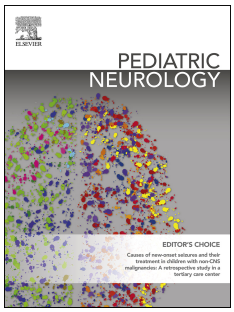

Copy Number Variation and epilepsy: state of the art in the era of high throughput sequencing, a multicenter cohort study

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Keywords (6): epilepsy, neurodevelopmental disorder, chromosomal microarray, copy number variations.

Word count: 3810 words

Highlights:

- The diagnosis rate is 14 % (35/250) using first-line microarray in the case of epilepsy mostly associated with neurodevelopmental delay or brain anomalies.
- The best results are observed in the case of associated neurodevelopmental disorders.
- 34% of reported CNV (12/35) contain genes that could have been found using targeted epilepsy gene panels.

Legends:

Figure 1: Inclusion flow chart of patients included in the analyses. Among the 3297 postnatal CMA performed between 2015 and 2021, 378 patients were included with the “epilepsy” keywords. We excluded 119 patients because of missing clinical information or no epilepsy treatment, and we excluded 9 patients with epilepsy-targeted gene panels performed before CMA with a positive result. Finally, 250 patients corresponded to our inclusion criteria with treated epilepsy and sufficient clinical information, 213 patients were considered as negative results (201 negatives, 12 VUS) and 38 positive results including 3 patients with incidental findings not linked with epilepsy (1 with *PMP22* deletion and 2 with Klinefelter syndrome) and 35 positive CNV results linked with epilepsy. CNV: copy number variation, CMA: chromosomal microarray, TGP: targeted gene panel, VUS: Variant of Uncertain Significance

Figure 2: Description of the 250 included patients and their genetic results: positive results corresponding to highly penetrant variants, negative results corresponding to VUS, or negative results. (A) associated epilepsy syndrome in each patient according to *ILAE 2022* classification (B) associated intellectual disability of each patient (C) size of head circumference at the time of the diagnosis for each patient.

DEE: Developmental and Epileptic Encephalopathy, EE-SAWS: epileptic encephalopathy associated with spikes and waves during sleep, EIDEE: early infantile developmental and epileptic encephalopathy, EMAtS: Epilepsy with Myoclonic Atonic Seizures, ID: intellectual disability, IESS: Infantile Epileptic Spasms Syndrome, MEI: Myoclonic Epilepsy of Infancy,

SeLECTS: Self-limited Epilepsy with Centrotemporal Spikes, SeLFNIE: Self-limited Familial Neonatal Infantile Epilepsy

Table 1: Thirty-five reported pathogenic CNV from our cohort with cytogenetics and clinical details. The 3 incidental findings of CNV are described at the end of the table.
We also separated the CNV according to the classification described in the text with 1. recurrent CNV enriched in epilepsy patients, 2.CNV related to a neurodevelopmental disorder (NDD) phenotype described in OMIM where epilepsy can occur 3. CNV included a gene already known in epilepsy 4. CNV based on size combined with de novo occurrence and detailed the ACMG class based on ISCN (International System for Human Cytogenomic Nomenclature) 2020.
CC: corpus callosum, CNV: copy number variation, DEE: Developmental and Epileptic Encephalopathy, DS: Dravet syndrome, dn: de novo, kb: kilobases, DEE: Developmental and Epileptic Encephalopathy, EE-SWAS: epileptic encephalopathy associated with spike and waves activation during sleep, EIDEE: early infantile developmental and epileptic encephalopathy, HPV: Highly Penetrant Variant, MRI: Magnetic Resonance Imaging, NP: not precise, PAGEM: Monogenic epilepsy gene panel, PVLM: periventricular leukomalacia, WM: white matter

Table 2: Comparison of clinical details from patients with positive CNV results and the one with negative CNV results.
DEE: Developmental and Epileptic Encephalopathy, EE-SWAS: epileptic encephalopathy associated with spike and waves activation during sleep, EIDEE: early infantile developmental and epileptic encephalopathy, EMAts: Epilepsy with myoclonic atonic seizure, GGE: Genetic Generalized Epilepsy, GEFS+: Generalized Epilepsy with Febrile Seizures, ID: Intellectual Disability, LGS: Lennox-Gastaut syndrome, MRI: Magnetic Resonance Imaging, NP: not precise

Table S1: Comparison of diagnosis rate depending on the type of epilepsy using CMA or other techniques (WES, WGS, TGP, Sanger) after negative CMA.

Supplemental file :
List of genes from the PAGEM list (PAGEM: Monogenic epilepsy gene panel).

ABSTRACT (max 250w):

Genetic epilepsy diagnosis is increasing due to technological advancements. While molecular diagnosis use increases, chromosomal microarray analysis (CMA) remains an important diagnostic tool for many patients. We aim to explore the role and indications of CMA in epilepsy, given the current genomic advances.

We obtained data from 378 epileptic described patients, who underwent CMA between 2015 and 2021. Different types of syndromic or non-syndromic epilepsy were represented. After excluding patients who were undertreated or had missing data, we included 250 patients with treated epilepsy and relevant clinical information. They mostly had focal epilepsy or developmental and epileptic encephalopathy, with a median start age of 2 years. Ninety percent of the patients had intellectual disability, more than two-thirds had normal head size, and 60% had an abnormal MRI. We also included ten patients with epilepsy without comorbidities.

In our cohort, we identified 35 pathogenic CNV (Copy Number Variation) explaining epilepsy with 9 recurrent CNV enriched in epilepsy patients, 12 CNV related to neurodevelopmental disorder phenotype with possible epilepsy, 5 CNV including a gene already known in epilepsy, 9 CNV based on size combined with *de novo* occurrence. The diagnosis rate in our study reached 14% (35/250) with first-line CMA, as previously reported.

While targeted gene panel sequencing could potentially diagnose some of the reported epilepsy CNVs (34% (12/35)), CMA remains a viable option as the first-line genetic test in cases where other genetic tests are not available, and as a second-line diagnostic technique if gene panel or exome sequencing yield negative results.

