

Improve your skills and knowledge in epileptology

NEW virtual campus and online learning environment



Work on your competencies

Explore the course portfolio

Track your progress

Earn a certificate

The International League Against Epilepsy (ILAE) introduces highly interactive, practice-oriented online courses for healthcare professionals worldwide who diagnose and treat epilepsy.

The ILAE Academy offers:

- Competency-based e-learning for different levels of expertise¹
- Content developed by ILAE experts
- Realistic cases covering most common epilepsies



EXPLORE AND REGISTER

www.ilae-academy.org

1. Blümcke, Ingmar, et al. "Roadmap for a competency-based educational curriculum in epileptology: report of the Epilepsy Education Task Force of the International League Against Epilepsy." *Epileptic Disorders* 21.2 (2019): 129-140.

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

Alessandro Vaisfeld¹  | Serena Spartano¹  | Giuseppe Gobbi² |
 Annamaria Vezzani³  | Giovanni Neri^{1,4} 

¹Institute of Genomic Medicine, Catholic University School of Medicine, Rome, Italy

²Residential Center for Rehabilitation Luce Sul Mare, Rimini, Italy

³Department of Neuroscience, IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy

⁴J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, SC, USA

Correspondence

Giovanni Neri, Institute of Genomic Medicine, Catholic University School of Medicine, Rome, Italy.
 Email: giovanni.neri43@gmail.com

Funding information

Ring 14 International; Ring14 Italia

Abstract

The ring 14 syndrome is a rare condition caused by the rearrangement of one chromosome 14 into a ring-like structure. The formation of the ring requires two breakpoints and loss of material from the short and long arms of the chromosome. Like many other chromosome syndromes, it is characterized by multiple congenital anomalies and developmental delays. Typical of the condition are retinal anomalies and drug-resistant epilepsy. These latter manifestations are not found in individuals who are carriers of comparable 14q deletions without formation of a ring (linear deletions). To find an explanation for this apparent discrepancy and gain insight into the mechanisms leading to seizures, we reviewed and compared literature cases of both ring and linear deletion syndrome with respect to both their clinical manifestations and the role and function of potentially epileptogenic genes. Knowledge of the epilepsy-related genes in chromosome 14 is an important premise for the search of new and effective drugs to combat seizures. Current clinical and molecular evidence is not sufficient to explain the known discrepancies between ring and linear deletions.

KEYWORDS

epilepsy-related genes, pharmaco-resistant seizures, Ring14 syndrome

1 | 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 because 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 pharmaco-resistant 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 by 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. This unexpected and unexplained finding could be due to: (a) a position effect on other genes on chromosome 14 not necessarily included in the deleted region; (b) the known instability of the ring, causing monosomy of chromosome 14 in a proportion of cells; or (c) a high level of monosomy in areas of the brain contributing to a potential epileptogenic focus. This proportion is known to be around 20% in peripheral blood cells.³ 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 syndromes, 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.

2 | 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

Key points

- Epilepsy is the most medically relevant manifestation in the ring 14 syndrome. Its cause and pathogenesis remain unclear
- Several genes on chromosome 14 may have a role in causing epilepsy. The effect of their deletion awaits investigation by multiple approaches
- Missense mutations of the *PACS2* gene, deleted in the ring 14 syndrome, cause a type of epilepsy similar to that observed in ring 14 patients
- A system biology investigation suggests that epilepsy in the ring 14 syndrome may be the result of complex interactions among a number of different genes

the chromosome loss, even in cases described as cytogenetically identical, is virtually impossible to prove.

3 | 14Q11-Q22 DELETION SYNDROME

Despite 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, and 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 al⁶ 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 al⁷ described a patient with a 5 Mb deletion at 14q12, encompassing the neurodevelopmental genes *FOXG1*, *PRKDI*, and *NOVA1*, and a phenotype described as “Rett-like”

with epilepsy. Critical among these three genes is *FOXG1*, the mutations of which cause a distinctive epilepsy syndrome including microcephaly, hypotonia, severe intellectual, and motor delay, also known as congenital Rett syndrome (OMIM #613454). The possible role of *FOXG1* in the r(14) syndrome is further discussed herein in the section dedicated to the epilepsy genes in chromosome 14. Torgykes et al⁸ 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, whereas 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 and 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 al⁹ 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 al¹⁰ 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 Kamasaran et al¹¹ with deletions involving the entire 14q11-q22 region.

4 | 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, and epilepsy in one case. More specifically, Nolen et al¹² described a boy with severe postnatal 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 digits 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 2 years. A brain magnetic resonance imaging (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 *KTNI* (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, whereas that of *BMP4* could be the cause of syndactylies.

The second case¹³ is that of a female born prematurely at 33 weeks with normal measurements and choanal atresia, velopharyngeal incompetence, insufficiency of the gastroesophageal sphincter, and frequent seizures. When re-examined at the age of 13 years, she was moderately delayed and had 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 second, third, and fourth fingers 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 2 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 an 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.

5 | 14Q24-Q31 DELETION SYNDROME

Only two cases from the literature can be firmly classified as having a 14q24-q31 deletion syndrome. Riegel et al¹⁵ 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 ~13.11 Mb within the 14q24.3-q31.3 region.

Nicita et al¹⁶ reported 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 single-nucleotide polymorphism (SNP) array analysis showed a de novo deletion of ~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.

