

The Role of New Genetic Technology in Investigating Autism and Developmental Delay

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INTRODUCTION

Children with developmental delay and dysmorphic features can present diagnostic and therapeutic challenges. One of the newer genetic technologies, known as chromosomal microarray, or array comparative genomic hybridization (aCGH) has revolutionized our diagnostic capabilities. We describe a patient who had global developmental delay and autistic features who did not receive a diagnosis until aCGH was obtained. Our case illustrates the way that current genetic testing may provide new information for a subset of patients with undiagnosed global delay, intellectual disability (previously termed mental retardation), and autism. To this end, we provide a brief background on the development and utilization of aCGH. Next, we discuss his particular diagnosis of 22q11 deletion syndrome as an example of the way that knowledge of a microdeletion syndrome may influence current medical management and future approaches. While 22q11 deletion syndrome is a well-known microdeletion, many syndromes of

similar consequence await further elucidation and case collection. We conclude by providing resources for physicians and families that may improve knowledge and support for those diagnosed with common and rare genetic syndromes.

CASE REPORT

A boy born to a 38 year-old G3 mother and 41 year-old father after a full-term uncomplicated pregnancy with a birth-weight of seven pounds and nine ounces appeared normal at birth, however, the parents became concerned about his development at around eighteen months. He spoke his first words after age two, and spoke in short phrases at age four. He began to walk independently at nineteen months. He was seen at the Children's Neurodevelopment Center (CNDC) at 20 months. Audiology examination did not reveal significant hearing loss, and the family was provided information on early intervention services. On follow-up visits at the CNDC, it was noted that he had echolalic language, a high activity level,

and poor attention span. His preschool teacher reported concerns about his lack of developmental progress, fleeting eye contact, and lack of awareness of his body in space. A formal psychological assessment at age four years, seven months indicated cognitive and adaptive skills at a 22 month level. Speech and language evaluation at four years, nine months found expressive and receptive language skills at a two year level. Behavioral observations revealed decreased social reciprocity and eye contact, limited functional communication and repetitive behaviors. On the Childhood Autism Rating Scale, the patient scored in the mildly-moderately autistic range. He was referred to Genetics Clinic.

Past medical history was significant for three febrile seizures. He had a history of several ear infections and a pilonidal cyst, for which no treatment was necessary. An MRI of his spine and head was normal. Review of systems was positive for episodes when he sometimes appeared to choke on his food.

