CASE REPORT

Cri-du-chat Syndrome: Application of Array CGH in Diagnostic Evaluation

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ABSTRAK

Genom manusia mengandungi banyak variasi genetik submikroskopik dari segi salinan nombor; ini termasuk delesi, duplikasi dan insersi. Walaupun analisis kromosom melalui teknik kariotip masih digunakan sebagai ujian utama dalam penyiasatan pesakit dismorfisma yang juga mengalami masalah terencat akal, teknologi molekular seperti hibridasi genomik perbandingan barisan (*array comparative genomic hybridisation*, aCGH) terbukti sensitif dan boleh dipercayai dalam mengenalpasti variasi genetik submikroskopik ini. Seorang bayi berumur tiga bulan dirujuk kerana masalah dismorfisma, mikrokefali dan rencatan perkembangan global. Ujian kariotip pertama adalah normal. Walaubagaimanapun, keputusan ini adalah berdasarkan kualiti metafasa yang kurang memuaskan. Ujian aCGH telah mengenalpasti delesi bersaiz 30.6Mb pada kromosom 5p15.33-p13.3. Kes ini menunjukkan keberkesanan aCGH sebagai ujian makmal bagi mengenalpasti masalah ketidakseimbangan kromosom.

Kata kunci: array comparative genomic hybridization, aCGH; kerencatan akal; sindrom cri-du-chat

ABSTRACT

The human genome contains many submicroscopic copy number variations which includes deletions, duplications and insertions. Although conventional karyotyping remains an important diagnostic tool in evaluating a dysmorphic patient with mental retardation, molecular diagnostic technology such as array comparative genomic hybridization (aCGH) has proven to be sensitive and reliable in detecting these submicroscopic anomalies. A 3 month-old infant with dysmorphic facies, microcephaly and global developmental delay was referred for genetic evaluation. Preliminary karyotyping which was confounded by the quality of metaphase spread was normal; however, aCGH detected a 30.6Mb deletion from 5p15.33-p13.3. This case illustrates the usefulness of aCGH as an adjunctive investigative tool for detecting chromosomal imbalances.

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Key words: array comparative genomic hybridization, aCGH; mental retardation; cri-du-chat syndrome

INTRODUCTION

Dysmorphism associated with developmental delay or mental retardation is a common indication for chromosomal analysis. Although the underlying aetiology may only be recognized in ~40 to 60% of cases, there is a need to obtain an accurate diagnosis as this will affect overall management in terms of counseling for recurrence and prognosis (Manning et al. 2007). Recent studies using array comparative genomic hybridization (aCGH) have shown that deletions and duplications are causative in 8 to 17% of such cases (Manning et al. 2007). We report an infant with craniofacial dysmorphism and global developmental delay who was diagnosed to have a terminal chromosomal deletion following aCGH.

CASE REPORT

A 3 month-old infant was referred for facial dysmorphism. The antenatal period was uneventful and she was born term via normal delivery. She was small for gestational age with birth weight 2.17kg. Her parents were non-consanguinous. She was admitted for neonatal jaundice at day 6 of life; newborn screening for hearing via otoacoustic emission (OAE) was abnormal. Follow-up with brainstem evoked response (BSER) revealed bilateral sensorineural hearing loss. Clinically she was microcephalic and had failure to thrive. She had fair skin pigmentation. The ears were simple; there was hypertelorism and she had a high nasal bridge. Her nose was short; the lips were thin and the corners of her mouth were downturned (Figure 1). Her fingers were clenched and she had bilateral clinodactyly. There was also feeding difficulty i.e. she had swallowing incoordination and there was global developmental delay. MRI brain showed microcephaly with evidence of white matter volume loss and cerebral atrophy suggestive of perinatal ischaemic insult. Screening for inborn errors of metabolism was negative and the preliminary conventional karyotyping (of 4 metaphase spreads) was reported as normal. In view of the craniofacial dysmorphism and global developmental delay, blood was sent for aCGH. The investigation revealed a 30.6Mb deletion at 5p15.33-13.3 (Figure 2) which was confirmed by fluorescence-in-situ hybridization (FISH) using the Vysis LSI EGR1/D5S23, D5S721 Dual Color Probe as shown in Figure 3.

