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ABSTRACT

Background: The EPIGENE network was created in 2014 by four multidisciplinary teams composed of geneticists, pediatric neurologists and neurologists specialized in epileptology and neurophysiology. The ambition of the network was to harmonize and improve the diagnostic strategy of Mendelian epileptic disorders using next-generation sequencing, in France. Over the years, five additional centers have joined EPIGENE and the network has been working in close collaboration, since 2018, with the French reference center for rare epilepsies (CRéER).

Results: Since 2014, biannual meetings have led to the design of four successive versions of a monogenic epilepsy gene panel (PAGEM), increasing from 68 to 144 genes. A total of 4035 index cases with epileptic disorders have been analyzed with a diagnostic yield of 31% (n = 1265/4035). The top 10 genes, *SCN1A, KCNQ2, STXBP1, SCN2A, SCN8A, PRRT2, PCDH19, KCNT1, SYNGAP1*, and *GRIN2A,* account for one-sixth of patients and half of the diagnoses provided by the PAGEM.

Conclusion: These results suggest that a gene-panel approach is an efficient first-tier test for the genetic diagnosis of Mendelian epileptic disorders. In a near future, French patients with "drug-resistant epilepsies with seizure-onset in the first two-years of life" can benefit from whole-genome sequencing (WGS), as a second line genetic

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screening with the implementation of the 2025 French Genomic Medicine Plan. The EPIGENE network has also promoted scientific collaborations on genetic epilepsies within CRéER.

1. Introduction

Identification of genes causing Mendelian epileptic disorders has long been made difficult by several factors, especially the high frequency of *de novo* variants in epileptic and developmental epileptic encephalopathy and the high prevalence of epilepsy in the general population, resulting in a high proportion of phenocopies. In addition, most of the common epilepsies, named genetic generalized epilepsy or idiopathic generalized epilepsies, do not follow a Mendelian inheritance pattern but result from multifactorial inheritance. A few genes were identified in the late 1990's and were associated with recognizable familial phenotypes, such as autosomal dominant frontal-lobe epilepsy, epilepsy with auditory features, and genetic epilepsies with febrile seizures plus. Over the past decade, the identification of genes involved in Mendelian epileptic disorders has grown exponentially, thanks to technological advances, first in cytogenetics (array-CGH, SNP-array) and then in next-generation sequencing (Lesca and Depienne, 2015; Bayat et al., 2021).

In the early 2010's, two University Hospital departments were involved in the diagnosis of epileptic disorders in France, each working on a limited number of genes: in Paris Pitié-Salpétrière for fever-related epilepsies (e.g. *SCN1A*, *PCDH19*) and in Lyon for Lafora disease (*EPM2A*, *NHLRC1*), Unverricht-Lundborg disease (*CSTB*), benign familial neonatal/infantile focal epilepsies (*KCNQ2*, *SCN2A*), and epilepsy-aphasia syndrome (*GRIN2A*). In 2014, a working group called the EPIGENE network was created including these two labs and two others: Marseille laboratory, involved in the field of genetic research for early-onset epileptic encephalopathies and Strasbourg laboratory that had developed the first gene panel for the diagnosis of neurodevelopmental disorders in France (intellectual disability). Each of these four teams was multidisciplinary, including molecular and clinical geneticists, pediatric neurologists and epileptologists. Their objectives were to rationalize and homogenize the genetic strategy using next-generation sequencing for the etiological diagnosis of Mendelian epileptic disorders in France. Over the years, additional multidisciplinary centers joined the EPIGENE network: Reims (2016), Paris Necker and Grenoble (2018), and more recently, Amiens and Rennes (2020). Each of these centers had a multidisciplinary team and most of them have been involved in the identification of genes causing Mendelian epilepsies (Ishida et al., 2013; Lesca et al., 2013; Barcia et al., 2012; Milh et al., 2013). Upon joining the network, each new center signed the network charter for data sharing and collaboration rules.

In 2018, this initiative was acknowledged by the French Reference Center for Rare Epilepsies (CRéER) dedicated to research, education, and to the care of patients with rare epilepsies. The CRéER includes 7 Reference centers and 20 competence centers in France and is itself part of the DéfiScience rare diseases healthcare network, which brings together resources and expertise in the field of rare neurodevelopmental diseases.

