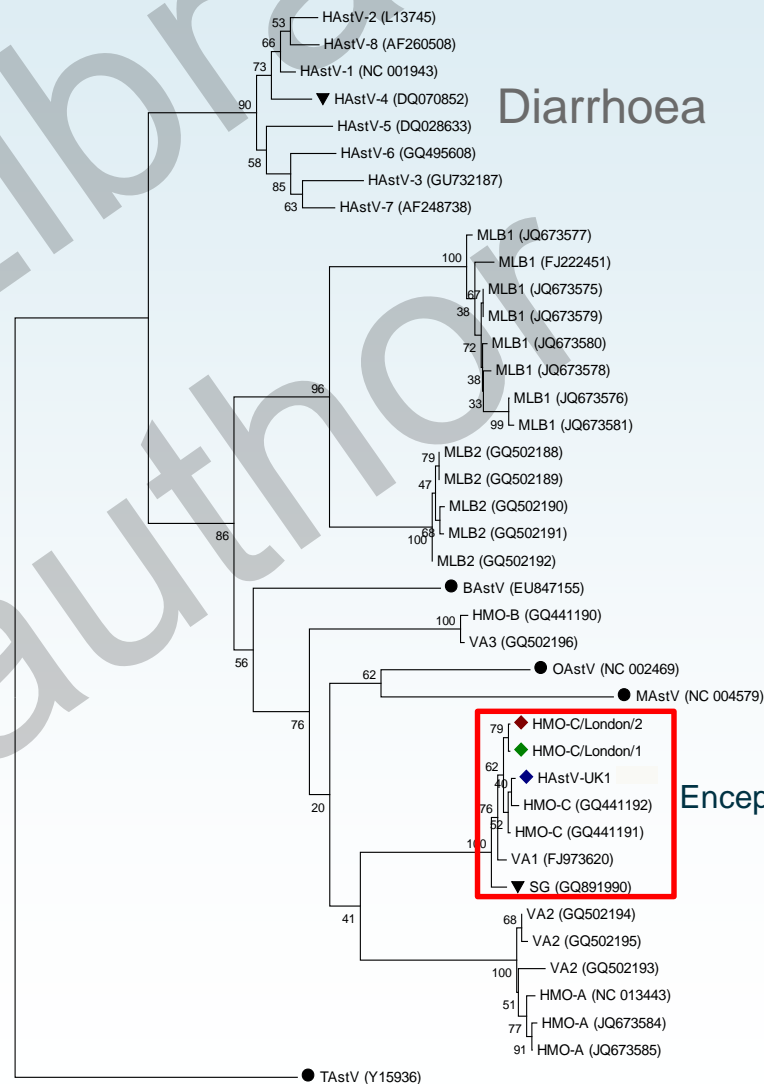
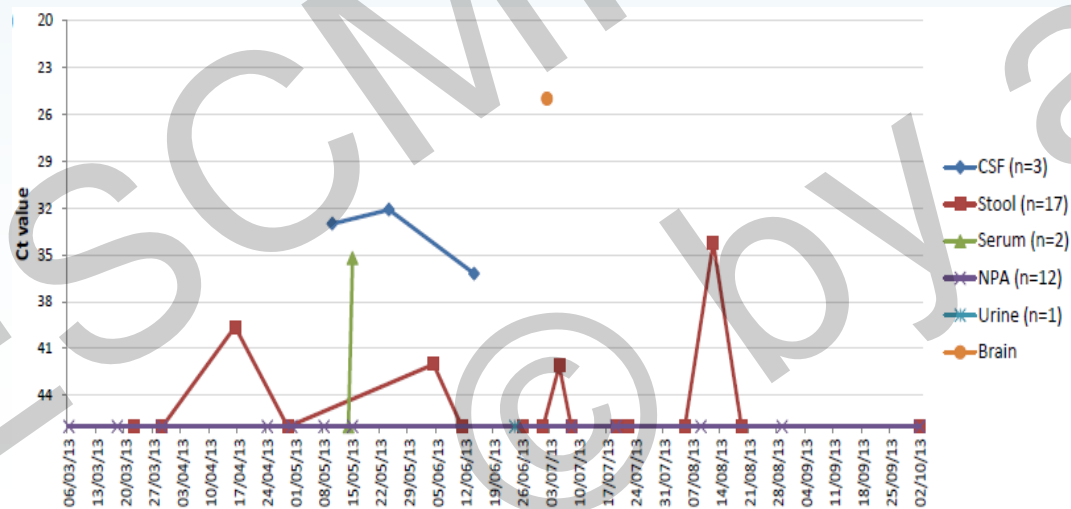
Designed real-time PCR assays

Astrovirus VA1/HMO-C



Crystalline inclusions

Specific staining of neuronal cytoplasm



Diarrhoea

Encephalitis

0.1



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Insights into Pathogenesis

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Case 2

16 month old boy

Severe Combined Immunodeficiency

RAG1 mutation (Recombination Activating Gene 1)

Delayed diagnosis until 4 months after MMR



Case 2

Treated with a CD34-selected haploidentical allogeneic stem cell transplant supported by infusions of donor lymphocytes carrying a suicide gene

