Successful Adaptation of Targeted Gene Panel Next-Generation Sequencing in Regional Hospital in Hong Kong: Genomic Diagnosis of *SCN2A*-Related Seizure Disorder

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To the Editor: To date, epilepsy is one of the most common neurological disorders globally, affecting more than 50 million people worldwide. It is debilitating and represents a large health-care and economic burden, especially when treatment may often be expensive and not necessarily effective. It has been suggested that the burden of lifetime epilepsy has been significantly increasing in China in recent decades.[1] The International League Against Epilepsy has defined epilepsy in 2014 as a neurological disorder with at least two unprovoked seizures occurring more than 24 h apart, one unprovoked seizure and a high probability of further seizures, or diagnosis of an epilepsy syndrome. Although the condition could also be caused by structural, infectious, metabolic, and/or immune etiologies, it has recently been estimated that a genetic etiology could be established in up to 40% of such patients.[2] Molecular testing with a genomic approach would allow delineation of the precise genetic cause, treatment stratification, as well as further detection of pharmacogenetic variants to avoid severe adverse drug reactions toward antiepileptic drugs, which is of particular clinical importance in Asian populations. A precise genetic diagnosis of the patient would also guide genetic counseling on the prognosis. The numerous genes associated with epilepsy and the significant genetic heterogeneity necessitated a high-throughput technique for clinical laboratories in the genomic era. Here, we reported a case of *SCN2A*-related seizure disorder, in which the likely pathogenic variant was only possible to be detected with the use of targeted gene panel with next-generation sequencing. This exemplary case demonstrated the value of genetic testing with a genomic approach in this group of genetically heterogeneous disorder.

Our patient was a male born full-term as the first child to nonconsanguineous Chinese parents. There was uneventful antenatal history and no remarkable family history. At 24 months of age, he first presented with recurrent afebrile convulsions, which were apparently more severe during episodes of febrile illness. Semiology ranged from generalized tonic-clonic convulsions to myoclonic jerks. He was also noted to have autistic features with severe language delay and depressed social interaction. Physical examination showed no focal neurological deficit or dysmorphic feature. Electroencephalography at 25 months once captured a clinical attack with ictal discharges beginning at bilateral anterior regions and evolving to generalized epileptiform discharges followed by generalized suppression of activities. Biochemical investigations, including urine organic acids, plasma and cerebrospinal fluid amino acid profiling, cerebrospinal fluid neurotransmitters, plasma and urine creatine, and guanidinoacetate, were all unrevealing. Previous genetic testing for common mitochondrial DNA point mutations was negative.

Approval for the study was obtained from the Kowloon West Cluster Clinical Research Ethics Committee, Hospital Authority (No. KW-EX-09-155), and written informed consent was obtained from the family. Next-generation sequencing was performed with targeted gene capture using TruSight One Sequencing Panel (Illumina; San Diego, CA, USA), which covers more than 4800 genes associated with specific clinical phenotypes, on a MiSeq Sequencing System (Illumina; San Diego, CA, USA). Target regions of interest were restricted to the coding regions and the 10-bp flanking regions of the selected genes. The sequencing data were aligned to GRCh37/hg19, and variants were identified within 74 selected genes associated with genetic epilepsies using NextGENe sequencing analysis software (version 2.4.1; State College, PA, USA). Variants identified were annotated with VariantStudio software (version 2.2.1; Illumina; San Diego, CA, USA) and filtered with Excel spreadsheet (2010; Microsoft, Richmond, VA, USA). Pathogenic and likely pathogenic variants were subsequently confirmed by Sanger sequencing in the proband and in the mother. Since the parents were divorced, no sample could be collected from the father.

