SHORT REPORT

Early myoclonic encephalopathy caused by a disruption of the neuregulin-1 receptor ErbB4

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The tyrosine kinase receptor ErbB4 (erythroblastic leukemia viral oncogene homolog 4) plays a crucial role in numerous neurobiological processes in the developing and adult brain. Moreover, recent molecular genetics studies implicate ErbB4 in the pathophysiology of schizophrenia. However, the phenotypic consequences of haploinsufficiency of *ErbB4* are not known, as no coding mutations have been identified until now. Here, we present a patient with early myoclonic encephalopathy and profound psychomotor delay with a *de novo* reciprocal translocation t(2;6)(q34;p25.3), disrupting the *ErbB4* gene. This patient represents the first case of haploinsufficiency for one of the ErbB family members of tyrosine kinase receptors.

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Introduction

'Early myoclonic encephalopathy' (ILAE; OMIM 609304) is one of the catastrophic epilepsies starting in the neonatal period. Prognosis is generally poor with early death in half of the patients. The other half usually develops a therapy-resistant epilepsy with numerous mainly myoclonic seizures and developmental arrest.¹ In most cases, the etiology is unknown and rare familial cases have been described.²

ErbB4 is a member of the epidermal growth factor receptor (EGFR) family of tyrosine kinase receptors that regulate cell growth, proliferation and differentiation. This family comprises four members that share structural and functional similarities (EGFR, ErbB2-4), and interact with a variety of ligands and with each other.³ *ErbB4* spans 1.15 Mb on chromosome 2q34 and has four different isoforms because of alternative splicing.⁴ ErbB4 is widely

expressed in many adult and fetal tissues, with high expression in developing brain and heart.⁵ Targeted inactivation of *ErbB4* in a mouse results in midembryonic lethality because of failed myocardium development.⁶ Rescued *ErbB4* mutant mice reach adulthood but display mammary gland defects, aberrant cranial nerve connections and increased cerebellar interneurons.⁷ One of the most important and well-studied ligands of ErbB4 is neuregulin-1 (NRG1), and it was shown that NRG1–ErbB4 signaling is essential for neurobiological processes, such as neurogenesis, migration, synaptic plasticity and differentiation of neurons and glia.⁸ After the identification of *NRG1* as a candidate for schizophrenia, different schizophrenia-associated SNPs in *ErbB4* were also identified.⁹ However, in humans, the phenotypic consequences of haploinsufficiency of *ErbB4* are not known, as no coding mutations or deletions have been identified until now.

Here, we present a patient with epileptic encephalopathy and profound psychomotor delay with a *de novo* translocation t(2;6)(q34;p25.3), disrupting the *ErbB4* gene. This patient represents the first case of haploinsufficiency for one of the ErbB family members of tyrosine kinase receptors.

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Figure 1 EEG taken during sleep at the age of 18 months showing hypsarrhythmia with periods of suppression-burst pattern. Longitudinal montage calibration 70 μ V, 1 sec between lines.

Patient report

The girl is the second child of normal parents, born at term with normal birth parameters after normal pregnancy. Family history is negative. Because of poor sucking, she was hospitalized at day 11, when a unilateral multicystic kidney and tubular acidosis type 4 were diagnosed.

During hospitalization, seizures were noted for the first time and initially fenobarbital was administered. These seizures were reported as typical 'erratic myoclonic seizures'. In addition, she had two generalized tonic-clonic convulsions during fever. The first EEG performed at day 15 was reported normal. However, in the following days and weeks, the EEG's changed, showing a pattern of suppression bursts initially more accentuating during sleep, but later also during wakefulness. Despite treatment, the frequency of seizures increased dramatically during the following months, necessitating frequent hospitalizations and additive therapy consisting of valproic acid and topiramate. Her development was severely delayed with pronounced hypotonia, absent eye contact, limited spontaneous movements, no speech development and progressive microcephaly. The therapy-resistant seizures led to her being admitted, at the age of 18 months, to an epilepsy center for children. On the basis of the patient's history, clinical observation and EEG registration, she was diagnosed with symptomatic early myoclonic encephalopathy. EEG at that time revealed a hypsarrhythmic pattern during

wakefulness with periods of suppression-burst pattern during sleep (Figure 1). As expected with this diagnosis, there was almost no progress in psychomotor development and she persisted in having multiple erratic myoclonic seizures. Tonic seizures were not seen, and generalized myoclonic seizures were rare. Additional investigations, including a metabolic work-up and skin biopsy, were normal. MRI of the brain showed moderate cortical and subcortical atrophy. Chromosome analysis revealed a *de novo* reciprocal translocation involving chromosomes 2q and 6p, t(2;6)(q33.1;p23).