Reconstitution complicated by enteropathy and autoimmune haemolysis



Case 2

Following discharge

Behavioural problems

Hearing impairment

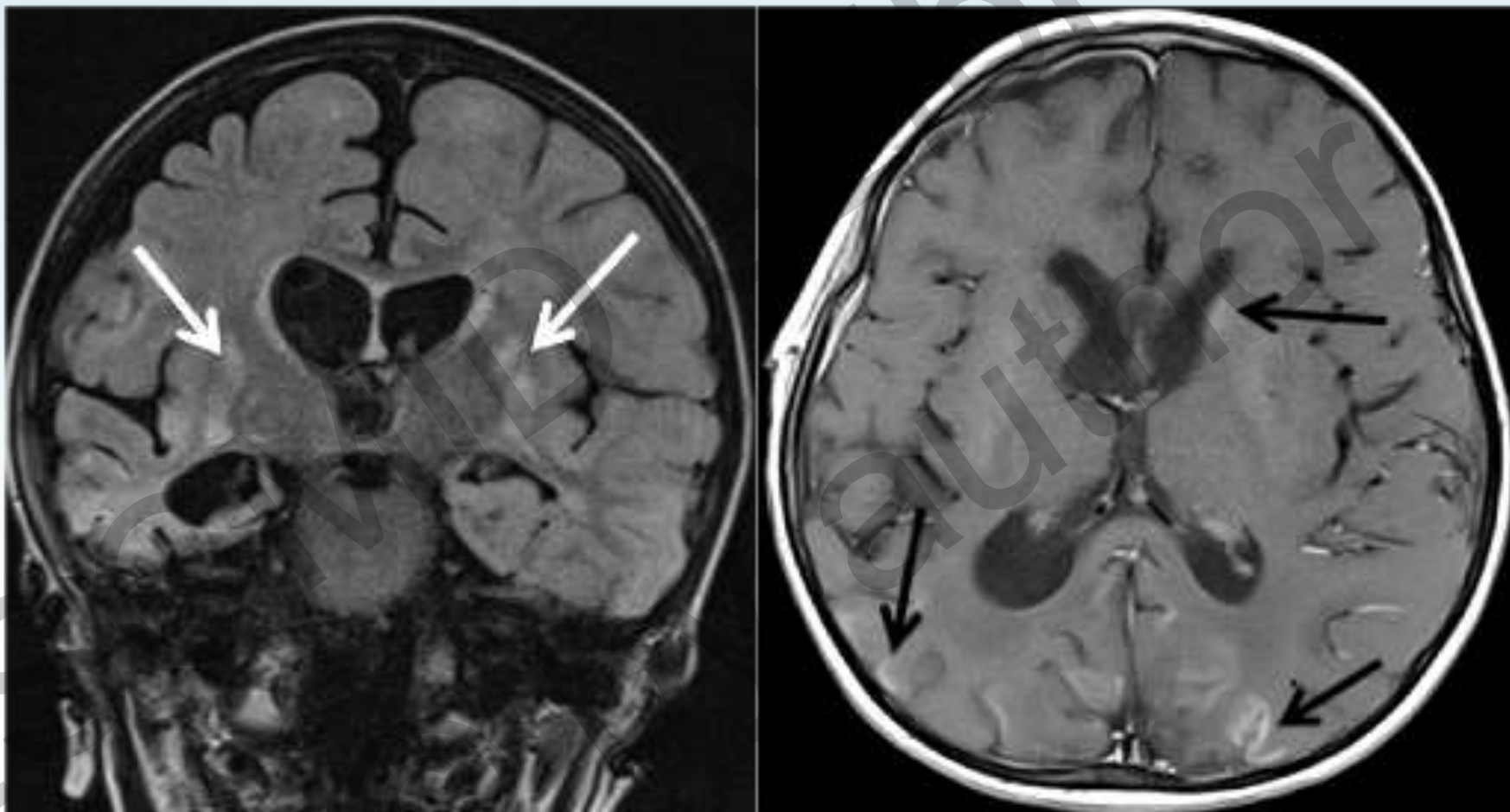
Language delay

Stable but after 2 year

Increasing refractory seizures

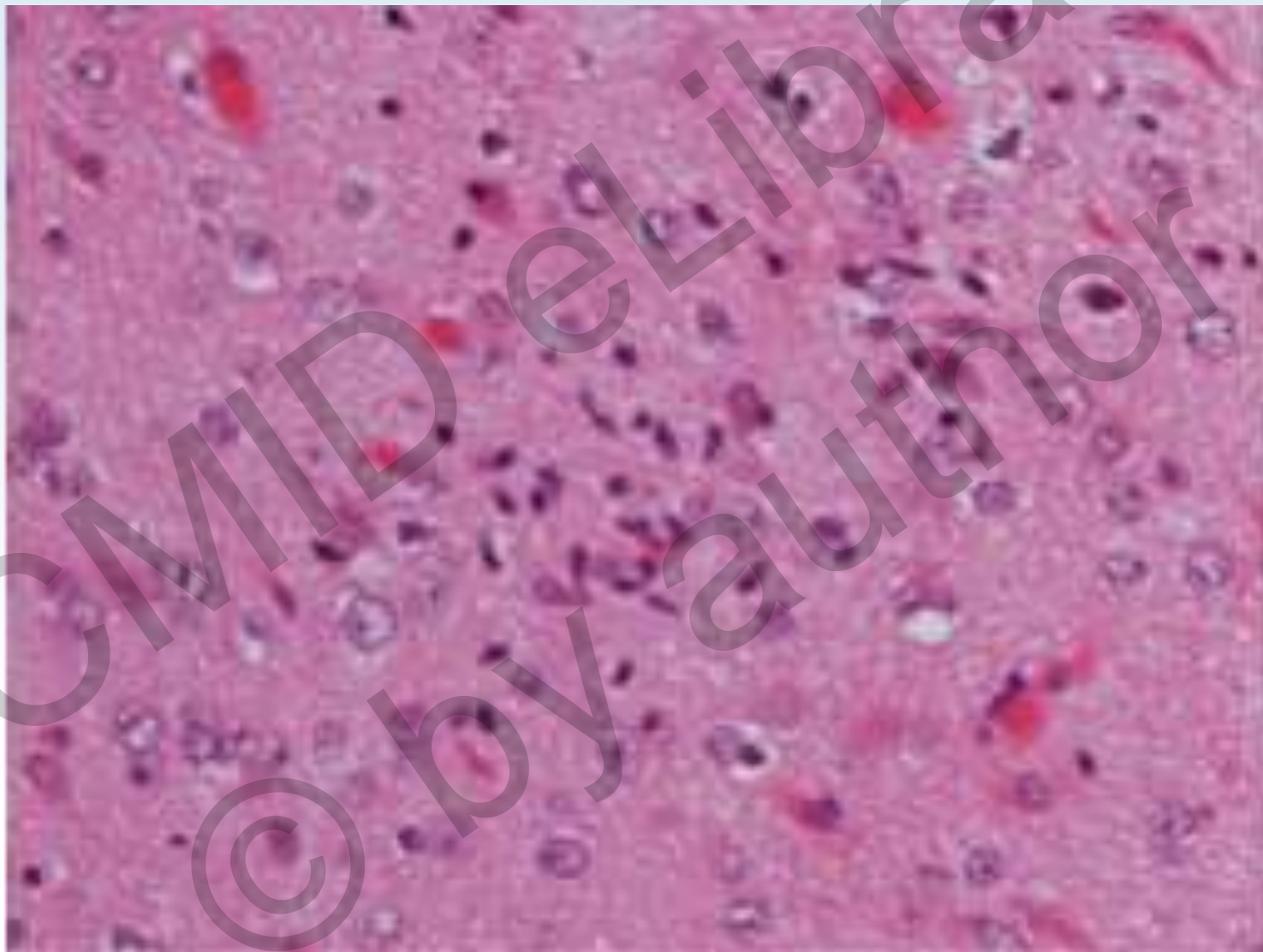
Over the following months:

