

Chromosome Microarray and Undiagnosed Seizures in a Pediatric Patient

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Chromosome microarrays have revolutionized conventional cytogenetics due to their greatly increased resolution, resulting in a greater number of chromosome abnormalities detected. Chromosome microarray analysis is the recommended first tier diagnostic test for children with mental handicap, congenital anomalies/dysmorphisms and autism¹ and results in a much higher diagnostic yield (15%-20%) than a conventional G-band karyotype (approximately 3%, excluding Down syndrome and other recognizable chromosomal syndromes)¹.

Numerous conventional chromosome disorders, such as the Wolf-Hirschhorn (4p-), Angelman (15q-), Miller-Dieker (17p-), Klinefelter (XXY), and Down (+21) syndromes, have been reported in association with various neurological disorders, including seizures and epilepsy². However, many children with a neurological disorder, such as severe epilepsies, have a normal G-band karyotype with no diagnosis, although a genetic etiology is often suspected³. Chromosome microarray has recently been reported to detect clinically significant chromosome abnormalities in approximately 9% of patients with a broad range of neurologic phenotypes of unknown etiology, including severe epilepsy³. For example, a variety of seizures types were reported in association with the newly identified 15q13.3 microdeletion syndrome, detectable only by microarray².

A conventional G-band karyotype will not reliably detect deletions or duplications smaller than approximately 5 Mb (megabases). Chromosome microarray allows for the detection of unbalanced DNA copy number variations or changes, i.e. deletions and duplications, at resolutions much less than 1 Mb, with the lower limits of resolution in the hundreds of Kb (kilobases) at gene rich regions. DNA segments from across all chromosomes are used as substitutes for metaphase chromosomes, analogous to a molecular karyotype¹. In addition, approximately 40% of patients with a clinical phenotype and an apparently balanced translocation by conventional cytogenetics have been shown, by chromosome microarray, to carry a cryptic imbalance that would be consistent with their clinical phenotype⁴. In prenatal diagnosis, therefore, chromosome microarray diagnosis is recommended for fetuses with a *de novo* chromosome rearrangement⁵.

We report an apparently balanced, *de novo* translocation detected prenatally by conventional karyotype in a patient with epilepsy and neurodevelopmental dysfunction of unknown etiology. A postnatal chromosome microarray revealed a cryptic microdeletion at one of the translocation breakpoints. The results of a prenatal microarray would have suggested consideration of

the diagnosis and altered management. We report this as an example of the usefulness of early microarray analysis in children with epilepsy of unknown etiology.

CLINICAL HISTORY

An eight month-old boy presented with recurrent seizures with his first event of a tonic-clonic, afebrile seizure occurring at five months-of-age. He was the first born to a non-consanguineous healthy 35-year-old mother and 41-year-old father. The patient has a brother and paternal half sister, both of whom are clinically normal. Prenatal and perinatal histories were unremarkable. The description of subsequent seizures varied and included episodes of arching, staring, twitching, eye rolling, unresponsiveness, cyanosis and collapse in various combinations. Periods of apparent regression (increased difficulty with gait and speech) were followed by recovery of these skills. Several episodes of sleepiness unassociated with hypoglycemia but seemingly improving with a "sweet drink" were described shortly before the diagnosis was established. Seizures were not controlled despite multiple anti-epileptic medications. Physical examination revealed microcephaly with an OFC (Occipital Frontal Circumference) of less than the 5th centile; and weight and height, both at approximately the 25th centile. Neuroimaging, including MRS (magnetic resonance spectroscopy), was non-diagnostic.

BIOCHEMICAL AND CYTOGENETIC INVESTIGATIONS

An extensive biochemical work-up on plasma, urine, cerebrospinal fluid and whole blood was performed. Prenatal and postnatal cytogenetic analysis was performed according to standard protocols, at a band resolution of 450 to 550 bands per haploid genome, respectively (Figure 1). Chromosome

