Utility of genetic testing in pediatric epilepsy: Experience from a low to middle-income country

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ABSTRACT

Monogenic epilepsies are a significant etiology of pediatric epilepsy. These are now more easily identified due to advances in genetic testing. However, the utility of genetic testing in low to middle-income countries (LMICs) has not been fully explored. A retrospective review was carried out in Karachi, Pakistan. Patients with symptoms suggestive of genetic epilepsy underwent next-generation sequencing (NGS). Seventy-seven patients were tested, of which 27% (n = 21) initially had pathogenic (P) or likely pathogenic (LP) results. This increased to 32% (n = 25) after clinical reclassification of some variants of uncertain significance (VUSs) based on American College of Medical Genetics and Genomics (ACMG) guidelines. Initially, 6% of patients (n = 5) had no P/LP or VUS, and 66% (n = 51) had at least one VUS. After variant resolution and reclassification, results were negative for 25% (n = 19) and 43% (n = 33) had VUSs. Genetic testing was positive in one-third of our population. The proportion of P/LP variants found in SCN1A is higher than that found in other populations, and we report two novel variants in SCN1A. The yield of genetic testing in our population is comparable to that found in North America. Initially, a higher proportion of our population had inconclusive results, indicating the need for better characterization of the South Asian genotype.

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Introduction

Pediatric epilepsy encompasses a family of disorders consisting of recurrent unprovoked seizures in children. These seizures may be secondary to environmental insult (brain trauma, hypoxia etc.), congenital malformations, (focal cortical dysplasia, hemimegalencephaly, subcortical band heteropia, lissencephaly etc.) or have a primary monogenic cause (Dravet syndrome, progressive myoclonus epilepsy, benign familial neonatal, infantile convulsions, X-linked infantile spasms etc.) [1,2]. Frequently, the etiology remains unknown [3].

Genetics is thought to play a significant role in the etiology of many forms of epilepsy due to observations such as a higher concurrence of epilepsy amongst twins and within families [4,5]. However, it was not until recently that causative genes and pathogenic (P) or likely pathogenic (LP) monogenic epilepsies were identified.

The diagnostic yield of genetic testing has increased, providing a specific etiology for epilepsy in up to 57% of patients in certain populations [6]. Advances in genomic sequencing have allowed the identification of new genes associated with epilepsy, increasing the utility of genetic testing [7]. Several genetic testing modalities exist, including conventional karyotyping, chromosomal microarray analysis (CMA), targeted next-generation sequencing (NGS) based panel testing, exome sequencing (ES) and genome sequencing (GS). The diagnostic yield of these tests vary significantly, karyotyping having yield as low as 3%, compared to ES with a higher yield, ranging between 10% and 60% [8–10]. There is currently no established guideline as to which modality of testing should be used in individual presentations of epilepsy. Reports have supported NGS testing as an effective first-tier test reflecting higher yields in severe, early onset epilepsy and for CMA testing in patients presenting with dysmorphic features or congenital abnormalities [11–13]. Therefore, the decision of which testing modality to use is made after a patient’s clinical manifestations are assessed (phenotyping) by treating physicians, usually in consultation with medical geneticists and genetic counselors.
Though epilepsy affects 70 million people worldwide, a disproportionately high burden lies in low and middle-income countries (LMICs) [14]. There is a serious knowledge gap about the underlying hereditary etiology of epilepsy and the subsequent utility of genetic testing for epilepsy in LMICs like Pakistan. Due to a high birth rate, combined with the fact that approximately 60% of unions are consanguinous [15], the genetic contribution to epilepsy may be high in Pakistan. Unfortunately, due to the dearth of population-based genetic studies as well as limited access to clinical genetic testing in academic centers, these questions remain unanswered. Although it is now considered standard of care to offer genetic testing if certain clinical criteria are met, patients in LMICs are unable to benefit from these advances, primarily due to lack of awareness, resources and access to genetic testing [16].

