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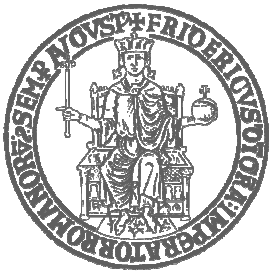

GENETIC ANALYSIS OF COPY NUMBER VARIATIONS AFFECTING ION CHANNEL GENES IN FAMILIAL IDIOPATHIC GENERALIZED EPILEPSY

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1 INTRODUCTION

Epilepsy is one of the most common neurological disorders in human, with a prevalence of about 1% and a lifetime incidence of up to 3% [1].

The epilepsies present with a broad range of clinical features, and over 50 distinct epilepsy syndromes are now recognized. Seizure disorders can roughly be divided into idiopathic or symptomatic epilepsies. While symptomatic epilepsies are due to an identifiable cause such as metabolic disorders, brain trauma or intracranial tumors, idiopathic seizure disorders occur in the absence of identifiable causal factors and are thought to have a strong genetic contribution [1].

Idiopathic Generalized Epilepsy (IGE) is a group of disorders characterized by recurrent spontaneous seizures which onset simultaneously by both cerebral hemispheres in absence of detectable brain lesions or metabolic abnormalities. IGE has a prevalence of about 0.4% in the general population and constitutes

nearly one third of all epilepsies as well as the most common group of inherited seizures [2].

IGE syndromes usually start in childhood or adolescence, but some of them have adult onset. They manifest with typical absences, myoclonic jerks, and generalized tonic−clonic seizures, alone or in varying combinations and severity. Most seizures occur on awakening, particularly after sleep deprivation.

Epidemiological studies indicate that genetic factors play a pivotal role in the etiology of IGE even though its mode of inheritance is complex. Recent progress in epilepsy research showed that IGE includes several single−gene traits showing Mendelian inheritance. A number of highly−penetrant genes have been localized or identified by studying large pedigrees through the classical approach based on linkage analysis and positional cloning. These studies led to the definition of novel clinical entities and to the recognition of neuronal ion−channel genes as major players in the genetics of IGE [3]. Mutations in genes encoding voltage−gated sodium channel subunits − SCN1A, SCN1B − and $GABA_A$ receptor subunits − GABRγ2 and GABRδ − have been identified in Genetic Epilepsy with Febrile Seizures plus (GEFS+) [4−7]. In

addition, mutations in genes encoding the α1 and β3 $GABA_A$ receptor subunits and voltage−gated calcium channel β4 subunit have been identified in families with IGE [8−10]. Ion channel genes are also involved in different familial forms of idiopathic focal epilepsy, including nocturnal frontal lobe epilepsy and benign neonatal and infantile seizures [11−15]. Taken together these findings provide evidence that primary defects in ion channels underlie epileptogenesis by altering the electrophysiological excitability of neuronal cell membranes and the communication between neurons [16]. Beside ion channel genes only few other specific pathophysiological pathways involved in IGE definitively emerged from genetic studies.

Mutations in EFHC1 have been found in a proportion of families with autosomal dominant Juvenile Myoclonic Epilepsy (JME), a subtype of IGE [17] and is the most frequent cause of hereditary grand mal seizures. We previously mapped and narrowed a region associated with JME on chromosome 6p12-p11 (EJM1). EFHC1 gene encodes a protein with an EF-hand motif, and mutations in EFHC1 cosegregate with epilepsy or EEG polyspike wave in affected members of unrelated families with JME [17]. Recently,

EFHC1 mutations have been shown to impair migration of cortical neuronal progenitors, suggesting a link between IGE and malformations of cortical development [18].

A metabolic mechanism underlying idiopathic generalized epilepsy is suggested by the identification of mutations in the gene encoding the glucose transporter 1 (SLC2A1), in families affected by movement disorders and generalised epilepsy [19] and more recently in a proportion of patients with early onset idiopathic generalized epilepsy with absences, and in families with autosomal dominant idiopathic generalized epilepsies [20,21].

On the other hand the identification of susceptibility alleles involved in common sporadic forms of IGE has been proven difficult, most likely due to high degree of genetic heterogeneity. Indeed, affected sib−pairs and association studies led to the detection of several candidate loci, but none of hem found a clear−cut confirmation [22,23]. Thus, alternative approaches are required to dissect the complex genetics of IGE.

The structural variation of the human genome is commanding a great deal of attention [24,25] as small, submicroscopic, genomic deletions and duplications (size 1 kb to 10 Mb) occur frequently

throughout the genome in healthy subjects. These Copy Number Variations (CNVs) result in the usual two copies of a gene changing to a single copy in a heterozygous deletion or more than two copies in duplications. Surprisingly, these submiscroscopic rearrangements often have no phenotypic expression, but they can act rare variants predisposing to complex diseases. In fact, they constitute up to 15% of all mutations underlying human monogenic diseases [26,27].

More recently it became evident that the CNVs and gene dosage effects could be also involved in the etiology of common neurological traits, well beyond rare mendelian disorders. In particular, some neurological diseases involved are Charcot−Marie−Tooth neuropathy type 1A [28], Smith−Magenis [29], Miller−Dieker [30], and Angelman syndromes [31]), such as autism spectrum disorders [32] and schizophrenia [33].

In idiopathic mental retardation, for instance, submicroscopic rearrangements are identified in about 5−10% of patients with otherwise normal cytogenetic analysis [34].

The role of microrearrangments in the pathogenesis of epilepsy has been initially recognized for complex syndromic epileptic phenotypes, leading to the definition of novel clinical entities, as 6q

terminal deletion syndrome, 15q24 microdeletion syndrome and a specific syndrome related to a genomic rearrangements of 17q12, associated with renal disease, diabetes, and epilepsy [35−37].