lethargy, disorientation, agitation, ataxic gait and visual loss

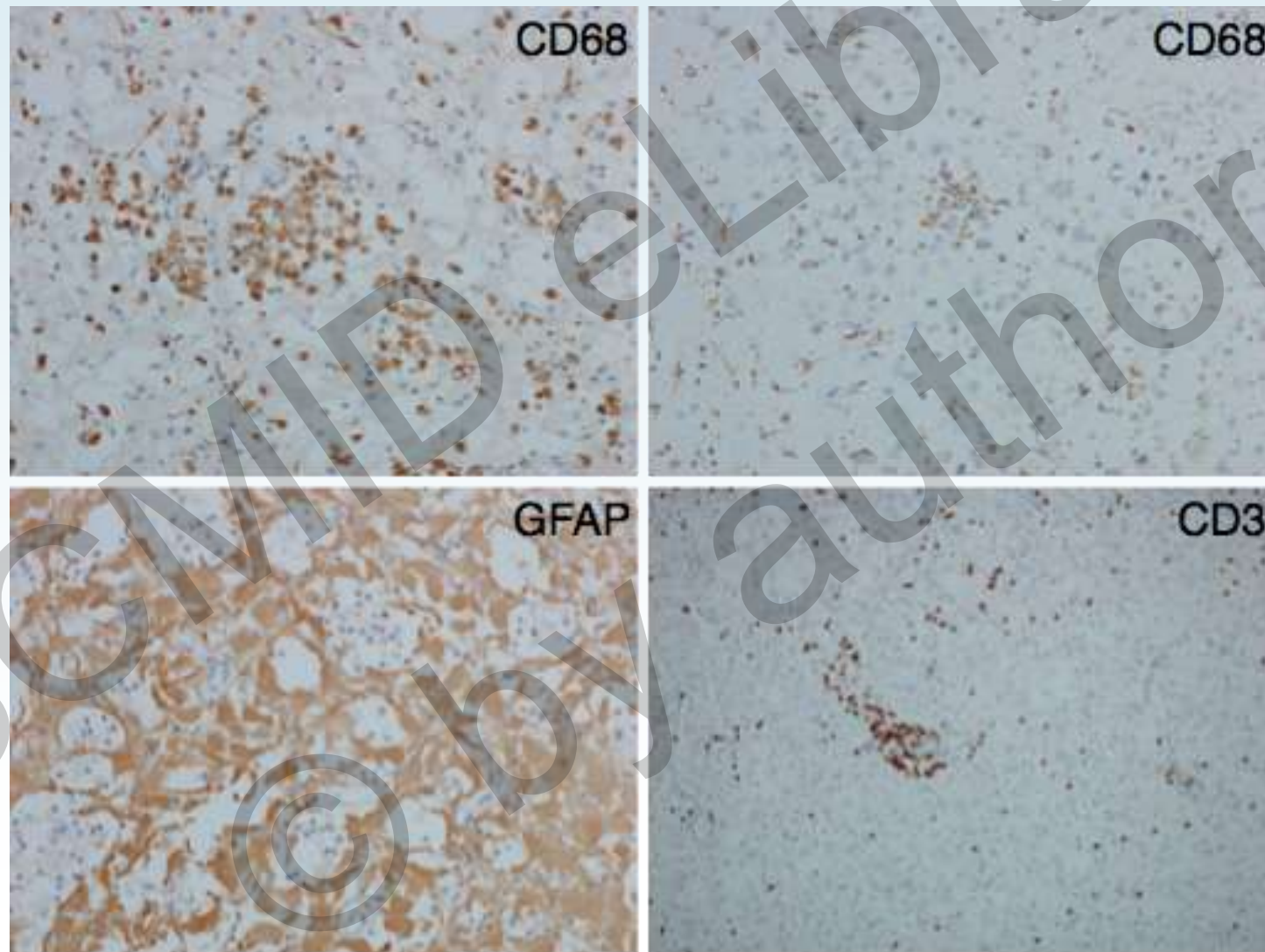




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Virus closest to Jeryl-Lynn mumps vaccine



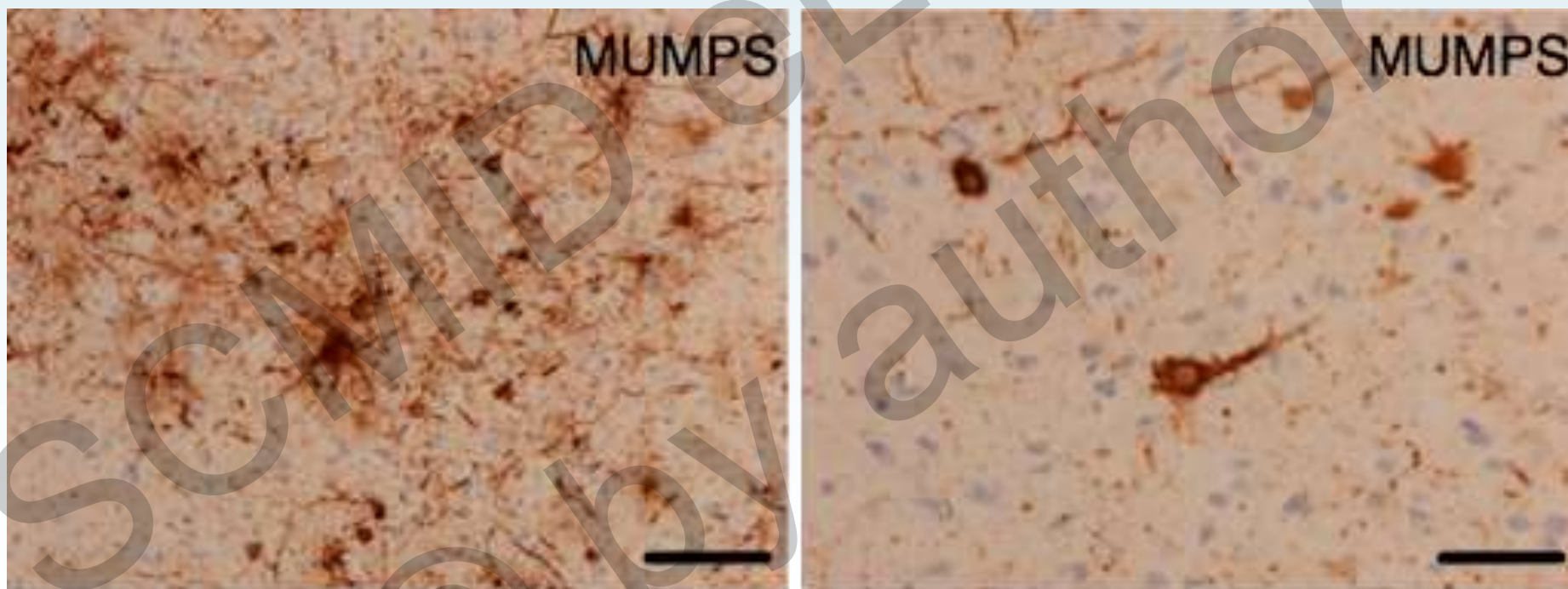
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Immunohistochemistry confirms the presence of Mumps virus