Genetic testing may provide useful prognostic information and potential therapeutic targets that may optimize management. For instance, in pyridoxine dependent epilepsy, seizures which are resistant to standard anti-seizure medications (ASM) can be effectively treated with lifelong therapy with vitamin B6. Similarly, in Dravet syndrome, drugs that block sodium channels such as lamotrigine and carbamazepine are avoided [17]. First-line anti-seizure medications include valproate and clobazam [17]. Treatment should be initiated with one of these agents and the other is added if control remains suboptimal. Topiramate and stiripentol are usually added as second-line drugs. The ketogenic diet is moderately effective for seizure control and could also be considered as second line management in patients with suboptimal response to clobazam and valproic acid. Recent studies also show that newer medications like cannabinoids and fenfluramine would be beneficial in conditions where first-line medications do not help [18–20].

Focused genetic counseling can also be provided once a genetic diagnosis is established. Thus, it is evident that genetic testing in epilepsy not only optimizes patient care, but also provides opportunities to identify family members at risk and offers the family options of reproductive decision making.

Given the fact that genetic testing is expensive, not offered in routine clinical labs in LMICs, and requires specific expertise to interpret genetic test results, it becomes even more important to determine its feasibility in resource-constrained countries like Pakistan. Our study was conducted at a quaternary care hospital in Pakistan, and aimed to explore the utility of genetic testing in patients with pediatric epilepsy. Doing so could help patients and families understand their diagnosis, as well as providing important prognostic information and impacting treatment [21].

### Methods

This study is a retrospective patient chart review, carried out at a quaternary care hospital based in Karachi, Pakistan. It received approval from the Aga Khan University Ethical Review Committee, ERC# 2021-5846-16866 and the requirement for informed patient consent was waived.

The study was conducted over a 28-month period, from June 2018 to November 2020. Our study population was retrospectively selected from pediatric patients who underwent NGS based genetic testing for epilepsy. Patients who presented to our Pediatric Neurology clinics as well as those seen by neurologist from other hospitals and were referred to the genetics clinic for evaluation were included. The charts of these patients were reviewed for their age of onset of symptoms, presentation of epilepsy, associated symptoms, family history, results of EEG and MRI and response of epilepsy to treatment. Additionally, any clinical and laboratory investigations that were conducted to identify any underlying cause were also evaluated.

Patients who were found to have drug-resistant epilepsy, defined as failure to become seizure free after adequate trial of two ASMs with or without dysmorphic facial features, and with no other identifiable causes of the condition, were referred by the pediatric neurologist to the genetics clinic. The protocol for referral from the Pediatric Neurology clinic was universally followed, as shown in Fig. 1.

In the Genetics clinic, patients were offered pre-test counselling on the possible outcomes and benefits of clinical genetic testing for epilepsy. The genetic test selection was done at this stage. It was decided, if epilepsy panel was the most appropriate first-tier genetic test to order, by assessing if presenting patient features were (i) not part of a chromosomal (microdeletion/duplication syndrome) and (ii) not part of another monogenic syndrome which was not covered by the epilepsy panel. After detailed counselling, patients who did not agree to genetic testing were referred to their primary neurologist for the continuation of treatment, and these patients did not proceed with genetic testing.

A total of 77 patients agreed to genetic testing and the cost of the test ($250.00 USD) was borne by the families of the patients.

A sample of blood was drawn from each index patient and outsourced to Invitae Genetics Lab, USA for testing for a panel of 187 genes associated with epilepsy [22]. The assay is validated for single nucleotide variants (SNVs) as well as exon-level deletions and duplication analysis, achieving an analytic sensitivity and specificity of over 99% [23].

Since this was a retrospective chart review of only those patients whose tests were sent, we do not have records available for patients who were advised testing but did not go ahead with it.

All the patients who had undergone testing were seen back in the Genetics clinic for post-test counselling. Based on results of the genetic testing, further counselling, testing or changes to management were advised. Subsequently, all patients were referred to their primary neurologist for the continuation of treatment.

### Results

Of the 77 patients who underwent testing, 27% (n = 21) harbored a pathogenic (P) or likely pathogenic (LP) variant. Six percent of patients (n = 5) had no P/LP or VUS resulting in a negative genetic test result while 66% (n = 51) had one or more VUSs.

81% (n = 17/21) of the cases with a P/LP result, were attributed to genes leading to autosomal dominant epilepsy, including SNC1A (n = 10), CNAO1 (n = 2), PCDH19 (n = 1), TSC2 (n = 1), DEPDC5 (n = 1), KCNQ3 (n = 1), KCN1T1 (n = 1). Four patients had biallelic (homozygous or compound heterozygous) variants in genes associated with autosomal recessive epilepsy, namely ADLH7A1 (n = 3) and NHLRC1 (n = 1).