1. **INTRODUCTION**:

Epilepsy is one of the most frequent neurological impairments affecting 0.5-1% of the population worldwide and encompassing different types of phenotypes and multiple etiologies (1,2). Although antiseizure medication has improved recently, almost 30% of patients still suffer from recurrent seizures. The neurological pathways involved have been broadly discovered, leading to better genetic diagnoses of these epilepsies and a deeper understanding of the neurobiological processes underlying these phenomena. As a result, specific epileptic treatment can be adapted in some cases. It is currently estimated that between 50 and 70% of epilepsies are linked with a genetic etiology (3,4).

In the last decades, advances in the field of genetics have taken a major turn. Firstly, genetically associated in 1995, with *CHRNA4* pathogenic variations (5); now more than 500 genes have been described as associated with one or more epileptic phenotypes (6) implicated in single-ion channels, metabolic pathways, or signaling pathways(7). The genetic diagnosis may lead to treatment improvement with personalized medications (8), specific gene therapy (*SCN1A*), or adapt surgical indications (9). It allows also, specific genetic counseling, adaptive follow-up, and more information to the families on supposed evolution (10). Recent studies have highlighted that one-third of patients with confirmed genetic epilepsy could benefit from personalized medicine (11). Seizures are prevalent among individuals with neurodevelopmental disorders. They affect 21.5% of those with autism and intellectual disability, while 8% are affected by autism without intellectual disability (12).

In France, genetic diagnosis for epilepsy is conducted using PAGEM, a gene panel designed for monogenic epilepsies. This panel consists of 144 genes that have been identified after national expertise collaboration. By using this core panel, a diagnosis rate of 31.3% has been achieved for patients with epilepsy (1).

Chromosomal microarray (CMA) is a genetic technology that plays a special role in diagnosing epilepsy. Copy number variation (CNV) is known to be involved in different types of epilepsy. We categorized 4 different types of copy number variations (CNVs) implicated in epilepsy: 1. recurrent CNV enriched in epilepsy patients (13), 2. CNV related to a neurodevelopmental disorder (NDD) phenotype described in OMIM where epilepsy can occur 3. CNV including a gene already known in epilepsy (14), 4. CNV based on size combined with *de novo* occurrence. Copy number variations known as cause or risk factors are described in 5 and 12% of patients with epilepsy by different studies (15–20).

Establishing a genetic diagnosis of epilepsy is crucial for treatment, follow-up, and counseling. Various techniques can be used, such as karyotype for ring chromosome 20 syndrome (21) to whole genome sequencing. With the advancement of genomic techniques, we aim to better understand the current role of chromosomal microarray techniques (such as SNP array and CGH array) in the context of recent genetic diagnostic developments. Specifically, we are exploring the following question: "What is the current significance of CMA in epilepsy genetic diagnosis odyssey?"

We focused on our multi-center experience with common genetic laboratory analyses, with DNA sampling from different hospitals. We selected patients with confirmed treated epilepsy and detailed their phenotype. Then we looked carefully at their cytogenetic analyses and classified them with the *ILAE 2022* classification. Finally, we compared these results to those obtained with an epilepsy-targeted gene panel sequencing, to establish the diagnostic performance regarding molecular analyses.

2. **MATERIAL AND METHODS:**

2.1. **Selected patients**

We have analyzed patients in our cytogenetic laboratory using CGH array and SNP array techniques. From this pool, we have selected patients who underwent an array between 2015 and 2021 and were treated in the hospitals of Strasbourg, Colmar, and Mulhouse. We

specifically focused on patients who had reported symptoms of "epilepsy", "seizures", "encephalopathy", or "febrile seizures" in their clinical records.

We have classified patients based on the ILAE 2022 classification (22) and gathered information related to their intellectual disability, head circumference, neurological and extra-neurological impairment, brain MRI, age at the cytogenetic analyses, dysmorphic features, and age at epilepsy onset. We have obtained this information from the hospitalization or consultation reports, including the specialty of the clinician who prescribed the CMA. For head circumference, we have used raw data and the WHO reference growth chart (23) to detect micro or macrocephaly. Additionally, some patients have undergone metabolic investigations that yielded negative results.

2.2 **Cytogenetic analysis**

Cytogenetic analyses were performed using the CGH-array technique (array comparative genomic hybridization) for 64 patients and using SNP-array techniques (array single nucleotide polymorphism) for 195 patients. In the case of consanguinity, a SNP array was preferred.

In the proband, DNA was extracted from blood samples using QIAamp DNA mini kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. Chromosomal microarray analysis (CMA) was performed with the human genome CGH Microarray 180K kit (Agilent Technologies®) or Infinium HumanCytoSNP-12 BeadChip (Illumina®). Results were analyzed using CytoGenomics V3.0 software (Agilent Technologies®) to CGH or Genome viewer from the GenomeStudio® software to SNP and genomic positions referred to the human genome version GRCh37 (hg19). All quality criteria were obtained and CNVs were considered with a minimum size of 200 kb in postnatal (according to French cytogeneticists’ recommendations).

Familial segregation was performed when parents' blood samples were available.

2.3 **Molecular analyses**

For the targeted gene panel, libraries have been prepared and capture was performed using probes including at least the PAGEM list v3(2) **(supplemental file)** (SureSelectQXT, Agilent or Roche Nimblegen) and then sequenced (Illumina HiSeq ou Nextseq). Pipeline STARK was used for sequence alignment and variant identification. Varank (24) and AnnotSV (25) were used for variant ranking and annotation.

Variants classified as pathogenic or probably pathogenic were confirmed through Sanger sequencing. Familial segregation analysis was performed if parent samples were available.

2.4 **Variant interpretation**

Interpretation was validated using ACMG recommendation (26,27) and AChro-Puce recommendations allowing us to classify results in 5 classes from benign to pathogenic. Predisposing factor associated as defined by AChro-Puce (28) network defined as CNV with susceptibility to neurodevelopmental disorder with incomplete penetrance and/or variable expressivity. VUS (Variations of Uncertain Significance) have been defined by Richards et al. in 2015 as insufficient or conflicting evidence about a molecular alteration's role in disease, with a probability of pathogenicity between 10 and 90% (26). To detail the genes included in each CNV we used the AnnotSV software (25).