PCR: Brain positive . CSF negative

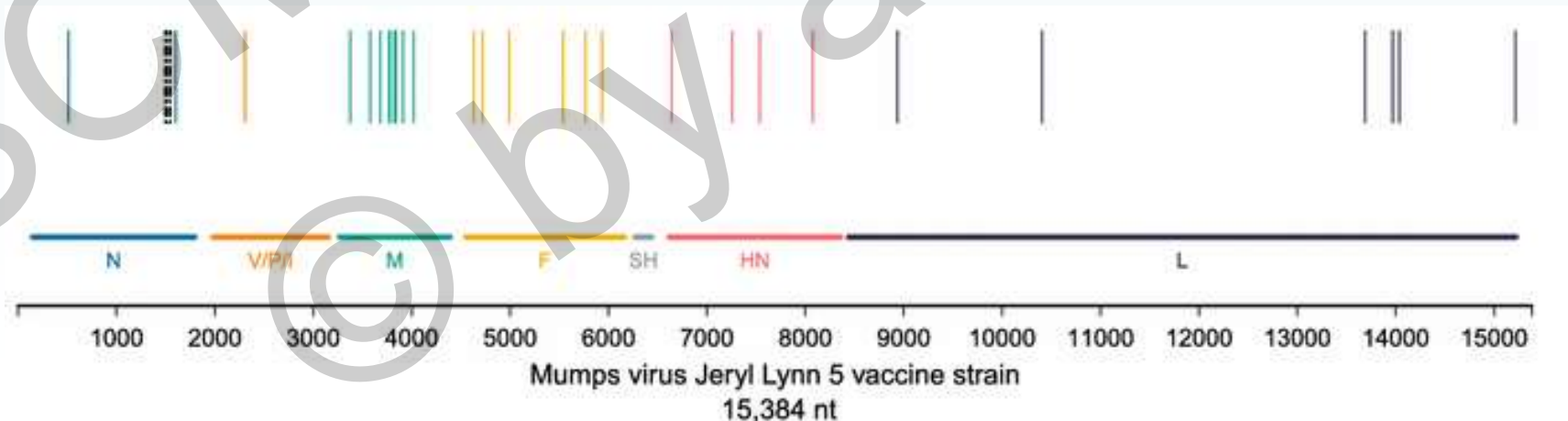
Case 3

Mumps virus

- Mumps vaccine: live attenuated vaccine, administered at 1 year of age (MMR)
- Contra-indicated in immunocompromised patients
- Given to patient before diagnosed with primary immunodeficiency
- Sequenced virus from same lot # administered to child



→ 99.6% identical to vaccine sequence



Summary of features of brain virus



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Minor variant present in the vaccine

Reversion of His140 in Matrix gene to wild type Tyr (associated with neurovirulence)

43 new mutations, 28 coding for amino acid substitutions
32% in the Matrix Protein – significantly more than by chance ($p=2.8 \times 10^{-4}$)
T to C hypermutation typical of adenosine deaminase (ADAR)

} Similar to findings for Measles virus in Subacute Sclerosing Pan Encephalitis in Measles Inclusion Body Encephalitis

Only identified by deep sequencing of brain biopsy (CSF PCR negative)

RARE AS NO EVIDENCE FOR MEASLES OR MUMPS VIRUSES FOUND INCIDENTALLY IN OTHER CASES WHERE DEEP SEQUENCING OF BRAIN MATERIAL HAS BEEN UNDERTAKEN

Detection of pathogens in encephalitides of unknown origin



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		Sample	Origin			Hx	Confirm	Published	
1	AP	BAL	GOSH	13M3410	Insufficient RNA	Fatal respiratory illness in Italy	n/a		
2	BS	CSF	UCLH	14V24130	Insufficient RNA		N/A	n/a	
3	RB	Brain biopsy e	Barts Health	14V24525	RNA degraded		N/A	n/a	
4	RF	Brain biopsy	GOSH	14M2468	No pathogens identified	Degenerative brain condition		n/a	response to steroids
5	IB	Brain biopsy	GOSH	13M1974	Astrovirus VA1/HMO-C	Fatal encephalopathy	compatible with viral encephalitis	IHC, PCR	CID
6	MA	Brain biopsy	GOSH	14V26125	Mumps vaccine	BMT	compatible with viral encephalitis	IHC, PCR	Acta neuropathologica
7	GC	Brain biopsy	GOSH	13M2664	Coronavirus OC43	Fatal undiagnosed encephalopathy (2011)	compatible with viral encephalitis	IHC, PCR	NEJM
8	EH	Oesophageal biopsy	UCLH	14V25758	No pathogens identified	Oesophageal ulcers		n/a	Re-analyse using 16S database
10	DP	Brain biopsy	Kings	14M3206	Astrovirus	Brother NFKB2	compatible with viral encephalitis	PCR & IHC	
11	AM	Brain biopsy	Royal Manchester Children's Hospital	14M3270	Astrovirus HMO-C	Died with encephalopathy following BMT	compatible with viral encephalitis	qPCR and sanger	Transpl Infect Dis.
12	RB	Brain biopsy	Barts Health	14M3205	RNA degraded	BMT	N/A		Same sample as 14V24525
13	SM	Brain biopsy	Northern Ireland Beaumont Hospital	14M3207	Mumps vaccine , rubella vaccine , HHV6	Fatal MMR related encephalitis in patient with new immunodeficiency mutation	compatible with viral encephalitis	confirmed by PCR	Sci translational medicine
14	AW	Brain biopsy	GOSH	15M0217	No pathogens identified	BMT	Acute encephalitis	n/a	response to high dose steroids
15	JM	Brain biopsy	GOSH	15M2326	No pathogens identified	Pre-BMT	no evidence of encephalitis	n/a	presumed non infectious
16		Brain biopsy	elsewhere	16V23590	Toxoplasmosis	BMY		PCR	Ribodepletion
17		Brain biopsy	Kings	16V11091	Astrovirus	brother of 10		PCR	FFPE Ribodepletion
18		Brain biopsy	Sidney	15V29279	No pathogens identified	BMT			Ribodepletion
19	DO	Brain biopsy	GOSH	15V30906	No pathogens identified	BMT			Ribodepletion
20	PH	Brain biopsy	ION	16V21500	No pathogens identified	Chronic Encephalitis			FFPE Ribodepletion
21	LF	Kdney biopsy	Belgium	15V34283	Aichivirus	XLA Chronic gastroenteritis . Renal deterioration.	?viral cause	pending	?response to Niasoxanide
22	JN	Brain biopsy	GOSH		No pathogen identified	BMT			FFPE

Publications



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The NEW ENGLAND
JOURNAL of MEDICINE

CORRESPONDENCE

Human Coronavirus OC43 Associated with Fatal Encephalitis

N Engl J Med 2016; 375:497-498 | August 4, 2016 | DOI: 10.1056/NEJM1509458

Clinical Infectious Diseases

Astrovirus VA1/HMO-C: An Increasingly Recognized Neurotropic Pathogen in Immunocompromised Patients