6 | DICER1 DELETION SYNDROME

This is a special case, deserving separate attention 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 neoplasia such as cystic nephroma, medulloblastoma, and rhabdomyosarcoma (OMIM #601200). van Engelen et al¹⁹ 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 al²⁰ 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 1 year, a cystic nephroma was removed from the left kidney, at 2 years and 5 months the left eye was removed for the presence of a malignant ciliary body medulloepithelioma, and during the postoperative 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 al²¹ reported on two patients with 14q32 deletions involving *DICER1*. One of these was a 15-year-old female patient 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.

7 | 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 12 literature cases, whose deletion was characterized by CGH.^{22–32} 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, and 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, and 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 9 and 10 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 in only three and four cases of the linear deletion syndrome, respectively. There are no reports of retinal abnormalities. In addition to these 11 cases, Piccione et al²⁶ 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.

8 | 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 cell-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 on the protein activity.³³ 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.³⁴ Olson et al⁵ found the same de novo missense variant p.Glu209Lys in 14 patients, whereas Dentici et al⁴ 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 very 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.

9 | 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 cisterna 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. Epilepsy in patients with r(14) syndrome is characterized by early onset, polymorphic, and drug-resistant seizures. In addition, focal secondarily

TABLE 1 Phenotype of published patients with linear 14q terminal deletions

(a) Facial features	1 ²²	2 ²³	3 ²³	4 ²⁴	5 ²⁵	6 ²⁶	7 ²⁷	8 ²⁸	9 ²⁹	10 ³⁰	11 ³¹	12 ³²
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												
Oligohydramnios												+
Premature birth				+	+					+	+	
SGA								+			+	
Failure to thrive		+		+	+	+					+	+
Psychomotor delay	+	+	?	+	?	+	+	+	+	+	+	+

(Continues)

(b) Other features	1 ²²	2 ²³	3 ²³	4 ²⁴	5 ²⁵	6 ²⁶	7 ²⁷	8 ²⁸	9 ²⁹	10 ³⁰	11 ³¹	12 ³²
Language delay				+		+			+			+
Hypotonia		+	+							+		
Weak cry										+	+	
Microcephaly	+					+		+				
GE reflux				+								
Nistagmus										+		
SN hearing loss										+		
Small hands/feet												+
Bicuspid aortic valve												+
Seizures					+		+	+	+			

Note: 1 Van Karnebeek et al²²; 2) Schlade-Bartusiak et al [patient 1658]²³; Schlade-Bartusiak et al [patient 1363]²³; 4) Maurin et al²⁴; 5) Schlade-Bartusiak et al²⁵; 6) Piccione et al²⁶; 7) Wu et al²⁷; 8) Cheon et al²⁸; 9) Holder et al²⁹; 10) Youngs et al³⁰; 11) Youngs et al³¹; 12) Teck Wah Ting et al³². Abbreviations: GE, gastro-esophageal; p.f., palpebral fissures; SGA, small for gestational age; SN, sensory-neural.

generalized seizures, seizure cluster tendency, frequent status epilepticus, and a rather typical epilepsy evolution were noted. Electroencephalography (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.³⁵ 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, and EEG characteristics. There are also differences to be noted, namely, the neonatal onset of seizures and a less severe evolution in cases of PACS2 syndrome. A summary of the compared traits is reported in Table 2.

10 | EPILEPSY GENES IN CHROMOSOME 14

This section describes 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 earlier. 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, the genomic location of which is graphically represented in Figure 1: We first cross-checked a list of epilepsy-associated genes from Human Phenotype Ontology (HPO) with the NCBI list of genes located on chromosome 14.^{36,37} We then added to this rough list of 43 genes another 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.^{38–43} As a 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; 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 database⁴⁴ and visualized with NetworkAnalyst 3.0.⁴⁵ 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: (a) haploinsufficiency should be the primary, although not necessarily the only pathogenic mechanism leading to epilepsy; (b) the association should not be anecdotal: epilepsy should be a well-established component of the clinical phenotype; and (c) 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 seven genes, reported in Table 3 in bold italic, whose contribution to epilepsy is described in detail in the next section.

11 | 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 with other clinical features, such as macrocephaly, gastrointestinal problems, regression of acquired skills, intellectual disability, 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.⁴⁶⁻⁵⁰

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 al⁵¹ and Douzgou et al⁵²) 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,

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	+					

Note: Blank spaces indicates absence of information. Abbreviations: SGA, small for gestational age.

a more complex mechanism, in which *CHD8* low expression may have a role, could be envisioned in r(14) epilepsy, whereas 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 seizure 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.^{53–56} As noted previously,^{3,34} *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. Second, 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.^{57,58}

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,⁵⁹ 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's 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.⁶⁰ As far as we know, there are no autopsy reports for r(14) patients documenting neuropathologic features associated with presenilin-1 dysfunction.⁶¹

Epileptic seizure 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 seizure types, including infantile spasms, and EEG patterns. Other clinical features are mostly neurological, with a high prevalence of speech delay, neurodevelopmental regression, ataxia, and brain/cerebellar atrophy on MRI.^{62,63} 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,⁶⁴ 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