3. RESULTS

3.1. Selection of patients with active epilepsy

Between 2015 and 2021, the cytogenetic laboratory of Strasbourg University Hospital conducted 3,297 cytogenetic postnatal array analyses (SNP or CGH array). Out of these, 378 patients were referred to the laboratory with a clinical questionnaire mentioning "epilepsy" (refer to Methods). After excluding patients with no anti-seizure medication (70 patients) and no confirmed epilepsy (49 patients), retrospective data for 259 patients were gathered. **(Figure 1)**.

The number of cytogenetic analyses requested per year varied between 26 and 50, and they were primarily prescribed by a pediatric neurologist (177), followed by a geneticist (66) and an adult neurologist (14). The age of patients at the time of the request for cytogenetic analysis ranged from birth to 64.6 years, with a mean age of 10.6 years. We excluded 9 patients from statistical analysis who had undergone epilepsy-targeted gene panel testing before CMA and had received a positive result.

3.2 Clinical characteristics of the selected patients

We collected data from about 250 patients aged from 0 to 775 months (64.5 years) [median: 7.7 years] at the age of the genetic prescription, 135 males (54%) and 115 females (46%). Epilepsy started between 0 and 720 months (60 years) [median 2 years]. **(Figure 1)**

The most common type of epilepsy was developmental and epileptic encephalopathy (DEE) (64 patients/26%), followed by focal epilepsy (59 patients/24%) and febrile seizures/GEFS+/Dravet syndrome (30 patients/12%). Twenty-four patients presented with infantile epileptic spasms syndrome (10%) and 22 patients with Genetic Generalized Epilepsy (GGE) (9%). Early infantile DEE and self-limited epilepsy accounted for 20 and 14 patients respectively (8% and 6%). The other phenotypes (progressive myoclonus epilepsy, epilepsy

with myoclonic-atonic seizures, Lennox-Gastaut syndrome, or myoclonic epilepsy in infancy) affected less than 10 children each of them. **(Figure 2A)**

Most of the patients presented with associated intellectual disability (ID) (227 patients/91%), ranking from mild ID for 81 patients, moderate ID for 61 patients, and severe ID/profound ID for 53 patients. Information was missing for 6 patients, 5 were too young for evaluation and 13 had ID with no ranking specified. Twenty-three patients presented with epilepsy without reported ID. **(figure 2B)**

In the reported clinical form, the head circumference measure was also considered, 43 patients had microcephaly (25%) whereas 13 patients had macrocephaly (7%), and 118 had a normal head size (68%). **(figure 2C)**

MRI analyses were performed for 206 patients (82%), 83 patients had a normal MRI (40%). Among the 123 patients with abnormal MRI nonspecific anomalies were found (white matter anomalies, corpus callosum anomalies, cortical malformation…).

In our cohort, 57% (94/165) of the described patients presented with extra-neurological anomalies such as cardiomyopathy, scoliosis, and cutaneous impairment, some of them oriented the diagnosis as in *NF1* microdeletion or Turner syndrome.

3.3 Genetic analyses

Genetic testing was conducted on 250 epilepsy patients, and 38 of them (15%) were diagnosed with a CNV through chromosomal microarray testing. It should be noted that this percentage does not include patients who had already been diagnosed through gene panel testing. Among the 38 positive cases, one patient had a *PMP22* deletion, while two other patients were diagnosed with Klinefelter syndrome, which was unrelated to their epileptic phenotype and was considered an incidental finding.

There were 35 positive array results. Out of these, 24 indicated a loss while 11 indicated a gain of copy. Three results showed gonosomal anomalies, including X trisomy, one with Turner syndrome, and one with unbalanced translocation concerning the Y chromosome.

Sixteen patients were reported as de novo, while four were inherited, two paternally and two maternally.

We choose to precise the pathological mechanism CNV in epilepsy as 1. 9 patients presented with recurrent CNV enriched in epilepsy patients, including 2 with highly penetrant CNV (22q11.2 duplication and 22q11.2 deletion) and 7 predisposing factors (16p11.2 deletion (n=3), 7q11.23 duplication, 15q13.3 deletion (n=2), and 15q13.3 duplication…) 2. 12 CNV related to NDD phenotype where epilepsy can occur (*NF1* deletion, *SHANK3* deletion, 1p36 deletion, 10q deletion, 10q trisomy…) 3. 5 CNV included a gene already known in epilepsy (5q14.3 deletion including *MEF2C* promoter, *STXBP1* or *SOX5* deletion…) and 4. 9 CNV classified as pathogenic based on their size and their de novo occurrence with 5 CNV affecting gonosomal chromosomes (Turner syndrome, and unbalanced translocation between X and Y chromosomes). Two mosaic aneuploidies which are trisomy 14 and a 9p tetrasomy have been reported. Detailed data about reported pathogenic CNV can be found in **Table 1.**

Among the 35 positive results patients **(table 2)**, 37% presented with DEE (13/35) and 20% with focal epilepsy (7/35). However, epilepsy syndromes such as progressive myoclonic epilepsy, infantile epileptic spasms syndrome, or Lennox-Gastaut syndrome could not be diagnosed using CMA in our cohort. Most of the patients with positive results had a mild (n=13/88: 15%) or moderate intellectual disability (n=10/60: 17%) as in the whole cohort, severe ID had the lowest diagnosis rate using CMA (n=6/53 11%). MRI results were similar. Head circumference anomalies do not seem to be associated with a specific diagnosis rate.

Out of the 19 patients who underwent CMA and had a pathogenic CNV or a variant of uncertain significance (VUS), 11 also had a targeted gene panel performed. However, none of them received a definitive diagnosis. Among these 11 patients, 6 had a VUS in genes associated with neurodevelopmental disorders (NDD), while the remaining 5 were considered negative after undergoing NDD gene panels, which were specific to epilepsy or intellectual disability.

Among the 201 patients with negative array techniques, 112 had another exploration including whole exome sequencing, epilepsy, or ID gene panel or targeted *MECP2* sequencing (one patient). Among them, 32 had a positive diagnosis corresponding to a diagnosis rate of 28,6% (Variation of Unknown Significance excluded). *KCNQ2*, *SCN1A,* and *STXBP1* were the most frequently reported as in previously reported cohorts, being the 3 most reported genes in epilepsy diagnosis(2) (3 *KCNQ2*, 3 *SCN1A,* and 3 *STXBP1* pathogenic or likely pathogenic variations). Other reported genes are also the most frequently reported in developmental epileptic encephalopathies with ion channels and receptors (*SCN2A, GABRB2, GABRB3, KCNB1*…) and in synaptic support proteins such as *STXBP1.* Twenty-two patients were reported with VUS using a specific epilepsy gene panel.