Julianne R. Brown,^{1,2} Sofia Morfopoulou,³ Jonathan Hubb,⁴ Warren A. Emmett,³ Winnie Ip,⁵ Divya Shah,² Tony Brooks,⁴ Simon M. L. Paine,^{7,9} Glenn Anderson,⁷ Alex Virasami,⁷ C. Y. William Tong,⁶ Duncan A. Clark,⁴ Vincent Plagnol,³ Thomas S. Jacques,¹⁵ Waseem Qasim,⁵ Mike Hubank,⁸ and Judith Breuer^{1,8}

Science Translational Medicine

Human IFNAR2 deficiency: Lessons for antiviral immunity

Christopher J. A. Duncan,^{1,2*} Siti M. B. Mohamad,^{1,3} Dan F. Young,⁴ Andrew J. Skelton,⁵ T. Ronan Leahy,⁶ Diane C. Munday,⁴ Karina M. Butler,⁶ Sofia Morfopoulou,⁷ Julianne R. Brown,^{8,9} Mike Hubank,¹⁰ Jeff Connell,¹¹ Patrick J. Gavin,⁶ Cathy McMahon,¹² Eugene Dempsey,¹³ Niamh F. Lurch,¹⁴ Thomas S. Jacques,¹⁵ Manoj Valappil,¹⁶ Andrew J. Cant,^{1,17} Judith Breuer,^{7,8} E. Randall,⁴ Sophie Hambleton^{1,17*}

Springer

ACTA NEUROPATHOLOGICA

springer.com

Acta Neuropathol (2017) 133:139–147
DOI:10.1007/s00401-016-1629-y



CASE REPORT

Deep sequencing reveals persistence of cell-associated mumps vaccine virus in chronic encephalitis

Sofia Morfopoulou¹ · Edward T. Mee² · Sarah M. Connaughton² · Julianne R. Brown³ · Kimberly Gilmour⁴ · WK 'Kling' Chong⁵ · W. Paul Duprex⁶ · Deborah Ferguson⁷ · Mike Hubank⁷ · Claran Hutchinson⁸ · Marios Kalliakatsos⁹ · Stephen McQuaid^{10,11} · Simon Paine^{8,12} · Vincent Plagnol¹³ · Christopher Ruis¹ · Alex Virasami⁸ · Hong Zhan¹⁴ · Thomas S. Jacques^{8,15} · Silke Schepelmann² · Waseem Qasim^{16,17} · Judith Breuer^{1,3}

TRANSPLANT INFECTIOUS DISEASE

AN OFFICIAL JOURNAL OF THE TRANSPLANTATION SOCIETY

Received: 18 April 2016 | Revised: 11 June 2016 | Accepted: 29 June 2016
DOI: 10.1111/itd.12807

CASE REPORT

WILEY

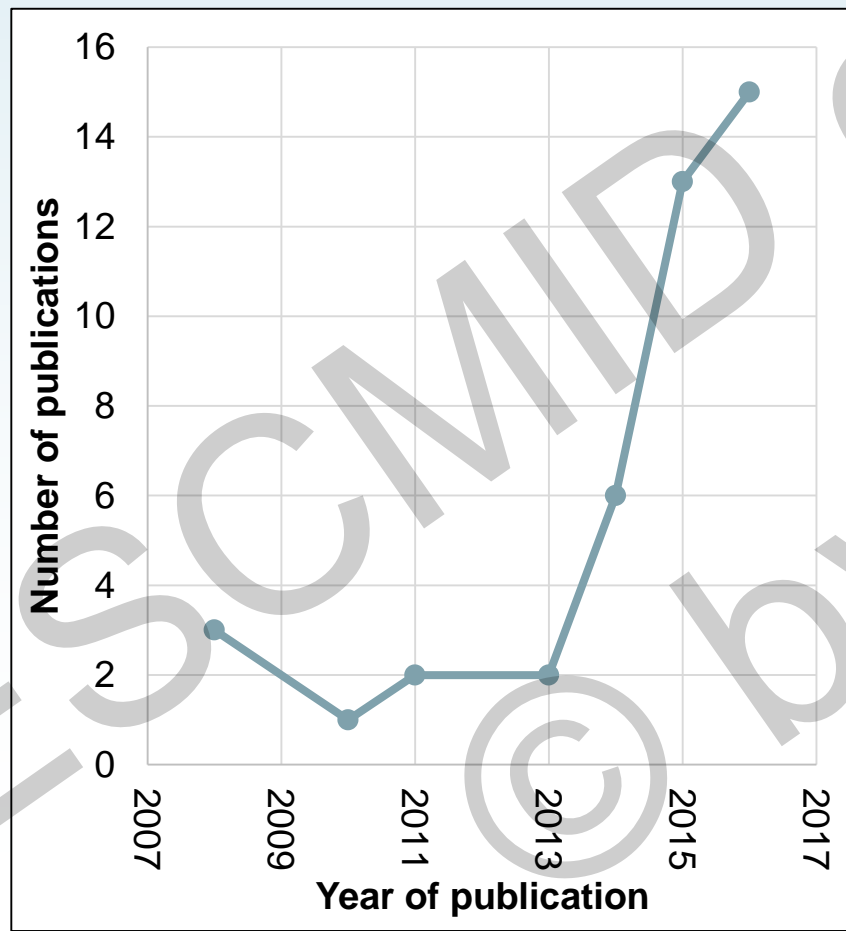
An emerging opportunistic infection: fatal astrovirus (VA1/HMO-C) encephalitis in a pediatric stem cell transplant recipient

Su Han Lum¹ | Andrew Turner² | Malcolm Gulver² | Denise Bonney¹ | Timothy Martland³ | Emma Davies² | Melanie Newbould⁴ | Julianne Brown⁵ | Sofia Morfopoulou⁶ | Judith Breuer⁶ | Robert Wynn¹