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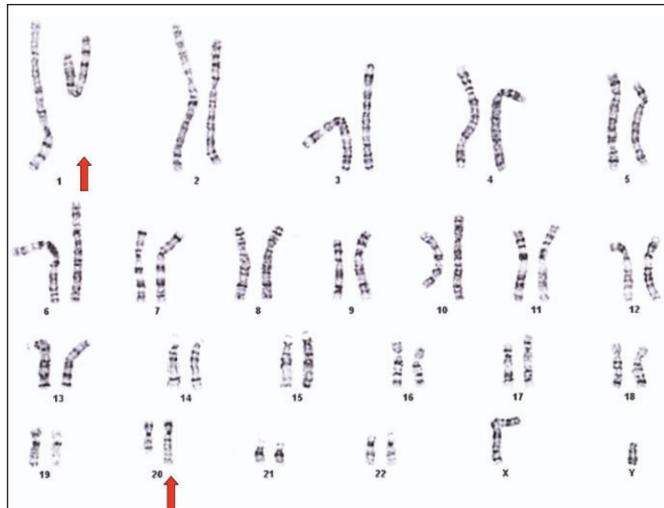


Figure 1: G-banded karyogram of the patient showing the *t(1;20)(p34.1;q13.1)dn* (red arrows).

microarray analysis was performed at CombiMatrix Diagnostics (CMDx, Irvine, CA) using the oligonucleotide 180K microarray (NCBI build 36, March 2006) (Figure 2). All results were confirmed by fluorescent in situ hybridization (FISH) analysis.

RESULTS

An amniocentesis had been performed because of advanced maternal age (AMA). A *de novo*, apparently balanced

Table: Oligonucleotide chromosomal microarray analysis

Loss of 1p34.2p34.1 (42,871,391-44,074,069)

[Genes (from centromere to telomere: CCDC30 (partial), PPIH, YBX1, CLDN19, LEPRE1, C1orf50, LOC100129924, CCDC23, ERMAP, ZNF691, SLC2A1, SLC2A1-AS1, FAM183A, EBNA1BP2, WDR65, TMEM125, C1orf210, TIE1, MPL, CDC20, ELOVL1, MED8, SZT2, HYI, PTPRF, KDM4A, KDM4A-AS1, ST3GAL3 (partial)]

The translocation breakpoint on the der(20)t(1;20) could not be determined by this assay as it did not involve a copy number change.

translocation was found: 46,XY,t(1;20)(p34.1;q13.1)dn. The karyotype was repeated at the child’s first clinic presentation, with the same conclusion of a balanced, reciprocal translocation (Figure 1). Metabolic work-up revealed a cerebrospinal fluid (CSF) to blood glucose ratio of 0.378 (normal ratio: 0.65+/-0.01), suggesting a biochemical diagnosis of glucose transporter type 1 (GLUT1) deficiency syndrome (De Vivo disease). The results of the chromosome microarray indicated a 1.2 Mb microdeletion of the 1p34.2-p34.1 region of the der(1)t(1;20) chromosome, including the SLC2A1 gene (Table): arr 1p34.2p34.1(42,871,391-44,074,069)x1. This cryptic microdeletion is below the level of classic cytogenetic resolution (approximately 5 Mb). The microdeletion was confirmed by