More specifically, in this CMA's first investigation negative population, the rates of pathogenic monogenic variation are also high in cases of focal epilepsy or DEE. However, it should be noted that 5 patients with ISS had a molecular diagnosis using second line TGP whereas none with first-line CMA, and no patients in the GEFS+ category had a diagnosis whereas there were 5 with CMA, considering patients with CMA-first. One patient with LGS, 1 with PME, and 1 with EMAtS had a diagnosis using TGP or WES whereas none with this type of epilepsy had a CMA diagnosis. **(table S1)**

3.4 Combined cytogenetic analyses and high throughput results.

We then looked carefully at the 35 positive CMA and crossed the data with the gene from the PAGEM panel (formerly known as the reference for epilepsy panel in France)(1).

Twelve patients had a CNV that included specific genes from the gene panel list such as *ALG13, ARHGEF9, ARX, CASK, CDKL5, CHRNA4, CLCN4, CNKSR2, EEF1A2, FOXG1, GPHN, GABRA1, GABRB2, GABRG2, IQSEC2, KCNQ2, MECP2, MEF2C, PACS2, PCDH19, PIGA, PIGO, PRRT2, SLC9A6, SLC35A2, STAMBP, STXBP1, SYN1, WDR45* **(table 1).** These patients presented mostly with developmental and epileptic encephalopathy (4/12), or

focal epilepsy (2/12) also reported as the most frequent epilepsy syndrome in our global cohort.

Since CNV could be analyzed on TGP data, 34% of our positively reported patients could have benefited from an initial diagnosis only using the PAGEM epilepsy gene panel (12/35).

For the positive patients with pathogenic CNV not containing a gene from the PAGEM list, multiple reasons can be described: seven patients presented with CNV enriched in epilepsy patients such as 15q13.3 microdeletion (29) without specific gene linked with epilepsy included in these deletions or 22q11.2 deletion with candidate genes known to be brain-related disease genes such as *TBX1* or *LZTR1* (30). In certain TGP designs, these specific regions could be incorporated. Ten patients presented with CNV where epilepsy can occur consistently such as *SHANK3* deletion or *NF1* deletion. Two patients had *MEF2C* promoter or *SOX5* deletion known to be implicated in epilepsy but rarely and not formally included in epilepsy gene panels. Four patients presented with large, de novo CNVs not linked to epilepsy genes responsible for NDD.

4. DISCUSSION

With advanced genetic techniques, we can estimate that around 50% of epileptic encephalopathy cases have a genetic origin (31), including single nucleotide variations and copy number variations (32). The purpose of this study is to evaluate the role of array testing in the realm of genetic tests for epilepsy, particularly considering advancements in genomic techniques, and to compare it with cytogenetic analyses. We have focused on patients who have been diagnosed with epilepsy and excluded those who have experienced fainting or movement disorders that can be mistaken for epilepsy, particularly in patients with neurodevelopmental disorders (33). One-third of patients with febrile seizures presented with NDD (34), especially with ADHD. This study excluded untreated patients with febrile seizures, as this type of seizure typically affects 3-5% of healthy children and may not require long-term treatment.

4.1. The rate of diagnosis is consistent with the one reported earlier.

A recent review focused on the diagnosis rate in epilepsy depending on the type of explorations. In this review, 43 articles have been reviewed with 5654 epileptic patients (31). With chromosomal microarray, the diagnosis rate is between 2 and 50% depending on the number of included patients and their inclusion criteria. Also, the categorization of VUS could differ in some publications, we chose to consider them as negative results but in some publications, they were classified as positive results (16). In our study, we diagnosed 14.8% of patients, which is consistent with previously reported cohorts. However, it should be noted that our patient selection was not based on epilepsy type or age of onset.

We conducted a CMA test on 39 patients who were over 18 years old. However, as these patients were relatively older, some clinical information could be missing, such as their age of walking and occipitofrontal circumference. In addition, the diagnosis rates may be limited due to the clinical heterogeneity among patients, which has been observed in both our studies and previously reported studies.

4.2. Associated developmental disorders seem to be associated with a higher CNV diagnosis rate.

We know from previous cohorts that pathogenic CNV can be identified in 1–4% of individuals with epilepsy without comorbidities and more than 10% of those with seizures and neurodevelopmental disorders (30).

All the patients included in this report were diagnosed with neurodevelopmental disorders (NDD), which included intellectual disability, micro/macrocephaly, or brain anomalies, except 10 patients who were diagnosed with epilepsy alone. As previously published, most of the patients in this study could be classified as "epilepsy plus" patients, which is a unique type of NDD that is frequently associated with pathogenic CNV (17,35). All the patients reported in our analyzed cohort presented with comorbidities mostly intellectual disability by a higher diagnosis rate. Recently Sheidley et al. reported a higher diagnosis rate in genetic epilepsy in case of associated neurodevelopmental disorder or case of developmental and epileptic encephalopathy. Whatever the type of genetic test used (exome, panel, or CMA) diagnosis rate is higher in the case of associated neurodevelopmental disorder 22% compared to the one without NDD (8%) (31).

The ILAE recommendation about genetic epilepsies in 2022 (36) recommended the use of exome or genome sequencing in case of epileptic encephalopathy or syndromic epilepsies. In case of isolated generalized or focal epilepsy, genetics investigations were recommended only if neurodevelopmental disorders were associated, or familial history or pharmacoresistant epilepsy. WGS or WES are recommended as a first-line diagnosis, but we know that these analyses could not be performed in all the epilepsy centers.

In our cohort, the global diagnosis rate was approximately 15%. This rate was more relevant in the case of developmental epileptic encephalopathy, which is a specific type of epilepsy where intellectual disability is the main issue. Among our cohort, 34 patients with positive CNV showed mainly intellectual disability, along with some seizures. This type of cohort had the

highest rate of diagnosis already reported (17). All the positive CNV patients presented either with intellectual disability, MRI anomalies, or head circumference anomalies; nearly most of them presented with the association of this 3-impairment associated with positive CNV. None of the patients with epilepsy without comorbidities had pathogenic results using CMA.