TABLE 3 Epilepsy-related genes on chromosome 14

Subgroup	Cytogenetic location	Gene symbol	Gene name	
14q11-q22	14q11.2	<i>CHD8</i>	chromodomain helicase DNA binding protein 8	
	14q11.2	<i>OSGEP</i>	O-sialoglycoprotein endopeptidase	
	14q12	<i>AP4S1</i>	adaptor related protein complex 4 subunit sigma 1	
	14q12	<i>FOXP1</i>	forkhead box G1	
	14q12	<i>NUBPL</i>	nucleotide binding protein like	
	14q21.1	<i>TRAPPC6B</i>	trafficking protein particle complex 6B	
	14q21.3	<i>L2HGDH</i>	L-2-hydroxyglutarate dehydrogenase	
	14q21.3	<i>MGAT2</i>	mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase	
	14q22.1	<i>NIN</i>	Ninein	
	14q22.1	<i>PTGER2</i>	prostaglandin E receptor 2	
	14q22.2	<i>BMP4</i>	bone morphogenetic protein 4	
	14q22.2	<i>GCH1</i>	GTP cyclohydrolase 1	
	14q22-q23	14q22.3	<i>OTX2</i>	orthodenticle homeobox 2
		14q23.1-q23.3	EIG2 (genetic locus)	epilepsy, idiopathic generalized, susceptibility to 2
14q23.3		<i>FUT8</i>	fucosyltransferase 8	
14q23.3-q24.1		<i>GPHN</i>	gephyrin	
14q24-q31	14q24.1	<i>PIGH</i>	phosphatidylinositol glycan anchor biosynthesis class H	
	14q24.1	<i>ZFYVE26</i>	zinc finger FYVE-type containing 26	
	14q24.2	<i>SLC8A3</i>	solute carrier family 8 member a3	
	14q24.2	<i>PSEN1</i>	presenilin 1	
	14q24.3	<i>COQ6</i>	coenzyme Q6, monooxygenase	
	14q24.3	<i>EIF2B2</i>	eukaryotic translation initiation factor 2B subunit beta	
	14q24.3	<i>FLVCR2</i>	feline leukemia virus subgroup C cellular receptor family member 2	
	14q24.3	<i>IRF2BPL</i>	interferon regulatory factor 2 binding protein like	
	14q24.3	<i>NPC2</i>	NPC intracellular cholesterol transporter 2	
	14q24.3	<i>POMT2</i>	protein O-mannosyltransferase 2	
	14q31.3	<i>GALC</i>	galactosylceramidase	
14q32-qter	14q32.11	<i>CALM1</i>	calmodulin 1	
	14q32.11	<i>TDP1</i>	tyrosyl-DNA phosphodiesterase 1	
	14q32.11-q32.12	<i>CCDC88C</i>	coiled-coil domain containing 88C	
	14q32.13	<i>DICER1</i>	DICER1, ribonuclease III	
	14q32.13	<i>GLRX5</i>	glutaredoxin 5	
	14q32.2	<i>BCL11B</i>	BAF chromatin remodeling complex subunit BCL11B	
	14q32.2	<i>EML1</i>	EMAP like 1	
	14q32.31	<i>DYNCH1</i>	dynein cytoplasmic 1 heavy chain 1	
	14q32.31	<i>RAGE</i>	renal tumor antigen	
	14q32.31	<i>TECPR2</i>	tectonin beta-propeller repeat containing 2	
	14q32.31-q32.32	<i>RCOR1</i>	REST corepressor 1	
	14q32.33	<i>AKT1</i>	AKT serine/threonine kinase 1	
	14q32.33	<i>PACS2</i>	phosphofurin acidic cluster sorting protein 2	

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

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 I (Mental Retardation AD type 13; OMIM #614563) with mild dysmorphic features, cortical malformations (defective gyration of the frontal lobes and focal cortical dysplasias), and intractable epilepsy manifesting as infantile spasms.⁶⁵ 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.^{66,67}

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 with 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 14q32qter deletion syndrome interval.

PACS2 (OMIM *610423) is a *PACS1* paralog, encoding a multifunctional sorting protein mainly expressed in the brain. Thomas et al⁶⁸ 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 seems 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.

In addition, considering that rare patients with linear deletions including *PACS2* do have epilepsy, it is possible that deletion of the gene has low penetrance.⁵ Current knowledge does not allow for formulating and testing of more specific hypotheses.

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 Appendix S1.

12 | 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 3.0,⁴⁵ 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 (Figure 2). 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 (Figure 3).

Pathway analyses were performed on all genes listed in Table 3, highlighting interesting interactions among some of them, participating in 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 (eg, leading to transcription of target genes, or direct modifications in neuronal excitability).⁶⁹ The most relevant of them (*CALM1*, *PSEN1*, and *AKT1*) are represented in Figure S2 as blue dots.

These findings, although limited, demonstrate that when looking for the epileptogenic role of genes on chromosome 14, it is not sufficient to consider only the action of their protein products, but it is necessary to explore the effects that their interaction with other proteins may have. This is a field wide open to new studies.

13 | DISCUSSION

The discussion addresses separately the physical/functional phenotype of the reviewed cases and 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,