The coding exons and flanking regions (10-bp) of the 74 selected genes were sequenced with a mean depth of coverage of 93.8

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reads, and 93.9% of the targeted bases were covered by at least 20 reads. The patient was found to harbor a heterozygous insertion-deletion NM_021007.2:c.2350_2365delinsTGTACTATCCAACAGATACT (NP_066287.2:p.(Thr784Cysfs*45)) in the *SCN2A* gene, which was confirmed by Sanger sequencing [Figure 1]. The variant was not detected in the mother, who did not have a history of convulsion or autism. This variant was not previously reported as disease-causing and not listed in the Human Gene Mutation Database (Professional 2018.1) and ClinVar at the time of reporting. This likely pathogenic variant was predicted to cause a frameshift and premature termination of the protein. At the time of reporting, the variant was absent from controls in the Exome Sequencing Project, 1000 Genomes Project, and Genome Aggregation Database. Although the variant was not reported and functional study data were not yet available, other frameshift and null variants in *SCN2A* were described previously in patients with seizures and considered disease-causing.[3] This variant was not found in the proband's mother. Unfortunately, genotyping of the father was not performed due to unavailability. The variant was thus considered likely pathogenic according to the standard variant interpretation guidelines.

SCN2A encodes the alpha subunit of voltage-gated sodium channel $Na_v1.2$, which is predominantly expressed in the brain. *SCN2A*-related disorders are inherited in an autosomal dominant manner with incomplete penetrance and variable expressivity, while *de novo* variants are not uncommon.[3] Pathogenic variants in *SCN2A* have been associated with a phenotypic spectrum that includes benign neonatal/infantile seizures, Ohtahara syndrome, epilepsy of infancy with migrating focal seizures, West syndrome, Lennox-Gastaut syndrome, myoclonic-atonic epilepsy, electrical status epilepticus during sleep, and intellectual disability and/or autism without epilepsy.[3] The diagnosis of a *SCN2A*-related disorder in this patient could explain his epilepsy as well as his autism and developmental delay. Sodium channel blockers, for example, phenytoin, carbamazepine, and lamotrigine usually represent the first-line treatment for *SCN2A*-related epileptic disorder, although recently it has been suggested that null variants in *SCN2A* might be associated epilepsies with a later onset and treatment resistance to sodium channel blockers.[3] This molecular-level diagnosis also facilitates appropriate genetic counseling, screening of at-risk family members, and prenatal diagnosis for future pregnancies in the family.

The Department of Pathology, Princess Margaret Hospital, has been providing clinical molecular testing service for more than 20 years. Our laboratory was among the first clinical laboratory to provide targeted gene panel next-generation sequencing since June 2016 within the Hospital Authority of Hong Kong, China, which

