

Array CGH Analysis and Developmental Delay: A Diagnostic Tool for Neurologists

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ABSTRACT: Developmental delay occurs in 1-3% of the population, with unknown etiology in approximately 50% of cases. Initial genetic work up for developmental delay previously included chromosome analysis and subtelomeric FISH (fluorescent *in situ* hybridization). Array Comparative Genomic Hybridization (aCGH) has emerged as a tool to detect genetic copy number changes and uniparental disomy and is the most sensitive test in providing etiological diagnosis in developmental delay. aCGH allows for the provision of prognosis and recurrence risks, improves access to resources, helps limit further investigations and may alter medical management in many cases. aCGH has led to the delineation of novel genetic syndromes associated with developmental delay. An illustrative case of a 31-year-old man with long standing global developmental delay and recently diagnosed 4q21 deletion syndrome with a deletion of 20.8 Mb genomic interval is provided. aCGH is now recommended as a first line test in children and adults with undiagnosed developmental delay and congenital anomalies.

RÉSUMÉ: Puce d'hybridation génomique comparative et retard de développement : un outil diagnostique pour les neurologues. Le retard de développement survient chez 1 à 3% de la population et son étiologie est inconnue chez à peu près 50% des cas. L'évaluation génétique initiale pour un retard de développement incluait antérieurement une analyse chromosomique et une analyse par FISH (hybridation *in situ* en fluorescence) de régions subtélomériques. La puce d'hybridation génomique comparative (CGHa) est devenue un outil de détection des changements du nombre de copies géniques ainsi que de la disomie uniparentale et elle est le test le plus sensible pour fournir un diagnostic étiologique dans le retard de développement. Le CGHa permet d'offrir un pronostic et un risque de récurrence, améliore l'accès aux ressources, aide à limiter les évaluations et peut modifier le traitement médical dans bien des cas. Le CGHa a mené à la définition de nouveaux syndromes génétiques associés à un retard de développement. À titre d'exemple, nous décrivons le cas d'un homme âgé de 31 ans qui présentait un retard de développement global depuis longtemps et chez qui un syndrome associé à une délétion 4q21 a été diagnostiqué récemment, soit une délétion de 20,8 Mb. Le CGHa est maintenant recommandé comme test de première ligne chez les enfants et les adultes présentant un retard de développement et des anomalies congénitales.

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Developmental Delay and Etiological Diagnosis

Global Developmental Delay (GDD) is a condition in which etiology is known only in 40 to 60% of cases¹. The families face considerable challenges in managing adult patients with cognitive delay who have no underlying diagnosis. Etiological diagnosis helps with providing recurrence risk and prognostic information, and avoids further unnecessary invasive investigations. Knowing the underlying basis also helps in providing anticipatory guidelines for proper management, and allows for the family to access community supports². Clinical dysmorphology skills of the physician as well as laboratory and cytogenetic testing have been the mainstay of diagnosis for many decades. Previous recommendations stated that testing should consist of at least a G-banding karyotype and fragile X testing in both genders¹. Metabolic testing and neurologic imaging are also now included if suggestive findings were present on history and examination. In undiagnosed GDD, the etiological yield of a dysmorphologist and neurologist exams have been noted to be 39-81% and 42%, respectively³. Previous studies on the detection rate of routine cytogenetic testing in the undiagnosed found chromosomal abnormalities in 2.93-11.6%⁴⁻⁹ with an overall yield of 3.7% in individuals with undiagnosed GDD¹⁰.

Traditional Cytogenetic Investigations

The classic cytogenetic test is by G-banding analysis¹¹. It has been especially useful in detection of aneuploidy (abnormalities in chromosome number) and large structural chromosomal abnormalities such as deletions, duplications, and unbalanced chromosomal rearrangements. With improved technology, it is sensitive for changes between 3 and 5 Mb (mega bases) in size. Many smaller genetic changes, however, cannot be detected by traditional karyotyping.

The development of fluorescent *in situ* hybridization (FISH) technology allows for detection of smaller chromosomal imbalances. Fluorescent *in situ* hybridization uses fluorescent probes to hybridize to the complementary DNA sequences. With the selection of an appropriate probe, the presence of certain deletions at loci could be detected with a sensitivity of ~100 kb.

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It has been especially useful for diagnosis of many contiguous gene deletion syndromes such as 22q deletion and Williams syndrome¹². Subtelomeric deletions have also been recognized as an important cause of developmental delay in 7–8% of individuals with developmental delay¹³. Fluorescent *in situ* hybridization, however, cannot tell the size of a deletion or duplication or molecular breakpoints involved¹⁴. Fluorescent *in situ* hybridization is not suited to whole genome screening.