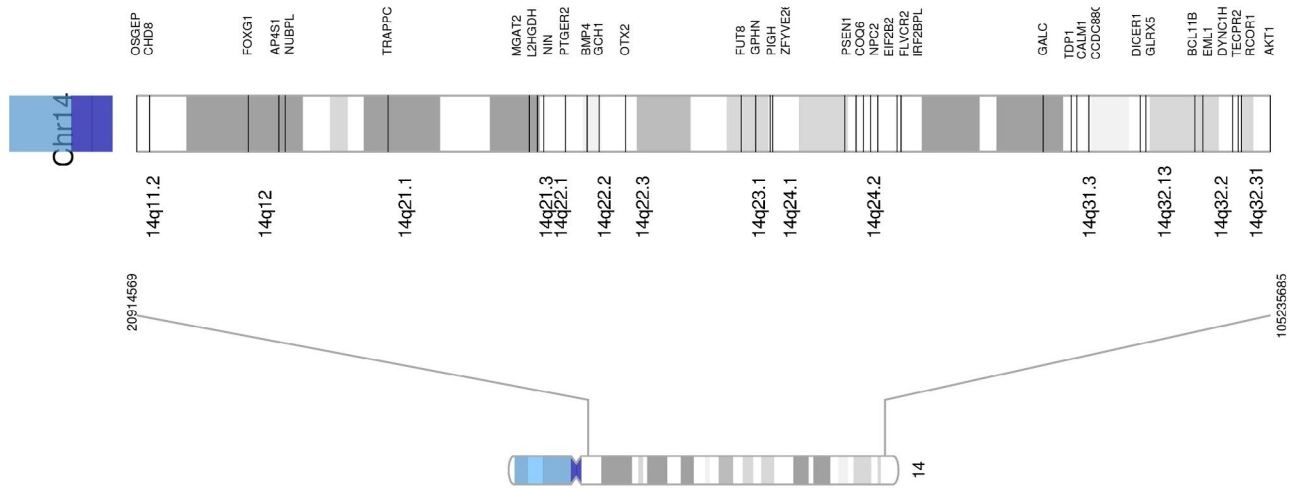


FIGURE 1 Ideogram of chromosome 14 with the position of the genes listed in Table 1

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. Despite the reported similarities, in our experience it is very difficult to diagnose any one of the reviewed conditions based on their *gestalt*. 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 the 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, and 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 its 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.^{70,71} Rings can form from any chromosome, causing ring syndromes, most of which are characterized by failure to thrive and intellectual delay. In addition to the r(14) syndrome, epilepsy has been reported in r(7), r(17), r(18), r(20), r(21), and r(22) syndromes (<http://www.orpha.net>), suggesting that the presence of a ring within the nucleus may by itself disrupt the balance of gene expression.

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

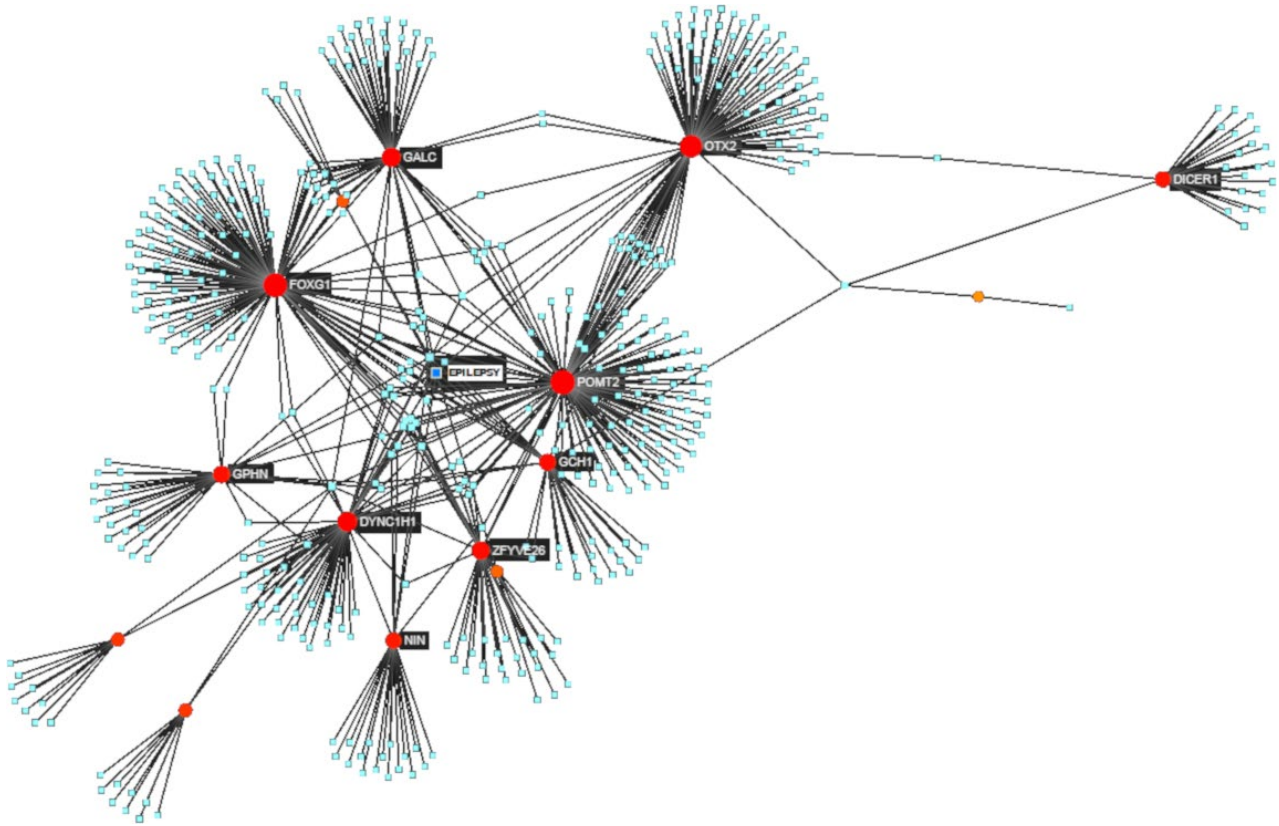


FIGURE 2 .Graphic rendition of the interactome of proteins encoded by epilepsy-related genes

lost and replaced by duplication of the normal homologue in iPS cultures.⁷² The reason that iPS cultures containing a ring chromosome are difficult to induce is likely to be the instability of the ring itself, a problem that may be solved in the future by improvement of the iPS-induction techniques.