Re-evaluation at the age of 4 years and 10 months shows a profoundly retarded girl showing no contact with her environment and acting at a developmental level of 4 months (Figure 2). She is microcephalic, unable to sit, with pronounced axial hypotonia and peripheral hypertonia with brisk tendon reflexes. She still has frequent daily fits of the myoclonic type, therapy resistant, compatible with early myoclonic encephalopathy.

Materials and methods

The protocol was approved by the appropriate Institutional Review Board of the University Hospital of Leuven, Belgium, and informed consent was obtained from the parents of the affected patient.

Figure 2 Clinical pictures of the patient at age 18 months (left), and 4 years and 10 months (right).

Chromosome and array comparative genome hybridization (CGH) analysis

Karyotyping was performed according to routine protocol. Arrays used in this study were constructed for total genome coverage using a 1 Mb clone set. Clone preparation, hybridization and data analysis were performed as described previously.¹⁰

Fluorescence *in situ* hybridization (FISH) was used to confirm results of array CGH as well as for mapping the breakpoints.

Mutation analysis of the *ErbB4* and GDP-mannose 4,6-dehydratase (*GMD5*) genes

Primers were designed to amplify all coding exons and intron–exon boundaries of the *ErbB4* and *GMD5* genes. Sequences are available on request. Amplification, sequencing and analyses were performed according to routine protocol.

Results

Routine karyotyping revealed a t(2;6)(q33.1;p23) in the patient (Figure 3). Karyotypes of both parents were normal. Because apparently balanced translocations can be more complex, genomic DNA of the patient was analyzed on 1 Mb array. Besides a known copy number variation, no other imbalances were detected. Subsequent FISH analysis demonstrated that the breakpoint on chromosome 2 is located in clone RP11-694B12 that shows a split signal in 2q34 and maps within the first intron of *ErbB4*. Therefore, the breakpoint is located between coding exons 1 and 2, disrupting the *ErbB4* gene (Figure 3). Gene expression analysis was not performed because *ErbB4* is not sufficiently expressed in Epstein–Barr transformed lymphoblastic cell lines (EBV-LCL). The breakpoint on chromosome 6p25.3 is located in the overlapping ends of

clones RP11-323F15 and RP11-707D01, disrupting the *GMD5* gene (Figure 3). Quantitative RT-PCR of *GMD5* mRNA levels in EBV-LCL of the patient showed 50% reduction in comparison with normal controls (data not shown). The corrected karyotype based on these data is t(2;6)(q34;p25.3). The disrupted genes are orientated in opposite directions, making it unlikely that a fusion transcript is generated. Subsequent mutation analysis showed no mutations in the normal *ErbB4* and *GMD5* alleles.

Discussion

We present a patient with severe epileptic encephalopathy, diagnosed as early myoclonic encephalopathy, carrying a *de novo* reciprocal translocation disrupting the *GMD5* and *ErbB4* genes.

The *GMD5* gene plays a role in the conversion of GDP-mannose to GDP-fucose. Deficiencies in the biosynthesis of GDP-fucose lead to leukocyte adhesion deficiency type II (LADII), which is a rare autosomal recessive disorder characterized by recurrent infections, persistent leukocytosis and severe mental and growth retardation (MIM 266265). Although it was first assumed that deficient GDP-fucose biosynthesis would cause LADII, Luhn *et al*¹¹ showed that LADII is caused by a defect in a specific transporter of GDP-fucose into the Golgi. Thus far, the *GMD5* gene has not been associated to a specific disease, although one can assume that this disease would resemble LADII and be recessive in inheritance. Hence, we sequenced the other *GMD5* allele, but did not detect a mutation. Moreover, recurrent infections, immune deficiency, growth retardation and persistent leukocytosis are absent in our patient. Disruption of *GMD5*, therefore, most likely has no phenotypic effect, and the phenotype in the patient can be attributed to the disruption of *ErbB4*. Given the absence of mutation on the second *ErbB4* allele, haploinsufficiency is the proposed pathogenic mechanism. The position of the breakpoint within the gene, as well as the absence of alternative splice forms starting downstream from the breakpoint, predicts a loss of function.

