Unexpected discovery of syndromic epilepsy during the genetic exploration of a case of leukemia.

Audrey Grain[1], adeline normand[1], caroline Thomas[1], camille debord[1], olivier pichon[1], Marie Bene[1], Marion Eveillard[1], and olivier theisen[1]

[1]Centre Hospitalier Universitaire de Nantes

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Abstract

A three-year-old girl with a history of epilepsy seizures was diagnosed with acute lymphoblastic leukemia. A comprehensive genetic study of blast cells led to the discovery of a constitutional deletion of the PCDH19 gene. This description underlines how modern techniques of molecular investigations in hematological disorders may lead to unexpected findings.

Introduction

Currently, the diagnosis of hematological malignancies is increasingly considered an integrated process collecting information from several different methodologies. The four major ones are morphology (either cytology or pathology), flow cytometry, cytogenetics and molecular analyses. Depending on the extent of the latter, it is increasingly likely to disclose constitutive anomalies with potential pathogenic impact. This may lead to changes in patient management as reported in this case.

Methods and Results

A three-year-old girl with a history of epileptic seizures was admitted in the pediatric emergency unit for deterioration of her general condition with lower limbs pain and epistaxis. Clinical examination revealed multiple bruises and splenomegaly. Blood tests showed a hemoglobin concentration at 40 g/L, white blood cell count at 3.11 x 10^9/L, polymorphonuclears at 0.33 x 10^9/L and platelet count at 19 x 10^9/L. Examination of a blood smear disclosed 7% of circulating blasts. The bone marrow was infiltrated by 97% homogeneous blast cells (figure 1). Immunophenotyping identified the blastic population as CD19+, CD22+ CD10+ and cytoplasmic CD79a+. There was no expression of immunoglobulins nor intracytoplasmic mu chains. All myeloid and T-lineage markers tested were negative. A diagnosis of B-II[1] acute lymphoblastic leukemia (ALL) was concluded.

Cytogenetic analysis was performed on bone marrow cells after R banding. The karyotype was abnormal and revealed a hyperdiploid clone at 56 chromosomes, with classical gains i.e. trisomy of chromosomes 4, 6, 8, 10, 14, 17, 18 and X associated to tetrasomy 21. In order to explore for other prognostic features, single nucleotide polymorphism (SNP)-array was performed (Cytoscan HD, Affymetrix®, figure 2) on DNA extracted from the bone marrow blasts (Quiagen, Hilden, Germany). This confirmed the chromosome gains already detected but also revealed a 2.6MB deletion in the long arm of chromosome X. Only 1 coding gene, *PCDH19* , is present in the deleted region. In this case, only 1 copy of this region was present since trisomy X comprised 1 normal and 2 altered chromosomes X. To confirm the hypothesis that this deletion could be constitutional, FISH analysis was performed after PHA-stimulated lymphocyte culture on a blood sample obtained after the child had reached complete remission. A homemade FISH probe, flanking the*PCDH19* gene (figure 3) was used to achieve this exploration. It confirmed a deletion of *PCDH19* in all cells and thus its constitutional origin.

While the ALL was taken care of, leading to ongoing complete remission, the clinical history of the patient was reviewed. Her parents are in good health and are not related. She is the youngest of a family of six children. The pregnancy went on without any incident. The child's development was normal and she displays no dysmorphic feature. Her neurological examination was unremarkable. However, she had presented multiple tonic-clonic seizures since the age of sixteen months, most of the time during her sleep, and often associated with fever. Seizures were short-lasting, less than a few minutes, but happened in clusters. Magnetic resonance imaging (MRI) had been performed when she was eighteen months and disclosed no anomaly. The little girl was treated by 300 mg/d of levetiracetam with mild improvement. Since the beginning of her seizures she had been presenting a delay in intellectual growth especially in language acquisition and was receiving speech therapy. Her mother reported frequent bouts of anger. In spite of the fact that only this child was affected in the family, a constitutive disorder had been evoked, but the parents had refused genetic investigations.

Discussion

Protocadherin19 has been shown to be implicated as a major actor of brain development. Alterations of the *PDCH19* gene are known to be linked to the EFMR syndrome (Epilepsy, Female restricted, with Mental Retardation) described for the first time in 1971 by Juberg and Hellman[2] and fully characterized in 2008 by Dibbens et al.[3]. The overall incidence of the disease is estimated at one per million. Variants (mutations) of *PCDH19* have mostly been described as associated with the EFMR syndrome, but deletions have also been reported[4]. *PCDH19*alterations play a major role in epilepsy. It is the second common gene, after *SCN1A,* implicated in childhood epilepsy[5,6]. About 70 % of *PDCH19* alterations occur *de novo* , but the variant can be transmitted from an asymptomatic father (18%) or asymptomatic mother (7%)[5].