Many authors showed that genomic microdeletions are also common in different monogenic forms of epilepsy such as Severe Myoclonic Epilepsy of Infancy or Dravet syndrome (DS), [38], Lafora Disease [39] and BFNS (Benign Familial Neonatal Seizures) [40].

So far, the identification of CNVs underlying neurological diseases has been challenged by methodological limitations.

CNVs are invisible on routine karyotyping, and classical cytogenetic techniques allow the genome−wide analysis of the entire genome but at low resolution and genomic aberrations below the resolution of about 10 Mb cannot be detected. Conversely, molecular techniques such as FISH and MLPA [41] allow the identification of small rearrangements but only at specific loci.

Recent technology developments now enable the analysis of the entire human genome at high resolution and allow the efficient detection of novel disease−causing CNVs.

These techniques rely on the ability of synthesizing on a single slide (array) thousands of short oligonucleotides complementary to unique genomic sequences. CNVs are identified through a competitive hybridization between a test and control DNA (array−CGH) or by the typing of single nucleotide polymorphisms (SNP−array) [42].

Recently genome−wide surveys of genomic disorders led to the detection of microdeletions and then the identification of the causative genes STXBP1 and PCDH19 − for two epileptic encephalophaties, the Ohtahara syndrome and epilepsy in female patients with clinical findings compatible with Dravet syndrome, respectively [43,44].

About idiopathic generalized epilepsy the most frequent of these CNVs identified to date is the 15q13.3 microdeletion.

It was first identified in 0.3% of patients with mental retardation, dysmorphic features and seizures. It then emerged that this same microdeletion occurs in 0.2% of patients with schizophrenia compared with 0.01% of controls. Finally, and with even higher frequency, the 15q13.3 microdeletion is present in 1% of patients with idiopathic generalized epilepsy [45]. It is observed in a range

of idiopathic generalized epilepsy subsyndromes including childhood absence epilepsy, juvenile myoclonic epilepsy, juvenile absence epilepsy and generalized tonic–clonic seizures alone [45].

Family studies reveal that the 15q13.3 microdeletion behaves as would be expected for a susceptibility variant; it is found in some but not all family members with idiopathic generalized epilepsy and some unaffected members also carry the copy number variant [45]

The 1.5 Mb critical region spanned by 15q13.3 deletions harbours 7 genes. Among these, CHRNA7 encoding the a7 subunit of the acetylcholine receptor represents the most plausible candidate for the seizure phenotype, as suggested by the involvement of α2, α4 and β2 subunits of the same receptor in autosomal dominant nocturnal frontal lobe epilepsy [11−13].

In the same cohort of patients 15q11.2 and 16p13.11 deletions have been detected at 2−3 fold frequency than controls suggesting that these CNVs might also predispose to IGE [46].

Hence, the detection of a pathogenic CNV in a single patient or family affected by a genetic disorder may represent a critical step toward the identification of a disease gene and high−density arrays now allow the genome−wide detection of CNVs sized less than 100

Kb. However, a number of studies indicate that CNVs are found at high frequency also in control populations, and most of them represent phenotypically neutral genomic variations [47].

Thus, the interpretation of a founded CNV, the biological consequences of genomic variations and their pathogenetic significance could be not easy. Furthermore, current data indicate that the etiology of IGE is highly heterogeneous and defined by several rare alleles with different penetrance and mode of inheritance.

The search of genomic rearrangements in multiplex families showing a Mendelian pattern of inheritance circumvents the issue of statistical significance raised by linkage analysis and association studies and may represent a straightforward genetic approach to identify epilepsy genes in IGE and disclose novel important insights into the complex pathogenesis of this group of disorders and epileptogenesis.

In this complex scenario we focused our CNV survey on candidate genes for IGE such as ion channels. In particular, we develop a specific experimental strategy to assess the role of DNA copy

number variations in familial idiopathic generalized epilepsy by array−CGH and to identify novel epilepsy genes.

The study has a specific focus on ion channel genes, a large gene family with a key role in the regulation of neuronal excitability and epileptogenesis.

2 SUBJECTS AND METHODS

2.1 Subjects

2.1.1 Selection of patients with idiopathic generalized epilepsy

We selected families affected by IGE through the Italian League against Epilepsy (LICE) network.

Seizure types and epilepsy syndromes were classified according to International League Against Epilepsy (ILAE) definitions and classifications and subsequent revisions [48].

In particular were selected patients affected by:

-Childhood absences epilepsy;

-Juvenile absences epilepsy;

-Juvenile myoclonic epilepsy;

-Generalized tonic-clonic seizures only/on awakening;

-Generalized epilepsy with febrile seizures plus (GEFS+);

All subjects were selected both prospectively or retrospectively.

Patients with neuropsychiatric diseases and/or mental retardation (according to DSM IV, 1994) were excluded, such as patients with brain structural anomalies and/or metabolic causes of epilepsy (symptomatic epilepsy).

All patients were asked to participate in a study of epilepsy during routine clinical appointments at each site, in accordance with institutional standards and an informed written consent was obtained from patients or their legal representatives.

We selected 160 multiplex families with at least two first−degree relatives or three relatives of different degree affected by IGE or GEFS+.

All patients underwent accurate examination in each participating Center.

Patients' evaluation included full details of clinical features of epilepsy, detailed familial and personal history with particular attention to the psychomotor development milestones or other significant pathologic conditions. The clinical evaluation include neurological examination and revision of neuroimaging studies.

For comparison, we used 165 control subjects from healthy blood donors (aged 40–60 years) related to Transfusion Center of the Giannina Gaslini Institute (Genoa, Italy). All subjects were surveyed through a structured questionnaire to exclude seizure disorders [49].