Evolution of CGH and aCGH

In the early 1990's comparative genomic hybridization (CGH) was developed. This involved the co-hybridization of test and reference DNA that had been "differentially labeled" with fluorescent probes¹⁵, and allowed for whole genome screening for copy number differences between the test and reference. It used metaphase chromosomes with a limited resolution of 5 to 10 Mb¹⁶.

The next development was microarray or array CGH (aCGH). It is similar to CGH in that two "differentially" labeled specimens are hybridized together, using a variety of different clones. In contrast to CGH, aCGH is not done in metaphase chromosomes but with DNA on a glass slide^{17,18}. This allowed for the slides to be scanned and analyzed by software which detects the different fluorescent intensities or cytogenomic copy number variation (CNV) between the patient and reference DNA.

The advantages of this technique are multiple. First, it allows for whole genome screening at resolution of 50 kb – 1 Mb (can be as small as ~10 kb) depending upon probe density on the array¹⁹. Second, aCGH can detect copy number variation in subtelomeric regions²⁰. Third, recent development of aCGH with SNP (single nucleotide polymorphism) capacity can detect not only deletion, duplication and amplification but also uniparental disomy (UPD)^{21,22}. Uniparental disomy of a whole chromosome or a segment of a chromosome has been associated with GDD and other phenotypes in many cases^{21,22}. Finally, aCGH has almost 100% concordance with G-banding¹⁹ and/or FISH²³ in detecting clinically significant genomic imbalances.

By the early 2000's, aCGH had been verified as a tool to identify copy number differences. It was originally used in tumor studies and has since been a method of providing etiological diagnosis for patients with developmental delay and other clinical features. Etiological duplications or deletions have been found in ~17% of individuals with undiagnosed GDD and/or other clinical phenotypes^{23–34}. This detection rate proves aCGH to be much more sensitive than routine cytogenetic methods.

Furthermore, in most studies, patients have already been investigated, with karyotype, FISH, and/or neuroimaging. All diagnostic rates were in excess of 1%, which is the diagnostic threshold stated by the American Academy of Neurology and the Child Neurology Society³¹ in evaluating a diagnostic test for developmental delay. Array CGH (aCGH), therefore, is an improvement over previous diagnostic techniques^{27,35} in undiagnosed developmental delay.

Cost Effectiveness of aCGH in Clinical Practice

Array CGH as an effective diagnostic tool in GDD has entered clinical practice in recent years. Positive aCGH may need further molecular cytogenetics tests on parents. With this

extra testing, some have questioned its cost effectiveness. Array CGH in Ontario can cost up to 750 Canadian dollars for one analysis. Newman et al. reported no significant cost differences between routine cytogenetics and aCGH³⁶. They concluded that aCGH in appropriately selected patients would have "minimal economic implications". More recently, Trakadis and Shevell analyzed the cost effectiveness of aCGH as a first line test versus initial karyotype and other diagnostics³⁷. They found that the cost of aCGH was much lower at their university hospital network compared to private enterprise. With this lower cost, aCGH was in fact less expensive than the previous diagnostic route. They concluded that aCGH as a first line test is cost-effective. A similar recommendation was made by Miller et al as they stated that aCGH is already less expensive than G banding karyotype followed up with a "customized" FISH test and aCGH is higher yield³⁸. As well, the reduction in follow up visits following diagnosis with aCGH provides further savings.

The issue then becomes who constitutes an appropriate patient for aCGH testing. As aCGH is the highest yield test for developmental delay without etiological diagnosis, both the American College of Medical Genetics and Genomics (ACMG) and the Canadian College of Medical Geneticists (CCMG) have published recommendations regarding use of aCGH³⁹. Array CGH is recommended as a first line investigation in patients meeting one of three criteria: multiple anomalies not specific to a known syndrome, patients with apparently non-syndromic developmental delay, or autism spectrum disorders. They also recommended performing a karyotype first if a common syndrome is suspected (Trisomy 21 or 18, sex chromosome aneuploidy). New directions for aCGH are also emerging in the field of prenatal diagnosis. Recent recommendations on this practice have been published by the CCMG and the Society of Obstetricians and Gynaecologists of Canada (SOGC)⁴⁰.

With this new technology, there have been recommendations published as to when a medical genetics referral is necessary. Following aCGH, a referral is indicated for clinical evaluation and counseling³⁹. Genetics referral is also indicated, as per ACMG guidelines in adults with a recognized genetic disorder or pediatric patients with congenital anomalies, failure to thrive, abnormal brain MRI findings, a known metabolic or chromosomal condition, or born to a parent with a known chromosomal abnormality, among others⁴¹.