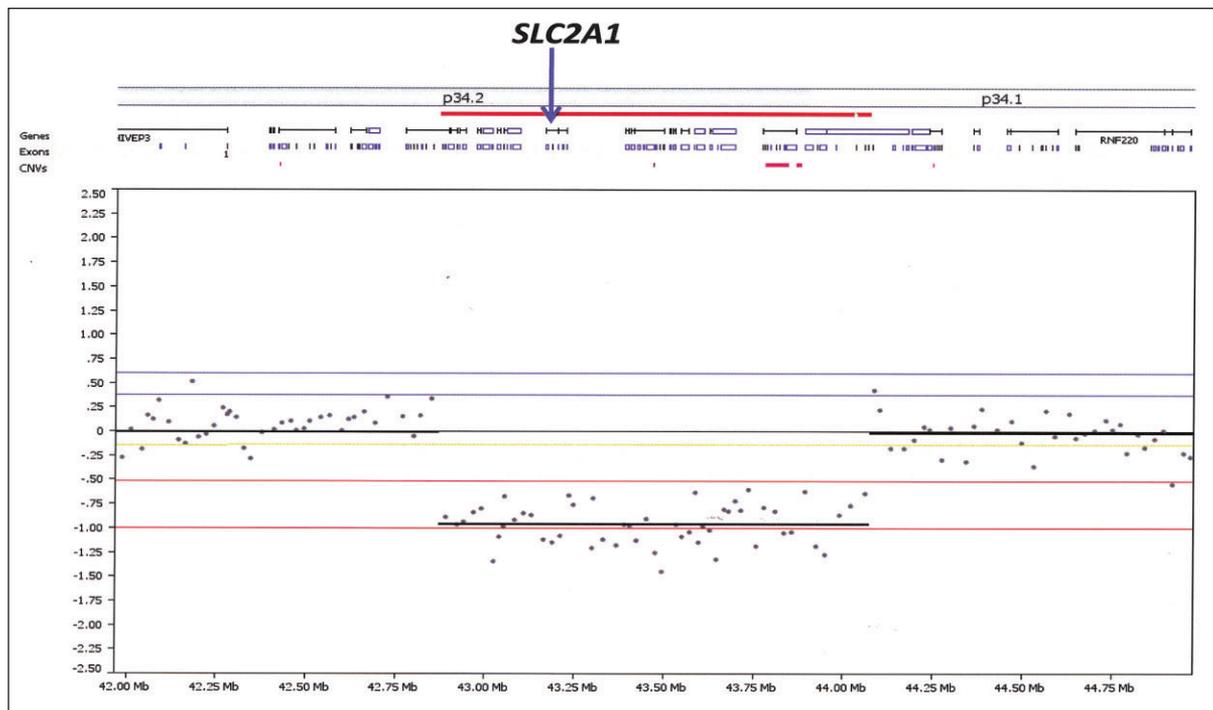


Figure 2: Oligonucleotide 180K microarray analysis showing a 1.2 Mb microdeletion of the 1p34.2-p34.1 region (red line) of the *der(1)t(1;20)*, including the *SLC2A1* gene (blue arrow).

FISH analysis with probes corresponding to loci mapped to 1p34.2 (data not shown). Parental karyotype and microarray analyses revealed normal results (data not shown).

DISCUSSION

The patient's 550 G-band prenatal and postnatal karyogram appeared to have a balanced, reciprocal translocation: 46,XY,t(1;20)(p34.1;q13.1)dn. Microarray analysis was not performed prenatally at the time as it was not available for prenatal samples at that time. The postnatal chromosome microarray revealed a cryptic, 1.2 Mb microdeletion of 1p34.2->p34.1, which was confirmed by FISH analysis. Parental karyotypes and microarray analysis were normal, confirming that the microdeletion was *de novo*. The deletion is the result of an unbalanced translocation between 1p and 20q; and includes OMIM genes *SLC2A1*, *CLDN19*, *LEPRE1*, and *MPL* (Table). Heterozygous mutations and deletions of the *SLC2A1* gene are responsible for GLUT1 deficiency, an autosomal dominant disorder characterized by infantile seizures refractory to anticonvulsants, decelerated head growth, mental and motor developmental delay, with variable degrees of cognitive impairment. The three other OMIM genes (*CLDN19*, *LEPRE1*, *MPL*) are associated with autosomal recessive disorders. The translocation breakpoint on 20q could not be determined by this microarray since it did not involve any copy number changes.

The patient has been on a ketogenic diet for the past two years. He has generally been well and has been making considerable developmental progress. His seizures remain under good control and his head growth is well maintained. This case clearly demonstrates the value of performing chromosome microarray analysis in the investigation of children with developmental delay and epilepsy by providing increased resolution to detect cytogenetic rearrangements, which lead to correlation of clinical manifestations with specific changes in gene copy number¹. Specifically, in our patient, chromosome microarray analysis detected a condition that was treatable. Furthermore, a prenatal microarray to confirm the apparently balanced, *de novo* t(1;20) detected by conventional karyotype would have led to a specific diagnosis of GLUT1 deficiency and altered management from birth.

CONCLUSIONS

Approximately 40% of reciprocal, cytogenetically balanced *de novo* translocations associated with abnormal phenotypes have a microdeletion at either one of the breakpoints or elsewhere and should be followed up by chromosome microarray⁴. The prenatal detection of apparently balanced, *de novo* chromosome rearrangements by conventional cytogenetic techniques should necessarily be followed up with microarray analysis to evaluate the possibility of an imbalance⁵. Chromosomal microarray is now a recommended first tier clinical test for the evaluation of individuals with developmental delay or congenital anomalies¹. Notably, in our patient, chromosomal microarray analysis identified not only the etiology of the disease, but also a specific treatment, which resulted in an improved clinical outcome for our patient. We therefore support the recommendation that chromosome microarray should also be a first tier diagnostic test in patients with severe epilepsy, neurologically abnormal neonates and patients with neurological disorders of unknown etiology³.

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