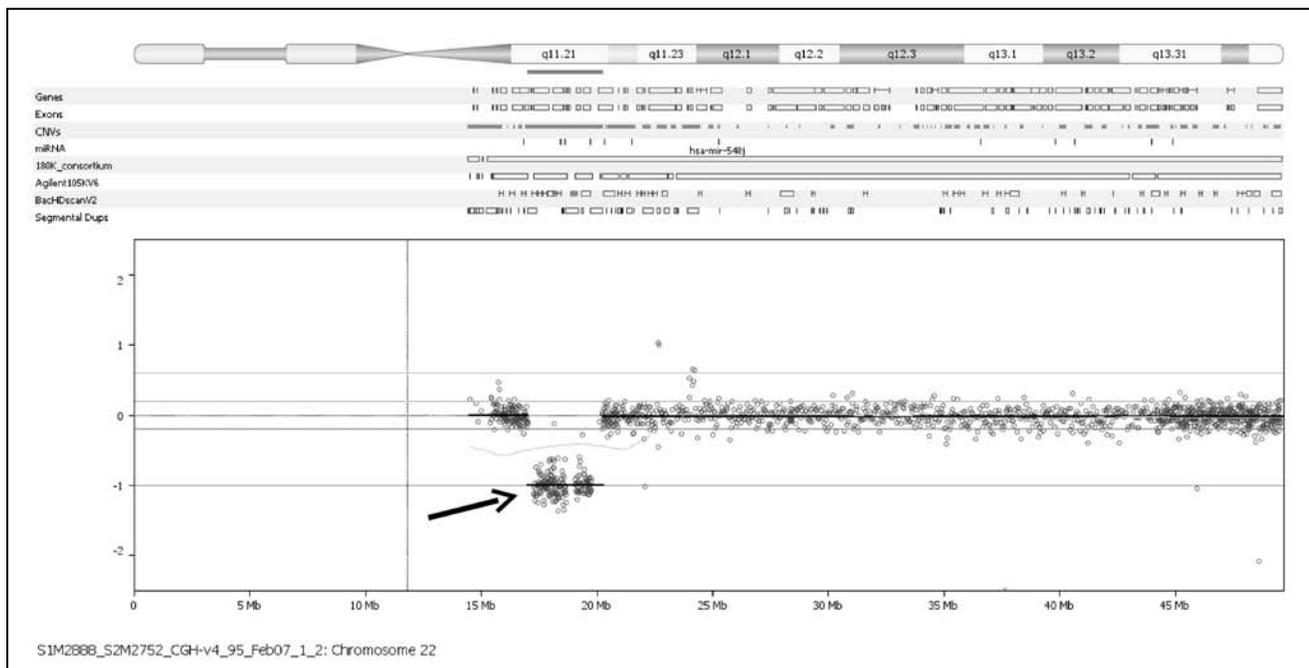


Figure 1. High density array CGH analysis of patient sample showing a single copy 3 Mb deletion of chromosome 22 including the *TBX1*, *COMT*, *PROH*, and *DGCR8* genes. The arrow points to where there is more reference than patient DNA, indicating a deletion of patient DNA.

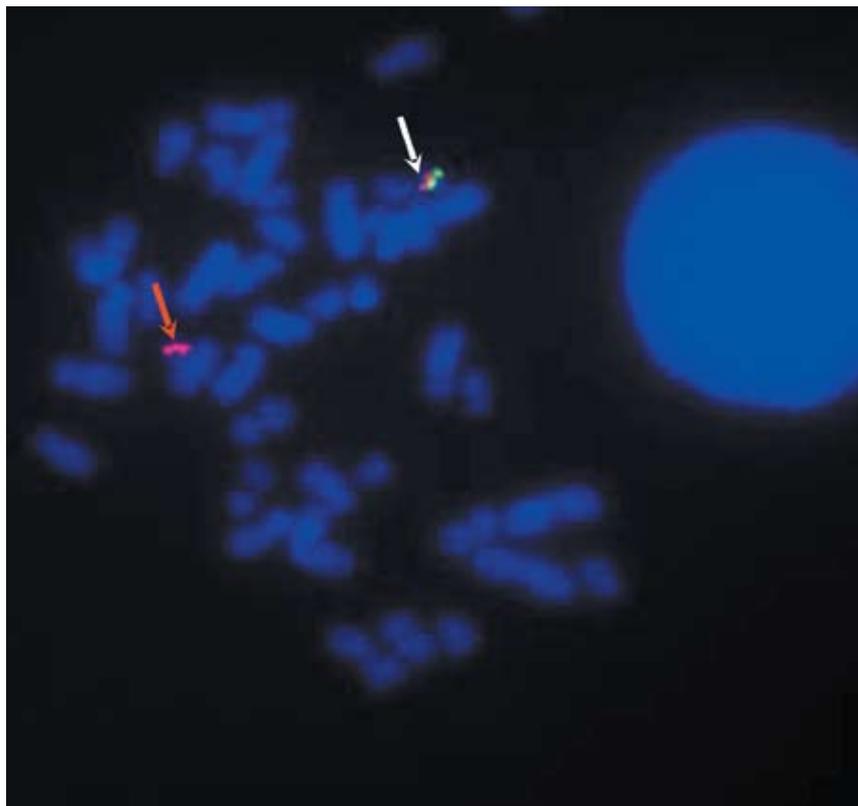


Figure 2. Example of 22q deletion by metaphase FISH analysis. The white arrow points to the chromosome with both the red control probes and the green probes that light when binding to 22q11. The red arrow points to the chromosome where the green probe does not light since 22q11 is absent.

