Chromosome 14 deletions, rings, and epilepsy genes: A riddle wrapped in a mystery inside an enigma

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CROMOSOME 14 DELETIONS, RINGS AND EPILEPSY GENES: A RIDDLE WRAPPED IN A MYSTERY INSIDE AN ENIGMA

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Introduction

The ring 14 syndrome (r(14) syndrome, OMIM #616606) is a rare condition caused by the rearrangement of one chromosome 14 into a ring-like structure. The typical karyotype of an affected person is 46,XY or XX, r(14). The formation of the ring requires two chromosome breakpoints, one on the short arm and one on the long arm. The former has received little scrutiny since it occurs within the heterochromatin of the short arm, devoid of protein coding genes. The latter is more relevant, causing loss of the gene-rich terminal band of the long arm. The deletion can usually be detected by Comparative Genomic Hybridization (CGH) assay, varying in size between 0.3 and 5 Mb. However, in a minority of cases the deletion is too small to be detected by CGH and the ring appears to be "complete" [1].

Clinically, the r(14) syndrome phenotype consists of shortness of stature, a distinctive, although not highly typical face, microcephaly, ocular abnormalities, mainly altered retinal pigmentation, abnormal macula and strabismus, intellectual disability, with aggressive and hyperactive behavior in some cases, and pharmacoresistant epilepsy [1]. The medical management of the affected persons is mostly concerned with the containment of seizures[2], with a strong need for new and more effective drugs. Knowledge of the gene(s) responsible for epilepsy would greatly help in designing a precision medicine based strategy for the discovery and development of new drugs targeting the proteins or cell signalings affected specific mutations. Genes located within the terminal region of chromosome 14q, which is lost in the ring, appear to be likely candidates. However, patients who have a linear deletion of the same region, without ring formation, do not have epilepsy, or only rarely. An explanation of this unexpected finding is lacking and it may reside in the involvement of other genes on chromosome 14 not necessarily included in the deleted region. This could be due to the known instability of the ring, causing monosomy of chromosome 14 in a proportion of cells. This proportion is known to be around 20% in peripheral blood cells[3], but it could be higher in areas of the brain contributing to a potential epileptogenic focus. Another possibility is that epilepsy genes located anywhere in chromosome 14q are dysregulated by position effect, due to the altered topology of the ring compared to that of the homologous linear chromosome. Special attention should also be paid to the potential role of the *PACS2* gene, located on chromosome band 14q32.33. Two recent reports show that de novo missense variants of this gene cause neonatal-onset developmental and epileptic encephalopathy by disrupting the regulatory functions of the gene[4,5]. In this article we review known cases of linear deletion of chromosome 14q and analyze their phenotype, as well as the epilepsy genes contained in each deletion interval, focusing on terminal deletions, overlapping those found in rings. We then compare the phenotype of cases with terminal linear deletions with that of cases with ring or *PACS2* missense variants, underlying similarities and

differences. We decided not to consider the possible role of genes within the 14q32.2 region subject to imprinting, because of the peculiar mechanism of uniparental disomy, the virtual absence of epilepsy in the Temple and Kagami-Osaka syndrome, as well as evidence that in cases that were investigated, uniparental origin of the ring and the normal homolog was excluded[3].

The purpose of this analysis is to facilitate future efforts to discover the cause(s) of epilepsy in the r(14) syndrome.

The phenotype of 14q deletion syndromes

We subdivided the literature cases with a CGH definition into five different groups, based on the position of the deleted region but also on some distinctive clinical peculiarities, plus a separate group for cases with a *PACS2* missense variant. Admittedly, this classification is somewhat arbitrary, has mainly practical purposes and does not imply an identity of cause or pathogenesis for cases assigned to the same group (except for the *PACS2* group). This is inevitable when dealing with deletion syndromes, given that the perfect identity of the chromosome loss, even in cases described as cytogenetically identical, is virtually impossible to prove.

14q11-q22 deletion syndrome

In spite of the size of the deletion interval (approximately 35 Mb), OMIM lists this entity as a single contiguous gene syndrome (#613457) clinically characterized by failure to thrive, hypotonia, severe psychomotor and language delay, epilepsy (rare), microcephaly, absence or hypoplasia of the corpus callosum and a characteristic face of triangular shape with deep set eyes, short palpebral fissures, hypertelorism, flat nasal sella and short bulbous nose, long philtrum, micrognathia, cupid bow shape of the upper lip, low set ears.

Although there is some consistency in this description, if one considers different case reports it is obvious that this contiguous gene syndrome is causally heterogeneous and clinically variable. Yasin et al.[6] describe a del 14q11 syndrome with a phenotype that differs from that just described for the presence of macrocephaly, gastrointestinal dysfunction and sleep disturbances. The deletion causes haploinsufficiency of the *CHD8* gene, thought to causally define this syndrome, given that point mutations of this gene result in the same clinical presentation. *CHD8* encodes a protein involved in chromatin remodeling and is thought to affect the expression of genes that are involved in brain development. In particular, the CHD8 protein and the genes it regulates likely help control the development of neural progenitor cells and the growth, proliferation and differentiation of neurons.

Vineeth et al.[7] described a patient with a 5 Mb deletion at 14q12, encompassing the neurodevelopmental genes *FOXG1, PRKD1* and *NOVA1*, and a phenotype described as “Rett-like” with epilepsy. Torgyekes et al.[8] described two cases and reviewed another 15 from the literature, all

carriers of a 14q12-q13.1 deletion. Microcephaly and agenesis/hypoplasia of the corpus callosum were highly prevalent in this group of patients, while epilepsy was reported only in three cases.

Worthy of special mention is the case of the Brain-Lung-Thyroid syndrome (BLTS, MIM #600635), consisting of benign chorea, interstitial lung disease and hypothyroidism, and caused by sequence variants or deletion of the *NKX2-1* gene, located in 14q13.3. This gene encodes a protein called homeobox protein Nkx-2.1, which functions as a transcription factor and is particularly involved in the development and function of the brain, lungs, and thyroid gland. In the brain, homeobox protein Nkx-2.1 regulates genes that play a role in the development and migration of interneurons to their proper location.

Cases of BLTS were also reported in association with larger deletions within the 14q13.3 sub-band, usually presenting with a more complex phenotype. Gentile et al.[9] described a case of BLTS accompanied by poor growth, dysmorphic face and oligodontia. The patient carried a 4.08 Mb deletion of the 14q13.2-q21.1 region encompassing the *NKX2-1* gene, plus several other mendelian genes, including *PAX9*, encoding a member of the paired box (PAX) family of transcription factors required for normal fetal development of various organs, likely to be the cause of oligodontia. Villafuerte et al.[10] described a female patient who, in addition to the BLTS triad, also had developmental delay, joint hyperlaxity, oligodontia and immune deficiency. She was carrier of a 3.2 Mb deletion in 14q13.2-q21.1 resulting in the loss of 20 mendelian genes, including *NKX2-1, PAX9, NFKB1A* and *PPP2R3C*, the latter two genes respectively encoding a protein that regulates the transcriptional activity of nuclear factor-kappa-B and a regulatory subunit of the serine/threonine phosphatase, protein phosphatase 2. These two genes are probably involved in the defective immune response. What is surprising is the lack of the BLTS triad in any of the cases reported under the OMIM heading of 14q11-q22 deletion syndrome, particularly those described by Kamnasaran et al.[11] with deletions involving the entire 14q11-q22 region.

