Letters

RESEARCH LETTER

Diagnostic Yield of Clinical Next-Generation Sequencing Panels for Epilepsy

During the past 2 years, next-generation DNA sequencing (NGS) has become a widespread diagnostic tool in neurology. Several studies have addressed the diagnostic yield and cost of NGS relative to other types of DNA testing. G-banded karyotyping identifies chromosomal aberrations and has a 3% diagnostic yield for unexplained developmental disabilities or other congenital anomalies.[1] In comparison, chromosomal microarrays detect gene copy number variations and have a yield of 15% to 20% for the same disorder categories.[1] Next-generation DNA sequencing, in the format of whole-exome sequencing (WES), can be diagnostic in 25% of neurogenetic cases.[2] Similarly, whole-genome sequencing (WGS) with NGS has a reported diagnostic yield of 27% in children and adults with a broad variety of diseases.[3] In contrast to WES and WGS, targeted NGS panels focus on subsets (dozens to hundreds) of genes associated with specific phenotypes. For example, targeted NGS directed at a single disease category, such as congenital glycosylation disorders, has a reported diagnostic yield of 14.8%.[4] Given the prevalence of pediatric epilepsy, we set out to critically assess the diagnostic yield of an NGS panel for epilepsy in a pediatric tertiary care hospital.

Methods | The University of Texas Southwestern Medical Center institutional review board approved this retrospective study; patient informed consent was waived. We conducted a 1-year retrospective review of all patients with epilepsy treated at our institution who received targeted NGS on peripheral blood by either the 2012 GeneDx Comprehensive (53 genes) or Infantile (38 genes) Epilepsy Gene Panels. The patients' clinical histories were reviewed to determine the relevance (ie, diagnostic yield) of the targeted NGS test results. In addition, we compared the cost of the targeted NGS panels, subsets of single-gene sequencing tests, and WES (**Table**).

Results | In 2012, 28 patients were tested using either the GeneDx Comprehensive or the Infantile Epilepsy Gene Panels. Six patients harbored pathogenic or likely pathogenic mutations in 5 epilepsy-associated genes (*TCF4*, *SCN1A*, *CDKL5*, *KCNQ2*, and *POLG*) and 11 patients were found to have novel missense variants that were classified as variants of unknown significance in 8 genes (*GABRG2*, *MECP2*, *PNPO*, *SCN1A*, *SCN2A*, *SCN1B*, *SLC9A6*, and *TSC2*). All of the pathogenic mutations had been previously characterized as such in the literature; novel variants that were likely pathogenic were reported as variants of unknown significance. The diagnostic yield of these disease-targeted NGS panels was 21.4% (6 of 28 patients), on par with WES or WGS.[2-4] If the GeneDx criteria for prior reporting in diagnosing pathogenicity had been used in a recent study of clinical WES, the WES diagnostic yield would have been only 18%[2]; therefore, with equivalent reporting criteria, these NGS panel tests for epilepsy would have a superior diagnostic yield compared with WES.

The Comprehensive and Infantile Epilepsy Gene Panels cost $5750 and $4780, respectively. Whole-exome sequencing costs up to $15 129.[6] In contrast, examining a subset of 3 commonly tested epilepsy genes by Sanger sequencing (*SLC2A1*, *MECP2*, and *SCN1A*) costs $7300.[6] We concluded that there is a cost advantage in the use of NGS technology compared with traditional Sanger sequencing.

Discussion | Whole-exome sequencing analyzes, with varying quality, more than 20 000 genes, but the Online Mendelian Inheritance in Man and the Human Gene Mutation Database currently include information for 3131 and 6137 genes, respectively.[7,8] Indeed, a recent study of WES reporting a diagnostic yield of 25% included 48 novel variants that had not been previously reported.[2] In this review of targeted NGS panels for epilepsy, the diagnostic yield was, in aggregate, similar to WES and achieved at a lower cost. In summary, targeted NGS gene panels are a cost-effective alternative to both Sanger sequencing of individual genes and WES for the genetic diagnosis of epilepsy.

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Table. Comparison of NGS Panel, WES, and Sanger Sequencing

Sequencing Panel	Cost, $	Hypothetical Cost for Patients in this Study (N = 28), $	No. of Genes	Diagnostic Yield, %	Turnaround Time, wk
NGS[a]	5265	147 420	53	21.4	10-12
WES	15 129	423 612	>22 000	16-25	24-28
Limited Sanger	7300	204 400	3	1.5 for *MECP2*[5]	2.5-4

Abbreviations: NGS, next-generation DNA sequencing; WES, whole-exome sequence.

[a] Comprehensive Epilepsy Gene Panel in 2012; in 2013, after this study was performed, the panel was expanded to 70 genes.

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Study concept and design: All authors.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: All authors.
Critical revision of the manuscript for important intellectual content: All authors.
Administrative, technical, or material support: All authors.
Study supervision: Pascual, Park.

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COMMENT & RESPONSE

Self-Reported Sleep and β-Amyloid Deposition in Older Adults

To the Editor Spira et al[1] reported the association between self-reported sleep variables and neuroimaging evidence of β-amyloid deposition in 70 community-dwelling older adults (33 women and 37 men). The mean (SD) age of the patients was 76.4 (8.0) years. Among them, the numbers of participants with sleep medication, elevated cortical distribution volume ratio, and elevated precuneus distribution volume ratio were 7, 24, and 16, respectively. The authors used sleep duration as measured by a standardized interview and the 5-item Women's Health Initiative Insomnia Rating Scale.[2,3] They concluded that shorter sleep duration and poorer sleep quality are associated with greater β-amyloid burden by multiple regression analysis. Their study is important for presenting neuroimaging evidence in addition to biomarkers of Alzheimer disease.[4]

I have some concerns on their research outcome. First, they used self-reported sleep variables but the percentages of worse sleep quality and other variables on poor sleep were relatively small. For example, the numbers of participants with restless sleep and mean sleep duration of 5 or fewer hours were 7 (10%) and 4 (7%), respectively. Although the percentage of elevated cortical distribution volume ratio and precuneus distribution volume ratio were 34% and 23%, respectively, the main purpose of their study cannot be determined by the small number of participants who experienced poor sleep.

Second, β values with significance of several adjusted variables are also informative for understanding the mechanism of the association between sleep β-amyloid deposition. There is a report that subjective sleep duration for individuals with poor sleep quality was negatively related to depressive state evaluated by the Patient Health Questionnaire, irrespective of having neurological disease.[5]

Finally, coefficient of determinations (adjusted R^2) to predict 2 types of elevated distribution volume ratio should be presented. In addition, 70 participants were available to be included in the multiple regression analysis, and this statistical procedure requires at least 10 individuals per variable for keeping stable estimates.[6] To confirm their conclusion, more samples are required for their study.

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In Reply Dr Kawada raised several concerns with our article.[1] First, we acknowledge that there were relatively few participants reporting restless sleep or extremely short sleep duration in our sample. However, we treated sleep quality and duration as continuous variables in our regression models, and our sample provided adequate power across the range of values to detect significant associations between reports of worse sleep and greater β-amyloid deposition. Although further research is warranted, our results may indicate that sleep need not be extremely restless or of very short duration to show an association with β-amyloid burden.