Observations reported by the parents strongly suggested a diagnosis of EFMR in the propositus. Indeed, her constitutional *PCDH19*deletion could explain her cognitive/neurological phenotype, since the EFMR syndrome is characterized by seizures beginning in early childhood, between 3 months and 3 years of age. All types of seizures, i.e. generalized tonic-clonic seizures, focal seizures or atypical absence seizures may happen and coexist in a patient[7,8]. These neurological manifestations frequently occur in a context of fever as in this child. When seizures begin, a variable degree of mental retardation is common, especially a delay in language acquisition[7,9]. Seizures tend to regress over time, but the EFMR syndrome is frequently associated with behavioral disorders, hyperactivity, aggressiveness and the presence of autistic spectrum manifestations[10,11].

The clinical expression of *PCDH19* alterations presents an unusual pattern. Indeed, although *PCDH19* is located on chromosome X (Xq22.1), affected males are asymptomatic while female heterozygotes present an EMFR syndrome. A few cases of male subjects presenting mosaicism of a mutated *PCDH19* have been reported, these patients displaying an EFMR-like symptomatology[12-14.] The mechanisms of this presentation are not yet totally elucidated.*PCDH19* encodes protocadherin 19, a cell adhesion protein involved in many intracellular pathways of neurogenesis, neuronal migration, neuronal tissue architecture and neuronal cells maturation[15]. One of the hypotheses to explain this peculiar X-linked inheritance is the coexistence, in females and because of the lyonisation phenomenon, of wild and mutated (or absent in case of deletions) PCDH19 proteins. Depending on the activated X chromosome in a given cell, the protein will be functional or not, leading to abnormal synaptic connections. The presence of two populations of neurons could thus be responsible for the symptomatology of the EMFR syndrome[16]. Recently, a deficit in allopregnanolone was discovered in PCDH19 related-epileptic patients, providing some therapeutic issues.[17] Indeed a phase III trial evaluating the efficacy and safety of ganaxolone (an analog of allopregnanolone) in female children with PCDH19-related epilepsy (NCT03865732) is ongoing.

This chance diagnosis of an EFMR syndrome disclosed upon genetic examination of ALL blast cells is an original presentation. It points out how progress in the exploration of molecular anomalies in such diseases as malignant hematological disorders may lead to the disclosure of genetic features associated to other diseases or predispositions. This possibly raises ethical questions. Of note, in this case, the family had initially declined genetic explorations, which led to underdiagnose the child's disorder. Yet, the child will now be able to benefit from therapeutic advances in this specific epilepsy subgroup.

Conflict of Interest: Authors have indicated they have no potential conflicts of interest to disclose.

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Figure legends.

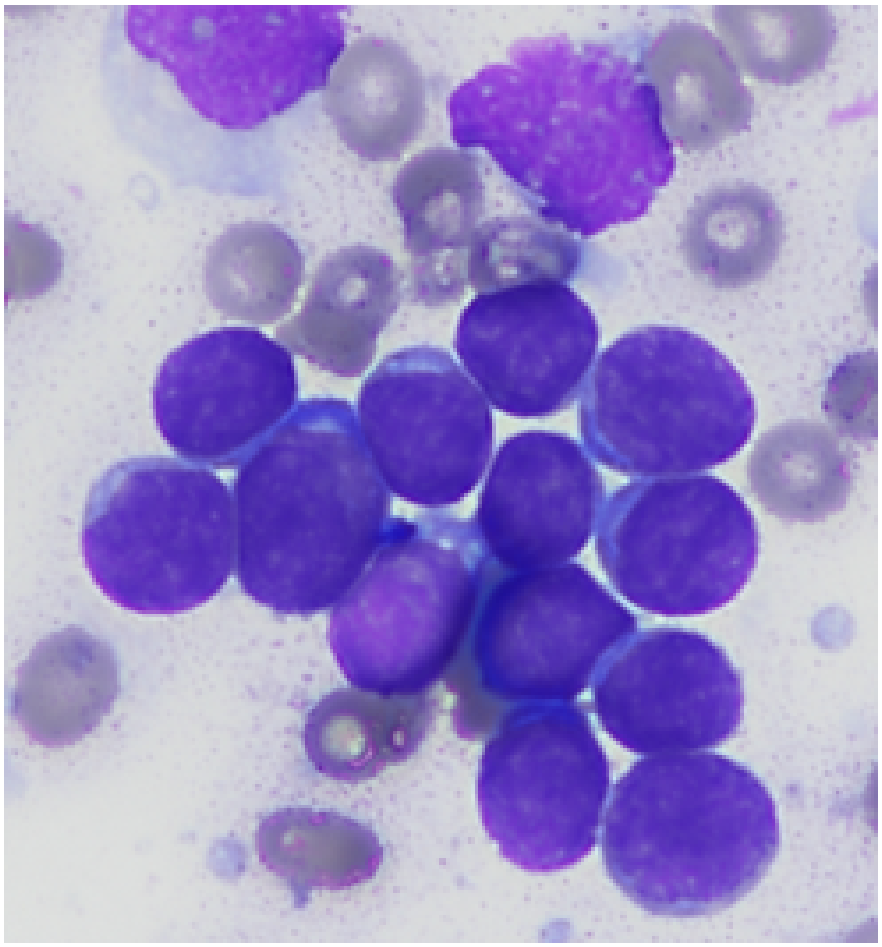

Figure 1. Cytology of bonne marrow aspiration showing the abundant infiltration by leukemic blasts.