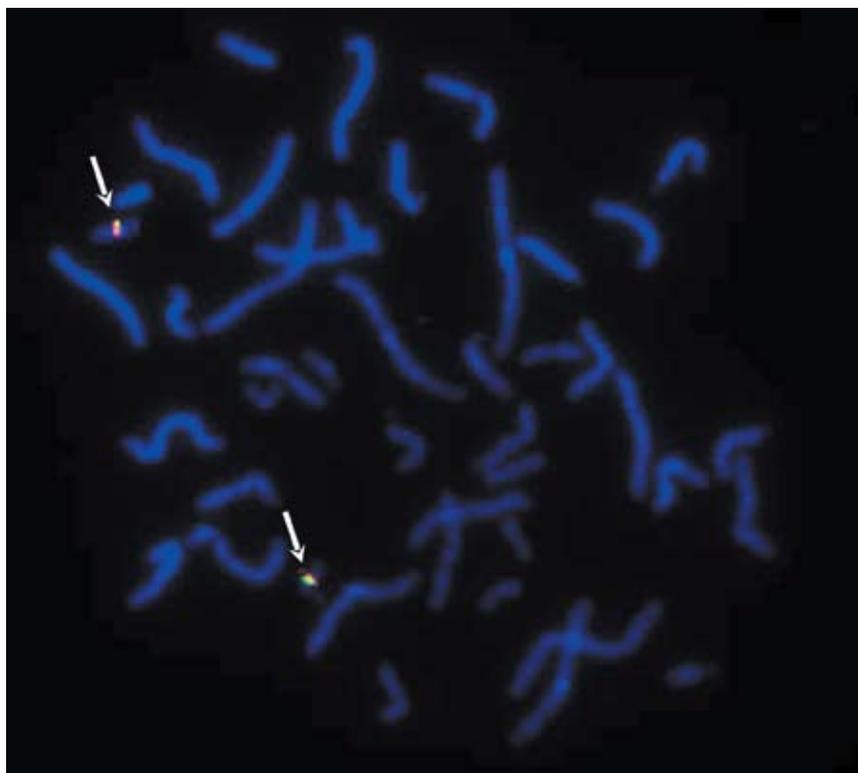


Figure 3. FISH analysis of paternal sample showing two normal copies of chromosome 22 with both chromosomes containing red control probes and green probes that light on each chromosome since 2 copies of 22q11 are present, as in the normal case.

Family history did not reveal any major learning problems in either of his parents. His father graduated from high-school and took college courses. Currently, he was taking care of his son full-time. His mother worked as an accountant. She had two sons from a previous relationship, one of whom required some help in school for motor problems. There were no other family members on either side with learning difficulties or birth defects.

On physical examination, he had mildly dysmorphic features including a slightly prominent nose and slight asymmetry of his mouth when crying, but otherwise he resembled his father. He had a faint strawberry hemangioma on his right arm. Neurological examination revealed hypotonia and awkward gait. He had hypernasal speech. The rest of the examination was normal.

A definitive diagnosis could not be made based on history and physical examination, and aCGH was ordered, which revealed a 22q11 deletion (Figure 1); current standards recommend confirmation of aCGH with fluorescent in situ hybridization probe (FISH) (Figure 2). The parents were tested by FISH and did not have the 22q11 deletion (Figure 3).

As per the management guidelines for 22q11 deletion syndrome, he was screened for hypocalcemia and immune deficiency; had a renal sonogram and was referred to a cardiologist. Chest X-ray suggested a right aortic arch, which echocardiogram confirmed. The patient had no evidence of dysphagia or cough with liquids or solid oral intake, so he did not require intervention. He was also referred to Craniofacial Clinic where evaluation documented a palatal zona pellucida and his speech was confirmed as hypernasal. His uvula appeared intact, but his posterior nasal spine could not be adequately examined after he bit the plastic surgeon's examining finger. Nevertheless, the tentative diagnosis of palatal submucous cleft was made, which may explain his hypernasal speech. He continues to be followed by a multidisciplinary team.

DISCUSSION

In recent years, aCGH has at least doubled our diagnostic ability in the work-up of patients with global developmental delays or intellectual disabilities.¹ Recently, the International Standard

Cytogenomic Array (ISCA) Consortium concluded that chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies and offers a much higher diagnostic yield (15%-20%) for genetic testing of individuals with unexplained developmental delay than a G-banded karyotype (approximately 3%, excluding Down syndrome and other recognizable chromosomal syndromes).² Thus, aCGH has revolutionized clinical cytogenetics, as it provides a relatively quick method to scan the genome for gains and losses of chromosomal material with significantly higher resolution and far greater clinical yield than was previously possible with karyotype and other techniques.³

Five years ago, before aCGH became a first-tier test in clinical genetics, the work-up for patients who had dysmorphic features and/ or unexplained global developmental delay or intellectual disability began with karyotype, a representation of the chromosomes, which had a five percent diagnostic yield, and in many cases, Fragile X testing, with a yield around 1%.⁴ The limit of a conventional karyotype is its ability to detect small gains or losses. Typically, for an abnormality to be seen on karyotype, a deletion or duplication would have to span at least 5-10 Mb (of the 3000 Mb human genome).