Published case reports of encephalitis and NGS



(44 cases since 2008) – most from us and one US lab

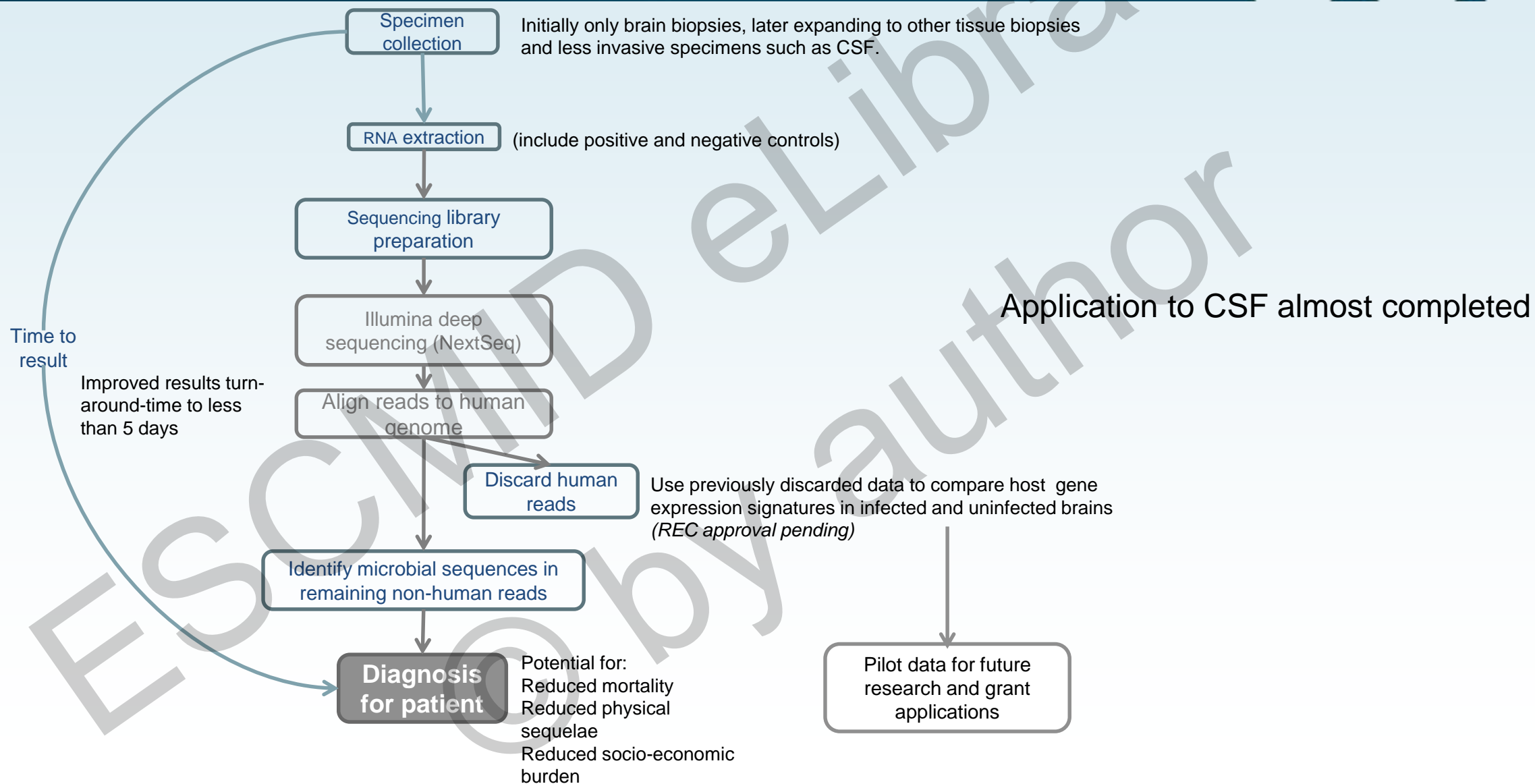


- **16 known, common causes** (HSV, entero, measles, VZV, EBV, JC, *M. tuberculosis*, mumps)
- **5 rare causes** (*Balamuthia mandrillaris*, *Brucella melitensis*, *Candida tropicalis*, *Leptospira santarosai*), **5 unexpected organisms** (PARV4, corona, astro MLB1-astrovirus, mumps vaccine)
- **14 novel organisms**
 - **astro VA1/HMO-C (n=5)**, squirrel bornavirus in squirrel breeders (n=3), *Bornavirus*, arenavirus in organ recipients (n=2) *Densovirus*, *cyclovirus*, *gemyrcircularvirus* (n=7) - ? incidental



Delivering a clinical service

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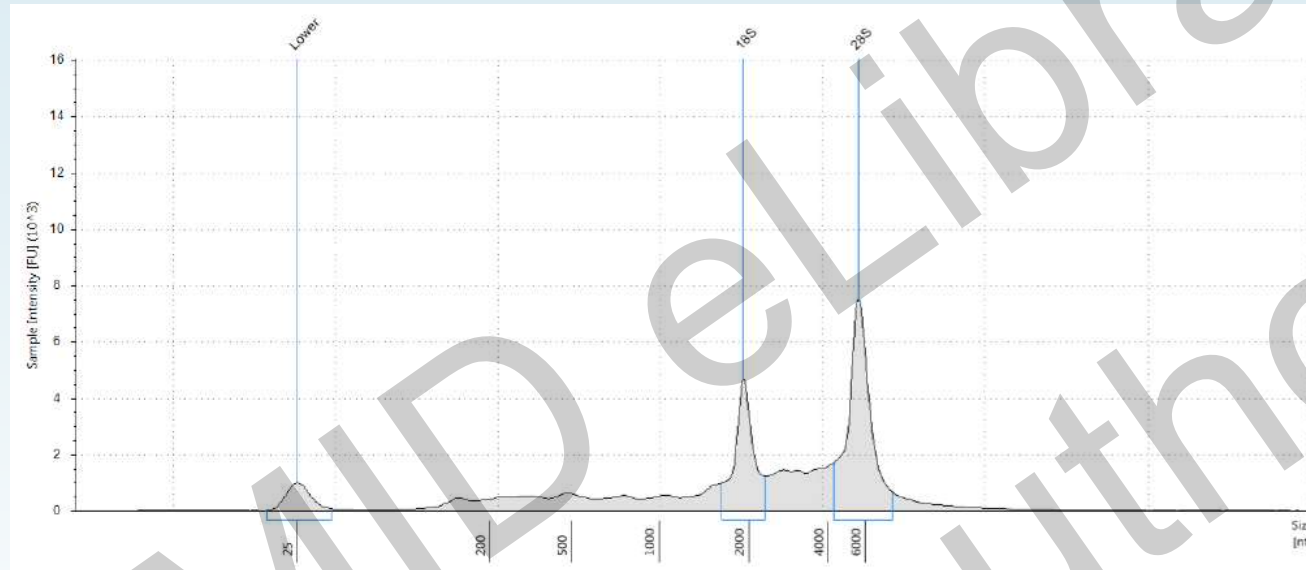


