Genetics in Epilepsy “plus”: Focus on the Role of CGH Array

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Summary

Up to 30 - 40% of children with epilepsy are refractory to treatment, and despite numerous invasive and costly investigations, approximately 30% of cases will not identify the cause. Array comparative genomic hybridization (CGH array) as a diagnostic tool in molecular genetics has facilitated recognition of microdeletions and microduplications as risk factors for both generalized and focal epilepsies. There is evidence that many microdeletions/duplications or so called copy number variants (CNVs) predispose to a range of epilepsy plus at least one other neurological/psychiatric disorder including developmental delay, neuropsychiatric intellectual disability, autism or multiple congenital anomalies (epilepsy “plus”). Studies suggest a diagnostic yield of 15 - 20% of CGH array for this type of condition. Furthermore, CGH array can lead to the discovery of candidate epilepsy or other disease associated genes, providing insight into new treatment and control of seizures.

The identification of pathogenic CNVs implicated in epilepsy can have a significant impact on early diagnosis and successful treatment of childhood epilepsy. Proper management of seizures can prevent negative effects on a child’s brain development, offer the possibility of genetic counselling for families and avoid costly testing for other rare forms of seizure disorders.

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Key words: Array Comparative Genomic Hybridization (CGH array), Copy Number Variant (CNV), childhood epilepsy, Intellectual Disability (ID), Autism Spectrum Disease (ASD)

Genetische Abklärung bei Epilepsie “plus” mit Fokus auf die Rolle von CGH Array-Analysen

Mehr als ein Drittel der Kinder mit Epilepsie leiden unter einer therapierefraktären Form, und bei vielen dieser Patienten findet man trotz unzähligen invasiven und teuren Zusatzuntersuchungen keine Ursache. Die Entdeckung der diagnostischen Methode der sogenannten CGH array (Comparative Genomic Hybridization)-Analyse hat es möglich gemacht, dass genetische Veränderungen in Form von Mikrodeletionen und Mikroduplikationen als Ursache beziehungsweise Risikofaktoren sowohl für generalisierte als auch für fokale Epilepsien gefunden wurden. Es gibt Hinweise darauf, dass viele dieser sogenannten CNVs (Copy Number Variants) zu einem breiten Spektrum von Epilepsie plus mindestens einer anderen neurologischen oder psychiatrischen Erkrankung wie generelle Entwicklungsverzögerung, geistige Behinderung, Autismus-Spektrum-Störung oder multiple kongenitale Anomalien führen (Epilepsie “plus”). Studien lassen den Schluss zu, dass die diagnostische Ausbeute einer CGH array bei dieser Art von Symptomkomplex 15 - 20% betragen kann. Forschungsmässig konnte mehrfach gezeigt werden, dass mittels CGH array-Analysen neue Epilepsiekandidaten oder andere krankheitsassoziierte Gene entdeckt wurden.

Die Ermittlung einer potenziell pathogenen CNV kann grossen Einfluss haben auf eine frühe Diagnose und bestenfalls entsprechend erfolgreiche Behandlung der Epilepsie, was sich wiederum positiv auf die kindliche Hirnentwicklung auswirkt. Ausserdem kann den Familien eine spezifische genetische Beratung angeboten werden, und andere Zusatzuntersuchungen können vermieden werden.

Schlüsselwörter: Array-CGH (Comparative Genomic Hybridization), CNV (Copy Number Variant), kindliche Epilepsie, geistige Behinderung, Autismus-Spektrum-Störung

Tests génétiques en cas d'épilepsie "plus": rôle de la CGH-Array

Jusqu'à 30 - 40% des enfants avec épilepsie sont réfractaires au traitement. Dans environ 30% des cas la cause reste inconnue, malgré de nombreux tests invasifs et coûteux. L'arrivée de la CGH array (Comparative Genomic Hybridization) a permis d'identifier des modifications génétiques telles que microdélétions et microduplications, concernant aussi bien les épilepsies généralisées que focales. Il semblerait que de nombreuses microdélétions ou duplications (aussi appelées CNVs, Copy Number Variants) soient un facteur de prédisposition pour toute une série d'épilepsies accompagnées d'au moins un autre symptôme neurologique ou psychiatrique, tel que retard du développement, retard intellectuel, autisme, ou autre anomalie congénitale (épilepsie « plus »). Des études suggèrent que la CGH array peut contribuer au diagnostic dans jusqu'à 15% des cas. De plus la CGH array peut conduire à la découverte de nouveaux syndromes ou gènes associés à une maladie, ce qui augmente notre connaissance du traitement et du contrôle des crises.