In the mid 1980s, a new advance allowed for smaller deletions or duplications to be detected than could be seen on routine karyotype, which is known as Fluorescent in Situ Hybridization (FISH). This technique uses fluorescent DNA probes that bind to regions of interest, typically those spanning around 1-4 megabases of genetic material. Probes for several common syndromes, such as Williams, Wolf-Hirschhorn, Smith-Magenis, and 22q11 deletion syndrome (previously called velocardiofacial syndrome or DiGeorge Syndrome) became available. These syndromes can often be diagnosed clinically by facial and other features, and FISH testing can be sent for confirmation. However, patients with mild or atypical cases have often been missed by this approach.

To understand why aCGH offers a more comprehensive test than FISH, it is useful to review the technology behind

the technique. In aCGH, slides are arrayed with small segments of DNA as the targets for analysis. DNA is extracted from a test sample (e.g., blood, skin, fetal cells). The test DNA is then labeled with a fluorescent dye of a specific color, while DNA from a normal control sample is labeled with a dye of a different color. With the original technique, the two genomic DNAs, test and reference, are mixed together and applied to a chip. In updated versions, the chip contains the reference DNA to which the patient DNA is applied. Because the DNAs have been denatured, they are single strands that attempt to hybridize with the arrayed single-strand probes. Next, digital imaging systems are used to capture and quantify the relative fluorescence intensities of the labeled DNA probes that have hybridized to each target. The fluorescence ratio of the test and reference hybridization signals is determined at different positions along the genome. The result demonstrates the relative copy number of sequences in the test genome as compared to the normal genome. For example, the control DNA could be labeled red and the patient DNA green. If the spots that represent 22q11 appear to have a higher red intensity, then that means that patient DNA is missing.

The first commercially available arrays used targeted bacterial artificial chromosomes (BACs) that contained DNA isolated from large insert clones that range in size from 150 – 200 kb and only included areas of the human genome that contain known microdeletion or duplication syndromes. The production of BAC DNA was labor-intensive, and resolution remained limited to 50 – 100 kb. Newer arrays, known as oligonucleotide arrays, use artificially synthesized vectors that represent each area of the genome. They are considered preferable to BACs, because they have extremely high resolution, up to 1 kb of the human genome. Since oligoarrays are considered more comprehensive, BAC arrays are already considered obsolete but are still used by some laboratories for confirmation of aCGH results.

In most cases, the microdeletion is considered causative for the patient's problem based on two major criteria: first, many genes are missing or added in the region and some may be associated with

neurodevelopmental or other problems, and second, the same deletion or duplication has been reported in similarly affected individuals.

In some cases, copy number variants are found with DNA missing or added that are also present in the general population, and therefore they are not considered causative. As more testing is performed, better delineation of copy number variants versus pathogenic changes awaits. While a lengthy explanation is beyond the scope of the paper, we refer the reader to a reference that addresses the issue.⁵

Results can also return with findings called variants of unknown significance (VUS)—changes found on aCGH that have not been routinely found in the general population but are not definitively associated with medical problems. In these cases, parental testing may be helpful. If one parent has the same VUS, it is assumed that it is less likely to be diagnostic (unless the parent has the same condition as the child). If neither parent has the same VUS, the de novo occurrence in the child increases suspicion of a causative role. However, a definitive diagnosis cannot be given, until more cases and information become available.

Challenges of aCGH include: cost and insurance approval for testing, variability in laboratory interpretation, and difficulty in providing adequate genetic counseling for complex test results. Many microdeletions/duplications discovered on aCGH have rarely been described, thus the impact on the child's prognosis or even care may be uncertain. Families often respond with bewilderment when told of a diagnosis such as "a 15.24.1 three megabase microdeletion" or "15q11 four megabase duplication." With education, most parents end up successfully understanding and navigating information currently available.

Despite the fact that aCGH is not a perfect test, this state-of-the-art technology has considerable diagnostic power and has become the gold standard for patients who require genetic evaluation. The American College of Medical Genetics currently recommends the use of aCGH for patients with undiagnosed intellectual disability, global developmental delay, multiple congenital anomalies, and autism.⁶ Recently, the ways in which aCGH

can change medical management—including leading to improvements in medical diagnosis, increased utilization of appropriate specialist referrals, avoidance of unnecessary diagnostic testing, avoidance of muscle biopsy, and improved access to services—has been published in *Genetics in Medicine*.⁷