2.1.2 Collection and storage of biological samples

Six to ten ml of peripheral blood was been collected from each patients and 1st degree family members and sent to Gaslini Institute for genomic DNA extraction. A database containaing information of each stored sample related to code number, personal data, diagnostic status, biological material and sending institution have been established according to the Oviedo Agreement recommendations, and in conformity to D.M. n.196/2003.

So far are already available 580 DNA, including 160 familial cases and their relatives and 165 control subjects.

2.2 Methods

2.2.1 Array−CGH assays

For array−CGH we used the validated IonChannel CGH−Array, specifically designed for the detection of CNVs affecting 429 candidate genes, according to the Gene Ontology (GO) Database. This array was been synthesized by Agilent on the basis of an our custom design. The proband of each family was been assayed by Array−CGH as follow: 500ng of purified DNA of a patient and of a control was been double−digested with RsaI and AluI for two hours at 37° degrees. Each digested sample was been labeled for two hours using Cy5−dUTP for the patient DNA and Cy3−dUTP for the control DNA. Labeled products was been columns purified and prepared according to the Agilent protocol. After probe denaturation and pre−annealing with 5ml of Cot−1 DNA, hybridization was been performed at 65° degrees with rotation for 40 hours. After two washing steps the arrays was been analyzed with the Agilent scanner. Array−CGH data was been analyzed with a new R package we are currently developing. A beta version of this package − called TASSO, Tools for Automatic Segmentation

and Statistical significance Of array−CGH − has been already implemented and applied for the analysis of 60 array−CGH assay. We initially investigated genomic database to explore whether putative CNVs match polymorphic genomic variants. Then, validation of putative CNVs was been performed by Dye−reversal experiments with reciprocal labelling of the test and reference DNA. In addition, we tested by array−CGH the available family members to perform segregation analysis and assess the penetrance and clinical expressivity of the identified CNVs.

To assess the impact of identified CNVs in IGE etiology we screened additional 135 controls by Ion Channel CGH−Array and real−time quantitative PCR (Q−PCR).

2.2.2 Statistical analysis and bioinformatics

The TASSO package was been initially read the raw array-CGH data, preprocesses and normalizes them. The pre-processing step allows to detect and filter out poor quality spots. To accommodate poor quality flagged spots or missing information, the package provided an implementation of knn imputation [50]. In terms of

normalization, the package used a lowess fit to remove spurious and systematic intensity−dependent variations in the data. After normalization the log2−ratios of signal intensities of the sample and test was been sorted against the physical location of the probes on the genome. The segmentation algorithms included in our package will be SLM and CBS [50], while the calling step was achieved by FastCall, a novel procedure that aims to give aberration probabilities in a very fast and accurate way [50]. Once the analyses were completed, our package organized the results in a database and created an intuitive html interface that allowed the user to browse them. The interface included the following: a plot displaying the segmentation overlapped to the log2−ratio data [50]; a plot reporting FastCall results and the probability of aberration; the cytogenetic ideograms with predicted aberrations; a tabular summary of the predicted aberrations with link to UCSC and Genomic Variants databases; zooms of the genomic profile nearby the predicted aberrations.

Sample power estimates were calculated assuming an α error of .05 and a β error of 0.2 (ie, a power of 80%). Two-tailed unpaired *t* test

and Fisher exact tests were used for categorical sample and phenotype correlations; the nonparametric Mann-Whitney*U* test was used to evaluate numerical data.

3 RESULTS

We selected 160 families affected by IGE and collected 1576 DNA samples of the probands and available relatives. Among these, we collected 137 multiplex families accounting for 73 family members and for a 2,6 average number of affected individuals/family.

We designed and tested a custom CGH−array specifically designed to explore 429 candidate genes at very high density (IonChannel array).

Genes were been selected through the BIOMART tool (www.ensembl.org/biomart/martview) using the following GO IDs: GO:0015267 (channel activity), GO:0005326 (neurotransmitter transporter activity) and GO:0042625 (ATPase activity, coupled to transmembrane movement of ions). The initial list of genes was been manually elaborated to avoid redundancies, pseudogenes and genes with unclear genomic organization and inconsistent function.

The final list of candidate genes includes 429 genes (Table 1). The array includes 60K probes covering the exons and introns of each

gene at the density of 100 bp and 1.000 bp, respectively. In addition, the array includes the 44K genome−wide probe set implemented in the widely used Agilent 44B commercial kit. Thus, the IonChannel array is expected to detect CNVs sized only few Kbs within the selected candidate genes.

We initially tested the IonChannel array in control and aneuploidic DNA samples and analyzed the assay using the novel SLM algorithm implemented in the FastCall software e grouped into 24 families, mostly involved in the transmembrane transport of ions (Table 1). Subsequently, we screened a first set of 137 cases affected by different familial forms of IGE and we identified these data: there are 68 patients (50%) with CNVs, and 69 patients (50%) without CNVs; of these 43 rearrangements are deletions and 266 are duplications, and the deletion/duplication ratio is 0.16; the mean CNVs size in patients is 395, 939 Kb; of these, 5 patients have CNVs clearly pathogenic (n=5), including 1 deletion and 4 duplications (Table 2). Four out of five (80%) rearrangements involve genes we specifically selected for high resolution analysis (KCNE1, KCNE2, KCND2, GABRG2, TRPV2). Chromosome 21 duplication affecting potassium channel genes KCNE1 and KCNE2

spans a polymorphic CNV. Two of these CNVs are intragenic and disrupt the genomic organization of a single ion channel gene, GABRG2 encoding the γ2 subunit of the $GABA_A$ receptor on chromosome 5 and KCND2 encoding the voltage−gated potassium channel Kv4.2 on chromosome 7 (Figure 1, 2). Segregation analysis in available family members showed that dup21, dup9 and dup17 do not co−segregate with IGE. Intragenic CNVs affecting GABRG2 and KCND2 were also detected in the affected parents (Figure 3).