14q22-q23 deletion syndrome

We are aware of only three cases reported in the literature characterized by growth and psychomotor delay and hypotonia. Microphthalmia/anophthalmia were present in two cases, choanal atresia in two cases, partial syndactyly of fingers and toes in two cases, epilepsy in one case. More specifically, Nolen et al[12] described a boy with severe post-natal growth delay, global developmental delay, severe hypotonia and a distinctive face with fused eyelids and sunken eyes, prominent forehead, hypoplastic nasal sella, short nose with a bulbous tip, downturned corners of the mouth, small ears of triangular shape and very narrow external auditory canals. There was partial syndactyly of the third and fourth digit on the right hand, and of toes two to five bilaterally. Genitalia were male, with undescended testes. There was growth hormone deficiency, treated with

growth hormone from the age of two years. A brain MRI scan showed absence of the eye globes and of the optic nerves and severe hypoplasia of the corpus callosum. Audiology assessment demonstrated high frequency hearing loss bilaterally. The patient had a de novo 6.99 Mb deletion of chromosome 14q resulting from a t(3;14)(q28;23.2) translocation, including mendelian genes *KTN1* (encoding a membrane protein that is a member of the kinectin protein family, primarily localized to the endoplasmic reticulum membrane and possibly involved in intracellular organelle motility), *OTX2, SIX6, SIX1* and *SIX4*, belonging to the family of homeobox proteins transcription factors, *BMP4* (encoding a secreted ligand of the TGF-beta proteins superfamily). These genes play a role in the proliferation and survival of precursor cells during early embryonic development in numerous tissue to control the formation of many body structures. Haploinsufficiency of *OTX2* is the likely cause of the optic bulbs and nerves deficiency, while that of *BMP4* could be the cause of syndactylies.

The second case[13] is that of a female born prematurely at 33 weeks with normal measurements and choanal atresia, velopharingeal incompetence, insufficiency of the gastro-esophageal sphincter and frequent seizures. When re-examined at the age of 13 years, she was moderately delayed and had a hypernasal speech. The face was long, hypotonic and expressionless with apparent hypertelorism, small alae nasi and a pointed chin. There was bilateral proximal syndactyly between the 2nd, 3rd, and 4th finger and between the equivalent toes. Metacarpals and metatarsals appeared thin on X-ray. The patient carried a 6.5 Mb deletion within bands 14q22.3-q23.2, encompassing 27 mendelian genes. *OTX2* and, surprisingly, *BMP4*, were not among these.

The third case is a boy reported by Picchiecchio et al[14]. Noted at birth were enophthalmia with right blepharophimosis, cryptorchidism and scrotal hypoplasia. Brain and orbital MRI showed right microphthalmia and homolateral agenesis of the optic nerve and hemi-chiasm, cerebellar vermis hypoplasia, and normal pituitary gland. Left choanal atresia was diagnosed at two months. The patient was hypotonic, growth and psychomotor development were severely delayed. A repeated brain MRI at an older age showed corpus callosum and pituitary gland hypoplasia, hemispheric white matter reduction and ventricular enlargement. CGH demonstrated the presence of a de novo 6.41 Mb deletion at 14q22.2-q23.1, including the *OTX2* gene.

These three cases, plus additional three published before the advent of CGH and reviewed by Picchiecchio et al.[14] demonstrate that in addition to global delays, microphthalmia/anophthalmia, choanal atresia and finger and toe partial syndactyly, other recurrent manifestations of the 14q22-q23 deletion syndrome are pituitary gland and growth hormone deficiency, gonadal underdevelopment and a face characterized by high forehead, downturned corners of mouth, micrognathia and ear anomalies.

14q24-q31 deletion syndrome

Only two cases from the literature can be firmly classified as having a 14q24-q31 deletion syndrome. Riegel et al.[15] described a boy who had normal growth parameters, but was hypotonic and developmentally delayed. Facial examination showed hypertelorism, bushy eyebrows, short nose with anteverted nostrils, deep nasolabial furrows, small mouth with an open bite, a prominent cupid bow of the upper lip and a prominent and everted lower lip. Ears were low-set with thick helices and lobules. Molecular cytogenetic analysis demonstrated the presence of a de novo deletion of approximately 13.11 Mb within the 14q24.3-q31.3 region.

Nicita et al.[16] reported on a 2-year-old boy with axial hypotonia, mild developmental and speech delay, recurrent seizures and a dysmorphic face characterized by arched eyebrows, downslanting palpebral fissures, anteverted nostrils, depressed nasal bridge with bulbous tip of nose, wide philtrum, and arched thin upper lip. A SNP array analysis showed a de novo deletion of approximately 5.5 Mb at 14q24.3-q31.1 region, including 14 mendelian genes, responsible in most cases of autosomal recessive conditions. These authors reviewed another 13 cases from the literature, carriers of 14q23-q32 deletions. It is worth noting that two of these[17,18] with deletions located within the 14q24-q31 region had a phenotype which is typical of the Holt-Oram syndrome, namely congenital heart defect and radial ray hypoplasia, suggesting that a gene for this syndrome may be located on chromosome 14q. The Holt-Oram syndrome is normally caused by mutation of the *TBX5* gene on chromosome 12.

DICER1 deletion syndrome

This is a special case, deserving to be dealt with separately, because of its peculiar presentation. The *DICER1* gene, a member of the ribonuclease III (RNaseIII) family, is involved in the generation of microRNAs (miRNAs), which modulate gene expression at the posttranscriptional level.

Mutations of *DICER1*, a cancer predisposing gene located in 14q32.13, cause an autosomal dominant condition characterized by pleuropulmonary blastoma and a number of other neoplasias such as cystic nephroma, medulloblastoma and rhabdomyosarcoma (OMIM #601200). van Engelen et al[19] reviewed a cohort of patients referred for evaluation of possible DICER1 syndrome. A significant proportion of these tested positive for a pathogenic variant. One patient, referred for a pleuropulmonary blastoma and a cystic lesion of the lung, was tested by CGH and found to be carrier of a large deletion of 14q32.11q32.2.

de Kock et al.[20] reported on a child described as hypertonic and developmentally delayed. The physical phenotype was characterized by dolichocephaly, long philtrum, thin upper lip, low set and protruding ears, bilateral epicanthal folds, high arched palate with bifid uvula, retrognathia, thin and "coarse" hair, flat feet, bilateral single palmar crease, and cryptorchidism. At one year a cystic

nephroma was removed from the left kidney, at two years and 5 months the left eye was removed for the presence of a malignant ciliary body medulloepithelioma, and during the post-operative period he was diagnosed with a brain high-grade spindle-cell sarcoma with myogenous differentiation. The child died soon after surgery. Molecular cytogenetic analysis by CGH demonstrated the presence of a de novo 5.82 Mb deletion at the 14q32.13q32.2 region, causing haploinsufficiency of *DICER1*.

Herriges et al.[21] reported on two patients with 14q32 deletions involving *DICER1*. One of these was a 15-year-old female described as having autism and "coarse" facial features. She was diagnosed with a Sertoli-Leydig cell tumor and a Wilms tumor. SNP microarray testing identified a 5.0 Mb deletion from 14q32.11 to 14q32.13 including *DICER1* and another 51 protein coding genes. The other case was a 6-year-old boy with a history of global developmental delays, including speech and fine and gross motor delays. Clinical findings included mild hypotonia, macrocephaly and tall stature. SNP microarray testing showed a 1.4 Mb deletion spanning from 14q32.12 to 14q32.13, encompassing 22 protein coding genes, including *DICER1*. No tumors were found in this boy, but his mother, a normally developed person, had a history of multiple thyroid tumors and was eventually found to be carrier of the same 14q deletion as in her son. Her family history was positive for thyroid, lung and pancreatic cancer.