L'identification de CNVs pathogènes impliquées dans l'épilepsie peut avoir un impact important sur le diagnostic précoce et le traitement de l'épilepsie chez l'enfant. Une thérapie adéquate des crises peut empêcher les effets néfastes sur le développement du cerveau de l'enfant, offre des possibilités de conseil pour des familles, et évite des examens coûteux pour d'autre maladies rares.

Mots clés: CGH array (Comparative Genomic Hybridization), CNVs (Copy Number Variants), épilepsie chez l'enfant, retard intellectuel, autisme

Introduction

About one percent of children are affected by epilepsy and in most cases onset occurs in infancy or early childhood. Unfortunately, 30 - 40% of children with epilepsy will be refractory to treatment, and despite numerous invasive and costly investigations, 30% of cases will not identify the cause [1]. Prolonged uncontrolled seizures especially in the developing immature brain can impair the functional development, resulting in disorders such as developmental delay/intellectual disability, psychiatric illnesses (epilepsy "plus"). However, it is likely that the underlying genetic cause also explains the developmental encephalopathy, as many of the associated epilepsies are suspected to be caused or influenced by genetic factors [2].

Clinical cytogenetic testing, in the past, relied on g-banded karyotyping. This technique only detects abnormalities in about 3% of patients with unexplained global developmental delay/intellectual disability (GDD/ID), autism spectrum disorder (ASD), or multiple congenital anomalies (MCA), whereas with using CGH array (array comparative genomic hybridization), a molecular cytogenetic method for analysing copy number variations (CNVs), the diagnostic yield rises to 15 - 20% [3]. Prospective and observational studies of patients with genetic generalized, idiopathic focal epilepsies, or epileptic encephalopathies have provided similar diagnostic yields of CGH array [4]. However, a case-control study has shown that CNVs were more common in individuals with a combination of intellectual disability (ID) and genetic generalized epilepsy (GGE) than in those with either phenotype alone [5]. These findings reflect data that have been published very recently, where Borlot et al. found that in a large cohort of adults with childhood-onset epilepsy and intellectual disability pathogenic and/or likely pathogenic copy number variations in 16.1% of the 143 probands investigated using CGH array. In this cross-sectional study eight non-recurrent rare CNVs that overlapped one or more genes associated with intellectual disability, autism, and/or epilepsy were identified [6]. In addition, the method of CGH array has a significant impact on epilepsy research and could lead to the discovery of candidate epilepsy genes.

To illustrate the complexity and broad variability of genotype-phenotype correlations this brief overview will focus on recurrent and rare microdeletion and duplication syndromes and their association with epilepsy "plus". The genetic variability of epilepsy "plus" associated with CNVs e.g. involving known epilepsy genes will be addressed by providing recent examples from the literature.

Methodical aspects of CGH array

CGH array, also referred to as the "molecular karyotype", has replaced the routine karyotype, because the resolution of CGH array is 100 - 1000 times greater than that of routine karyotyping, meaning that CGH array can detect CNVs as small as ~1 kb. CNVs could contain zero up to several genes and be both, normal genetic variants and or pathogenic mutations. The technique of CGH array is based on competitive hybridization of reference and patient DNA to an immobilized target sequence on a glass platform or other flat surface [7]. Copy number variants (CNVs) are deletions and duplications ranging from 1 kilobase (kb) to an entire chromosome. They are an important source of normal genomic variation, but some act as risk factors or causes of disease. The interpretation of results may be challenging for different reasons, first of all one has to consider technical aspects of the analysis such as the applied platform, accuracy of reference databases, filter settings determining threshold values (e.g. 200 kb) for deletions and 400 kb for duplications and number of probes (e.g. 50 markers). In terms of potential pathogenicity of a deletion or duplication many factors have

to be considered: location, size, contented gene(s), parental inheritance, presence or absence in control studies and of course any of the individual's phenotypes are all very relevant. Furthermore, one has to consider an extensive phenotypic heterogeneity as well as incomplete penetrance, variable expressivity and susceptibility loci of CNVs [8]. In general, de novo CNVs, larger CNVs (> 500 kb) and deletions more often than duplications have a higher likelihood to be possibly pathogenic. CNVs are usually categorized as either pathogenic, benign or variants of unknown significance (VUS).