DISCUSSION

Chromosomal analysis is often requested as a first-line investigation in a child with mental retardation, multiple congenital malformation and dysmorphism. Based on data from published research, mental retardation affects approximately 3% of the general population; of these, less than 50% have an identifiable cause of which 40% is attributable to chromosomal anomaly (Manning et al. 2007). In a review by Sharkey et al. 2005, 0.8% of liveborn infants have chromosomal anomalies of whom half are associated with an abnormal phenotype (Sharkey et al. 2005). With conventional moderate level karyotyping (at 400 to 500 banding), the detection rate of chromosomal anomalies

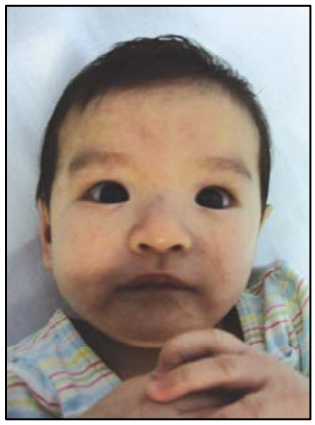

Figure 1: Frontal view of the patient

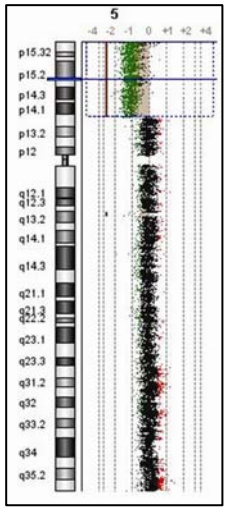

Figure 2: Array CGH revealed a 30.6Mb deletion at 5p15.33-13.3

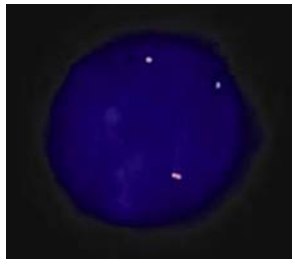

Figure 3: Interphase FISH analysis using the Vysis LSI EGR1/D5S23, D5S721 Dual Color Probe shows one (1) green and two (2) orange signal pattern, indicating the deletion of one 5p15.2 region. The 5p15.2 region (D5S23, D5S721) is labelled with SpectrumGreen-dUTP while the EGR1 region is labelled with SpectrumOrange-dUTP.

is 13.3%; 3.8% was for structural and 7.8% for numerical anomalies (Sharkey et al. 2005).

In general, both conventional and spectral karyotyping (SKY) is unreliable in detecting copy number changes of less than 5Mb (Bar-Shira et al. 2006). Array CGH is a high resolution, whole genome based technology that has a better diagnostic detection rate of these submicroscopic chromosomal aberrations underlying patients with multiple anomaly syndromes; a resolution of ≤1Mb and up to 35kb has been reported (Manning et al. 2007 & Bar-Shira et al. 2006). With aCGH, metaphase spreads are replaced by cloned (bacterial artificial chromosomes, BACs) or synthesized (oligonucleotides) DNA fragments across the genome that are immobilized on a glass surface, the exact chromosomal locus of which is known (Manning et al. 2007). Both sample and control DNA are then hybridized onto the DNA fragments spotted onto the array; copy number variations are then measured via computer analysis based on the diferrences in hybridization pattern intensities.

With aCGH, the detection rate of unbalanced chromosomal rearrangements has been shown to be between 10% to 24% (Bar-Shira et al. 2006). At present, there are two types of array platform: constitutional or targeted array, and whole genome array (Manning et al. 2007 & Baldwin et al. 2008). The targeted approach incorporates common microdeletion or duplication syndromes, telomeric regions and centromeres and selected Mendelian disorders (Baldwin et al. 2008). The development of such a custom made array has been likened to performing a '6000 band karyotype'. Whereas, a whole genome array has a wider coverage over the human genome. When first introduced, the whole genome array contained ~2400 BAC clones distributed over the genome; the newer generation of arrays are now even more

dense. The resolution of aCGH is limited by the size and distance between the DNA fragments that are spotted onto the array (Manning et al. 2007). In a series by Baldwin et al. 2008, the genome-wide array coverage identified an additional 4.7% of clinically significant abnormalities that would not be detected by current targeted array platform. The average size of imbalances was ~3.7Mb and contained an average of 17 known genes (Baldwin et al. 2008). In this study, array-based CGH analysis was performed using commercially available oligonucleotide microarrays containing about 244, 000 60-mer probes with an average resolution of about 8.9 kb (Human Genome CGH Microarray 244A kit, Agilent Technologies). The deletion was subsequently revealed by the use of array-based CGH, a molecular cytogenetic technique with an extremely high resolution. All these data confirm the importance of performing a more sophisticated investigation in patients in whom a complex phenotype is strongly suggestive of the presence of a chromosomal aberration; indeed, aCGH is a powerful technique for identifying chromosomal rearrangements where conventional cytogenetics has failed.