14q32-qter deletion syndrome

This condition was analyzed in great detail, given that linear deletions extending from 14q32 to terminus are similar to those found in the r(14) syndrome. We considered only 11 literature cases, whose deletion was characterized by CGH[22-31]. The facial phenotype of this syndrome is in general characterized by high and narrow forehead, hypoplastic nasal sella, short nose with bulbous tip and anteverted nares, short palpebral fissures with blepharophimosis and epicanthic folds, large and flat philtrum, thin upper lip, micrognathia, low-set and posteriorly angulated ears. More details are given in Table 1a, where blank spaces are not to be interpreted necessarily as absence of that given trait, considering that in some cases a detailed clinical description of the patient was missing. Even though the described facial phenotype has some consistency, it does not have an easily recognizable "gestalt", when one looks at the few published photographs. There are, in any case, similarities with the facial features of r(14) patients which include high forehead, short palpebral fissures, short nose with bulbous tip, long philtrum[1]. Other manifestations recurring in the 14q32-qter linear deletion syndrome are psychomotor delay, present in all reported cases, and failure to thrive. More details are given in Table 1b, also showing that cases 8 and 9 are more severely affected compared to the others and suggesting that the group, even if restricted, may not be homogeneous. Notably, microcephaly and epilepsy, nearly constant features of the r(14) syndrome, are reported only in

three and two cases of the linear deletion syndrome, respectively. There are no reports of retinal abnormalities. In addition to these 11 cases, Piccione et al.[26] reviewed another 12 cases of 14q32-qter linear deletion studied by traditional cytogenetic methods, whose phenotypes are essentially in agreement with those studied by CGH.

PACS2 syndrome

The epileptic encephalopathy of neonatal-onset, caused by sequence variants of the *PACS2* gene, located on chromosome band 14q32.33[4,5] and referred to here as PACS2 syndrome, is worthy of special mention. *PACS2* encodes a multifunctional sorting protein involved in nuclear gene expression and pathway traffic regulation, it is transcribed in brain tissue where it is enriched in glial cells-enriched white matter. PACS2 has roles in both the nucleus and cytoplasm. In the nucleus, PACS2 inhibits SIRT1-dependent deacetylation of p53. The mutation may alter deacetylase functions, such as the control of p53, which may impact[32]. In the cytoplasm, PACS2 regulates endoplasmic reticulum (ER) homeostasis, ER-mitochondria communication, autophagy, and endosomal trafficking of ion channels, receptors, and enzymes. The mutation may therefore alter the function of one or more ion channels, contributing indirectly to channelopathies associated with excitability disorders. Finally, the mutation may affect mTORC2/Akt role in neuronal migration and dendritic arborization[5], and the mTOR complex is causally involved in various forms of genetic and structural epilepsies[33]. Olson et al[5] found the same de novo missense variant p.Glu209Lys in 14 patients, while Dentici et al[4] found missense variant p.Glu211Lys in another patient. PACS2 syndrome is a complex condition characterized by hypotonia, motor and intellectual delay, behavioral issues, dysmorphic face with hypertelorism, broad nasal sella and thin upper lip, minor distal limb abnormalities, cerebellar dysgenesis and early onset epilepsy. In general, the epilepsy starts as focal in the neonatal period, to become mixed focal and generalized over time, with status epilepticus in many affected subjects.

Comparing the r(14) with other deletion syndromes and with the PACS2 syndrome

As stated above, looking at published photographs of affected individuals falling within one of these three categories, one does not have the impression of a shared and recognizable facial *gestalt*. This is rather surprising, given that a combination of facial traits such as short and downslanted palpebral fissures, hypertelorism, broad and hypoplastic nasal sella, short nose with a bulbous tip, long philtrum and downturned corners of the mouth is seen not only in cases of r(14) and in those with a matching linear deletion, but also in some of the cases with different deletions. Perhaps even more surprising is the fact that a similar facial phenotype is also reported in cases of PACS2 syndrome. Expectedly, generalized muscular hypotonia, global developmental delay with intellectual disability and speech delay are common to all conditions. Another element of similarity

is the rarity of internal organ malformations. Distinctive phenotypes, such as anophthalmia/microphthalmia, as well as digit and toe syndactylies are associated with haploinsufficiency of *PAX2* and *BMP4*, both located within the 14q22q23 deletion interval. Dysplasias of the fundus oculi, namely abnormal macula, abnormal retinal pigmentation and retinitis pigmentosa are characteristic of the r(14) syndrome, although not attributable to single gene(s) loss or dysfunction. These defects are not reported in cases with matching linear deletions. Cerebellar vermis hypoplasia with foliar distortion of cerebellar hemispheres and mega cysterna magna are typical findings in the PACS2 syndrome.

Most important in the context of this review is the constant presence of epilepsy in the r(14) and in the PACS2 syndrome, but not (with a few exceptions) in the linear 14q32qter deletion syndrome. The characteristics of the PACS2 syndrome epilepsy were outlined above. The epilepsy in patients with r(14) syndrome is characterized by early onset, polymorphic and drug-resistant seizures. In addition, focal secondarily generalized seizures, seizure cluster tendency, frequent status epilepticus, and a rather typical epilepsy evolution were noted. EEG abnormalities consisted of slow background activity with pseudoperiodic bursts of generalized slow waves in the early stage, focal frontotemporal or temporoposterior slow waves with multifocal spikes interposed, and unusual rhythmic fast recruiting posterior spikes followed by secondary generalization. The degree of severity of the epileptic phenotype negatively influences child cognitive development[34]. From this description it appears that the r(14) syndrome epilepsy is similar to the PACS2 epilepsy in several respects: type of seizures, their high frequency at an early age with a negative impact on brain development, EEG characteristics. There is also a difference to be noted, namely a less severe evolution in cases of PACS2 syndrome. A summary of the compared traits is reported in Table 2.

Epilepsy genes in chromosome 14

This section will describe those epilepsy-related genes in chromosome 14 that are expected to be lost in patients with a linear deletion, in accordance with their location in any of the deletion intervals described above. The question is which genes can be considered bona fide epilepsy-related genes. Given the purpose of this review, we decided to be as inclusive as possible in order to analyze in detail the most interesting candidates.

Table 3 shows a selection of epilepsy-related genes: we first crosschecked a list of epilepsy-associated genes from Human Phenotype Ontology (HPO) with the NCBI list of genes located on chromosome 14[35,36]. We then added to this rough list of 43 genes other 5 genes (*PTGER2, DICER1, RAGE, SLC8A3* and *RCOR1*) located on chromosome 14, rarely associated with epilepsy and therefore not included in the HPO search, yet worthy of attention based on preclinical evidence in animal models of their involvement in seizure mechanisms and epilepsy-associated neurological

comorbidities[37-42]. As third step, we checked the epileptic involvement of the identified genes in the OMIM database of clinical synopses (Online Mendelian Inheritance in Man, OMIM®. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD). World Wide Web URL: https://omim.org/). If epilepsy/seizures were not reported as part of the phenotype, a more detailed research was carried out in pertinent literature. Eventually, 8 genes were excluded (*RPGRIP1, KIAA0586, MTHFD1, RDH12, MLH3, NEK9, SPATA7* and *ZC3H14*), leaving 40 genes as candidates for a role in causing epilepsy (Table 3).