CNVs predisposing to epilepsy in individuals in microdeletion syndromes associated with epilepsy

A way to classify CNVs in general is to group them in so called recurrent and non-recurrent CNVs. Recurrent CNVs are deletions and duplications which occur as a result of nonallelic homologous recombination at meiosis due to a predisposing sequence architecture being consisting with particularly unstable genomic regions. This mechanism explains that recurrent CNVs in two unrelated individuals with the same disorder have nearly identical breakpoints [9]. The occurrence of recurrent CNVs was initially noted in ID syndromes, such as Prader-Willi and Angelman syndromes. However, on proximal 15q, deletions and duplications involving various combinations of five BPs are associated not only with these syndromes but also with idiopatic genetic generalized (**Figure 1**) [10]. A selection of microdeletions and duplications identified as risk factors for neurodevelopmental disorders and epilepsy were summarized by Carvill and Mefford [11] (**Table 1**). With their study they highlighted that these microdeletions are associated with diverse phenotypes, including epilepsy as well as intellectual disability, autism and neuropsychiatric disorders [11].

For example, deletions and duplications of 16p11.2 have been shown to be associated with autism, ID, schizophrenia, but also epilepsy, as reviewed in a recent large case series. These patients presented a rather specific neurological phenotype as both, individuals with 16p11.2. microdeletion or duplication were found to have rather highly prevalent speech articulation abnormalities, hypotonia, abnormal agility, sacral dimples besides epilepsy. However, reciprocal phenotypic characteristics such as predominant hypo- versus hyperreflexia and macro- versus microcephaly may reflect opposite neurobiological abnormalities causing the functional impairments shared between 16p11.2 deletion and duplication carriers [12]. To make things even more complicated it has been shown, that the identical deletion on chromosome 16p11.2 (from genomic coordinates 29.5 Mb to 30.1 Mb) was found in children with developmental delay, mental retardation, or suspected ASD, as well as individuals with autism [13].

Rare, non-recurrent CNVs in epilepsy "plus"

There are several mechanisms for the generation of non-recurrent breakpoints that have been described; however, they seem to be often errors of replication. Non-recurrent CNVs with identical breakpoints are rare, but comparison of overlapping CNVs in similar phenotypes could reveal the "smallest region of overlap" that can highlight one or a few genes as primarily responsible for the phenotype. The technique of CGH array

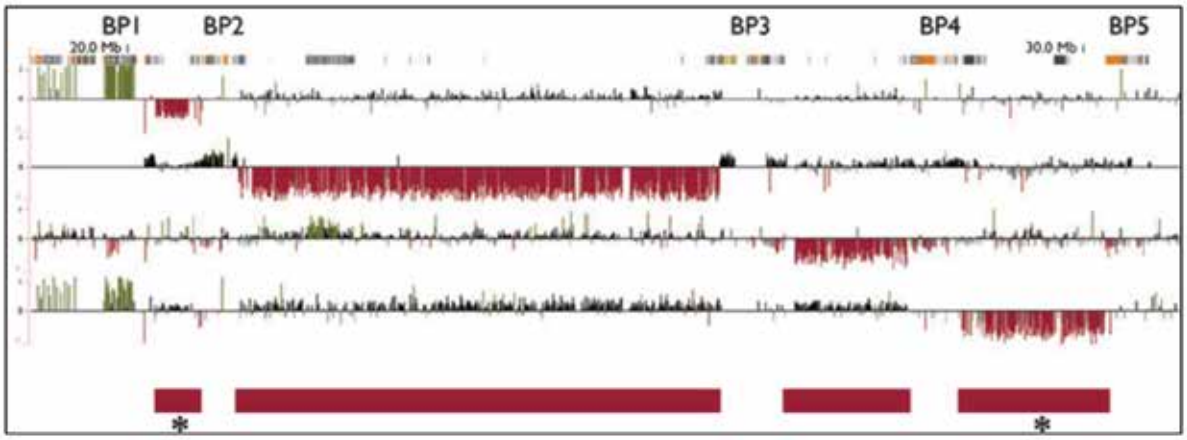

Figure 1. The current figure is taken from a publication by Mulley and Mefford, 2011. The authors demonstrated deletions and duplications involving various combinations of five BPs associated with Prader-Willi and Angleman syndromes (BP1–BP3 or BP2–BP3 deletions), autism (BP2–BP3 duplications), and genetic generalized epilepsy (BP1–BP2 and BP4–BP5 deletions) marked with an asterix. Blocks of segmental duplications, in which the BPs lie, are represented at the top by orange/yellow/gray blocks. Red bars represent the unique sequence deleted (or duplicated) between blocks of segmental duplications.