Some epilepsy syndromes were associated with no pathogenic CNV such as infantile spasms syndrome, a specific type of DEE starting before 1 year of age associated with a cognitive decline most of the time. In previous studies, the diagnosis rate was around 11% using CMA in patients with West syndrome mostly involving 15q CNV with a lower rate than the targeted gene panel with a diagnosis rate of 24% (37).

4.3 Precision about some genetic anomalies

Two patients have been reported to have Klinefelter syndrome, a genetic disorder that affects males. One of them has been associated with SeLECTS (Self-limited epilepsy with centro-temporal spikes) and the other with epileptic encephalopathy. Although the link between Klinefelter syndrome and epilepsy is not definitive, a 1998 article reported a higher risk of developing epilepsy in individuals with Klinefelter syndrome (38). For these patients, we considered that the genetic results are not linked with epilepsy, further genetic exploration would be necessary. Gonosomal anomalies seem to be frequently associated with epilepsy (39), in our cohort 6/38 patients had a chromosomal anomaly located on X or Y chromosome. A *PMP22* deletion was diagnosed using CMA, revealing incidental findings as a pangenomic technique. Prescribers, parents, and patients should be aware of this possibility.

Two patients had an *MEF2C* deletion, one comprised the *MEF2C* gene and was also detected using an epilepsy gene panel, but one patient had an *MEF2C* regulatory element deletion (40). CMA as a pangenomic technique could also highlight CNV with an implication of regulatory elements not always specified in targeted gene panels. In a specific version of PAGEM, the *MEF2C* regulatory element has been included in the pipeline to diagnose this peculiar anomaly.

Our study had some limitations. Firstly, we only analyzed samples in one genetic laboratory using the same method. This restriction could have resulted in a limited number of reported

patients. Secondly, we mainly focused on epilepsy patients who also had associated neurodevelopmental anomalies. This could reflect the common indication of genetic exploration in cases of epilepsy.

4.4 The CMA still has a place in the genetic epilepsy diagnosis odyssey

Genes implicated in epilepsy are widely known (41,42) with complex physiopathology mechanisms (loss of function, gain of function, dominant negative effect) especially considering channelopathy. The diagnosis rate in the case of exome sequencing or genome sequencing is higher than in the case of chromosomal microarray or targeted gene panel (TGP) (31). Previous studies have also shown that gene panels have higher diagnostic yields than CMA in patients with epilepsy (43). If an entire gene has been deleted or duplicated, confirmation of the size of the CNV and the number of contiguous genes affected is usually required through CMA, which was performed on nine excluded patients. Unlike TGP, CMA provides a whole genome analysis and can detect medium-size deletions or duplications. Additionally, TGP only supports coding sequences but not noncoding sequences such as promoter or UTR (untranslated region), unless they are specifically added, such as the *MEF2C* promoter region (40). Including some genes in this recurrent CNV such as *TBX1* in 22q11.2 could help diagnose this type of CNV for instance (29,30).

Twenty-three of the 38 patients presented with pathogenic CNV-affecting genes do not present on the PAGEM list. This patient would have a negative result using TGP. Exome or genome analysis could have reached the diagnosis (44). Genome sequencing could have made the diagnosis, with now the highest diagnosis rate of 48% (31). But this technique is still expensive not broadcast everywhere and a long analysis of up to 1 year is partially explained by the number of variants analyzed.

In cases of epilepsy, depending on the availability of local facilities, we could consider using chromosomal microarray analysis. Even after targeted gene panel testing, CMA can still be

useful for detecting copy number variations without known epilepsy genes, which accounts for up to 9% of our overall cohort (23 out of 250 cases). In addition, CMA can identify medium-sized CNVs that may not be covered in TGP or exome sequencing. CMA analysis also appears to be more effective in cases of epilepsy associated with multiple congenital anomalies, while TGP seems to be more efficient for phenotypes with predominant epilepsy.