Limitations and challenges of aCGH

Commercially available aCGH platforms generally cannot identify balanced translocations or inversions. Array CGH may miss mosaicism, depending upon the level of the mosaic cell line and the size and nature of the cytogenomic segments (e.g. small marker consisting mainly of centromeric repeats)^{42,43}. Interpretation of the clinical significance of some aCGH findings can be challenging, especially for variants of undetermined significance (VUS). Genome wide studies have found large scale copy CNV throughout the genome and the frequent occurrence of presumably benign CNVs in normal people^{44,45}. The challenge with aCGH is to determine whether a positive test is related to the clinical phenotype^{29,31}. A positive aCGH finding may necessitate confirmation by FISH or other DNA analyses of the patient as well as the parents^{39,46}. The follow-up investigations can determine if the copy number change is *de*

novo or inherited. If the parents test positive for the same copy number change and are unaffected, the change may be interpreted non-contributory. If the parents test negative and the copy number change is reported in multiple cases of GDD, it may be interpreted to be pathogenic or likely pathogenic. It should be pointed out that clinical interpretation of aCGH findings should take into consideration many factors, including gene content, size of the genomic interval, clinical history, and expressivity and penetrance of the inherited genetic changes.

The discovery of VUS may also cause anxiety to the family of the patient. There are various cytogenomic databases now available, including the University of California Santa Cruz (UCSC) Genome Browser, International Standard of Cytogenomic Array (ISCA) Consortium Database, Database of Genomic Variants (DGV), European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (EUCARUCA) and Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER)^{16,47}. These databases have been instrumental in interpretation of VUS and have helped improve aCGH as a clinical tool.

aCGH and its Effect on Clinical Management

An important aspect of any clinical test is its impact on patient management. As aCGH increases diagnostic rates, there are improvements in clinical management which are related to the inherent benefits in having a diagnosis. Saam et al⁴⁸ studied how aCGH changed management in a group of GDD patients with diagnostic copy number change detected by aCGH. Of their original population, 70.8% had some changes in management due to aCGH. Of those who had no change in management upon receiving an etiological diagnosis, the majority of families still felt "relieved" by the information. They concluded that with diagnosis, aCGH was able to clarify recurrence risk, increase access to resources, and guide future referrals.

There are several other positive impacts of aCGH on clinical practice. With the sensitivity of the technology, many novel deletion and duplication syndromes have been established. Furthermore, for previously described syndromes, aCGH can expand our knowledge of the phenotypic variability that exists³⁰. Finally, diagnosis can also provide families with a sense of control⁴⁹ and relief⁴⁹, as etiological diagnosis can be a long and drawn out process.

Array CGH has also been demonstrated to be useful in other clinical scenarios related to neurology. A recent study found aCGH to provide etiological diagnosis in 28% (7/25) of previously undiagnosed children with movement disorders⁵⁰. All of the positive cases had some level of learning disability. This suggests that aCGH also may play a role in providing etiological diagnosis in neurological clinical scenarios where developmental delay may only be part of the presentation.

Illustrative Case

This patient was originally evaluated by the genetics department at 1 and 13 years-of-age for global developmental delay. He was reevaluated at 31-years-of-age for global developmental delay, dysmorphic features, and concern surrounding the heritability of his condition. His parents are

nonconsanguineous of Scottish ethnicity. His mother was a 30-year-old gravida 1 and had amniocentesis during her pregnancy. Cytogenetic analysis was initially performed by low-resolution Q-banding in 1980, which was interpreted as a normal male karyotype (46,XY). The proband had a birth weight of 3.280 kg, head circumference of 37cm (greater than 97th percentile) and hypotonia was noted. At one year, his development was delayed and communicating hydrocephalus was found on cranial CT scan. This was treated with a ventricular peritoneal shunt and his development improved. A possible diagnosis of Prader-Willi syndrome was suspected at birth but was deemed unlikely due to his hydrocephalus, frontal bossing, and normal weight. His parents later had another male child born without dysmorphic features or developmental delay.

On examination, his height was 171.3 cm (25th centile) and he weighed 87.7 kg (75th-90th centile). He was non-verbal, and uses gestures for communication, in keeping with his documented intellectual disability. With receptive language, he understood verbal commands. His gait was unsteady and he was mostly confined to a wheel chair. On craniofacial exam, he had an enlarged skull, short palpebral fissures (2.5cm), high arched palate and low set, posteriorly rotated ears (Figure 1). His teeth were short. His hands were small and his fifth digit and third toe were were shortened bilaterally. His lower limb reflexes were brisk.

Array CGH was performed as the first line investigation as per our current guidelines, using DNA extracted from the blood and analyzed utilizing an Agilent oligonucleotide array (180K) and Bluegenome software. The aCGH analysis identified a 20.8



Figure 1: Views of the proband at the age of (from top left): 4 months, 2 years, 5 years, 8 years, 12 years and 29 years.