Finally, we evaluated the shared pathways and verified the potential contribution of the selected genes through pathway analyses, made by collecting the literature curated gene-disease association information from the DisGeNET database[43] and visualized with NetworkAnalyst 3.0[44]. As shown in Figure S1, such analyses for gene-disease associations strongly corroborated our selection.

We then restricted our search on the most promising genes using the following criteria: 1) haploinsufficiency should be the primary, although not necessarily the only pathogenic mechanism leading to epilepsy; 2) the association should not be anecdotal: epilepsy should be a well-established component of the clinical phenotype; 3) the gene should cause seizures mainly through a dominant effect. The characteristics of epilepsy were not taken into consideration, since the possible contribution of the candidate genes in the r(14) syndrome epileptic phenotype is probably not unique. This further selection yielded 7 genes, reported in Table 3 in bold italic, whose contribution to epilepsy is described in detail in the next section.

The epileptic phenotype of candidate genes

CHD8 (Chromodomain Helicase DNA Binding Protein 8; OMIM *610528) is considered a major autism spectrum disorder (ASD) susceptibility gene. Reported variants seem to act through a loss of function (LOF) mechanism. In addition to ASD, *CHD8* has been associated to other clinical features, such as macrocephaly, gastrointestinal problems, regression of acquired skills, ID, some recurring facial features and seizures. The gene encodes for the chromatin remodeling factor CHD8, which is a member of the chromodomain-helicase-DNA binding proteins, involved in chromatin dynamics, transcriptional regulation and cell survival[45-49].

We consider *CHD8* a good candidate for playing a role in the r(14) syndrome epileptogenic process even though the prevalence of seizure disorder among patients with LOF variants is low (20-30% according to Bernier et al.[50] and Douzgou et al.[51]) and the seizures lack a clinically recognizable, consistent pattern. Against a *CHD8* LOF effect in r(14) syndrome, where microcephaly is consistently present, is the high prevalence of macrocephaly in patients with disruptive mutations causing LOF (reported as 80-85%). However, a more complex mechanism, in which *CHD8* low

expression may have a role, could be envisioned in r(14) epilepsy, while head circumference should be considered a multifactorial trait unlikely to result from the action of a single gene.

FOXG1 (Forkhead Box G1; OMIM*164874) is a well-known epilepsy gene, encoding for a protein acting as a transcriptional repressor, therefore turning off the activity of certain genes with a master role on brain formation and development. In consideration of its pleiotropic role on brain functions, significant phenotypic differences have been correlated with type and position of the pathogenic sequence variants. In patients with LOF mutation, the core of the clinical phenotype includes microcephaly, psychomotor delay with lack of language development, dyskinesia, dystonia, stereotypic movements, structural cerebral defects and early-onset seizures. Epilepsy is reported as highly penetrant, with variable seizures types, often refractory to treatment. There is not a specific EEG pattern and therefore the epileptic phenotype associated with LOF of *FOXG1* is not categorized as a particular epilepsy syndrome[52-55]. As previously noted[3,33], *FOXG1* seems to be a good candidate for a causal role in the epilepsy of the r(14) syndrome for a number of reasons. In the first place, the core clinical features and the epileptic characteristics of the *FOXG1*- related syndromes resemble those of the r(14) syndrome. Secondly, its involvement in causing epilepsy in the r(14) syndrome could be due to silencing of the proximal region of chromosome 14q as a position effect caused by the ring formation. Incidentally, the same argument is also valid for the above mentioned gene *CHD8*. As originally proposed by Zollino et al.[3], a position effect mechanism on the 14q11q13 segment is worthy of special consideration, since this region harbors candidate genes not only for epilepsy but also for retinal dystrophy, another relevant manifestation of r(14) syndrome missing in the 14q32 linear deletions.

OTX2 (Orthodenticle Homeobox 2; OMIM*600037) is a homeobox gene required for specification of the developing forebrain and eye. Although clinical conditions linked to variants of this gene may include epilepsy (about 10% of reported cases), their most recurrent and typical manifestations are anophthalmia and pituitary anomalies, not found in the r(14) syndrome. Nevertheless, other manifestations included in the clinical synopsis are compatible with the r(14) syndrome spectrum, specifically eye and retinal abnormalities[56,57].

Considering the potential contribution of different mechanisms to the r(14) epileptic process, such as tissue-specific genomic imbalances, perturbation of the epigenetic state and the effect of simultaneous deletion of several genes, and noting that variable phenotypic effects of *OTX2* are described depending on the position of the sequence variant[58], a contribution of this gene to the r(14) epilepsy cannot be excluded.

PSEN1 (OMIM*104311) encodes for presenilin-1, which represents the catalytic domain of gamma-secretase. This is a multiprotein complex whose alterations are the most common cause of

autosomal dominant Alzheimer disease (AD, OMIM#104311), characterized by high variability of neurological manifestations.

Seizures are described in AD and they are likely often unrecognized due to the lack of routine EEG recordings in patients to detect focal seizures. Recent preclinical and clinical evidence have shown that seizure may occur early in the course of the disease possibly contributing to progressive cognitive impairment. The proposed mechanisms of epileptogenesis in AD are multifactorial and not merely consequent to severe structural brain lesions[59]. As far as we know there are no autopsy report for r(14) patients documenting neuropathologic features associated with presenilin-1 dysfunction[60].

Epileptic seizures onset, trend to become more frequent over time, pathophysiology and response to therapies in AD seem very different from the epileptogenic process described in the r(14) syndrome, making *PSEN1* an unlikely candidate.

IRF2BPL (Interferon Regulatory Factor 2 Binding Protein Like; OMIM*611720) encodes a transcriptional regulator predicted to be highly intolerant to LOF variants, as found in association with an early-onset developmental disorder characterized by an epileptic encephalopathy known as NEDAMSS (neurodevelopmental disorder with regression, abnormal movements, loss of speech and seizures; OMIM#618088). Epileptic manifestations resemble those of the Lennox-Gastaut type, generally of early-onset, severe and drug-resistant, with variable seizures types, including infantile spasms, and EEG patterns. Other clinical features are mostly neurological, with a high prevalence of speech delay, neurovelopmental regression, ataxia and brain/cerebellar atrophy at MRI[61,62]. Most of the reported *IRF2BPL* pathogenic variants are nonsense or frameshift; moreover, the gene belongs to a family of intronless genes that are known to possibly escape nonsense-mediated decay. To date, it is still unclear whether mechanisms other than haploinsufficiency may have a pathogenic role. Several copy-number variants are reported in online databases such as Decipher[63], including deletions; however, a clinical description of individuals carrying a deletion limited to *IRF2BPL* is not available. It is still worth mentioning the reported phenotype of two cases with a deletion that includes *IRF2BPL* and spanning less than 5 Mb: one case carries a paternally inherited 1.39 Mb deletion resulting in autistic behavior, cognitive impairment and seizures; the other has a *de novo* 3.21 Mb deletion associated with autistic behavior, delayed speech and EEG abnormality.