Two patients were found to be heterozygous carriers of P/LP variants in genes causing autosomal recessive epilepsy conditions, including one in ADSL and the other in ADLH7A1, which were not contributing to their phenotype.

Out of the 17 patients with proven autosomal dominant epilepsy, parental testing was performed for 6 families, (SCN1A = 3, KCNQ3 = 1, PCDH19 = 1 and TSC2 = 1). Out of them, in four families, the variants in SCN1A and KCNQ3 were confirmed to be de novo and the possibility of germline mosaicism with regards to the recurrence risk 1–7% was explained [24].

Patients harboring P/LP variants had varying presentations, including focal, generalized, focal with generalized seizures and epileptic encephalopathy. Patients also had other symptoms such as global delay (33%), microcephaly (14%), and movement disorders (14%). Age of onset varied between 1st day of life and 13 years, with a median age of 4 months. One (5%) of these patients had regression in milestones, thirteen (62%) had some degree of
developmental delay and nine (43%) had a family history of seizures. The details of the genotype and phenotype of the patients are shown in Table 1.

One patient with drug-resistant epilepsy who subsequently had a TSC2 positive result was initially referred for molecular testing because of high suspicion of tuberous sclerosis complex (TSC), even though clinical criteria of TSC diagnosis was not fulfilled. In three out of 51 patients with VUS results, the clinical suspicion was high for them to be disease-causing variants. When clinically correlated, the presenting patient phenotypes were
| Case No. | Gene | Nucleotide | Protein | Consequence | Zygosity | ACMG classification | Age of onset | ILEA Classification of Epilepsy | Clinical Features of Epilepsy | Other Features (development, dysmorphia, regression) | Family History of seizures | Consanguinity | Change to medical treatment made |
|----------|------|------------|---------|-------------|----------|---------------------|-------------|---------------------------------|-----------------------------|---------------------------------|-------------------|-------------|-------------------------------|
| 1        | ALDH7A1 | Exon 6, c.538dup | (p. Glu180Glyfs*48) | Frameshift (nonsense) Pathogenic | Homozygous | 3rd day of life | N/A | status epilepticus | N/A | (siblings, 1st cousin) | No | Yes | Yes |
| 2        | ALDH7A1 | Exon 11, c.1003C > T; Exon 14, c.1301_1302del | (p. Tyr344Cysfs*3) | Frameshift (nonsense) | Heterozygous | 8th day of life | Generalized | GTC | Mild GDD, torticollis in early childhood | No | No | Yes |
| 3        | ALDH7A1 | Intron 3, c.1312 + 1G > A | Splice donor site | Splice acceptor site | Homozygous | 2nd day of life | Generalized | Seizures clinically | GDD and hypotonia | No | No | Yes |
| 4        | DEPDC5 | Intron 6, c.364-2A > G | Splice donor site | Splice acceptor site | Homozygous | 8 years | Focal | Seizures from left temporal and spreading to the left hemisphere | Movement disorder, GDD | Yes (mother) | Yes | Yes |
| 5        | GNAO1 | Exon 6, c.596T > C | (p.Leu199Pro) | Missense | Heterozygous | 1 month | Generalized | Infantile spasms | Speech and motor delay | Yes (extended family) | Yes | N/A |
| 6        | GNAO1 | Exon 6, c.680C > T | (p.Ala227Val) | Missense | Heterozygous | 2 months | Focal | Infantile spasms | Dilated left atrium and left ventricle, hypotonia, large tongue, dysmorphism | Yes (1st cousin, extended family) | Yes | No |
| 7        | KCNQ3 | Exon 12, c.1657G > A | (p.Gly553Arg) | Missense | Heterozygous | 10 months | Generalized | Infantile spasms | Speech and motor delay | No | No | N/A |
| 8        | KCNT1 | Exon 15, c.1420C > T | (p.Arg474Cys) | Missense | Heterozygous | 1st day of life | Combined focal and generalized | Tonic | Dilated left atrium and left ventricle, hypotonia, large tongue, dysmorphism | Yes (1st cousin, extended family) | Yes | Yes |
| 9        | NGLRC1 | Exon 1, c.462dup | (p.Asp155*) | Nonsense | Homozygous | 13 years | Developmental and epileptic encephalopathy | Progressive myotonic and generalized tonic-clonic seizures | Altered behavior, hallucinations, paranoia, gait disturbance, hyperactivity, ataxia, regression of milestones | Yes (sister) | Yes | No |
| 10       | PCDH19 | Partial Deletion (Exons 1–3) | Truncated/absent protein | Truncated/absent protein | Homozygous | 10 months | Focal | GTC | Learning difficulties | No | Yes | No |
| 11       | SCN1A | Exon 3, c.459G > A | (p.Trp153*) | Large genomic rearrangement | Heterozygous | 11 months | Generalized | GTC with fever, occasionally without fever | Body tremors, fine hand and speech tremors, mild speech and motor delays, microcephaly | Yes (1st cousin) | No | Yes |
| 12       | SCN1A | Exon 10, c.1624C > T | (p.Arg542*) | Nonsense | Homozygous | 3 months | Generalized | GTC with fever | No | No | Yes |
| 13       | SCN1A | Exon 11, c.1972del | (p. Ser658Glnfs*14) | Frameshift (nonsense) | Heterozygous | 3.5 months | Combined focal and generalized | GTC with fever (complex febrile) | Yes (cousin) | Yes | Yes |
| 14       | SCN1A | Exon 18, c.3612G > A | (p.Trp1204*) | Nonsense | Homozygous | 4 months | Epileptic encephalopathy | ASD and partial anomalous pulmonary return | N/A | N/A | Yes |
| 15       | SCN1A | Exon 20, c.3985C > T | (p.Arg1329*) | Nonsense | Homozygous | 6 months | Combined focal and generalized | Focal with generalized | Speech delay | No | No | Yes |
consistent with genotypes caused by P/LP variants in genes identified, with supporting evidence from population databases, segregation studies and in-silico analysis. This led us to reclassify these variants as LP, based on the American College of Medical Genetics and Genomics (ACMG), variant classification criteria [25]. These cases are discussed in detail below.