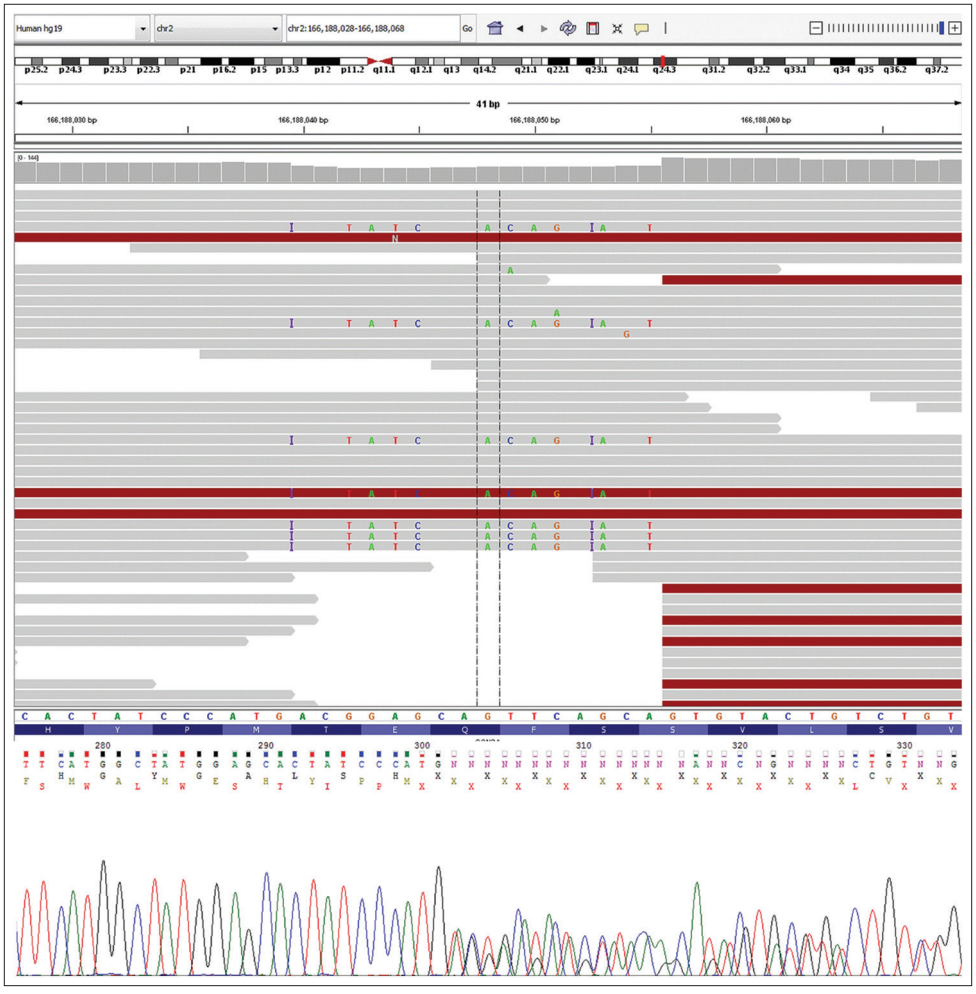

Figure 1: The variant is visualized in Integrative Genomics Viewer (Version 2.3.68; Broad Institute). Due to limitation of the software, the heterozygous indel variant (c.2350_2365delinsTGTACTATCCAACAGATACT) is detected as a combination of insertions and substitutions. The electrophoretogram showing confirmation of the variant c.2350_2365delinsTGTACTATCCAACAGATACT by Sanger sequencing. Note the insertion of TGTACTATCCAACAGATACT (20 nt) in place of ACGGAGCAGTTCAGCA (16 nt) on the variant allele.

serves the public sector and around 90% of patients in Hong Kong. Referrals for both inpatients and outpatients are accepted from clinicians or pathologists in Hong Kong with appropriate informed consent. Targeted genes are selected for each of the individual disease panels by chemical pathologists with on-going discussion with clinicians and continuous review of the current literature, with the aim to fill in the gaps within the existing diagnostic algorithms for the various clinical conditions. Costs of both the instrument and the consumables for next-generation sequencing have been drastically dropping and will continue to decrease in the near future.[4] Genomic testing approaches are recently believed to end diagnostic odysseys early, thus potentially be cost-saving; appropriate treatment might yield better control of the disorders, improve quality of life, and reduce health-care expenditure related to hospitalization.[5] The sequencing costs for each case were estimated to be around US 1000 dollars (or RMB 7000 Yuan), where minimal set-up costs and preparatory investments were involved. We routinely utilize commercial software for data analysis, which costs around US 3000 dollars (or RMB 21000 Yuan) per year, and is also backed up with an in-house Linux pipeline consisting of Burrows-Wheeler Aligner and Genome Analysis Toolkit. The advantages of utilizing commercial software include user-friendly interface and higher acceptability by technical staff and pathologists, as well as a large number of users with shared experiences worldwide, while the analysis could be performed on an average 64-bit personal computer costing less than US 1000 dollars (or RMB 7000 Yuan) without the need of a dedicated bioinformatician. Validation is nevertheless necessary and our laboratory has joined the external quality assessment scheme for next-generation sequencing by the European Molecular Genetics Quality Network and achieved satisfactory scores since 2014.

A "clinical exome" targeted capture kit is used for the various panels, so that sequencing is performed for all the 4811 genes associated with clinical phenotypes, where the uniform workflow aids easier quality assurance. Compared with whole exome sequencing, the tedious work of interpreting variants and secondary or incidental findings could be avoided by the tactfully designed workflow and submission of data only to the clinically relevant genes. A significantly shorter turn-around time that would suit routine clinical need could also be achieved. At the same time, existing data could be easily reanalyzed without additional costs when new clinical information becomes available from the patient, or when genes have been newly associated with the phenotype. The use of the commercial clinical exome kit is not without limitation: it generates more data than custom-designed targeted panels and analyses with a lower coverage with the same sequencing capacity. The genes included in the commercial kit are designed by the vendor, which might lack flexibility for customization, and supplementary techniques may be required if the region of interest is not covered by the panel.