A first version of the list of most wanted genes list was defined by the EPIGENE network in 2014 and was revised annually to add novel genes. Criteria for inclusion of a novel gene in the list are: i) epilepsy is a leader symptom of the disease or is frequently present in patients, ii) the mode of inheritance is Mendelian, iii) the role of the gene in the disease is supported by relevant data from the literature. The resulting gene panel, named PAGEM (*PAnel de Gènes pour les Epilepsies Monogeniques*, i.e. gene panel for epileptic monogenic epilepsies) was designed as a diagnostic tool for the pediatric neurologist, epileptologist and clinical geneticist following patients with epileptic disorders. We chose not to include the genes causing brain malformations, tuberous sclerosis and most genes for inborn errors of metabolism that were covered by other dedicated gene panels in France. Yet, a limited number of genes causing metabolic disorders, which are frequently associated with seizures and can benefit from specific treatments were included in the PAGEM gene panel: *SCL2A1* (GLUT1 deficiency), *CAD* (CAD deficiency), *BTD* (biotinidase deficiency), *SLC19A3* (thiamine transporter deficiency), *TPP1* (CLN2), as well as *ALDH7A1* and *PNPO* (deficiency of vitamin B6 metabolism).

Two centers (Lyon and Paris Pitié-Salpétrière), use the PAGEM as a specific panel whereas the other laboratories included the common gene list in a larger screening tool, including genes for other disease entities, with different library-building technologies, or used it as an *in-silico* panel on exome sequencing.

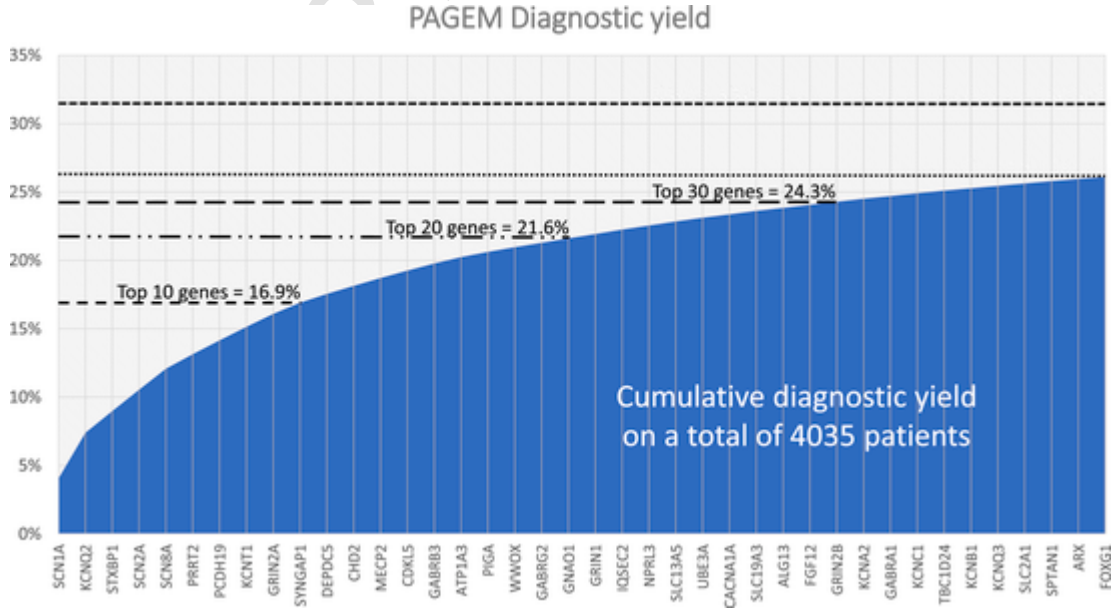

Fig. 1. Diagnostic yield of the French Mendelian epilepsy gene panel (PAGEM) for 4035 patients analyzed from 2015 to 2020.