A fourth patient had additional markers on X chromosome, on the NGS panel testing, that did not explain the seizure phenotype, however, it facilitated the diagnosis of this patient, as discussed below.

The clinical details and variant classification details of the first three patients are found in Table 2.

Case #22

A 3-year-old female harbored a homozygous VUS (R112H) in KCNT2; She was the product of a consanguineous union and presented with generalized seizures at 2 years of age, with progressive worsening of seizures and frank developmental regression. Her EEG showed continuous bilateral parasagittal discharges. These symptoms are consistent with the diagnosis of progressive myoclonic epilepsy, an autosomal recessive condition caused by a P/LP variant in KCNT2 (MIM#611726). At the variant level, the arginine residue is highly conserved through evolution of species. The MAF (minor allele frequency) of this variant (rs774626720) in population database is 0.0004 % and no homozygotes are reported in healthy population (gnomAD database) [26]. The in-silico tools including, SIFT, Polyphen and Mutation Taster, predict the variant to be disease-causing, and the variant is also identified in published literature and ClinVar [27,28]. All these factors, lead to the reclassification of the variant to be LP, based on the ACMG score (PS1, PP3, PP4, PP5).

Case #23

A 7-year-old female patient harbored a heterozygous VUS (P1748R) in SCN1A; She was also a product of a consanguineous union and presented with a history of generalized tonic-clonic seizures at 6 months of age initially with fever, but then having afebrile seizures as well. She displayed no developmental delay and had a normal Head MRI and interictal EEG. She was initially started on phenytoin and leviteracetam but once she started having afebrile seizures and the diagnosis of generalized epilepsy with febrile seizure plus syndrome (GEFS+) was considered, the phenytoin was tapered off and valproate was added. Considering GEFS+, genetic testing was done which showed a VUS in SCN1A (MIM# 604403). At the variant level, the proline residue is highly conserved through evolution of species. This variant is absent from the population database (gnomAD database); however, it is present at a mutational hotspot, due to the presence of LP variants in the region (p.D1742N) and (G1749E) [26,29–31]. The in-silico tools including, SIFT, Polyphen and Mutation Taster, predict the variant to be disease-causing. Parental testing also confirmed that the variant found in this proband was de novo. All these factors, lead to the reclassification of the variant to be LP, based on the ACMG score (PS1, PM1, PM2, PP3, PP4).