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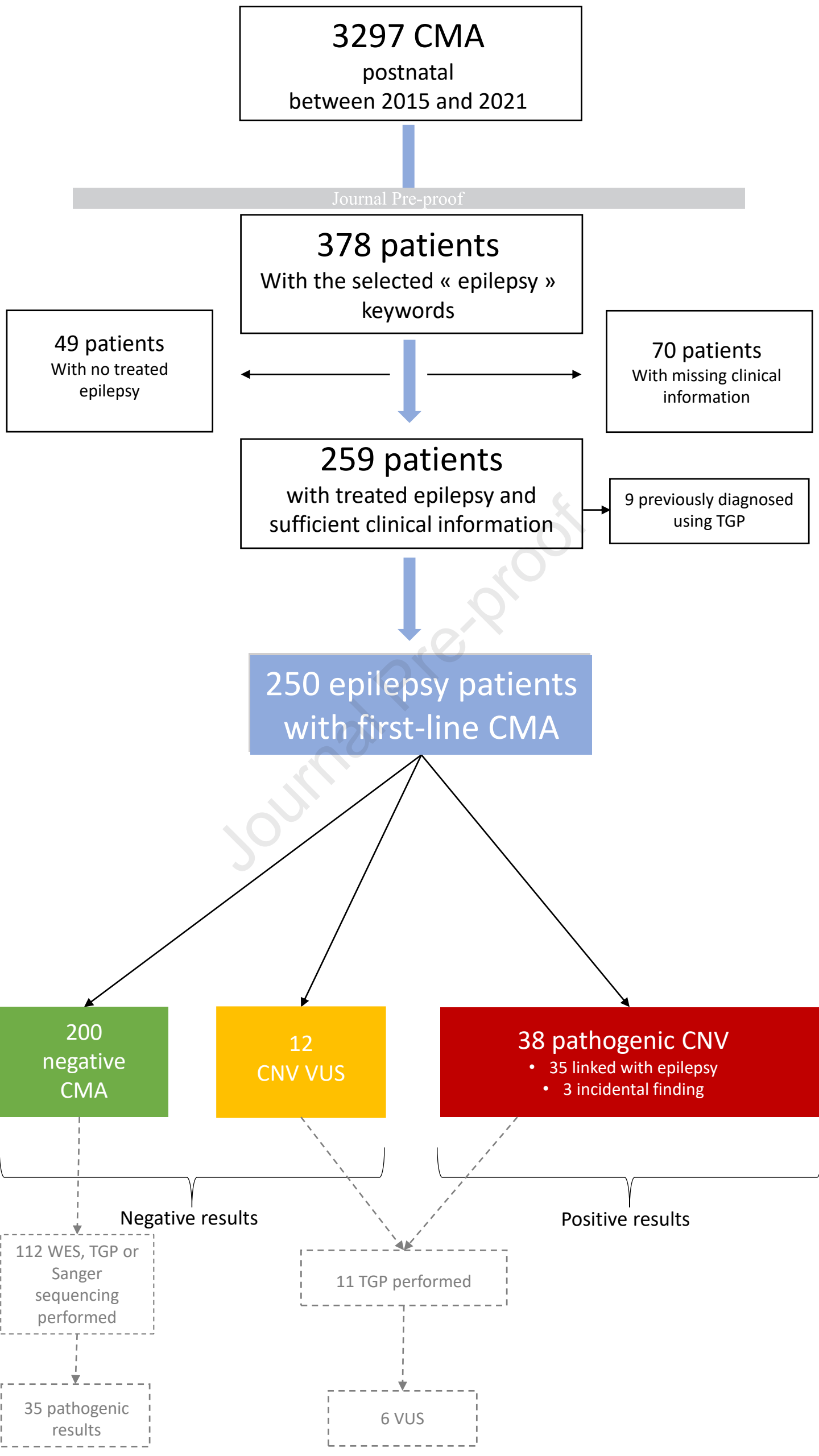
N°	Cytogenomic abnormalities (ISCN 2020)	Size of the CNV (kb)	Number of OMIM in the CNV	CNV	ACMG class	PAGEM	Age at first seizure	Epilepsy syndrome	Intellectual disability	MRI anomalies	Head circumference	Extra neurologic anomalies
1	arr[GRCh37] 1q44(246097984_249202755)x1 dn	3104	15	2	class 4	no	0	EIDEE	Severe	Multiple anomalies	Normocephaly	no
2	arr[GRCh37] 1p36.32p36.33(1627987_2580546)x1	952	17	2	class 4	no	NP	DEE	NP	NP	NP	NP
3	arr[GRCh37] 1p36.33p36.32(752566_5211338)x1 dn	4458	64	2	class 5	no	120	DEE	Severe	NP	NP	scoliosis, dysmorphism
4	arr[GRCh37] 1p36.33p36.32(752566_4456133)x3	3703	64	4	class 5	no	5	EE-SWAS	Moderate	PVLM	NP	NP
5	arr[GRCh37] 2p13.2p11.2(73131167_85181724)x1 dn	12050	54	2	class 5	*STAMBP*	42	Focal epilepsy	Mild	Normal	NP	no
6	arr[GRCh37] 2p22.3p22.1(36381404_41723262)x1 dn	5341	23	2	class 4	no	NP	DEE	Mild	WM anomalies	Microcephaly	NP
7	arr[GRCh37] 3p26.3(232496_1237776)x3 dn	1005	2	4	class 4 (PF)	no	51	DEE	Moderate	NP	NP	Leber amaurosis, tubulopathy
8	arr[GRCh37] 5q14.3(88088204_88253916)x1 dn	165	1	3	class 5	*MEF2C*	55	DEE	Moderate	NP	Normocephaly	no
9	arr[GRCh37] 5q14.3(88774328_91802402)x1	3028	5	3	class 5	no	76	DEE	Severe	CC anomalies	Macrocephaly	no
10	arr[GRCh37] 5q33.3q34(157182897_167392011)x1 dn	10209	24	3	class 5	*GABRB2, GABRA1, GABRG2*	88	Focal epilepsy	Learning difficulties	Normal	NP	no
11	arr[GRCh37] 6q22.1q22.31(117253609_119591307)x1 dn	2337	10	2	class 5	no	12	Febrile seizures / DS	Mild	Normal	Microcephaly	dysmorphism
12	arr[GRCh37] 7q11.23(72722981_74134911)x3 dn	1411	25	1	class 5	no	NP	GGE	Absent	Global cerebral atrophy	Macrocephaly	aortic dilatation, arachnodactyly
13	arr[GRCh37] 9q34.11(130308439_130504070)x1 dn	195	4	3	class 5	*STXBP1*	108	DEE	Severe	Cortical malformation	NP	no
14	arr[GRCh37] 9p24.3p13.1(46587_38771460)x2~4	38724	48	4	class 5	*PIGO*	22	Febrile seizures / DS	No possible evaluation	Normal	Normocephaly	no
15	arr[GRCh37] 10q26.12q26.3(122934825_135434178)x1 dn	12499	65	2	class 5	no	360	Focal epilepsy	Moderate	NP	NP	dysmorphism
16	arr[GRCh37] 10q11.22q11.23(47085844_51795395)x3	4709	20	2	class 4	no	36	DEE	Moderate	CC anomalies	NP	nephrotic syndrome
17	arr[GRCh37]12p12.1(23777466_23938667)x1 dn	161	1	3	class 5	no	56	DEE	Moderate	Normal	Microcephaly	No
18	arr(14)x2~3	86430	151	4	class 5	*GPHN, PACS2 FOXG1*	NP	Focal epilepsy	Learning difficulties	NP	Normocephaly	dilated myocardiopathy
19	arr[GRCh37] 15q13.3(32021733_32510863)x1	489	2	1	class 4 (PF)	no	NP	DEE	Mild	NP	NP	scalp aplasia