DYNC1H1 (dynein cytoplasmic 1 heavy chain; OMIM*600112) encodes a protein involved in intracellular motility including retrograde axonal transport, protein sorting between apical and basolateral surfaces, and redistribution of organelles like endosomes and lysosomes. This gene has been described in association with different neurological conditions, such as autosomal dominant spinal muscular atrophy with lower extremity predominance (SMALED; OMIM#158600), axonal

Charcot-Marie-Tooth disease type 20 (OMIM#614228) and a severe form of intellectual disability with intractable epilepsy manifesting as infantile spasms (Mental Retardation AD type 13; OMIM#614563). However, a few individuals have been reported with combined features, consistent with the notion that *DYNC1H1*-associated neurological phenotypes constitute a unique spectrum. Also in accordance with this idea is the functional role of the encoded protein DYNC1H1 as a crucial subunit of the dynein motor complex and of the microtubule-based transport system. In fact, several other microtubule transport proteins are known to cause neurological diseases with varying degrees of phenotypic overlap[64,65].

It is thought that functional impairment of DYNC1H1 domains (dominant-negative or gain-of-function effect), rather than haploinsufficiency, is the causal mechanism for the above mentioned neurological conditions. To our knowledge, LOF *DYNC1H1* variants have never been associated to an epileptic phenotype.

Although based on provisional evidence, the role of a hypothetical *DYNC1H1* LOF as the underlying cause of epilepsy in the r(14) syndrome seems unlikely. Nevertheless, it is worth stressing again that this gene is included in the 14q32-qter deletion syndrome interval.

PACS2 (OMIM*610423) is a *PACS1* paralog, encoding a multifunctional sorting protein mainly expressed in the brain. Thomas et al.[66] recently reviewed PACS protein as a model for evolutionary protein adaptation, and comprehensively illustrated the regulatory role of PACS2 in cytoplasmic membrane trafficking, interorganellar communication and nuclear gene expression.

As already mentioned, *PACS2* sequence variants cause a developmental epileptic encephalopathy characterized by early onset epilepsy, global developmental delay with variable autistic features, facial dysmorphisms and cerebellar dysgenesis. This phenotype seem to be linked to two similar missense variants, resulting in a reduced ability of the predicted autoregulatory domain to modulate the interaction between PACS2 and its client protein, which may dysregulate several cellular functions[4,5].

On the other hand, *PACS2* haploinsufficiency, occurring in cases with 14q32qter linear deletions does not seem to have a major epileptogenic role. However, through mechanisms already alluded to, it could acquire such role when the haploinsufficiency is consequent to the formation of a ring.

We are aware that other genes not included in the above short list may have a role in r(14) epilepsy and should not be discarded *a priori* from a more detailed analysis. Some of these, namely those whose altered function has been more tightly associated to hyperexcitability phenomena and therefore to the genesis of seizures, are reported in the Supplementary Information.

Pathway Analysis

In addition to analyzing the function of individual genes, it is of the utmost importance to consider the interactions of their protein products with other proteins. Protein-protein interactions (PPIs) and pathway analysis were performed using NetworkAnalyst 3.0[67], a web-based tool that offers integrative approaches for PPI network analysis and visual exploration.

This analysis clearly showed that some of our genes of interest form crowded networks among each other. Particularly interesting is the network connecting CALM3-AKT1-DYNC1H1-PSEN1 (Fig 1). This network includes several other epilepsy-related genes, three of which (*SMARCB1, YWHAE* and *ITPR1*) encode proteins that are strongly associated with and contribute to the epileptic phenotype (Fig 2).

Pathway analyses were performed on all genes listed in Table 3, highlighting interesting interactions among some of them, participating to the neurotrophin signaling pathway (Figure S2).

Neurotrophins are a family of secreted growth factors that control neuron development, function and survival. The neurotrophin signaling pathway is involved in the cellular response to growth factor stimuli and involves a series of molecular signals initiated by the binding of a neurotrophin to its receptor on the surface of a target cell, resulting in the regulation of a downstream signaling process (e.g. leading to transcription of target genes, or direct modifications in neuronal excitability[68]. The most relevant of them (CALM1, PSEN1 and AKT1) are represented in Figure S2 as blue dots.

Discussion

The discussion will deal separately with the physical/functional phenotype of the reviewed cases and with the role of individual genes in causing epilepsy.

Concerning the physical/functional phenotype, what is well known to clinical geneticists is that the repertoire of phenotypes is much more restricted than that of the causal genotypes, meaning that different genetic defects, chromosomal or single-gene, may result in similar phenotypes. That said, if we inspect the data reported in Table 1a,b, we conclude that there exists a 14q terminal deletion syndrome characterized by failure to thrive, congenital muscular hypotonia, developmental delay and a facial phenotype characterized by high and narrow forehead, short palpebral fissures with epicanthic folds, hypoplastic nasal sella, bulbous tip of nose, long philtrum with thin upper lip, micrognathia and low-set ears. If we then proceed to compare this phenotype with that of other 14q deletion syndromes, of the r(14) syndrome and of the PACS2 syndrome (Table 2), some similarities are still to be noted, along with distinctive features such as retinal abnormalities and scoliosis, only seen in the r(14) syndrome, and epilepsy, exclusive of the r(14) and the PACS2 syndrome, with rare exceptions. In spite of the reported similarities, in our experience it is very difficult to diagnose any one of the reviewed conditions based on a *gestaltic* impression. Even a mere diagnostic suspicion

would be difficult to formulate and the diagnosis will only be obtained by a genetic test. In the case of the r(14) syndrome, the classical karyotype will be the ultimate confirmatory test.

Concerning the role of individual genes in causing epilepsy, after thorough scrutiny of pertinent clinical and molecular evidence, the mystery alluded to in title of this review remains unsolved. With the exception of *FOXG1* and *PACS2*, none of the genes we have selected, either in the long or in the short list of Table 3, has a clear and unquestionable epileptogenic potential. Even *FOXG1* and *PACS2* are not the best candidates to explain epilepsy in the r(14) syndrome, the former because of its position outside the 14q32-qter region, the latter because of the pathogenic mechanism of its known mutations. Nevertheless a possible role of these two genes, as well as other genes on chromosome 14 linked to epileptic manifestations is worth exploring until the pathogenic complexities of the r(14) syndrome have been disentangled.

The epigenetic dysregulation of some of the genes contained in the more centromeric tract of the long arm of chromosome 14, including *FOXG1*, *NRL* and *RPGRIP1* as a consequence of the chromosomal rearrangement, is an interesting hypothesis[1]. The epigenetic status of this chromosomal region could radically change after ring chromosome formation, due to the changed distances among genes and to the possible repositioning of the entire chromosome inside the nucleus. To our knowledge this aspect has not been molecularly investigated. In the case of *FOXG1,* expression studies could validate the hypothesis that the formation of the ring inhibits this gene expression, resulting in heterozygous LOF, which is sufficient to cause microcephaly, psychomotor delay and epilepsy.

In the case of *PACS2*, it may be worth exploring whether haploinsufficiency has a minimally penetrant epileptogenic effect, which is enhanced by the formation of the ring. Admittedly, this hypothesis would not be easy to test.

Lacking knowledge of specific mechanisms related to the action of single genes, the discrepancies between linear and comparable ring deletions with respect to their phenotype, could be generically attributed to the well-known ring chromosome instability. Sister chromatid exchanges occurring during mitosis can result in the generation of dicentric or interlocked rings, or lead to ring chromosome loss, creating a mosaic of cells with different functional properties[69,70].