Other 5 CNVs not co-segregate with IGE and are showed in Table 2 and in Figure 4.

These CNVs identified are not recurrent and sized is >50 Kb (range 80 Kb-1.5 Mb)b.

We also identified 29 small recurrent CNVs (recurrent in ≥2 patients), showed in Table 3. They are 20 duplications and 9 deletions, with mean size 400 bp. Eighteen of these are not previously reported in Database of Genomic Variant and eight of these affecting interesting ion channel genes (KCND3, KCTD20, KCNH2, KVNJ6, KCNG2, GRM5, GRIK5, CACNA1A).

To assess the frequency of identified CNVs in ethnically−matched controls, we set−up TaqMan−based real−time assays. For each CNV we already tested 80−100 controls and found no positive individuals.

In particular, in 165 healthy subjects we identified: 74 subjects (45%) with CNVs and 91 subjects (55%) without CNVs; of these 35 rearrangements are deletions and 186 are duplications, and the deletion/duplication ratio is 0.18; the mean CNVs size in healthy subjects is 404, 224 Kb. In particular, in 165 control subjects were not found the five not recurrent- rearrangements including 1 deletion and 4 duplications found in patient with IGE (Table 2), as well as were not found the eight recurrent CNVs affecting ion channels showed in Table 3.

The genomic data of 137 patients and 165 healthy subjects are summarized and compared in Table 4 and in Table 5. Statistical analysis failed to show any significant difference regarding the frequency (*p=0.41*), the type (deletion vs duplication; *p=0.53*) of the identified CNVs between patients and controls.

Statistical analysis showed a significant difference regarding gene-enrichment, with an increased enrichment of ion channel genes in patients with IGE *(p=0.0037)*

4 DISCUSSION

Epilepsy is one of the most common neurological conditions and for most patients (approximately 65%) with either generalized or partial epilepsies, the underlying cause is unknown and the condition is referred to as being ‘idiopathic’[1]. Overall, 30% of all individuals with epilepsy have idiopathic generalized epilepsy. In the past two decades an increasing number of mutations in genes predisposing for various forms of IGE have been identified [3]. Most of the mutations are found in genes encoding for voltage-or ligand-gated ion channels; they account for most familial forms of these disease [3] and so the IGE is primarily a ‘channelopathy’.

The role of copy number variation (CNVs) in intellectual disability, autism and schizophrenia has been extensively investigated [32,33] and it has become increasingly clear that collectively rare variants contribute significantly to the genetic etiology of many neurological disorders, including epilepsy. In order to identify new genes that contribute to the genetic cause of epilepsy, we performed a high-resolution screening of CNVs in 137 patients with idiopathic

generalized epilepsy. Our studies showed that CNVs affecting ion channel genes are a common finding in familial IGE.

In particular, we identified a previously undescribed intragenic deletion on chromosome 5, affecting γ2 subunit of $GABA_A$ receptor (GABRG2), the major inhibitory neurotransmitter receptor in the Central Nervous System. The α1β2γ2S receptor is the most abundant receptor isoform and the γ2 subunit is critical for receptor trafficking, clustering and synaptic maintenance. To date, most $GABA_A$ receptors mutations associated with IGEs have been found in the γ2 subunit [51]. Phenotypes in the affected pedigrees are heterogeneous. Kang JQ et al [52] demonstrated that γ2 (Q351X) nonsense mutation, associated with genetic epilepsy with febrile seizures plus (GEFS+), had a dominant-negative effect on wild-type receptors by reducing their assembly, trafficking and surface expression. They supposed that this combination of loss of function and dominant-negative effect of the mutant subunits on the wild type subunits would result in considerable loss of inhibition and in associated GEFS+ phenotype. In other pedigrees the phenotype ranges from simple febrile seizures to the more severe seizures of the Dravet syndrome and Lanchance-Touchette et al. [51] reported

a family with a single point mutation P83S and a combination of febrile and childhood absence seizures.

According to these data this is a strong candidate gene for epilepsy, because signalling through $GABA_A$ receptor provides almost all inhibitory tone to the brain, balancing the tendency of excitatory neuronal circuits to induce convulsions. So, the high degree of penetrance of rearrangement in our family, including and disrupting γ2 $GABA_A$ receptor subunit gene, can contribute to the occurrence of IGE in this family.

The intragenic duplication disrupting the genomic organization of KCND2 in IGE is a novel intriguing finding and pinpoints a novel candidate gene for IGE. KCND2 encodes for a primary pore-forming (α) subunit member within the Shal subfamily of voltage-gated potassium channels, Kv4.2. Within the brain, Kv4.2 mediates a rapidly activating and inactivating (IA) Kv channels, contributing to action potential repolarization and repetitive firing, and affecting neuronal outputs [53]. In addition, somatodendritic IA channels modulate the back−propagation of action potentials in neuronal dendrites, and impact synaptic efficacy and plasticity [54].

Recently, it has been reported that attenuation of IA has been linked to acquired experimental temporal lobe epilepsy [55] and a Kv4.2 truncation mutant has been identified in a single patient with temporal lobe epilepsy [56].

Hence, rearrangement in our family, disrupting this physiologically important IA channel could lead to dysregulation of neuronal excitability, a hallmark of epilepsy. Our results suggests that KCND2 may segregate within pedigrees and may suggest that KCND2 may have a significant role in the etiology of IGE. The other CNVs do not segregate within pedigrees and may not be readily interpreted without further genetic and functional studies. However, CNV including KCNV2 gene is interesting. Kcnv2 encodes the voltage-gated potassium channel Kv8.2, which is a silent subunit as a homotetramer. Kv8.2 can form functional heterotetramers with Kv2 subunits and influence membrane translocation and channel properties [57]. Members of the Kv2 family are expressed in the nervous system and underlie the neuronal delayed rectifier K+ current, which is important for limiting membrane excitability, particularly under conditions of repetitive stimulation [57]. In the hippocampus, which is a region of

particular importance for seizure generation, Kv2.1 is the major contributor to the delayed rectifier potassium current and colocalizes with Kv8.2 in hippocampal pyramidal neurons.