Case #24

A 6-year-old girl, born to consanguineous parents, harbored a homozygous VUS (N83C) in CLN6 (MIM#601780) that is associated with autosomal recessive neural ceroid lipofuscinosis. The patient presented with sudden falls and generalized tonic-clonic seizures at the age of 4 years. She then began to have regression of developmental milestones including motor and speech milestones, with later development of ataxia, with diffuse cerebral atrophy observed.
on MRI. At the variant level, the aspartic acid residue is highly conserved through evolution of species. The MAF of this variant (rs774026720) in population database is 0.0008% and no homozygotes are reported in healthy population (gnomAD database). The in-silico tools including, SIFT, Polyphen and Mutation Taster, predict the variant to be disease-causing. Segregation studies showed that the unaffected sibling harbored a heterozygous CLN6 variants, suggesting that the phenotype in the proband did segregate with the homozygous variant in CLN6. Moreover, previously missense variants have been reported to be disease causing, with low rate of common benign missenses [32–34]. Based on these factors, we reclassify this variant as LP based on the ACMG score, (PM2, PP1, PP2, PP3, PP4).

**Case #25**

A 4-year-old male, adopted into a family with unknown family history of disease, presented with complaints of staring gaze and absence seizures at the age of 3 years. He visited the genetics clinic at the age of 3 years and 11 months, based on the presenting phenotype of absence seizures and limited family history. In the absence of features of common chromosomal disorders, NGS testing was ordered as the first-tier test that showed copy number variants (CNVs) in multiple genes that reside on X chromosomes. Later a karyotype was ordered that led to the diagnosis of Klinefelter syndrome (47, XXY) in this proband. No other P/PL variants were identified.

Thereby, after including these additional cases, the positive result rate increased from 27% (n = 21/77) to 32% (n = 25/77).

Out of the remaining 61% (n = 47) cases with VUS results, in 12 patients, the identified VUSs were in genes associated with autosomal recessive phenotypes. Since these patients harbored heterozygous variants, we considered them as having clinically negative results, decreasing the VUS rate to 46% (n = 35) from 61% (n = 47) and increasing the negative result rate from 7% (n = 5) to 22% (n = 17).

Going further, out of 46% (n = 35) remaining patients, variant resolution testing was offered in 23 cases, which was carried out in 12 patients. Subsequently, in four patients, the heterozygous VUSs associated with autosomal dominant phenotype did not segregate with the phenotype and based on our clinical judgement, deemed as not disease causing; however, from the laboratory, variants were identified. In 12 patients, Subsequently, in four patients, the heterozygous variants (CNVs) in multiples genes that reside on X chromosomes. Later a karyotype was ordered that led to the diagnosis of Klinefelter syndrome (47, XXY) in this proband. No other P/PL variants were identified.

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**Discussion**

In this study population, 27% (n = 21/77) received positive result on their genetic test. Furthermore, we considered three VUSs
cases#22, 23 and 24) to be LP, and further testing in another patient (case#25) led to the diagnosis of Klinefelter syndrome, as previously discussed; thus, improving the yield to 32% (n = 25/77). Subsequently, this has allowed patients to benefit from changes to treatment modalities and their families to benefit from guidance on making future reproductive decisions.

In our study, inconclusive results were found in 66% (n = 51/77) of those tested. However, after offering familial studies, reverse
phenotyping to establish phenotype-genotype correlation in the clinic and offering further testing (as in case #25), the inconclusive result proportion was reduced to 43% (n = 33/77).