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 2 and 3). Moreover, the *in silico* analysis underscored genes not included in the short list 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.

Finally, 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 (miRNAs) in the entire human genome. Some of these were found to play significant roles in brain development. For instance, miR-134 is expressed 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 al⁷³).

The application of “omics” technologies will add further valuable information that would be useful to ameliorate the pathway analysis discussed. In addition to the already mentioned expression studies (coding RNAs and proteins), consideration should be given to the study of the methylome and to a better definition of ring and linear deletions through long-read sequencing.

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.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of Ring 14 International, Ring 14 Italy, and of all the families who were instrumental in collecting some of the data discussed in this review.

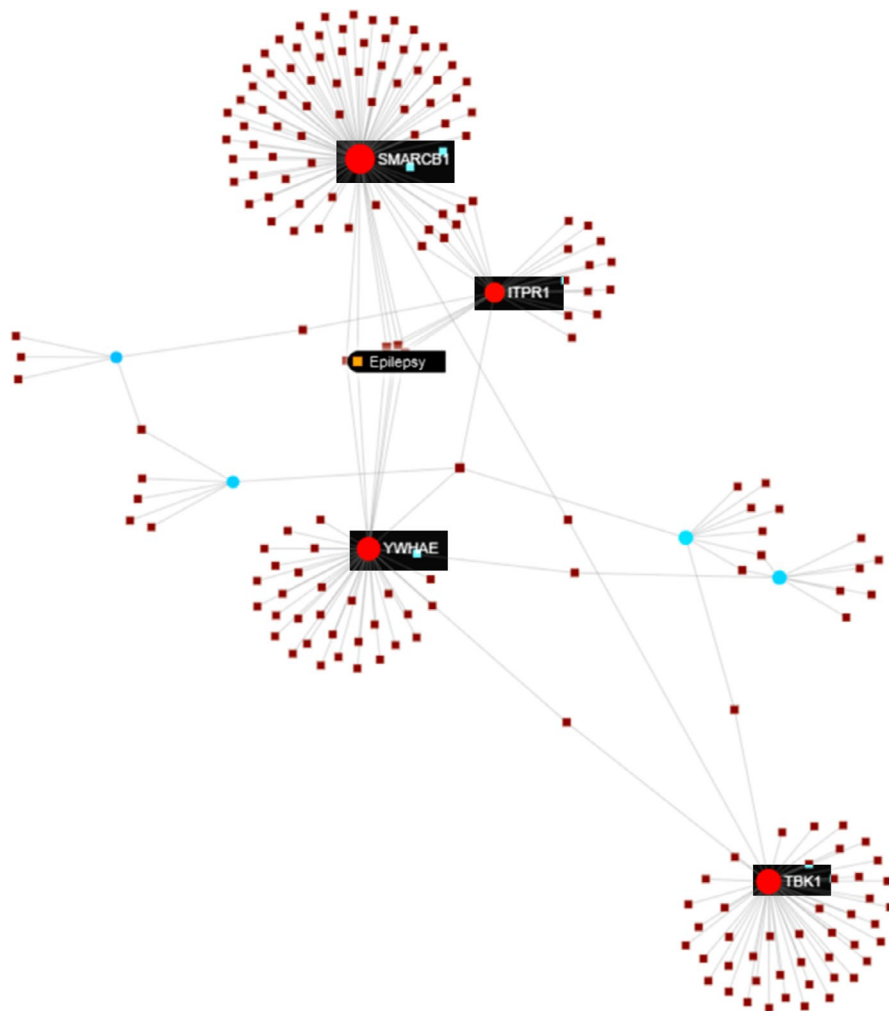


FIGURE 3 Protein-protein interaction analysis demonstrates that candidate genes on chromosome 14 interacts with other epilepsy-related genes. The size of dots indicates the level of involvement in causing epilepsy

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.

ORCID

Alessandro Vaisfeld  <https://orcid.org/0000-0001-8413-621X>

Serena Spartano  <https://orcid.org/0000-0002-7095-9968>

Annamaria Vezzani  <https://orcid.org/0000-0003-0573-6175>

Giovanni Neri  <https://orcid.org/0000-0001-7642-0130>

REFERENCES

- Zollino M, Ponzi E, Gobbi G, Neri G. The ring 14 syndrome. *Eur J Med Genet.* 2012;55:374–80.
- Rinaldi B, Vaisfeld A, Amarri S, Baldo C, Gobbi G, Magini P, et al. Guideline recommendations for diagnosis and clinical management of Ring14 syndrome—first report of an ad hoc task force. *Orphanet J Rare Dis.* 2017;12:69.
- Zollino M, Seminara L, Orteschi D, Gobbi G, Giovannini S, Giustina ED, et al. The ring 14 syndrome: clinical and molecular definition. *Am J Med Genet A.* 2009;149:1116–24.
- Dentici ML, Barresi S, Niceta M, Ciolfi A, Trivisano M, Bartuli A, et al. Expanding the clinical spectrum associated with PACS2 mutations. *Clin Genet.* 2019;95:525–31.
- Olson HE, Jean-Marçais N, Yang E, Heron D, Tatton-Brown K, van der Zwaag PA, et al. A recurrent de novo PACS2 heterozygous missense variant causes neonatal-onset developmental epileptic encephalopathy, facial dysmorphism, and cerebellar dysgenesis. *Am J Hum Genet.* 2018;103:631.
- Yasin H, Gibson WT, Langlois S, Stowe RM, Tsang ES, Lee L, et al. A distinct neurodevelopmental syndrome with intellectual disability, autism spectrum disorder, characteristic facies, and macrocephaly is caused by defects in CHD8. *J Hum Genet.* 2019;64:271–80.
- Vineeth VS, Dutta UR, Tallapaka K, Das Bhowmik A, Dalal A. Whole exome sequencing identifies a novel 5 Mb deletion at 14q12 region in a patient with global developmental delay, microcephaly and seizures. *Gene.* 2018;673:56–60.
- Torgykes E, Shanske AL, Anyane-Yeboah K, Nahum O, Pirzadeh S, Blumfield E, et al. The proximal chromosome 14q microdeletion syndrome: delineation of the phenotype using high resolution SNP oligonucleotide microarray analysis (SOMA) and review of the literature. *Am J Med Genet A.* 2011;155A:1884–96.