Functional in vitro studies of neurons derived from iPS cells could provide valuable information on why a ring chromosome triggers cellular modifications leading to seizures. Unfortunately, previous studies have shown that ring chromosomes tend to be lost and replaced by duplication of the normal homologue in iPS cultures[71].

The study of PPIs and the analysis of specific pathways support that all selected chromosome 14 genes are associated with epileptogenic pathways, and highlighted both the neurotrophin signaling

pathways and a network involving several epilepsy-related genes, including some located on 14q (Figures 1 and 2). Moreover, the in silico analysis underscored genes not included in the shortlist and that could be worth studying, such as *AKT1*, *CALM1*, *MAGAT2* and *POMT2*, as well as other epileptogenic genes not localized on chromosome 14, whose protein products are in close connection with several genes possibly disrupted in the r(14). In future transcriptomic analysis in patients and controls, it will be very important to correlate differential gene expression with the in silico predictions. The intermediate genes highlighted by pathway analysis, such as *CALM3* and *YWHAE,* foster further investigations, also considering their potential interactions with specific miRNAs and their downstream effects.

Lastly, this review has considered exclusively the weight of pertinent cytogenetic and genomic (single gene) evidence. Notably, there is essentially no literature concerning the possible role of untranslated RNAs in the r(14) syndrome, thus highlighting a gap in knowledge that should be addressed. In particular, the 14q32 region contains the largest cluster of microRNAs (miRNA) in the entire human genome. Some of these were found to play significant roles in brain development. For instance, miR-134 is specifically expressed in the brain and controls dendritic spine formation in vitro. MiR-495 was found to be expressed in prefrontal and parietal cortex and exhibited laminar specificity in human prefrontal cortex (reviewed by Benetatos et al[72]).

We conclude that the available evidence prompts further investigations especially addressing the expression and functional consequences of candidate pathogenic genes and the role of epigenetic mechanisms in simplified model systems.

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Disclosure of conflict of interest

None of the authors has any conflict of interest to disclose.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this research is consistent with those guidelines.

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Tables

Table 1. Phenotype of published patients with linear 14q terminal deletions

a) Facial features

	1	2	3	4	5	6	7	8	9	10	11
Shape											
Elongated		+							+		
Round				+							
Forehead											
High	+						+	+	+	+	
Low				+							
Narrow	+					+	+			+	
Eyes											
Short p.f.	+		+		+				+		
Upslanted p.f.				+		+					
Downslanted p.f.					+				+		
Epicanthus		+		+				+	+		
Ptosis	+		+								
Hypertelorism				+	+			+			
Telecanthus			+	+				+			
Myopia							+				
Strabismus						+	+				
Nose											
Sellae hypoplasia	+								+	+	+
Bulbous tip	+			+		+	+				+
Anteverted nares	+				+		+		+		
Philtrum											
Long	+	+		+	+	+					
Flat					+	+		+			
Mouth											
Rounded upper lip				+							
Downturned corners				+	+						
Thin upper lip				+	+	+		+	+		
Protruding lower lip				+							
Arched palate		+	+								
Micrognathia	+		+	+		+					
Ears											
Lowset				+		+			+	+	
Posteriorly rotated	+	+			+			+			
Small					+				+		
Cupped	+									+	

b) Other features

	1	2	3	4	5	6	7	8	9	10	11
Olygohydramnios										+	
Premature birth				+	+				+	+	
SGA							+			+	
Failure to thrive		+		+	+	+				+	+
Psychomotor delay	+	+	?	+	?	+	+	+	+	+	+
Language delay				+		+		+			+
Hypotonia		+	+						+		
Weak cry									+	+	
Microcephaly	+					+	+				
G-E reflux				+							
Nistagmus									+		
SN hearing loss									+		
Small hands/feet										+	
Bicuspid aortic valve										+	
Seizures							+	+			

1) Van Karnebeek et al., 2002; 2) Schlade-Bartusiak et al., 2005 [patient 1658]; Schlade-Bartusiak et al., 2005 [patient 1363]; 4) Maurin et al., 2006; 5) Schlade-Bartusiak et al., 2008; 6) Piccione et al., 2010; 7) Chong et al., 2011; 8) Holder et al., 2011; 9)Youngs et al., 2011; 10) Youngs et al., 2012; 11) Teck Wah Ting et al., 2016.

Table 2. Comparison of the defined clinical groups according to clinical manifestations

TRAIT	RING14	TERMINAL 14Q del	*PACS2*	14Q11Q22 del	14Q22Q23 del	14Q24Q31 del
Elongated face	+	+				
High forehead	+	+			+	
Narrow forehead		+				
Horizontal eyebrows	+					
Synophris			+			
Short palpebral fissures	+	+		+		
Downslanted palpebral fissures	+	+	+			+
Hypertelorism	+	+	+	+		+
Nose sellar hypoplasia		+	+	+		+
Bulbous nasal tip	+	+		+		+
Anteverted nares		+				
Long philtrum	+	+		+		+
Downturned mouth corners	+	+	+		+	
Thin upper lip		+	+			+
Everted lower lip		+	+			+
Micrognathia		+		+	+	
Strabismus/myopia	+		+			
Abnormal macula	+					
Abnormal retinal pigmentation	+					
Preterm birth		+	+			
SGA		+				
Failure to thrive		+		+	+	
Microcephaly	+	+		+		
Hypotonia	+	+	+	+	+	+
Psychomotor delay	+	+	+	+	+	+
Speech delay	+	+	+			
Behavioural issues	+		+	+/-		
Epilepsy	+		+			
Scoliosis	+					

Blank spaces indicates absence of information

Table 3. Epilepsy-related genes on chromosome 14

Subgroup	Cytogenetic location	Gene symbol	Gene name
14q11-q22	14q11.2	***CHD8***	chromodomain helicase DNA binding protein 8
	14q11.2	*OSGEP*	O-sialoglycoprotein endopeptidase
	14q12	*AP4S1*	adaptor related protein complex 4 subunit sigma 1
	14q12	***FOXG1***	forkhead box G1
	14q12	*NUBPL*	nucleotide binding protein like
	14q21.1	*TRAPPC6B*	trafficking protein particle complex 6B
	14q21.3	*L2HGDH*	L-2-hydroxyglutarate dehydrogenase
	14q21.3	*MGAT2*	mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
	14q22.1	*NIN*	Ninein
	14q22.1	*PTGER2*	prostaglandin E receptor 2
	14q22.2	*BMP4*	bone morphogenetic protein 4
	14q22.2	*GCH1*	GTP cyclohydrolase 1
14q22-q23	14q22.3	***OTX2***	orthodenticle homeobox 2
	14q23.1-q23.3	EIG2 (genetic locus)	epilepsy, idiopathic generalized, susceptibility to 2
	14q23.3	*FUT8*	fucosyltransferase 8
	14q23.3-q24.1	*GPHN*	gephyrin
14q24-q31	14q24.1	*PIGH*	phosphatidylinositol glycan anchor biosynthesis class H
	14q24.1	*ZFYVE26*	zinc finger FYVE-type containing 26
	14q24.2	*SLC8A3*	solute carrier family 8 member a3
	14q24.2	***PSEN1***	presenilin 1
	14q24.3	*COQ6*	coenzyme Q6, monooxygenase
	14q24.3	*EIF2B2*	eukaryotic translation initiation factor 2B subunit beta
	14q24.3	*FLVCR2*	feline leukemia virus subgroup C cellular receptor family member 2
	14q24.3	***IRF2BPL***	interferon regulatory factor 2 binding protein like
	14q24.3	*NPC2*	NPC intracellular cholesterol transporter 2
	14q24.3	*POMT2*	protein O-mannosyltransferase 2
	14q31.3	*GALC*	galactosylceramidase