Minimising RNA degradation

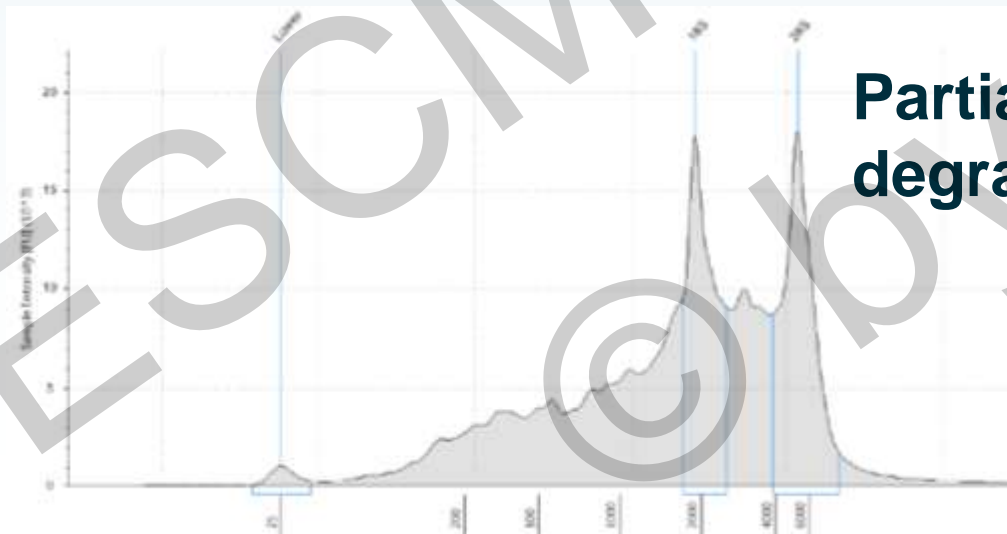


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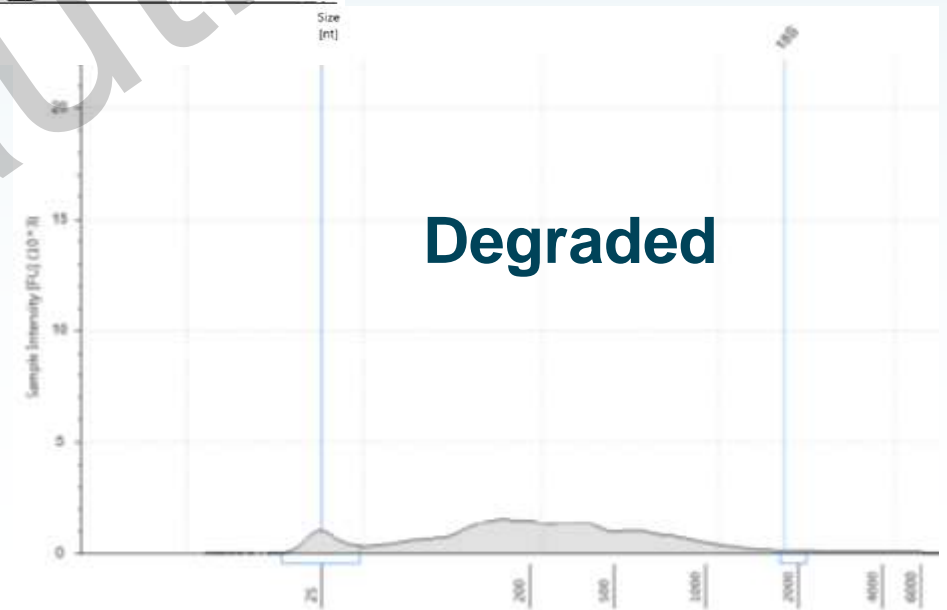
Intact RNA



Partially degraded



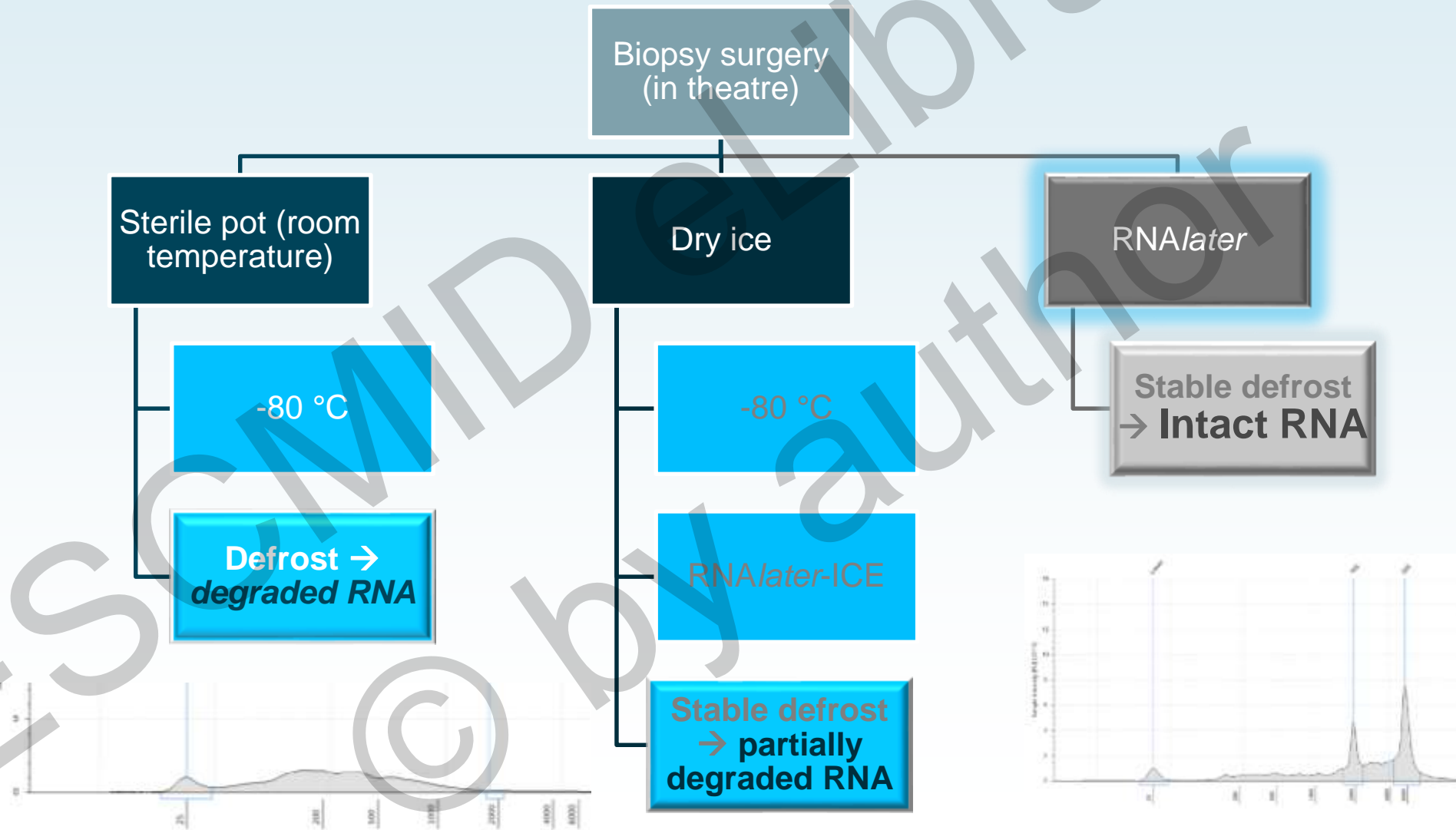
Degraded



Specimen collection (minimising RNA degradation)