9. Gentile M, De Mattia D, Pansini A, Schettini F, Buonadonna AL, Capozza M, et al. 14q13 distal microdeletion encompassing NKX2-1 and PAX9: Patient report and refinement of the associated phenotype. *Am J Med Genet A*. 2016;170:1884–8.
10. Villafuerte B, Natera-de-Benito D, González A, Mori MA, Palomares M, Nevado J, et al. The Brain-Lung-Thyroid syndrome (BLTS): a novel deletion in chromosome 14q13.2-q21.1 expands the phenotype to humoral immunodeficiency. *Eur J Med Genet*. 2018;61:393–8.
11. Kamnasaran D, O'Brien PCM, Schuffenhauer S, Quarrell O, Lupski JR, Grammatico P, et al. Defining the breakpoints of proximal chromosome 14q rearrangements in nine patients using flow-sorted chromosomes. *Am J Med Genet*. 2001;102:173–82.
12. Nolen LD, Amor D, Haywood A, St. Heaps L, Willcock C, Mihelec M, et al. Deletion at 14q22–23 indicates a contiguous gene syndrome comprising anophthalmia, pituitary hypoplasia, and ear anomalies. *Am J Med Genet A*. 2006;140:1711–8.
13. Martínez-Frías ML, Oejo-Vinyals JG, Arteaga R, Martínez-Fernández ML, MacDonald A, Pérez-Belmonte E, et al. Interstitial deletion 14q22.3-q23.2: genotype-phenotype correlation. *Am J Med Genet A*. 2014;164A:639–47.
14. Pichiecchio A, Vitale G, Caporali C, Parazzini C, Milani D, Recalcati MP, et al. New insights into the phenotypic spectrum of 14q22q23 deletions: a case report and literature review. *BMC Med Genomics*. 2018;29(11):87.
15. Riegel M, Moreira LM, Espirito Santo LD, Toralles MB, Schinzel A. Interstitial 14q24.3 to q31.3 deletion in a 6-year-old boy with a non-specific dysmorphic phenotype. *Mol Cytogenet*. 2014;7:77.
16. Nicita F, Di Giacomo M, Palumbo O, Ferri E, Maiorani D, Vigeveno F, et al. Neurological features of 14q24-q32 interstitial deletion: report of a new case. *Mol Cytogenet*. 2015;24(8):93.
17. Turleau C, de Grouchy J, Chavin-Colin F, Dore F, Seger J, Dautzenberg MD, et al. Two patients with interstitial del (14q), one with features of Holt-Oram syndrome. Exclusion mapping of PI (alpha-1-antitrypsin). *Ann Genet*. 1984;27:237–40.
18. Le Meur N, Goldenberg A, Michel-Adde C, Drouin-Garraud V, Blaysat G, Marret S, et al. Molecular characterization of a 14q deletion in a boy with features of Holt-Oram syndrome. *Am J Med Genet A*. 2005;134:439–42.
19. van Engelen K, Villani A, Wasserman JD, Aronoff L, Greer M-L, Tijerin Bueno M, et al. DICER1 syndrome: approach to testing and management at a large pediatric tertiary care center. *Pediatr Blood Cancer*. 2018;65:e26720.
20. de Kock L, Geoffrion D, Rivera B, Wagener R, Sabbaghian N, Bens S, et al. Multiple DICER1-related tumors in a child with a large interstitial 14q32 deletion. *Genes Chromosomes Cancer*. 2018;57:223–30.
21. Hériges JC, Brown S, Longhurst M, Ozmore J, Moeschler JB, Janze A, et al. Identification of two 14q32 deletions involving DICER1 associated with the development of DICER1-related tumors. *Eur J Med Genet*. 2019;62:9–14.
22. van Karnebeek CD, Quik S, Sluijter S, Hulsbeek MM, Hoovers JM, Hennekam RC. Further delineation of the chromosome 14q terminal deletion syndrome. *Am J Med Genet*. 2002;1(110):65–72.