HER4/*ErbB4* is a member of the type I receptor tyrosine kinase subfamily and serves as a receptor for the NRG family of growth factors. At the cellular level, *ErbB4* is expressed by somatodendrites in cerebral cortex and hippocampus from birth to adulthood.¹² The importance of this receptor during development is further demonstrated by the generation of knockout mice. These mice die during midembryogenesis because of failed development of the myocardium.⁶ Mice heterozygous for the inactivated *ErbB4* allele displayed no obvious defects in contrast to our finding in the patient.^{6,13} However, in a more recent study, Golub *et al*¹⁴ found that heterozygous nulls do display subtle neurological defects, such as delayed motor

Figure 3 Schematic representation of the translocation and the two breakpoints on 2q34 and 6p25.3: (a) Partial karyotype of the patient showing the reciprocal translocation between chromosomes 2q and 6p. (b) FISH analysis on metaphase spreads of the patient with BAC clone RP11-694B12 spanning the 2q34 breakpoint showing signals on chromosome 2, and derivatives 2 and 6. (c) Schematic representation of the 2q34 breakpoint. The breakpoint-spanning clone, RP11-694B12, maps within the first intron of the *ErbB4* gene, and therefore disrupts the *ErbB4* gene (forward slash). The orientation of *ErbB4* is indicated by the black arrow. The exons are indicated as black boxes. (d) Schematic representation of the 6p25.3 breakpoint. The breakpoint resides in the region covered by the BAC clones RP11-323F15 (signal on the derivative chromosome 2) and RP11-707D01 (signal on the derivative chromosome 6), and interrupts the *GMDS* gene between exons 4 and 8 (brackets). The orientation of *GMDS* is indicated by the arrow. The exons are indicated as black boxes. Positions of the genes and BAC clones are indicated in Mb and are based on the Ensembl view release 43, Feb 2007 (http://www.ensembl.org/Homo_sapiens/). EX exon.

development and altered cue use in a Morris maze learning and memory task. Interestingly, high ErbB4 expression was shown in precursors of interneurons migrating from the medial ganglionic eminence to the developing cortex.¹⁵ A crucial role in interneuron migration and differentiation is further corroborated by an elegant study done by Flames *et al.*¹⁶ They showed that NRG-1/ErbB4 interaction is necessary for short/long range attraction of tangentially migrating interneurons at different stages of their journey. ErbB4-expressing interneurons migrate to the cortex through a corridor where there is expression of NRG-1, which constitutes the attractant for the migration. Loss of ErbB4 and/or NRG-1 function perturbs interneuron migration and alters the number of GABA-ergic interneurons in postnatal cortex.¹⁶ These findings might explain the occurrence of severe epileptic encephalopathy in our patient because of a reduced number or function of

cortical GABA-ergic interneurons. Defects in interneuron migration and function have already been associated with epilepsy in humans, as is the case for *ARX*.^{17,18} Mutations in *ARX* are responsible for X-linked infantile spasms (ISSXs) and more recently for early infantile epileptic encephalopathy (EIEE) or Ohtahara syndrome.¹⁹ Another monogenic cause of early epileptic encephalopathy, almost exclusively restricted to female patients, are mutations in the X-linked *CDKL5* gene^{20–22} This gene was initially identified in female patients with the early seizure variant of Rett syndrome.^{23,24} In this respect, it is interesting to mention that Pescucci *et al.*²⁵ reported a girl, who was presented with severe epilepsy and Rett-like features carrying a deletion in 2q34 encompassing the *ErbB4* gene.

Although the *ARX* and *CDKL5*-associated epilepsy phenotypes can be clearly differentiated from the present case, it would be interesting to analyze *ErbB4* in a larger cohort

of patients presenting with unexplained early epileptic encephalopathy.

In contrast to the central nervous system defects, our patient does not have a cardiac defect or signs of cardiomyopathy, suggesting that the amount of residual ErbB4 protein during fetal heart development and post-natal heart function is sufficient. Alternatively, we cannot exclude an important or redundant role for other ErbB family members, especially ErbB2, during heart development in humans in contrast to mice. In addition, our patient has a unilateral multicystic kidney. Although two of the four known *ErbB4* isoforms, JM-a and CYT-2, are specifically expressed in the kidney, renal anomalies were not reported in the *ErbB4* $-/-$ mice, neither in the surviving rescued mice.^{6,7} Again, interspecies differences might account for this discrepancy, but we can not exclude other genetic or environmental factors.

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Conflict of interest

The authors declare that there are no conflicts of interest. URLs: Ensembl, <http://www.ensembl.org/>; ILAE, <http://www.ilae-epilepsy.org/>; OMIM, <http://www.ncbi.nlm.nih.gov/omim/>.

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