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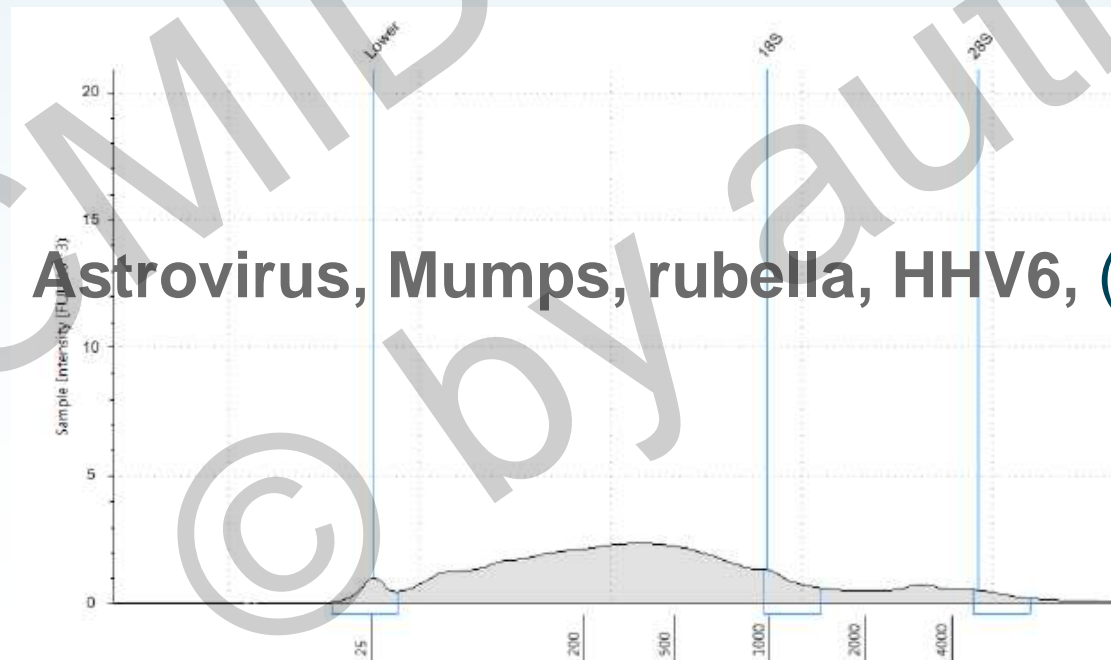


FFPE tissues



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- Formalin fixed, paraffin embedded
- Highly fragmented
- Post-mortem
- Histopathology \pm retrospective investigations
- ? Reduced sensitivity (further validation)



Astrovirus, Mumps, rubella, HHV6, (VZV)

DNA vs RNA sequencing



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Our brain biopsies: HHV6 and *Toxoplasma gondii*

Journal of the Pediatric Infectious Diseases Society

BRIEF REPORT

Neurobrucellosis: Unexpected Answer From Metagenomic Next-Generation Sequencing

Kanokporn Mongkolrattanothai^{1,2}, Samia N. Naccache^{2,3,5,*},
Jeffrey M. Bender¹, Erik Samayoa^{3,5}, Elizabeth Pham^{3,5}, Guixia Yu^{3,5},
Jennifer Dien Bard², Steve Miller^{3,5}, Grace Aldrovandi^{1,6}, and
Charles Y. Chiu^{3,5}

CSF sample	Raw reads	<i>Brucella</i> reads
DNA library	23,638,587	227 (0.0012 %)
RNA library	9,161,626	0

Likely reduced sensitivity for DNA organisms with RNASeq → DNA and RNA sequencing in parallel

Additional support



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Multidisciplinary Team meetings

monthly

- children at Great Ormond Street Hospital
- adults National Hospital for Neurological Disease Queens Square

Successes

Precision prescribing of antiviral and immunosuppressive agents

Trials of new antiviral agents

Timeline



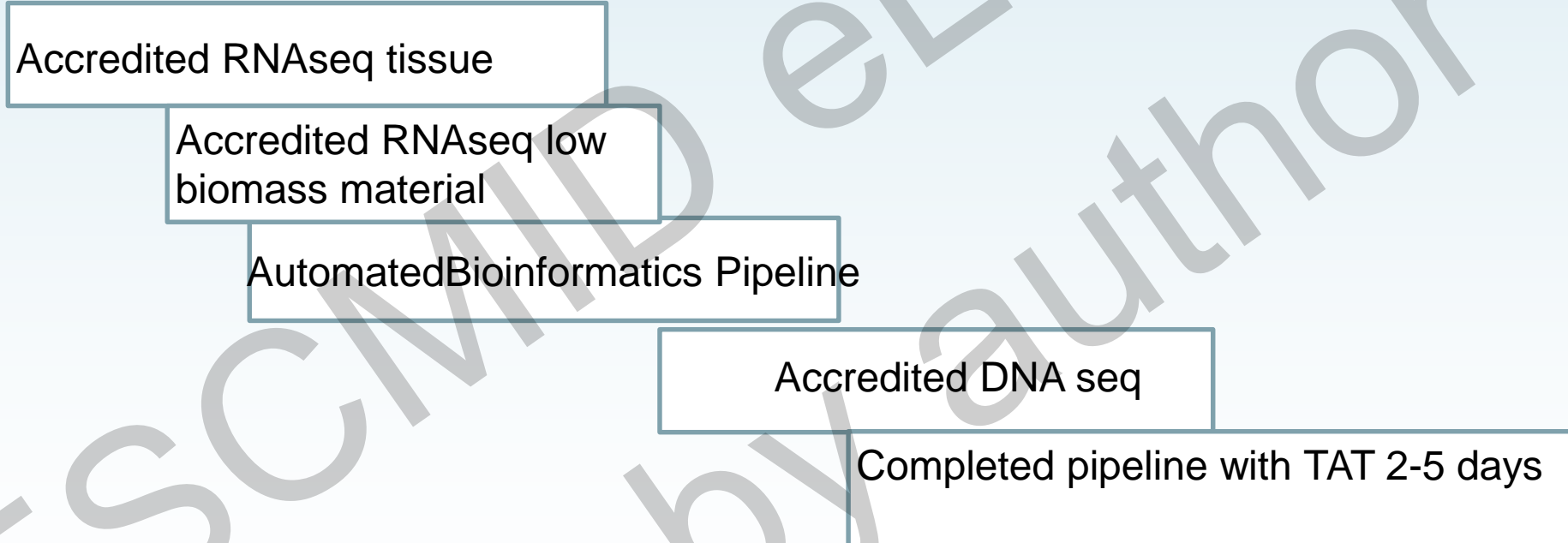
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Jan 2018

June 2018

Sept 2018

Dec 2018



Acknowledgements



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Dr Cristina Venturini

Dr Sunando Roy

Dr Rachel Williams

Helena Tutil

Erica Yara Romero

Charlotte Williams

x



Great Ormond Street 
Hospital for Children
NHS Foundation Trust

Reubens Foundation



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