Jorge BS et al [57] evaluated the *in vivo* effect of Kcnv2 variants in transgenic mice and, to determine whether the human ortholog contributes to human epilepsy, they screened epilepsy patients for genetic variation in KCNV2. They demonstrated that Kcnv2 transgene-mediates transfer of seizure susceptibility in mice, as well as human variants that alter delayed rectifier K+ currents. According to these authors, we propose KCNV2 as an epilepsy gene in humans.

We also identified 29 small recurrent CNVs. Eight of these are not previously reported in DGV and affect interesting ion channel genes (KCND3, KCTD20, KCNH2, KVNJ6, KCNG2, GRM5, GRIK5, CACNA1A).

CACNA1A mutations are associated to epilepsy with absences [58], and mutation in KCNJ6, encoding for GIRK 2 (Kir3.2), a G-protein-coupled inwardly rectifying K+ channel, have previously found to be associated with epilepsy both in mice and humans, above all in patient with juvenile myoclonic epilepsy [59]; KCND3

and KCNH2 encodes for ion channels involved in Brugada syndrome and long QT, both diseases perhaps linked to sudden death in epilepsy (SUDEP) [60,61].

Based on these findings these genes can be regarded as a common cause of epilepsy or allelic susceptibility to epilepsy, even if more comprehensive investigations will be needed to clarify the role of these ion channels in epilepsy. We showed that the cumulative incidence of rare CNVs is not significantly higher in patients with familial IGE and the type (deletions vs duplications) is not significantly different in patients with familial IGE and in healthy subjects. However, the number of genes involved in CNVs and the type of ion channel represent key factors underlying pathogenesis.

In our study, a significant enrichment of genes is observed within CNVs identified in IGE patients. Particularly, CNVs including KCND2, GABRG2, inherited from an affected parent, have not been found in healthy subjects; recurrent CNVs affecting KCND3, KCNH2, KVNJ6, CACNA1A genes have not been found in healthy subjects. The same, rare not co-segregating CNVs are found only in IGE patients, even if inherited from an unaffected parent.

Moreover, rearrangements inherited from a healthy parent cannot always be considered a benign variation. Indeed, it already was demonstrated that CNVs may represent susceptibility alleles for a number of neuropsychiatric disorders, including idiopathic epilepsy [45].

Then, it should be emphasized that CNVs detection is largely dependent on the type of platform (array-CGH vs. single-nucleotide polymorphism genotyping), the density and design of the array, and the setting of analytic parameters [62]. Most population-based CNV surveys have been performed by single-nucleotide polymorphism arrays, whereas array-CGH has become the elective routine diagnostic tool to identify genomic rearrangements in patients [62].

We identified 5 previously unreported segregating within pedigrees CNVs in IGE patients, indicating that recurrent polymorphic CNVs may have been missed by large scale population single-nucleotide polymorphism–based studies. Accordingly, CNVs screening should include appropriate internal controls, and heterogeneous data from a public database such as the Database of Genomic Variants should be considered with caution within a diagnostic context.

5 CONCLUSIONS

Our study confirm that impairment of neuronal ion currents represents a common pathophysiological mechanism in epileptogenesis. In particular, we identified 2 epilepsy-genes (KCND2, GABRG2) and 8 susceptibility-epilepsy genes (KCND3, KCTD20, KCNH2, KVNJ6, KCNG2, GRM5, GRIK5, CACNA1A). The custom CGH- array specifically designed to explore candidate genes encoding ion channels at very high density (IonChannel array) should be useful for other channalopathies (cardiac, neuromuscular, endocrinological and other neurological diseases). In addition, the characterization of a novel channels involved in epilepsy will define a biological target for the development of new antiepileptic drugs.

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TABLES AND FIGURES

GENES	
GENE FAMILIES	N° OF GENES
CHOLORIDE CHANNELS	19
CALCIUM CHANNELS	24
SODIUM CHANNELS	18
POTASSIUM CHANNELS	120
ACETYLCHOLIN RECEPTORS	16
GABA RECEPTORS	18
GLUTAMATE RECEPTORS	26
GLYCINE RECEPTORS	4
ATPase	71
GAP JUNCTIONS	18
SOLUTE CARRIERS	25
TRANSIENT RECEPTOR CHANNELS	21
PURINERGIC RECEPTOR CHANNELS	7
CYCLIC NUCLEOTIDE CHANNELS	6
ATP BINDING CASSETTE	4
AMILORIDE CHANNELS	5
BESTROPHIN	4
SEROTONIN RECEPTOR	5
MUCOLIPIN	3
PKD	5
ROPPORIN	3
ANION CHANNEL	3
TPCN	2
TMEM	2
TOTAL	429

Table 1 Candidate genes included in the IonChannel array, grouped by family

Pt. ID	RESULTS	BREAKPOINTS' LOCATION (bp)		SIZE	GENE(S)
E15	dup21	34.625.253	34.887.912	262.659	KCNE1, KCNE2
I15	dup7	119.153.841	119.807.574	653.733	KCND2
I31	del5	161.446.702	161.964.455	517.753	GABRG2
E11	dup9	26.639.443	28.203.364	1.563.921	C9orf82, PLAA, IFT74, LRRC19, TEK, NCRNA, MOBKL2B, IFNK, Corf72
I53	dup17	16.226.884	16.307.688	80.804	TRPV2, C17orf45, C17orf76
I104	del16p	14.852.060	16.807.117	2.119.422	ABCC6P2, NOMO1, NPIP, PDXDC1, NTAN1, RRN3, MPV17L, KIAA0430, NDE1, MYH11, ABCC1, ABCC6, NOMO3
I72	dup9p	2.706.657	2.822.700	116	KCNV2
I90	dup12q	130.430.646	131.706.489	1.666.762	U6, MMP17,ULK1, PUS1, P2RX2, GPR133, FBRSL1, MUC8, SNORA49, GALNT9, NOC4L, DDX51, EP400NL, EP400, SFRS8
I120	del9q	72.418.491	72.717.377	299.378	TRPM3
I124	dup18q	22.289.206	22.350.197	158.507	KCTD1