Table 1: Selected microdeletion and -duplication syndromes associated with phenotypic heterogeneity

Table adapted from Carvill and Mefford, Curr Opin Gen Dev 2013 [11]

Genomic location	Coordinates (hg19) for critical region (Mb)	Associated phenotypes, with focus on ID: Intellectual disability, EPI: epilepsy, ASD: Autism spectrum disorder, and MCA (multiple congenital anomalies
1q21.1	Chr1: 146.5–147.5	ID, EPI, MCA
3q29	Chr3: 195.8–197.4	ID, EPI
10q22q23	Chr10: 81.5–89.0	ID
15q11.2	Chr15: 22.8–23.1	ID, EPI, ASD
15q13.3	Chr15: 31.3–32.5	ID, EPI, ASD
15q24	Chr15: 74.4–75.5	ID, ASD
16p11.2	Chr16: 28.8–30.2	ID, ASD, specifically speech disorder
16p12.2	Chr16: 21.9–22.5	ID, EPI
16p13.11	Chr16: 15.0–16.3	ID, ASD, EPI
17q12	Chr17: 34.8–36.3	ID, ASD, EPI
17q21.3	Chr17: 43.7–44.3	ID
22q11.2 distal	Chr22: 21.8–23.7	ID, MCA
22q11.2 distal	Chr22: 21.8–23.7	ID, MCA

led to the detection of rare CNVs in 8.8 % (7/80) of the adults and children with ID or developmental delay, and childhood-onset epilepsy. The CNVs involved known microdeletion syndromes (16p11.2, 16p13.11 and 2q13) but also rare CNV encompassing known disease genes, such as SCN1A in four individuals. Such a finding is relevant, as deletions disrupting SCN1A might be associated with single gene abnormalities [14]. In the presence of SCN1A abnormalities certain anti-seizure medications should be avoided because they make seizures worse, and other medications or diet are more likely to be associated with improved seizure control.

As mentioned before, the importance of CGH array has been further increased through its role in the identification of disease-causing genes. The identification of regions of overlapping CNVs between patients with similar neurodevelopmental phenotypes and epilepsy, for example deletions of CDKL5 in girls with severe epilepsy and a Rett syndrome-like phenotype, might be a starting point toward identifying additional epilepsy genes [15]. The former only candidate epilepsy gene CHD2 was identified by being the only shared gene within several reported overlapping CNVs of the chromosome 15q26.1 region associated with complex

phenotypes including not only developmental delay but epilepsy with photosensitivity [16]. Another similar example is that of a de novo microdeletion at 9q33.3-q34.11 encompassing STXBP1 in a girl with mental retardation and Early Infantile Epileptic Encephalopathy (EIEE), characterized by tonic seizures, seizure intractability and characteristic suppression-burst pattern on EEG. After mutation analysis of the candidate gene STXBP1 four other unrelated EIEE patients revealed heterozygous missense mutations in the same gene [17].

Conclusion

CGH array is a method of genetic testing that has been shown to have a significant impact on epilepsy research and diagnosis, being that CNVs are an important genetic cause of epilepsy in children. Pathogenic CNVs are most likely to be found in cases where epilepsy is associated with developmental delay, intellectual disability or autism spectrum disorder, especially in the setting of dysmorphic features. CGH array testing to evaluate for potential pathogenic CNVs should be performed in patients with epilepsy "plus" (epilepsy combined with intellectual disability, malformations, dysmorphic stigmata, ASD or other features). However, evaluating the implications of a potentially pathogenic CNV for an individual patient can be challenging. To interpret the findings of a particular CNV and its possible degree of pathogenicity and the reproductive risk for the family the clinician should seek the assistance of a geneticist. Once a possibly disease causing or contributing CNV has been found the advantages to the patient and clinician include ending the often long diagnostic „odyssey", providing families with prognostic information and possible features of the syndrome that may be relevant for clinical management and in an increasing number of cases providing families access to syndrome-specific research or patient organizations.

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