In the era of genomic testing, it is of utmost importance to select suitable genes for testing to achieve optimal diagnostic sensitivity and specificity. Gene panels are designed in a way not only to address the clinical question but also to streamline with other investigation modalities that are locally available, which would aid to limit the size of individual panels. For example, genes associated with common inborn errors of metabolism that are routinely detected by metabolic screening in our laboratory are not included in the current epilepsy panel. This could ensure a shorter turn-around time, as well as avoid reporting variants of uncertain significance unnecessarily and causing confusion for clinicians, patients, and their families. The gene panels could also be further updated when local diagnostic pathways are modified.

The panel testing could be initiated as a referral by the case clinician or as part of investigations by pathologists. The chemical pathologists endorse or choose the panel based on clinical details provided by the clinician, as well as information accessible on electronic patient records, including results of biochemical tests, histopathology, electrophysiology investigations, and/or radiological imaging, as well as clinical response toward specific medications. Additional testing or reanalysis could be performed if deemed relevant before the panel testing.

Library preparation, sequencing on instrument, and postsequencing data analysis take 2.0 days, 1.5 days, and 0.5 day, respectively; thus, a list of annotated variants could become available for interpretation by chemical pathologist around 4.0 days after receiving the sample. When variants of uncertain significance are detected, further confirmatory tests could be promptly added, for example, further review of histological slides or blood films, or additional immunohistochemistry, with collaboration with other pathology specialties, so that reclassification of the variants is possible before reporting. Interpretative comments with clinical correlation could be included in the reports, for example, treatment strategies or suitability of reproductive options for the patient. Reflex testing of other panels could be initiated by the chemical pathologist if deemed appropriate, for example, when the first panel turns out unrevealing or when new clinical information becomes available. Cascade testing and genetic counseling could also be arranged, which may provide co-segregation data. Successful implementation of genomic testing in regional hospitals requires clinicians and various pathology specialties to work closely as a team.

In conclusion, we have successfully implemented targeted panel genomic testing by next-generation sequencing in clinical diagnostic service in a regional hospital in Hong Kong (China) with the use of commercially available clinical exome capture kit and bioinformatics software. This combination provides flexibility and could be exemplary for implementation of genomic testing in small-to-medium-scaled clinical diagnostic laboratory service.

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Conflicts of interest

There are no conflicts of interest.

References

1. Song P, Liu Y, Yu X, Wu J, Poon AN, Demaio A, *et al.* Prevalence of epilepsy in China between 1990 and 2015: A systematic review and meta-analysis. J Glob Health 2017;7:020706. doi: 10.7189/jogh.07-020706.
2. Berg AT, Coryell J, Saneto RP, Grinspan ZM, Alexander JJ, Kekis M, *et al.* Early-life epilepsies and the emerging role of genetic testing. JAMA Pediatr 2017;171:863-71. doi: 10.1001/jamapediatrics.2017.1743.
3. Wolff M, Johannesen KM, Hedrich UB, Masnada S, Rubboli G, Gardella E, *et al.* Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. Brain 2017;140:1316-36. doi: 10.1093/brain/awx054.
4. Christensen KD, Dukhovny D, Siebert U, Green RC. Assessing the costs and cost-effectiveness of genomic sequencing. J Pers Med 2015;5:470-86. doi: 10.3390/jpm5040470.
5. Córdoba M, Rodriguez-Quiroga SA, Vega PA, Salinas V, Perez-Maturo J, Amartino H, *et al.* Whole exome sequencing in neurogenetic odysseys: An effective, cost- and time-saving diagnostic approach. PLoS One 2018;13:e0191228. doi: 10.1371/journal.pone.0191228.