Table 2 Genetic details of CNVs identified by IonChannel array

Assay ID	Chr	N of Probes	Start Position (bp)	End Position (bp)	Length of region (bp)	Call type	Intron / Exon	Recurrent probe set	CNV	Genes
V27-1Q	6	5	36518172	36518583	411	GAIN	E			KCND20
V8-1Q	6	4	36518348	36518583	235	GAIN	E			KCND20
V31-1Q	6	4	36518348	36518583	235	GAIN	E			KCND20
V28-2Q	6	4	36518348	36518583	235	GAIN	E			KCND20
V27-2Q	6	4	36518348	36518583	235	GAIN	E			KCND20
V25-1Q	6	4	36518348	36518583	235	GAIN	E	4	-	KCND20
V24-1Q	6	4	36518348	36518583	235	GAIN	E			KCND20
V23-1Q	6	4	36518348	36518583	235	GAIN	E			KCND20
V21-2Q	6	4	36518348	36518583	235	GAIN	E			KCND20
V14-2Q	6	4	36518348	36518583	235	GAIN	E			KCND20
V8-1Q	7	3	150302727	150303488	761	GAIN	I/E	3	-	KCNH2
V14-2Q	7	3	150302727	150303488	761	GAIN	I/E			KCNH2
V10-2Q	10	10	50490334	50492169	1840	GAIN	I/E	8	-	CHAT/SLC18A3
V08-2Q	10	8	50491175	50492169	994	GAIN	I/E			CHAT
V8-1Q	11	5	87881702	87882547	845	GAIN	I/E			GRM5
V25-1Q	11	5	87881702	87882547	845	GAIN	I/E	5	-	GRM5
V24-1Q	11	5	87881702	87882547	845	GAIN	I/E			GRM5
V14-2Q	11	5	87881702	87882547	845	GAIN	I/E			GRM5
V12-1Q	19	6	13179721	13181315	1590	GAIN	I/E	3	-	CACNA1A
V15-2Q	19	3	13180475	13181315	840	GAIN	I/E			CACNA1A

Assay ID	Chr	N of Probes	Start Position (bp)	End Position (bp)	Length of region (bp)	Call type	Intron / Exon	Recurrent probe set	CNV	Genes
V30-1Q	1	3	112332967	112333041	74	LOSS	I/E	3	-	KCND3
V27-2Q	1	3	112332967	112333041	74	LOSS	I/E			KCND3
V31-1Q	8	3	106883442	107377047	494000	LOSS	I/E	3	-	ZFPM2/OXR1
V25-1Q	8	3	106883442	107377047	494000	LOSS	I/E			ZFPM2/OXR1
V24-1Q	8	3	106883442	107377047	494000	LOSS	I/E			ZFPM2/OXR1
V14-2Q	10	3	87370129	87371814	1680	LOSS	I	3	-	GRID1
V10-2Q	10	3	87370129	87371814	1680	LOSS	I			GRID1
V37-2Q	21	9	38076596	38081637	5040	LOSS	I	5	-	KCNJ6
V26-1Q	21	5	38076596	38078406	1810	LOSS	I			KCNJ6

Table 3 Recurrent CNVs in 137 pts with IGE

	CNVs	no CNVs	TOTAL
Cases	68	69	137
Controls	74	91	165
Total	142	160	302

Table 4 n° CNVs in cases vs controls; $p > 0.05$

	DELETIONS	DUPLICATIONS	TOTAL
Cases	43	266	309
Controls	35	186	221
Total	78	452	530

Table 5 Deletions and duplications in cases vs controls; ($p > 0.05$)

