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Nanopore and Illumina sequencing for pathogen metagenomics and host transcriptomics of cerebrospinal fluid in infantile central nervous system infections

Kazuhiro Horiba [1,4]*, Yuka Torii [2], Makoto Yamaguchi [2], Kazunori Haruta [2], Toshihiko Okumura [2], Takako Suzuki [2], Yoshihiko Kawano [3], Jun-ichi Kawada [2], Shinya Hara [3], Christian G. Giske [1], Tomoo Ogi [4], Yoshinori Ito [2,5]

[1] Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institute and Karolinska University Hospital, [2] Department of Pediatrics, Nagoya University Graduate School of Medicine, [3] Department of Pediatrics, TOYOTA Memorial Hospital, [4] Department of Genetics, Research Institute of Environmental Medicine, Nagoya University, [5] Department of Pediatrics and Child Health, Nihon University School of Medicine

Contact Information:
Kazuhiro Horiba 0000-0003-3017-299X
Karolinska Institute, 171 77 Stockholm, Sweden
E-mail: khoriba@med.nagoya-u.ac.jp
TEL: +46-76-563-6814 @cikazda

Background

Infantile central nervous system infections (CNSI) are particularly frequent for those under the age of one year and can be life-threatening and cause severe sequelae in encephalitis and bacterial meningitis[1,2].**Next-generation sequencing (NGS)** has enabled the simultaneous decoding of large-scale nucleotide sequences in a sample[3]. **Nanopore sequencing** (Oxford Nanopore Technologies, ONT), one of the long-read sequencing methods, is attracting attention because of its simplicity and rapidity for real-time sequencing in clinical diagnosis fields[4].

Objectives

(1) To detect a pathogen from CNSI clinical samples using Nanopore Sequencer
(2) To compare the performance of Nanopore and Illumina sequencer in pathogen detection
(3) To analyze host gene expression from cerebrospinal fluid (CSF) with CNSI using a Nanopore sequencer

Patients and Samples

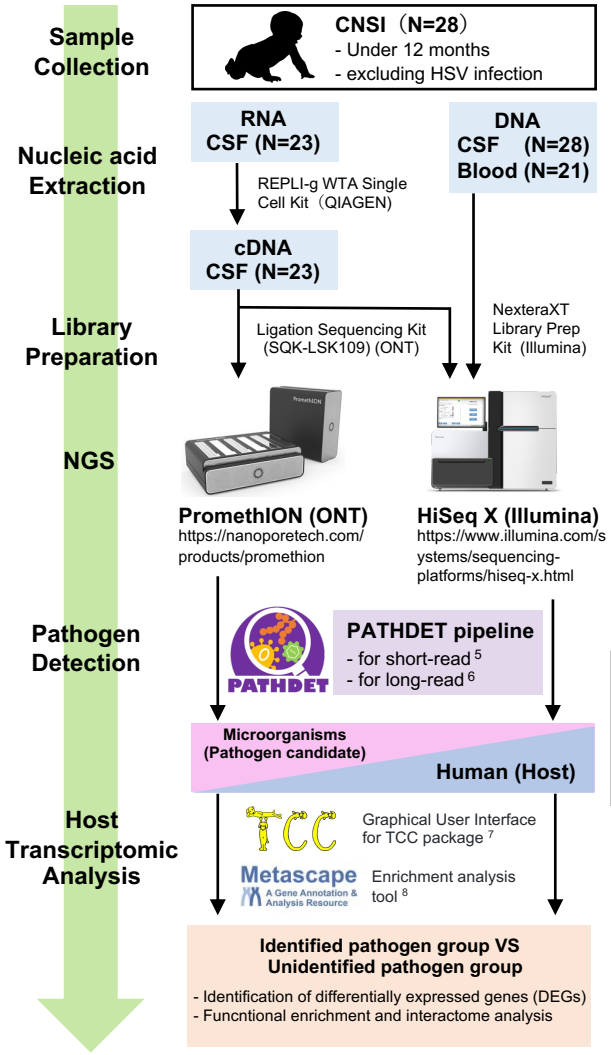
Twenty-eight CNSI patients (<12 months) were enrolled, and 49 clinical samples (28 CSF and 21 blood) were collected.

Table 1. Clinical characteristics of 28 patients

Characteristic	Infantile CNSI (n=28)	
Age, d, median (range)	23.5	(3 – 311)
Sex, male, no. (%)	13	(46)
Length of stay, d, median	8	(5 – 113)
Clinical signs and symptoms, no. (%)		
Depressed/altered level of consciousness	16	(57)
Poor feeding	13	(46)
Vomiting	2	(7)
Seizure	3	(11)
Bulging fontanelle	5	(18)
Pleocytosis*	23	(82)
Cerebrospinal fluid test, median		
Cell count, /μL	255	(1 – 1,301)
Neutrophil's count, /μL	46.5	(0 – 875)
Protein, mg/dL	84	(14 – 271)
Glucose, mg/dL	52.5	(19 – 203)
Blood tests, median		
White blood cell count, /μL	11,250	(1,100 – 47,500)
C-reactive protein, mg/dL	0.22	(0 – 6.8)

* Pleocytosis was defined as the following: CSF cell count > 30 /μL for newborn (0 – 8 weeks), > 5 /μL for infant (> 8weeks).