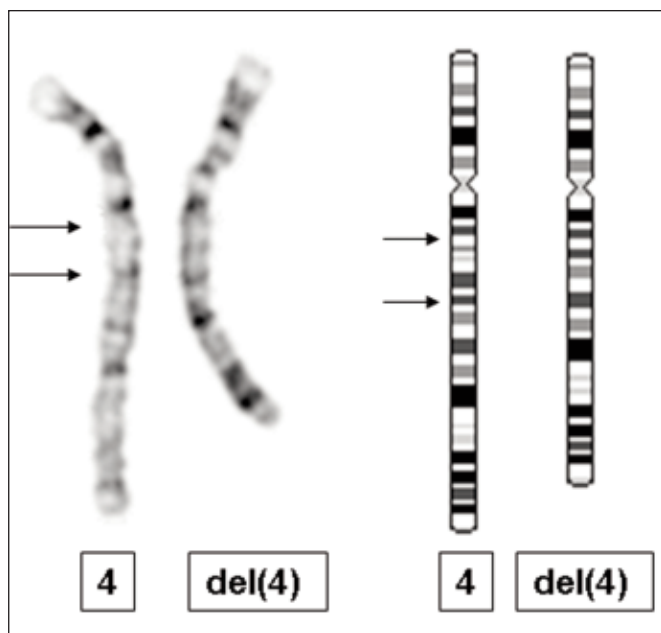


Figure 2: G-banding showing a normal chromosome 4 (left) and an abnormal chromosome 4 with a deletion of 4q21.1q22.3 (arrows). This result confirms aCGH finding.

Mb deletion in chromosome region 4q21.1q22.3 (chr4: 77,796,761-98,589,579 [hg18]). The 4q deletion involves 65 OMIM genes including 9 OMIM morbid genes (<http://genome.ucsc.edu>). In addition, the aCGH detected two smaller duplications at Xp22.33: 0.724 Mb (chrX:387,409-1,112,282 [hg18]) and 0.592 Mb (chrX:1,434,992-2,026,644 [hg18]). The 0.724 Mb duplication involves an OMIM morbid gene (SHOX); its duplication has been reported in patients with developmental delay, congenital anomalies and Asperger syndrome^{51,52}. However, this duplication may be inherited from an unaffected father and the clinical significance of this duplication is uncertain. The 0.592 Mb duplication has not been associated with any syndrome or disease and its clinical significance remains uncertain due to lack of publications on the duplication of this interval. Given the large 4q21 deletion, neither duplication was considered likely to significantly contribute to our patient's presentation. The 4q deletion was confirmed by G-banding analysis (Figure 2) and interpreted as *de novo* since the parents had a normal karyotype.

At least 15 patients have been reported with deletions involving the 4q21 region⁵³⁻⁵⁷. 4q21 deletion has been summarized as a syndrome of four features⁵³: severe mental retardation, severely delayed or absent speech, dysmorphic facial features, and short stature, and is an example of a novel syndrome that has been documented due to aCGH. The deletion in the present case is the largest among the deletions in this 4q region reported.

Efforts have been made to identify the genes responsible for the phenotype seen in 4q21 deletion syndrome. A region of shared overlap among previous cases was found to include two

genes: PRKG2 and RASGEF1B⁵³. PRKG2 encodes a protein kinase that is expressed in cartilage and other tissues. Mutations in PRKG2 are associated with a natural dwarf rat phenotype⁵⁸ and its deletion may result in short stature. RASGEF1B encodes a guanine nucleotide exchange factor for RAS proteins expressed in the central nervous system (CNS)⁵³. These genes may be responsible for the neurodevelopmental phenotype.

The patient's family decided to once again search for a diagnosis after giving up almost 20 years prior as the patient's unaffected sibling was considering starting a family. The resulting diagnosis provided closure as they had been part of a diagnostic odyssey for over 30 years. With the provision of an etiology, the family has been able to access internet resources and additional information about 4q21 deletion syndrome.

CONCLUSION

For patients with developmental delay, approximately only half are provided with etiological diagnosis. Array CGH can help significantly improve the diagnostic rate and therefore should be considered a first line investigation in developmental delay. It has also been shown to be useful in other clinical scenarios related to neurology where developmental delay is only part of the presenting phenotype. Pre-test genetic counseling is needed to discuss benefits as well as limitations of the aCGH testing. The expectations of the family need to be assessed and the clinician must ensure that they are in keeping with the expected benefits of a diagnosis. Array CGH has been proven as a valuable first line tool for neurologists as well as geneticists in investigation of developmental delay.

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FC drafted the illustrative case and review of array CGH and its implications for neurologists with the help from CP. JX contributed cytogenetic and array CGH analyses of the illustrative case. JJ and all others edited the manuscript.

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