23. Schlade-Bartusiak K, Costa T, Summers AM, Nowaczyk MJ, Cox DW. FISH-mapping of telomeric 14q32 deletions: search for the cause of seizures. *Am J Med Genet A*. 2005;15(138A):218–24.
24. Maurin M-L, Brisset S, Le Lorc'h M, Poncet V, Trioche P, Aboura A, et al. Terminal 14q32.33 deletion: genotype-phenotype correlation. *Am J Med Genet A*. 2006;1(140):2324–9.
25. Schlade-Bartusiak K, Macintyre G, Zurich J, Cox DW. A child with deletion (14)(q24.3q32.13) and auditory neuropathy. *Am J Med Genet A*. 2008;1(146A):117–23.
26. Piccione M, Antona V, Scavone V, Malacarne M, Pierluigi M, Grasso M, et al. Array CGH defined interstitial deletion on chromosome 14: a new case. *Eur J Pediatr*. 2010;169:845–51.
27. Wu YE, Ji T, Wang J, Xiao J, Wang H, Li J, et al. Submicroscopic subtelomeric aberrations in Chinese patients with unexplained developmental delay/mental retardation. *BMC Med Genet*. 2010;11:72.
28. Cheon CK, Park SJ, Choi OH. 14q32.33 Deletion Identified by array-CGH in a 5-year old-girl with Seizure. *J Genet Med*. 2011;8:62–6.
29. Holder JL Jr, Lotze TE, Bacino C, Cheung SW. A child with an inherited 0.31 Mb microdeletion of chromosome 14q32.33: further delineation of a critical region for the 14q32 deletion syndrome. *Am J Med Genet A*. 2012;158A:1962–6.
30. Youngs EL, Hellings JA, Butler MG. A clinical report and further delineation of the 14q32 deletion syndrome. *Clin Dysmorphol*. 2011;20:143–7.
31. Youngs EL, Dasouki M, Butler MG. 14q32 deletion syndrome: a clinical report. *Clin Dysmorphol*. 2012;21:42–4.
32. Ting TW, Brett MS, Cham BWM, Lim J-Y, Law HY, Tan EC, et al. DICER1 deletion and 14q32 microdeletion syndrome: an additional case and a review of the literature. *Clin Dysmorphol*. 2016;25:37–40.
33. Engel T, Tanaka K, Jimenez-Mateos EM, Caballero-Caballero A, Prehn JH, Henshall DC. Loss of p53 results in protracted electrographic seizures and development of an aggravated epileptic phenotype following status epilepticus. *Cell Death Dis*. 2010;1:e79.
34. Ostendorf AP, Wong M. mTOR inhibition in epilepsy: rationale and clinical perspectives. *CNS Drugs*. 2015;29:91–9.
35. Giovannini S, Marangio L, Fusco C, Scarano A, Frattini D, Della Giustina E, et al. Epilepsy in ring 14 syndrome: a clinical and EEG study of 22 patients. *Epilepsia*. 2013;54:2204–13.
36. Köhler S, Carmody L, Vasilevsky N, Jacobsen JOB, Danis D, Gourdine JP, et al. Expansion of the human phenotype ontology (HPO) knowledge base and resources. *Nucleic Acids Res*. 2018;47:D1018–D1027.
37. NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res*. 2018;46:D8–D13.
38. Iori V, Iyer AM, Ravizza T, Beltrame L, Paracchini L, Marchini S, et al. Blockade of the IL-1R1/TLR4 pathway mediates disease-modification therapeutic effects in a model of acquired epilepsy. *Neurobiol Dis*. 2017;99:12–23.
39. Vezzani A, Balosso S, Ravizza T. Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. *Nat Rev Neurol*. 2019;1:459–72.
40. Rojas A, Chen D, Ganesh T, Varvel NH, Dingledine R. The COX-2/prostanoid signaling cascades in seizure disorders. *Expert Opin Ther Targets*. 2019;23:1–13.
41. Henshall DC, Hamer HM, Pasterkamp RJ, Goldstein DB, Kjemis J, Prehn JHM, et al. MicroRNAs in epilepsy: pathophysiology and clinical utility. *Lancet Neurol*. 2016;15:1368–76.