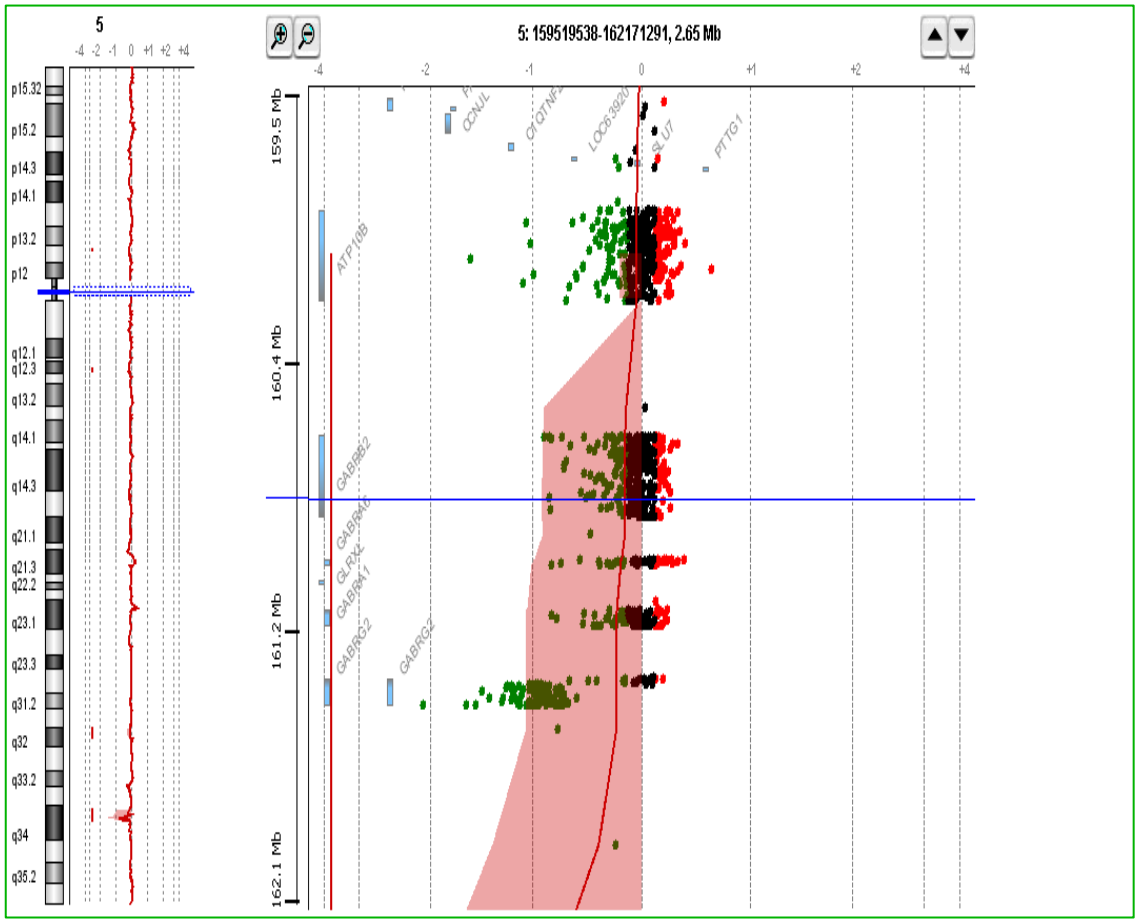

Figure 1 CGH-array in family ID I31. It showed a del5q, spanning 161.446.702-161.964.455, including GABRG2 gene

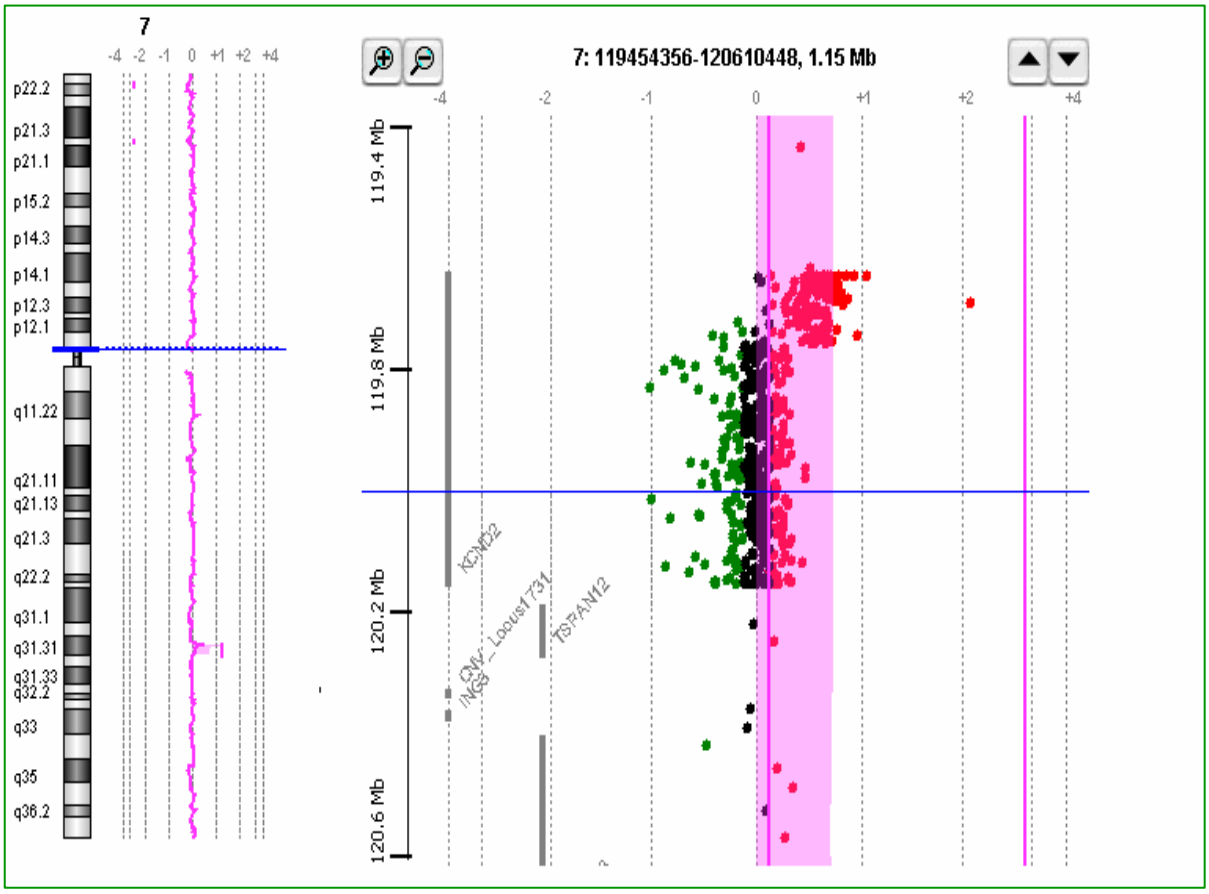

Figure 2 CGH-array in family ID I15. It showed a dup7q, spanning 119.153.841-119.807.574, including KCND2 gene