The testing yield for genetic panels for epilepsy vary based on the genes tested and the study population, with higher yields in populations that have been well studied. In high income countries (HICs), specifically the United States and Canada, the yield of P/LP variants ranges between 15 and 33% [8,27,38], and the percentage of patients with VUSs ranges from 42 to 75% [8,12,35], however further VUS resolution reduced the VUS rate to 39%, as showed by Truty et al, using the same expanded panel at Invitae Genetics. In our population, our yield of positive results was similar at 27% (or 32%) vs 33%. Moreover, our yield of VUSs was also comparable at 66% (or 60%) initially vs 75%, which after further VUS resolution went down to 43% vs 39% [35]. Despite comparable yield of positive results and VUSs, the pathogenicity of genetic variants found in our population largely remains uncharacterized, in a pediatric population that is highly suspected to have an underlying genetic etiology for epilepsy. This emphasizes the need for better characterization of our population’s genetic variation.

Amongst those who had positive results (excluding the patients with clinically suspicious VUSs) in our study, almost half (44% n = 11/25) had P/LP in the SCN1A, two of which were intronic variants. Out of the reported exonic variants, two were novel variants (case#13, 23), including one protein truncating variant (p.Ser658Clnfs*14) and one missense variant (p.Pro1748Arg). The presentations of epilepsy in these patients varied significantly, with patients presenting with focal, generalized, and focal with generalized epilepsy. The proportion of P/LP results involving the SCN1A in our population is higher than that found in HICs [6]. A higher prevalence of SCN1A mutations in our population may be the result of our small sample size, referral bias as severe SCN1A associated phenotypes may be more likely to be referred for genetic evaluations or it may indicate a difference in the genetic causes of epilepsy in our population. We feel all these are contributing factors in the data we present; and these factors need to be further explored with a larger sample size.

Our study population demonstrated high levels of consanguinity amongst the parents of the patients with clear (excluding the patients with clinically suspicious VUSs) P/LP variants, with the consanguinity reported in 38% (n = 8/21, data missing for one patient) of cases. However, as the mode of inheritance in six (out of eight) of these patients was autosomal dominant, thereby, consanguinity was not the primary contributor of epilepsy in these patients, which is comparable with other populations [39].

For the autosomal recessive phenotypes, four patients tested positive, and two were found to be carriers for autosomal recessive conditions and did not receive a genetic diagnosis. Of the four patients with confirmed diagnosis of autosomal recessive conditions, two were born to consanguineous parents. Interestingly out of the two patients born to non-consanguineous parents, one harbored P/LP homozygous variant in ALDH7A1, while the second patient was a compound heterozygote for two P/LP variants.

**Study limitation**

Given the retrospective nature of the study, the sampling was selective and therefore may have led to selection bias.

Referrals to the Genetic clinic from the Pediatric Neurology clinic are currently not electronically maintained, therefore the total number of patients referred; cannot be counted. This included (i) those families who did not come to the Genetics clinic either for pre-test counselling or those who visited the Genetics clinic for the pre-test counselling but did not go ahead with testing. Moreover, the reasons for the family’s denial for testing were not documented and need to be explored in further prospective studies, looking at both financial and psychosocial reasons for refusal of testing.

The ethnic background of the families was also not documented; therefore, the results may not be generalizable to the ethnically diverse Pakistani population. The study used NGS panel testing, which was not followed by expanded genetic testing such as ES and GS, therefore; the genetic etiology of epilepsy of patient was not fully understood, despite very high clinical suspicion of a monogenic disorder. The other limitation is the narrow spectrum of the population studied due to the cost as well as selection bias by the pediatric neurologist who generally only referred difficult to control epileptic patients.

**Conclusion**

NGS based genetic testing for epilepsy among our pediatric patients from Pakistan had a yield of P/LP variants of 32%. We report two novel P/LP variants in SCN1A, and based on the clinical correlation and segregation studies, we offer reclassification of variants, KCTD7, (R112H), SCN1A (P1748R) and CLN6 (N83G).

Compared with studies using similar expanded epilepsy panels, the VUS rate in our work was comparable, at 66% vs 75%. Sixty-eight percent of patients received no diagnostic results, indicating that our population may have genetic variants that have either not yet been classified as pathogenic using a NGS panel, or there may be novel genes underlying pediatric epilepsy leading to inconclusive results. This demonstrates that better characterization of south asian genomic data and a reference population from Pakistan studying carrier status and assessing autosomal recessive and X-linked conditions are urgently needed.

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**CRediT Author Contribution Statement**

Fizza Akbar: conceptualization, formal analysis, investigation, visualization, writing- review & editing.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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