42. McClelland S, Brennan GP, Dubé C, Rajpara S, Iyer S, Richichi C, et al. The transcription factor NRSF contributes to epileptogenesis by selective repression of a subset of target genes. *Elife*. 2014;3:e01267.
43. Hall AM, Brennan GP, Nguyen TM, Singh-Taylor A, Mun HS, Sargious MJ, et al. The role of Sirt1 in epileptogenesis. *eNeuro* 2017;4(1):ENEURO.0301-16.2017.
44. Piñero J, Bravo À, Queralt-Rosinach N, Gutiérrez-Sacristán A, Deu-Pons J, Centeno E, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res*. 2017;4(45):D833–D839.
45. Zhou G, Soufan O, Ewald J, Hancock REW, Basu N, Xia J. NetworkAnalyst 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. *Nucleic Acids Res*. 2019;47:W234–W241.
46. Xu Q, Liu Y-Y, Wang X, Tan G-H, Li H-P, Hulbert SW, et al. Autism-associated CHD8 deficiency impairs axon development and migration of cortical neurons. *Mol Autism*. 2018;19(9):65.
47. Wade AA, Lim K, Catta-Preta R, Nord AS. Common CHD8 genomic targets contrast with model-specific transcriptional impacts of CHD8 haploinsufficiency. *Front Mol Neurosci*. 2019;14(11):481.
48. Barnard RA, Pomaville MB, O’Roak BJ. Mutations and modeling of the chromatin remodeler CHD8 define an emerging autism etiology. *Front Neurosci*. 2015;17(9):477.
49. Ostrowski PJ, Zachariou A, Loveday C, Beleza-Meireles A, Bertoli M, Dean J, et al. The CHD8 overgrowth syndrome: a detailed evaluation of an emerging overgrowth phenotype in 27 patients. *Am J Med Genet C Semin Med Genet*. 2019;181:557–64.
50. Beighley JS, Hudac CM, Arnett AB, Peterson JL, Gerdtz J, Wallace AS, et al. Clinical phenotypes of carriers of mutations in CHD8 or its conserved target genes. *Biol Psychiatry*. 2020;87:123–31.
51. Bernier R, Golzio C, Xiong BO, Stessman H, Coe B, Penn O, et al. Disruptive CHD8 mutations define a subtype of autism early in development. *Cell*. 2014;17(158):263–76.
52. Douzgou S, Liang HW, Metcalfe K, Somarathi S, Tischkowitz M, Mohamed W, et al. The clinical presentation caused by truncating CHD8 variants. *Clin Genet*. 2019;96:72–84.
53. Murphy DB, Wiese S, Burfeind P, Schmundt D, Mattei M-G, Schulz-Schaeffer W, et al. Human brain factor 1, a new member of the fork head gene family. *Genomics*. 1994;21:551–7.
54. Seltzer LE, Ma M, Ahmed S, Bertrand M, Dobyns WB, Wheless J, et al. Epilepsy and outcome in FOXG1-related disorders. *Epilepsia*. 2014;55:1292–300.
55. Wong LC, Singh S, Wang HP, Hsu CJ, Hu SC, Lee WT. FOXG1-related syndrome: from clinical to molecular genetics and pathogenic mechanisms. *Int J Mol Sci*. 2019;26:20.
56. Hou P-S, hAilín DÓ, Vogel T, Hanashima C. Transcription and beyond: delineating FOXG1 function in cortical development and disorders. *Front Cell Neurosci*. 2020;25(14):35.
57. Ragge NK, Brown AG, Poloschek CM, Lorenz B, Henderson RA, Clarke MP, et al. Heterozygous mutations of OTX2 cause severe ocular malformations. *Am J Hum Genet*. 2005;76(6):1008–22. Erratum. In: *Am J Hum Genet*. 2005 Aug; 77(2):334.
58. Beby F, Lamonerie T. The homeobox gene Otx2 in development and disease. *Exp Eye Res*. 2013;111:9–16.
59. Schilter KF, Schneider A, Bardakjian T, Soucy J-F, Tyler RC, Reis LM, et al. OTX2 microphthalmia syndrome: four novel mutations and delineation of a phenotype. *Clin Genet*. 2011;79:158–68.
60. Vossel KA, Tartaglia MC, Nygaard HB, Zeman AZ, Miller BL. Epileptic activity in Alzheimer’s disease: causes and clinical relevance. *Lancet Neurol*. 2017;16:311–22.
61. Kelleher RJ 3rd, Shen J. Presenilin-1 mutations and Alzheimer’s disease. *Proc Natl Acad Sci USA*. 2017;114:629–31.
62. Tran Mau-Them F, Guibaud L, Duplomb L, Keren B, Lindstrom K, Marey I, et al. De novo truncating variants in the intronless IRF2BPL are responsible for developmental epileptic encephalopathy. *Genet Med*. 2019;21:1008–14.
63. Marcogliese PC, Shashi V, Spillmann RC, Stong N, Rosenfeld JA, Koenig MK, et al. IRF2BPL is associated with neurological phenotypes. *Am J Hum Genet*. 2018;6(103):456.
64. Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, et al. DECIPHER: database of chromosomal imbalance and phenotype in humans using ensembl resources. *Am J Med Genet*. 2009;84:524–33.
65. Willemsen MH, Vissers LE, Willemsen MA, van Bon BW, Kroes T, de Ligt J, et al. Mutations in DYNC1H1 cause severe intellectual disability with neuronal migration defects. *J Med Genet*. 2012;49:179–83.
66. Beecroft SJ, McLean CA, Delatycki MB, Koshy K, Yiu E, Haliloglu G, et al. Expanding the phenotypic spectrum associated with mutations of DYNC1H1. *Neuromuscul Disord*. 2017;27:607–15.
67. Laquerriere A, Maillard C, Cavallin M, Chapon F, Marguet F, Molin A, et al. Neuropathological hallmarks of brain malformations in extreme phenotypes related to DYNC1H1 mutations. *J Neuropathol Exp Neurol*. 2017;1(76):195–205.
68. Thomas G, Aslan JE, Thomas L, Shinde P, Shinde U, Simmen T. Caught in the act – protein adaptation and the expanding roles of the PACS proteins in tissue homeostasis and disease. *J Cell Sci*. 2017;1(130):1865–76.
69. Simonato M, Tongiorgi E, Kokaia M. Angels and demons: neurotrophic factors and epilepsy. *Trends Pharmacol Sci*. 2006;27:631–8.
70. Sodr  CP, Guilherme RS, Meloni V, Brunoni D, Juliano Y, Andrade J, et al. Ring chromosome instability evaluation in six patients with autosomal rings. *Genet Mol Res*. 2010;26(9):134–43.
71. Guilherme RS, Ayres Meloni VF, Kim CA, Pellegrino R, Takeno SS, Spinner NB, et al. Mechanisms of ring chromosome formation, ring instability and clinical consequences. *BMC Med Genet*. 2011;21(12):171.
72. Bershteyn M, Hayashi Y, Desachy G, Hsiao EC, Sami S, Tsang KM, et al. Cell-autonomous correction of ring chromosomes in human induced pluripotent stem cells. *Nature*. 2014;6(507):99–103.
73. Benetatos L, Hatzimichael E, Londin E, Vartholomatos G, Loher P, Rigoutsos I, et al. The microRNAs within the DLK1-DIO3 genomic region: involvement in disease pathogenesis. *Cell Mol Life Sci*. 2013;70:795–814.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Vaisfeld A, Spartano S, Gobbi G, Vezzani A, Neri G. Chromosome 14 deletions, rings, and epilepsy genes: A riddle wrapped in a mystery inside an enigma. *Epilepsia*. 2020;00:1–16. <https://doi.org/10.1111/epi.16754>