14q32-qter	14q32.11	*CALM1*	calmodulin 1
	14q32.11	*TDP1*	tyrosyl-DNA phosphodiesterase 1
	14q32.11-q32.12	*CCDC88C*	coiled-coil domain containing 88C
	14q32.13	*DICER1*	DICER1, ribonuclease III
	14q32.13	*GLRX5*	glutaredoxin 5
	14q32.2	*BCL11B*	BAF chromatin remodeling complex subunit BCL11B
	14q32.2	*EML1*	EMAP like 1
	14q32.31	***DYNC1H1***	dynein cytoplasmic 1 heavy chain 1
	14q32.31	*RAGE*	renal tumor antigen
	14q32.31	*TECPR2*	tectonin beta-propeller repeat containing 2
	14q32.31-q32.32	*RCOR1*	REST corepressor 1
	14q32.33	*AKT1*	AKT serine/threonine kinase 1
	14q32.33	***PACS2***	phosphofurin acidic cluster sorting protein 2

Further explored genes are in ***bold italics***. The list is ordered by cytogenetic location, from centromere to telomere.

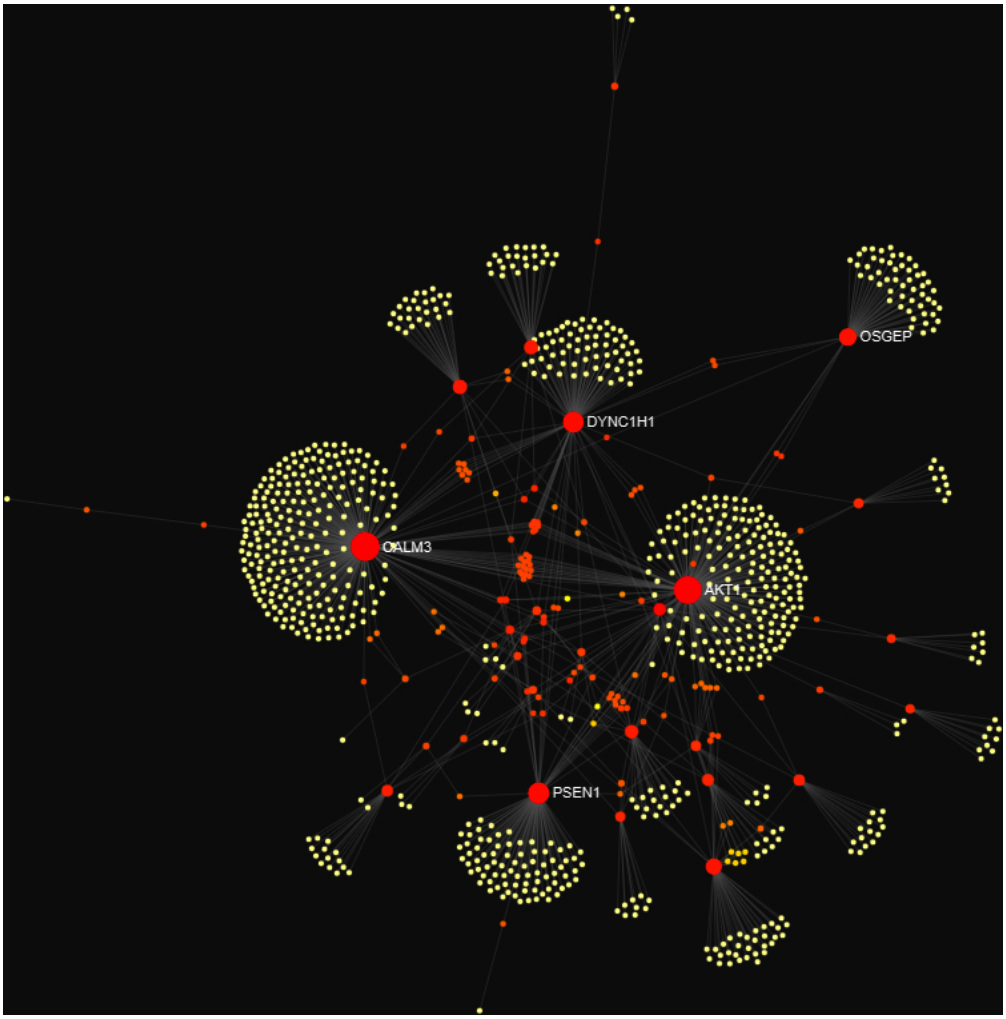

Figure 1.Graphic rendition of the interactome of proteins encoded by epilepsy-related genes.

63x64mm (300 x 300 DPI)