20	arr[GRCh37] 15q13.2q13.3(30955149_32515681)x1 dn	1560	6	1	class 4 (PF)	no	36	GGE	Mild	Normal	Normocephaly	NP
21	arr[GRCh37] 15q13.2q13.3(30955149_32513176)x3 pat	1558	6	1	class 4 (PF)	no	6	Focal epilepsy	Mild	Cortical malformation	Normocephaly	no
22	arr[GRCh37] 16p11.2(29652999_30198600)x1 pat	545	24	1	class 4 (PF)	*PRRT2*	NP	GGE	Yes	NP	Normocephaly	NP
23	arr[GRCh37] 16p11.2(29661217_30199805)x1	538	24	1	class 4 (PF)	*PRRT2*	0	EIDEE	Moderate	NP	Normocephaly	no
24	arr[GRCh37] 16p12.2(21956457_22431170)x1	474	4	1	class 4 (PF)	no	NP	IGE	Yes	NP	NP	NP
25	arr[GRCh37] 17q11.2(29032375_30366843)x1 dn	1334	13	2	class 5	no	NP	Focal epilepsy	Mild	NP	NP	bilateral inguinal hernia, café au lait spot
26	arr[GRCh37] 17p13.3(978653_1637777)x3 dn	659	14	2	class 4	no	48	DEE	Severe	Ventricular anomalies	Normocephaly	jaw malformation
27	arr[GRCh37] 19p13.3(2833562_4647231)x3 dn	1813	48	4	class 4	no	24	Febrile seizures / DS	Mild	Normal	Microcephaly	growth retardation
28	arr[GRCh37] 20q13.33(61360088_62909908)x1	1549	44	*2*	class 5	*KCNQ2, CHRNA4, EEF1A2*	4	DEE	Moderate	Ventricular anomalies	Normocephaly	no
29	arr[GRCh37] 22q13.31q13.33(46114266_51186249)x1	5071	46	2	class 5	no	NP	Febrile seizures / DS	Mild	Global cerebral atrophy	Macrocephaly	no
30	arr[GRCh37] 22q11.21(18894835_21464119)x3 dn	2569	44	1	class 5	no	0	EIDEE	No possible evaluation	Normal	Microcephaly	no
31	arr[GRCh37] 22q11.21(18895227_21462353)x1 mat	2567	44	1	class 5	no	13	Febrile seizures / DS	Learning difficulties	NP	Normocephaly	ASD
32	arr[GRCh37] Xp22.33q28(676109_154929279)x2,Yp11.2q11.23(6592845_28784095)x0	154253 22191	246	4	class 5	*ALG13, ARX, MECP2, WDR45, PIGA, ARHGEF9, CDKL5, PCDH19, SLC9A6, SLC35A2, CLCN4, SYN1, CASK, CNKSR2, IQSEC2*	NP	EE-SWAS	Severe	NP	NP	NP
33	arr(X)x3	154755	245	4	class 5	*ALG13, ARX, MECP2, WDR45, PIGA, ARHGEF9, CDKL5, PCDH19, SLC9A6, SLC35A2, CLCN4, SYN1, CASK, CNKSR2, IQSEC2*	3	EIDEE	Moderate	White matter	NP	no

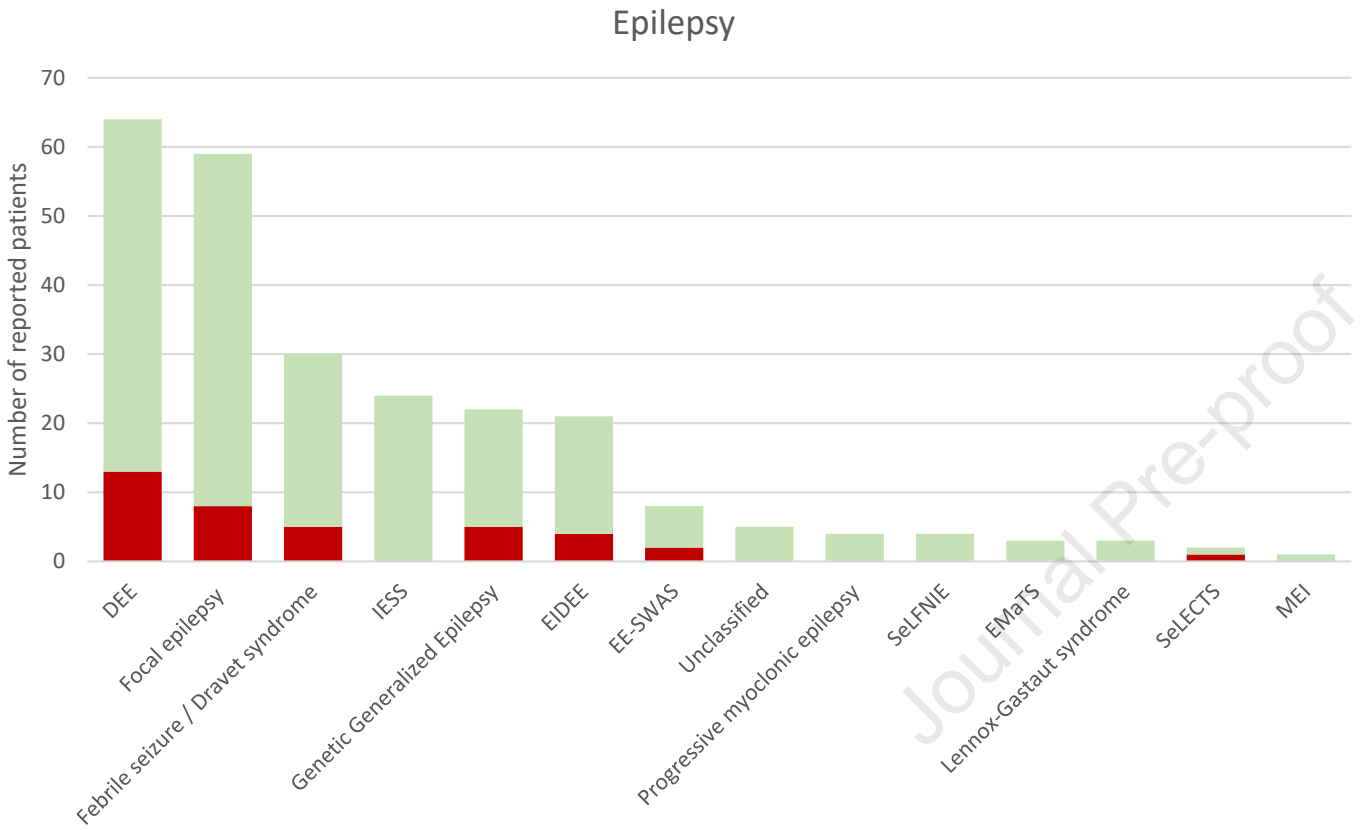
34	arr[GRCh37] Xp22.33p11.21(61091_56515434)x1,Xp11.21q21.1(56515434_77,378659)x1~2,Xq21.1q28(77378659_153023615)x1	154755	245	4	class 5	*ALG13, ARX, MECP2, WDR45, PIGA, ARHGEF9, CDKL5, PCDH19, SLC9A6, SLC35A2, CLCN4, SYN1, CASK, CNKSR2, IQSEC2*	120	GGE	Mild	Cortical malformation	NP	Turner syndrome
35	arr[GRCh37] Xq22.1(101186177_102631035)x1	1444	15	4	class 4	no	98	Focal epilepsy	Moderate	Multiple anomalies	Macrocephaly	scoliosis, macrosomia