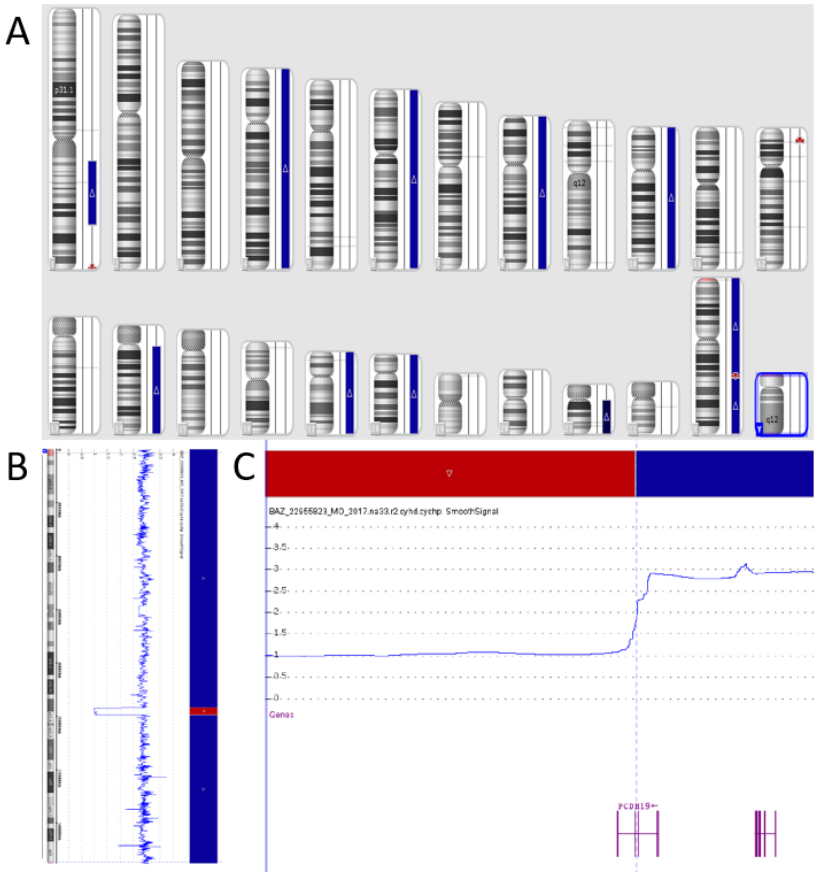

Figure 2. SNP-array profiles**.** A: nine chromosomes show hyperdilploidy (blue lines); B: focus on chromosome X confirms trisomy X and reveals a small deletion in the long arm of chromosome X; C: zoom on the distal breakpoint of the deleted region encompassing the*PCDH19* gene.

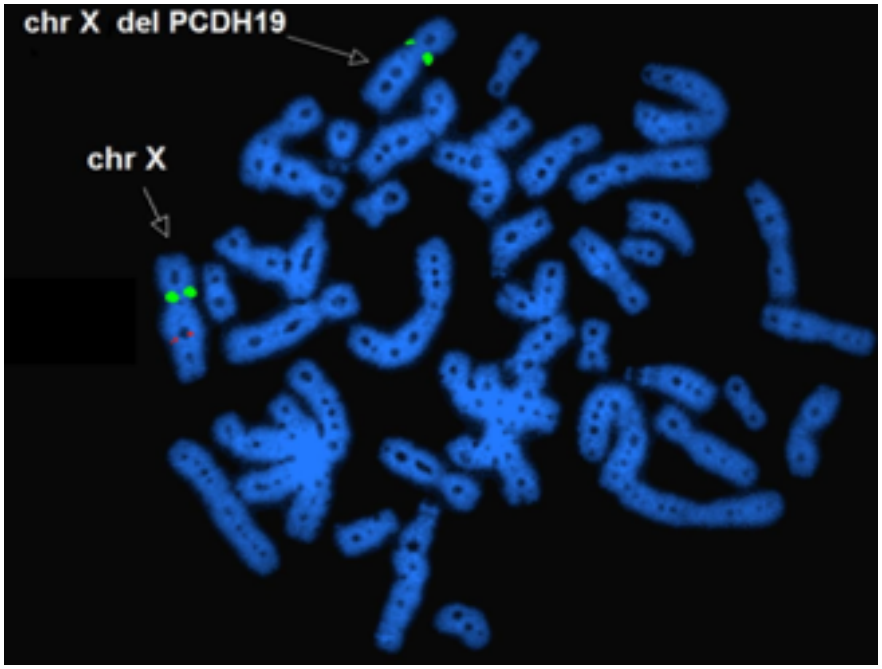

Figure 3. Constitutional FISH analysis confirmed *PCDH19*deletion. Red: deleted region Green : control probe [chromosome X centromere].