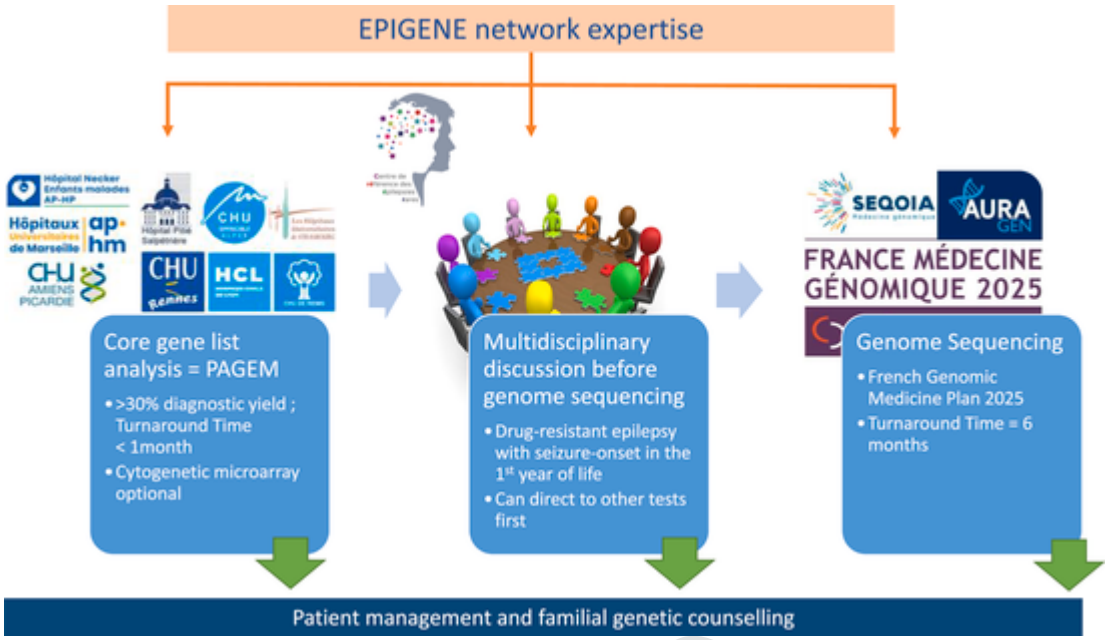

Fig. 2. Summary of the EPICARE network expertise and organization.

The objectives of the EPIGENE network were: i) to harmonize the practices (clinical questionnaire, core gene list), ii) to improve practices (inter-laboratory quality control, discussion of complex cases on a regular basis) and of cross knowledge between clinicians and geneticists, iii) to provide equal access to genetic testing throughout the country, and iv) to facilitate common research projects.

2. Results and discussion

From 2014 to 2020, four successive versions of the PAGEM gene panel were designed and included 68 (V1), 71 (V2), 116 (V3) and 144 (V4) genes, respectively. All the gene panels (or exome sequencing) used correspond to type C tests according to the European Society of Human Genetics guidelines (Matthijs et al., 2016), with gene panels covering more than 99% of the target. The regions with no or insufficient coverage are indicated in the report or were available upon request. In parallel, EPIGENE has organized an inter-laboratory quality control, annually since 2018 in order to improve practices, homogenize the interpretation and report of panel analysis nation-wide, from DNA sequencing to clinical report.

Between 2015 and 2020, 4035 index cases with epileptic disorders were analyzed with the PAGEM gene panel in a diagnostic setting, with the following distribution: Lyon: 1377, Paris Pitié Salpétrière: 974, Marseille: 691, Paris Necker: 530, and Strasbourg: 463. Data from centers that recently joined the network, or that used several lines of analyses before the PAGEM, were not included in this survey.

Fig. 1 shows the distribution, for each gene, of the pathogenic or likely pathogenic variants according to the ACMG classification (Richards et al., 2015). The global diagnostic yield was 31.3% (n = 1265/4035). No pathogenic or likely pathogenic variant was found in 34 genes, among which four were present since V1, two were added in V2 and the others had been added in the V4. Pathogenic or likely pathogenic variants were found for 94 genes (65.3%, 94/144) in at least two patients. The top 10 genes (i.e., *SCN1A, KCNQ2, STXBP1, SCN2A, SCN8A, PRRT2, PCDH19, KCNT1, SYNGAP1*, and *GRIN2A*) accounted for about 17% of the patients and more than 50% of the confirmed diagnoses (Fig. 1). These genes cause early-onset epileptic disorders in most of the patients. This top 10 representation has probably been reinforced in our cohort by historical interest in a few genes in each group, especially for genes such as *GRIN2A* (Lyon, Strasbourg), *SCN1A* (Paris Pitié-Salpétrière), *KCNT1* (Paris Necker), and *KCNQ2* (Marseille, Lyon).