36	arr[GRCh37] 17p12(14101029_15449627)x1	1348	4	incidental finding	class 5	incidental finding	60	Focal epilepsy	Mild	Normal	NP	no
37	arr(X)x2,(Y)x1	154755	245	4	class 5	*ARX, MECP2, WDR45, PIGA, ARHGEF9, CDKL5, PCDH19, SLC9A6, SLC35A2, CLCN4, SYN1, CASK, CNKSR2, IQSEC2*	5	DEE	Moderate	NP	Microcephaly	dysmorphism
38	arr(X)x2,(Y)x1	154755	245	4	class 5	*ALG13, ARX, MECP2, WDR45, PIGA, ARHGEF9, CDKL5, PCDH19, SLC9A6, SLC35A2, CLCN4, SYN1, CASK, CNKSR2, IQSEC2*	48	SeLECTS	Mild	NP	Normocephaly	no

		Diagnosis rate	
Epilepsy	DEE	12/63	19%
	Focal epilepsy	8/60	14%
	Febrile seizures / GEFS+	5/30	17%
	Infantile spasms syndrome	0/24	0%
	GGE	5/22	23%
	EIDEE	3/20	15%
	Self-limited epilepsy	2/13	15%
	Other type (unclassified, EMAtS, LGS, progressive myoclonic epilepsy, myoclonic epilepsy of infancy)	0/16	0%
Intellectual disability	No ID	1/23	4%
	Mild ID	13/88	15%
	Moderate ID	10/60	17%
	Severe ID	6/53	11%
	NP	1/6	17%
	Too young for evaluation	2/5	40%
	Present	2/13	15%
MRI	Normal brain MRI	8/83	10%
	Pathological brain MRI	14/123	8%
	NP	13/42	31%
Head circumference	Microcephaly	5/42	12%
	Macrocephaly	4/13	31%
	Normal head circumference	11/117	10%
	NP	16/76	21%



A.



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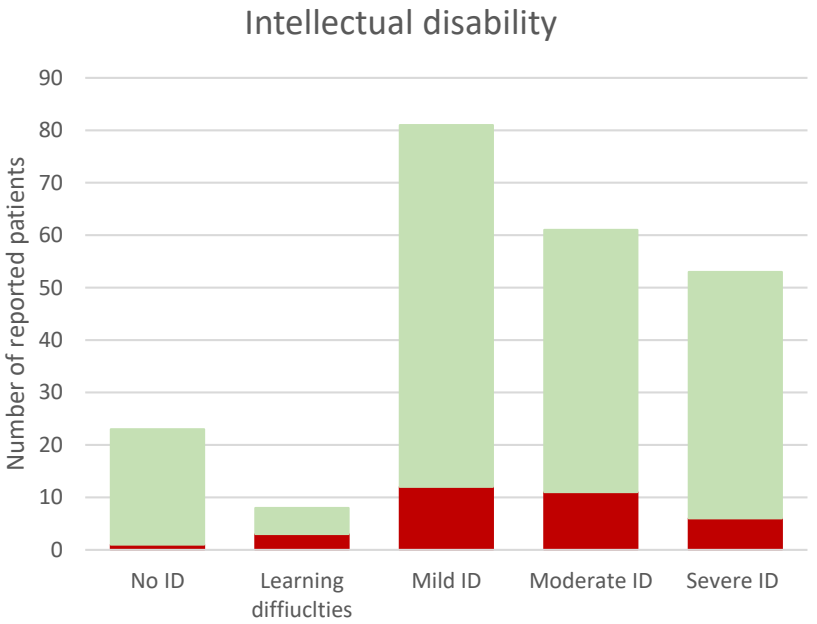

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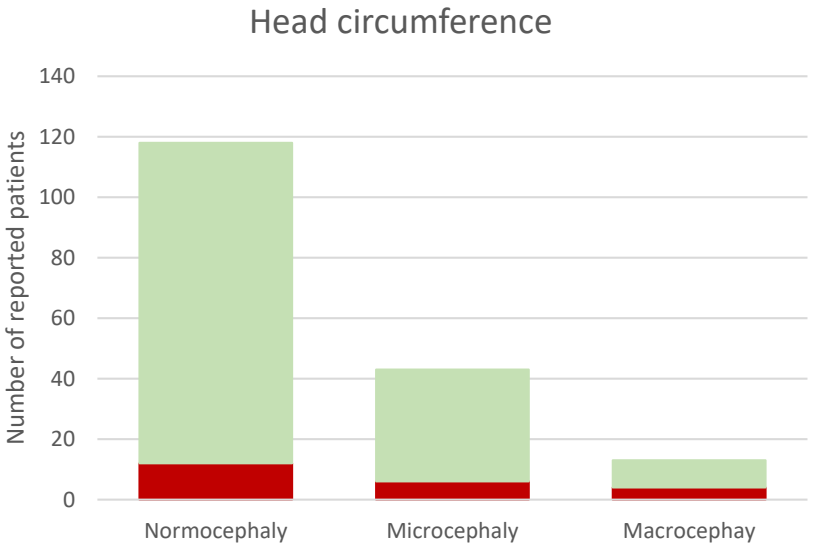

Positive results (predisposing factor + pathogenic CNV)

Negative results (VUS + negative CMA)

Disclosure of Conflicts of Interest :

None of the authors have any conflicts of interest related to this study to declare.