Methods



Results

Table 2. Pathogen candidates detected in CSF samples using NGS

Patient	Sequencing methods	Pathogen candidates (species)	Reads	Major serotype (Occupancy)	Hybrid assembly** (Accession number)
N01	Nanopore RNA	Enterovirus B	19,242	Echovirus E7 (98%)	Echovirus E7 (KU355273.1)
	Illumina RNA	Enterovirus B	5,956	Echovirus E7 (88%)	
N05	Illumina DNA	Primate erythroparvovirus 1	13,623	Human parvovirus B19 (79%)	NA
N13	Nanopore RNA	Enterovirus B	3,901	Coxsackievirus B2 (52%)	Coxsackievirus B2 (KU574632.1)
	Illumina RNA	Enterovirus B	7,428	Coxsackievirus B2 (58%)	
N14	Illumina DNA	Proteus mirabilis	49,284	NA	NA
N15	Nanopore RNA	Enterovirus B	937	Coxsackievirus B4 (26%)	Coxsackievirus B4 (MW015043.1)
	Illumina RNA	Enterovirus B	205	Coxsackievirus B4 (32%)	
N16	Nanopore RNA	Enterovirus B	6,576	Coxsackievirus B5 (99%)	Coxsackievirus B5 (MW015056.1)
	Illumina RNA	Enterovirus B	2,825	Coxsackievirus B5 (98%)	
N17	Nanopore RNA	Parechovirus A	1,650	Human parechovirus 3 (99%)	Human parechovirus 3 (LC043127.2)
	Illumina RNA	Parechovirus A	1,281	Human parechovirus 3 (90%)	
N18	Nanopore RNA	Enterovirus B	193	Coxsackievirus B5 (98%)	Coxsackievirus B5 (MW015056.1)
	Illumina RNA	Enterovirus B	67	Coxsackievirus B5 (100%)	
N19	Nanopore RNA	Enterovirus B	93	Coxsackievirus B4 (40%)	None
	Illumina RNA	Enterovirus B	71	Coxsackievirus B4 (51%)	
N20	Nanopore RNA	Enterovirus B	1,007	Coxsackievirus B5 (100%)	Coxsackievirus B5 (MW015056.1)
	Illumina RNA	Enterovirus B	357	Coxsackievirus B5 (100%)	
N23	Nanopore RNA	Enterovirus B	148	Coxsackievirus B5 (96%)	Coxsackievirus B5 (MW015056.1)
	Illumina RNA	Enterovirus B	84	Coxsackievirus B5 (100%)	
N26	Nanopore RNA	Enterovirus B	92	Coxsackievirus B4 (28%)	Coxsackievirus B4 (MN590273.1)
	Illumina RNA	Enterovirus B	94	Coxsackievirus B4 (33%)	

** Hybrid assembly were performed using metaSPAdes(https://cab.spbu.ru/software/meta-spades/)

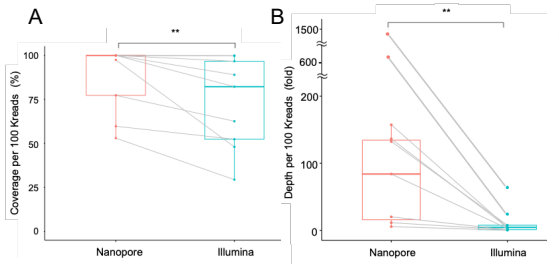


Figure 1. Comparison of pathogen genome mapping Nanopore sequencing generated significantly greater mapping coverage (A, $p = 0.008$) and mapping depth (B, $p = 0.008$) than Illumina sequencing.

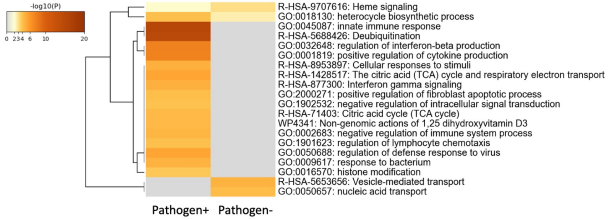


Figure 2. Enrichment analysis of DEGs from Nanopore sequencing Heatmap of enriched terms across DEGs lists from Nanopore sequencing, colored based on p-values. *MX1*, *ISG15*, and *OAS1* were DEGs in patients with identified pathogens via both Nanopore and Illumina sequencing, and were associated with antiviral roles in innate immunity.

Conclusion

The use of Nanopore sequencing for metagenomic diagnostics of CSF samples should help to understand both pathogens and host immune responses of CNSI and could shed light on the pathogenesis of these infections.

References

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