Reports of aCGH in identifying cytogenetic abnormalities missed by routine cytogenetic analyses have been reported in the literature and further support the sensitivity of aCGH (Shafer et al. 2006). In our patient, initial karyotyping was reported as normal; interpretation was however made based on a limited number of metaphase spreads, as correctly cited by the laboratory. The issue of obtaining an adequate number of metaphase spreads occurs every now and then; this may be the result of insufficient volume of blood extracted which is not uncommon amongst paediatric patients particularly. Application of other molecular technique such as Multiplex Ligation Probe-dependant Amplification (MLPA) method can improve resolution for detection of changes in DNA copy number but it is typically applied in a targeted manner that assesses one or several candidate loci at a time which limit the detection rate in syndromic cases. Thus, aCGH was subsequently requested following the 'normal' karyotype as the patient was seen to be very 'chromosomal', a statement often concluded by clinical geneticists. The latter investigation indeed eluded the underlying chromosomal anomaly which was subsequently confirmed by FISH analysis. FISH analysis is the recommended method for validating aCGH studies; furthermore, FISH is not only cost-effective but also has the advantage of demonstrating the mechanism of the imbalance (Baldwin et al. 2008).

Cri-du-chat syndrome is a relatively rare syndrome and affects between 1 in 40 000 to 50 000 live births (Cornish et al. 1999). The basic defect is due to a partial deletion, either terminal or interstitial, of 5p15.2-p15.3. Although the name of the syndrome is derived from the high-pitched, shrill cry heard in a larger series of patients, the cry is neither pathognomonic nor present in all patients. This anomaly is largely de novo (~85%) but may be the result of an unbalanced translocation inherited from either parent (Gorlin et al. 2001). In our patient, a 30.6Mb deletion was detected at 5p15.33-p13.3; of the 54 genes that spanned this region, 10 have been reported in association with clinical disorders. However, two of the latter have been identified to be the most significant; this was the CTNDD2 gene and SDHA gene which are associated with Cri-du-chat syndrome and Leigh syndrome. Phenotypically, our patient showed features consistent with the former syndrome. Parental karyotyping and aCGH were normal, thus the deletion was de novo. Prenatal diagnosis with FISH using uncultured amniocytes have been successfully reported in cases where the chromosomal anomaly is the result of a parent

being a balanced translocation carrier (Gorlin et al. 2001 & McKusick-Nathans Institute of Genetic Medicine 2010).

As anticipated, the larger the deletion, the more severe the phenotype. In addition to both somatic and mental retardation, patients with 5p deletion exhibit the following clinical features: microcephaly, increased inner canthal distance (75%), down-slanting palpebral fissures (60%), epicanthic folds, broad nasal bridge with prominent nasal root and a round face. Marked hypotonia and feeding difficulties are common as seen in our patient. Other major malformations include: congenital heart defects (30-50%), musculoskeletal and central nervous system anomalies [eg hypoplastic cerebellum, corpus callosum dysgenesis and arachnoid cyst] (Gorlin et al. 2001 & McKusick-Nathans Institute of Genetic Medicine 2010). The MRI brain of our patient showed no specific structural anomaly; she is presently on a multidisciplinary follow-up which involves regular monitoring by the developmental Paediatrician and Clinical Geneticist, rehabilitation by occupational therapy, physiotherapy and speech therapy, in addition to serial evaluation by audiology and ophthalmology.

Although aCGH is increasingly used as a diagnostic tool in patients with global developmental delay, one of the main drawbacks is its potential for identifying novel copy number variations that may not be responsible for the patient's global delay. Even if a variant is present in the affected individual but absent from "normal" parental genomes, it does not necessarily follow that it is a pathogenetic change, and it may instead represent an innocuous copy-number polymorphism (a normal variation in the human genome). Interpretation of aCGH is often a challenging process; to facilitate this process, several international databases have been established; these include DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans Using Ensemble Resources, http://www.sanger.ac.uk/PostGenomics/decipher/) and ECARUCA, the European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (http://www.ecaruca.net/). In a meta-analysis by Subramonia-Iyer et al. (2007) involving seven studies amongst patients with learning disabilities, an acceptable false-positive rate (for non-causal abnormalities) of between 5 to 10% was reported (Subramonia-Iyer et al. 2007).

CONCLUSION

Array CGH is a sensitive tool in detecting submicroscopic chromosomal aberrations that are undetectable by current cytogenetic tests. However, before being accepted as a routine diagnostic evaluation, certain issues pertaining to both clinical and laboratory aspects should be considered. These include the selection of patient phenotype, optimal array resolution and appropriateness of array platform, establishment of quality assurance in a clinical set-up, availability of genetic counseling and of course, cost-effectiveness as compared to existing genetic tests.

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