However, this peculiar distribution of genes in epileptic disorders was already observed in previous cohorts and is different from the distribution of genes causing intellectual disability without epilepsy or from diseases which epilepsy is a secondary feature (Heyne et al., 2019). In that latter case, the number of genes involved is far higher, many of them accounting for a small subset of patients. One of the reasons for the recurrent involvement of a subset of genes in epilepsy disorders is related to the role of ion channels that are globally rather intolerant to missense variants and the presence of multiple functional consequences (gain of function, loss of function, dominant negative, or more complex combinations of functional dysfunctions) (Heyne et al., 2018). Pathogenic or likely pathogenic variants were found in genes causing treatable inborn errors of metabolisms for 38 patients (3% of patients with a diagnosis), including *SLC2A1* (15), *ALDH7A1* (11), *PNPO* (7), *BTD* (3), and *SLC19A3* (2).

Our global results are quite consistent with those from previous studies and suggest that a gene-panel approach is an efficient first step for the genetic diagnosis of Mendelian epileptic disorders. This approach allows a rapid turn-over for very young patients who can benefit from treatment adaptation.

The EPIGENE network capitalized on groups' interests over the past few years and enable deep phenotyping collaborative studies and new epilepsy genes discoveries (Kuchenbuch et al., 2019; Mignot et al., 2019; Denis et al., 2019; Bar et al., 2020, 2021). The EPIGENE network enhances translational collaborations in genetic epilepsies and beyond within the reference center for rare epilepsies frame. A detailed study of the clinical and genetic data of the global EPIGENE series is in preparation.

In 2016, the French government launched a national effort - The *Plan France Medecine Genomique 2025* - to ensure that every patient with rare disease can access to new genomic medicine technologies in an equitable manner across the territory, in order to improve the way diseases are diagnosed, prevented and treated. Two first platforms performing whole-genome sequencing in cancer (somatic) and rare diseases (germline) were created in Lyon (AURAGEN) and in Paris (SEQOIA) and the first sequences were produced in 2020.

The CRéER and the EPIGENE network obtained that patients presenting "drug-resistant epilepsies with seizure-onset in the first two

years of life" are eligible for genome sequencing. This category of patients was prioritized because expected to be associated with the higher proportion of Mendelian disorders and the higher diagnostic yield. WGS can be performed in trios (affected patient and the unaffected parents), in quartets (two affected children and the unaffected parents) or any other family combination including several affected individuals. Patients without any pathogenic or likely pathogenic variants identified by the panel can now benefit from WGS in order to look for missed variants in frequent genes, to screen less commonly involved genes, and to identify non-coding pathogenic variants or novel genes causing Mendelian epilepsies.

The *Plan France Medecine Génomique* requested to add specific case discussion meetings corresponding to the geographical area of each platform (AURAGEN and SEQOIA), to validate the relevance of genomic testing and the results that require additional expertise (some variants of unknown significance, variants in unusual or novel genes …). This organization, based on our 6yearsexperience as a multidisciplinary network, aims to improve the genetic diagnosis of epileptic disorders and to disseminate our shared knowledge and practices to other French Centers (Fig. 2). The strength of the multidisciplinary video case discussions is to provide comprehensive overview of the patients' phenotype, including the expertise of video-EEG and correct brain MRI interpretation, to improve the accurate interpretation of the molecular data. The molecular geneticists and cytogeneticists of the EPIGENE network will be involved in the analysis of WGS data, including single-nucleotide variants, indels, copy-number variations and structural variants. After the validation step of this organization and of the scale-up of the sequencing workflow of both platforms, other categories of patents with epileptic disorders of yet unknown etiology will also benefit from WGS. A next step will be a national consensus in order to restrict the number of genes on the panel to core recurrent genes that will have to be tested quickly in patients with early-onset epilepsy. The proposal will be to leave for WGS the non-urgent cases and those with a negative gene panel.

This collaboration between expert clinicians and biologists enables the EPIGENE members with CRéER to be ready for the coming challenges of genome analysis, functional analysis based on collaborations at European level mainly ERN-EPICARE, and International level with the acceleration of the open data space.

Authors statement

All the authors contributed to conceptualization, data acquisition and curation, as well as writing and review. GL Drafted and edited the manuscript.

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