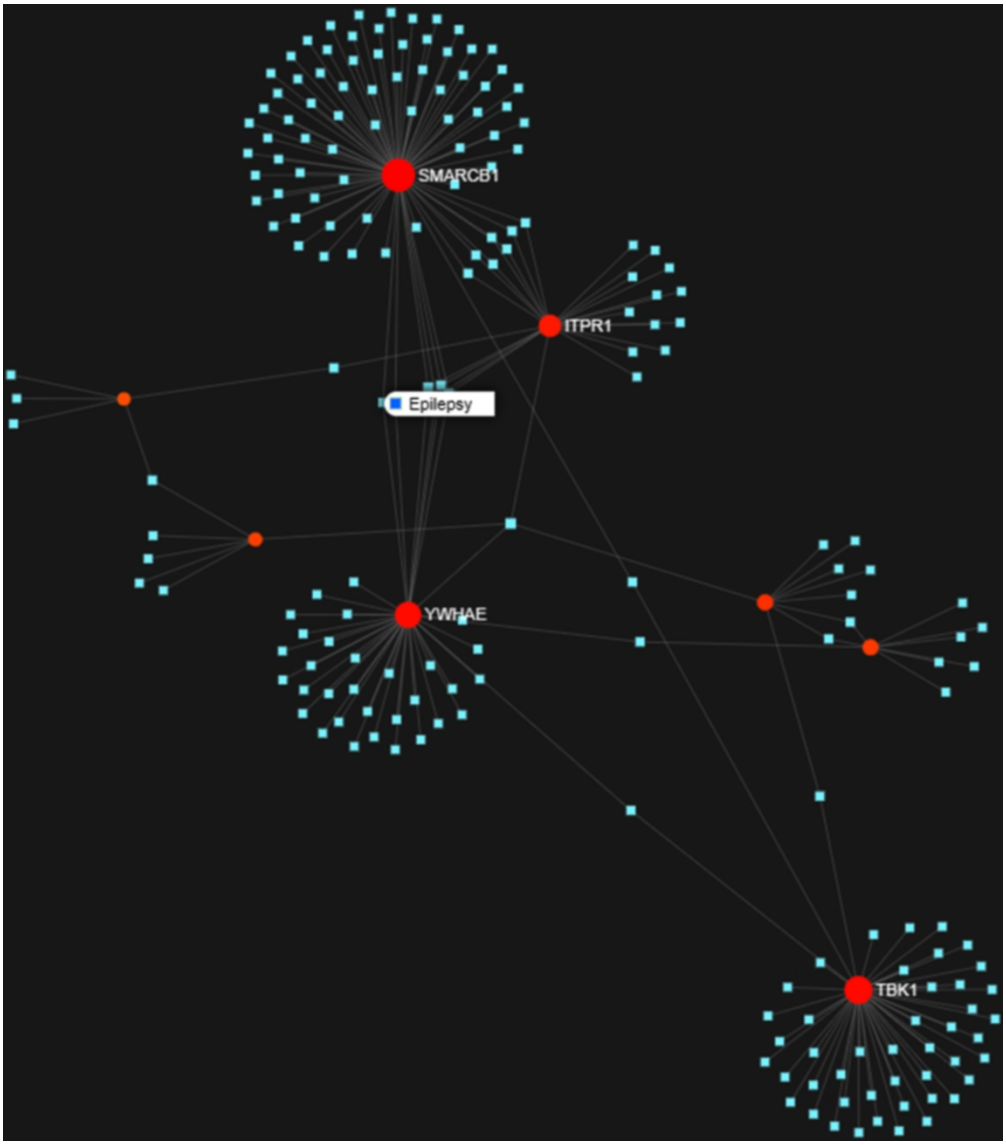

Figure 2. Protein-protein interaction analysis demonstrates that candidate genes on chromosome 14 interacts with other epilepsy-related genes. The size of dots indicates the level of involvement in causing epilepsy.

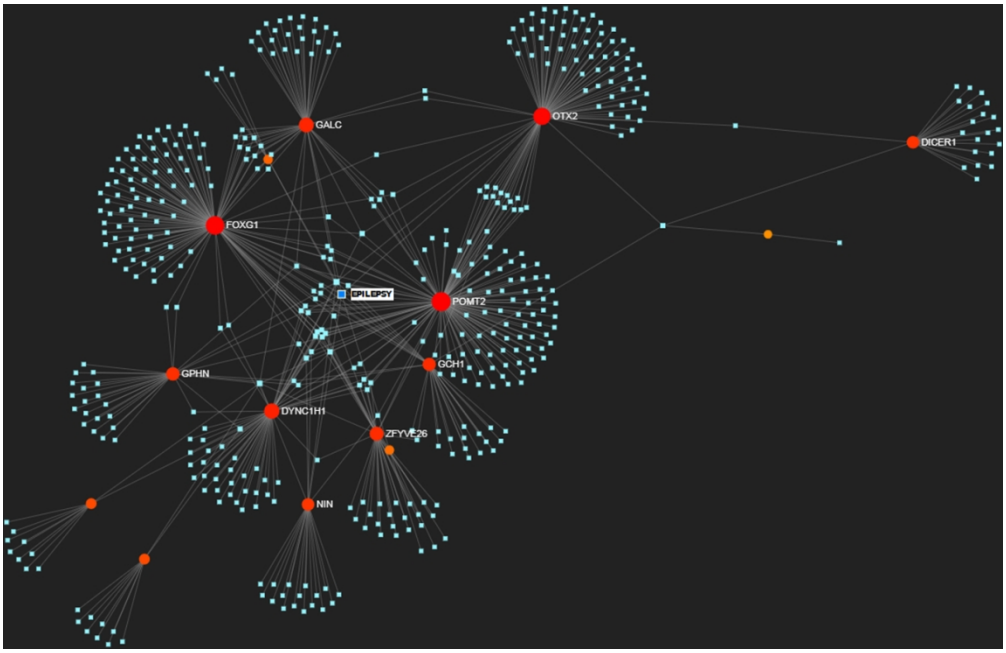

Figure S1. Graphic rendition of the central position of the epileptic phenotype in a network describing gene-disease association analysis for candidate genes listed in table 3.

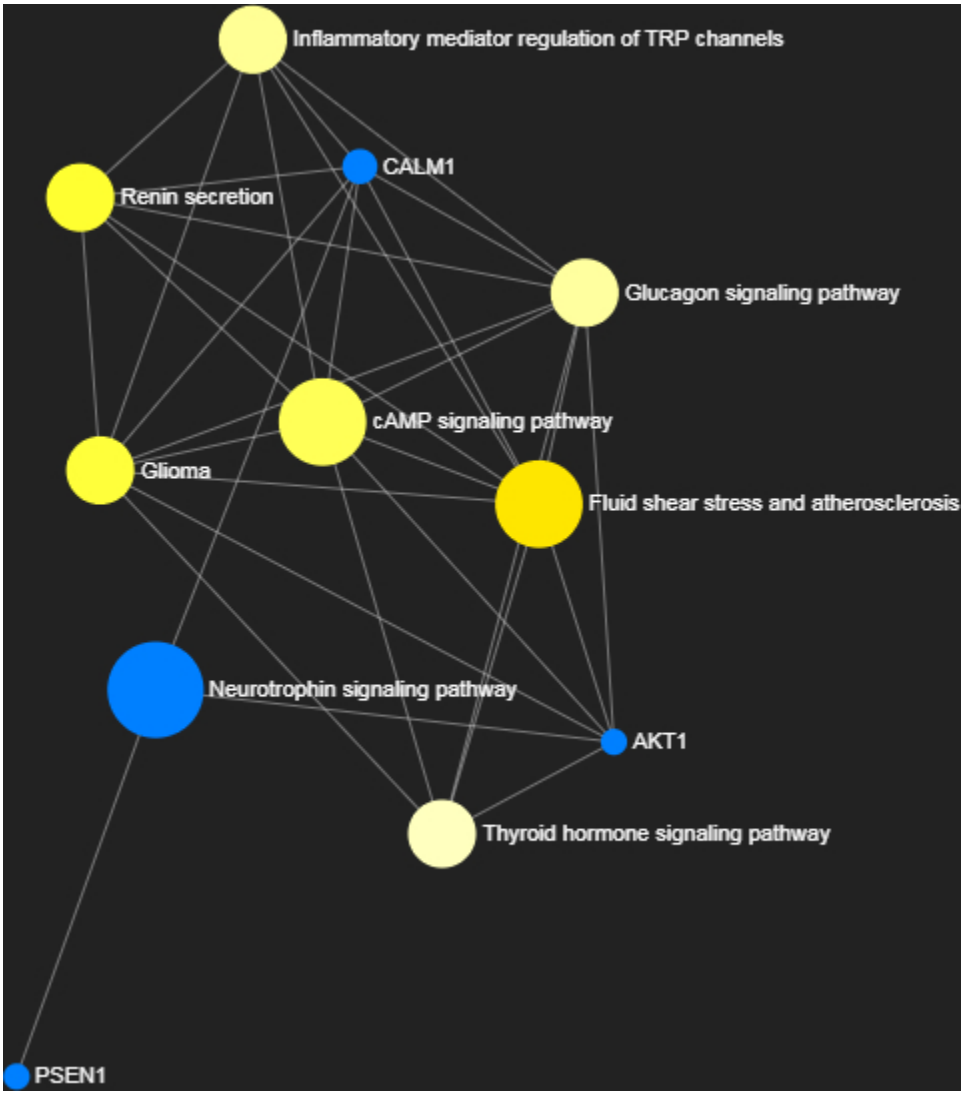

Figure S2: Graphic rendition of pathway analysis. The most relevant pathway is represented as topographical localization within the neurotrophin signaling pathways of three chromosome 14 epilepsy-related genes (blue dots).

168x191mm (72 x 72 DPI)