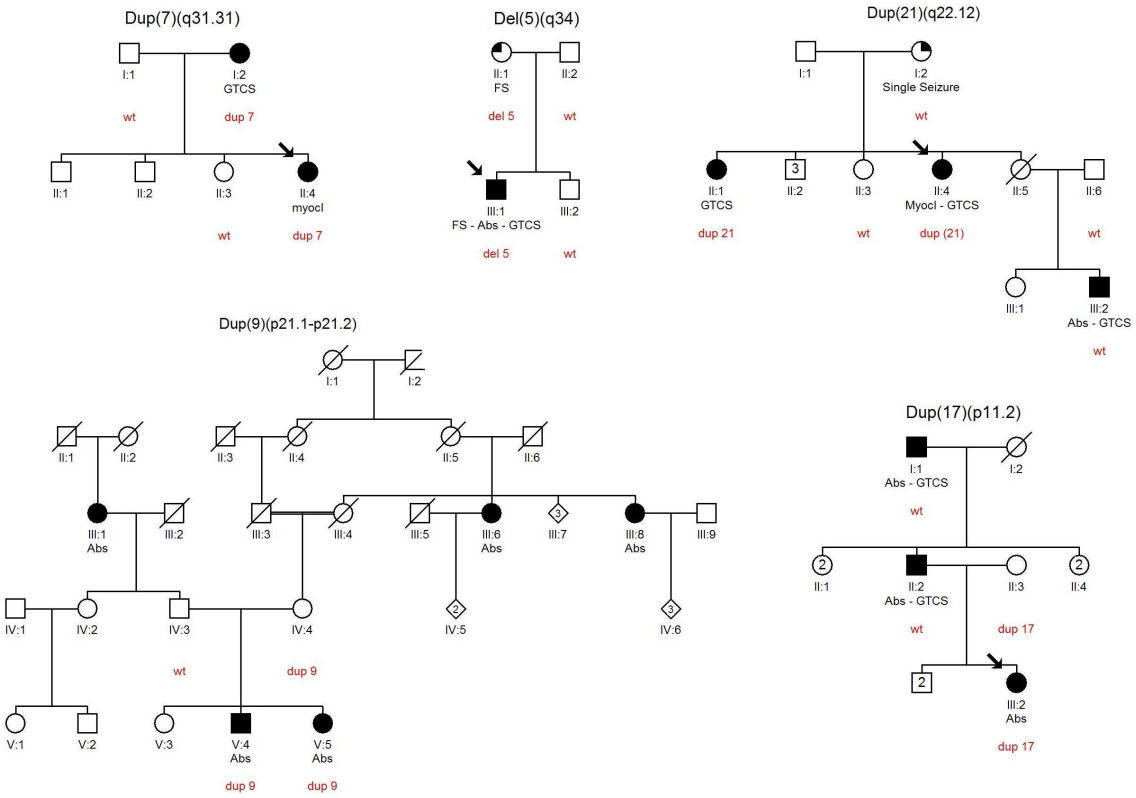

Figure 3 Segregation analysis of identified CNVs (1/2)

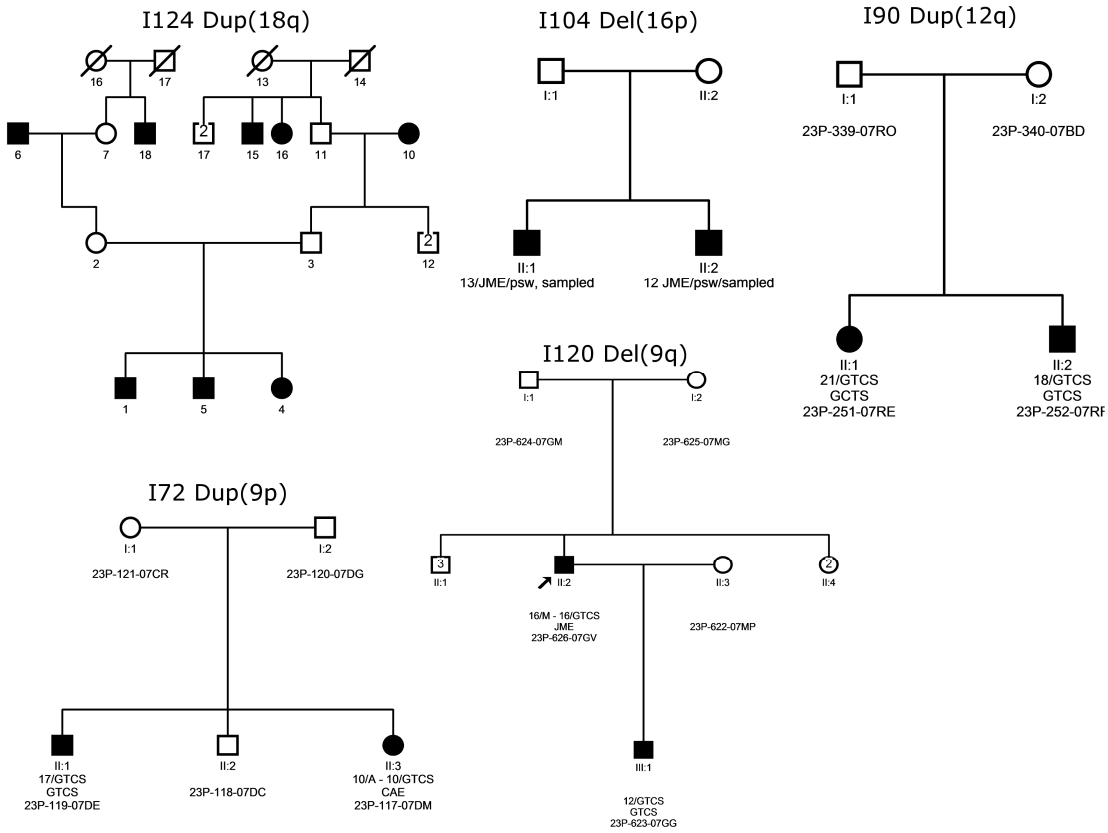

Figure 4 Segregation analysis of identified CNVs (2/2)