A DESCRIPTION OF 22q11 DELETION SYNDROME AS AN EXAMPLE OF A MICRODELETION DIAGNOSED BY ACGH

In our case of a boy with global delay and autistic features, the diagnosis of 22q11 deletion syndrome was made. This particular microdeletion has an interesting history which, in turn, gives insight on the wide variation between genotype and phenotype in patients with microdeletion or microduplication syndromes. A brief discussion of 22q11 deletion syndrome is particularly timely in commemoration of Dr. Angelo M. DiGeorge, who recently died at the age of 88, and who described the first cases. He authored over 230 articles, numerous text book chapters, received awards as one of the United States' best doctors, and he became famous in the 1960s after his groundbreaking description of a group of patients who shared common features: hypoparathyroidism and immune defects related to hypoplastic or absent thymus. In the 1970s, Robert Shprintzen, PhD, a speech pathologist described another set of patients with similar clinical features including cleft lip and/or palate, conotruncal heart defects, and characteristic facial features. Dr. Shprintzen named this group of features velocardio-facial syndrome, but the syndrome was also referred to as Shprintzen syndrome. In the 1980s, it was discovered that patients with features of DiGeorge, Shprintzen, and velocardio-facial syndromes all had a chromosome deletion in the region of 22q11.2. Multiple names had been given for the same syndrome, not only because different researchers had described it, but because individual patients who shared the same genetic abnormality had variable physical features. Today, the term 22q11.2 deletion syndrome is most widely accepted. A debate exists about using eponyms in genetics, but most geneticists describe newly discovered microdeletions or duplications based on the exact chromosomal location.

We have found that even well-characterized genetic syndromes have a wide range of expressivity, thus we have cast our net wider in considering which children may have syndromes.

Many patients with microdeletions or microduplications have additional problems beyond the neurocognitive. Thus, by making a diagnosis, we can screen children for medical problems that could otherwise go undiagnosed and make appropriate specialty referral. For example, in individuals with 22q11 deletion syndrome, congenital heart disease, palatal abnormalities, and learning disabilities are present in the majority. Almost 75% have congenital heart disease, particularly cono-truncal malformations (tetralogy of Fallot, interrupted aortic arch, ventricular septal defect, and truncus arteriosus). Due to complex cardiac anatomy in some patients, who may even have aberrant subclavian arteries, surgery and intubation can pose a risk, so knowledge of the diagnosis may be life-saving.⁸ About 70% of 22q11 deletion syndrome patients have palatal abnormalities, particularly velopharyngeal incompetence (VPI), submucosal cleft palate, and cleft palate. In some patients, VPI may have been missed prior to their diagnosis of 22q11 deletion syndrome. Once diagnosed, associated articulation difficulties and hypernasality may improve with intensive speech therapy but if refractory may require surgical intervention. In addition, over half of individuals have an immune deficiency; about 50% have hypocalcemia, approximately 30% have renal problems, and approximately 30% have conductive or sensorineural hearing loss. Other medical problems in some patients include laryngotracheoesophageal anomalies, growth hormone deficiency, seizures (without hypocalcemia), and skeletal abnormalities.⁹

Interestingly, subsequent evaluations in our case did not reveal any major medical anomalies. An extremely variable phenotype has been repeatedly described when following large cohorts.¹⁰ Like our patient, some individuals with 22q11 deletion syndrome have no systemic manifestations, and other individuals may have few obvious features. For example, researchers tested thirty relatives of affected individuals without any typical manifestations of 22q11 deletion syndrome who were found, by screening, to have a deletion on 22q11. Nineteen were adults ascertained only following the diagnosis in their child, 10 were children identified following the diagnosis in their sibling, and one was a child diagnosed prenatally following the diagnosis in her parent. Sixty percent of patients had no visceral anomalies.¹¹ Similarly, the range of presentation in all microdeletion syndromes is variable. The second point is that parental testing is considered optimal both for medical management of a potentially affected parent and for prenatal counseling for future pregnancies. In our case, as previously mentioned, the parent's tests were normal, and we counseled them that their recurrence risk was low.

The most concerning findings in our patient included behavioral difficulties and autistic features. Interestingly, a subset of patients with 22q11 deletion syndrome present with autistic spectrum disorder as a feature.¹² One current area of particular interest has been the effect of 22q11 deletion syndrome on neuropsychology. The psychiatric disorders most commonly reported in children and adolescents with 22q11 deletion syndrome have been attention-deficit/hyperactivity disorder, oppositional defiant disorder, anxiety disorders, and major depression. Psychotic symptoms have been observed in 14% to 28% of children with 22qDS. A 5-year follow-up study of 22qDS children with psychotic symptoms at baseline found they had an increased risk for a subsequent psychotic disorder. In particular, an increased rate of schizophrenia is widely recognized.¹³ By knowing that patients with 22q11 deletion syndrome are at risk for psychiatric disorders, physicians can recognize signs and symptoms earlier and provide treatment more promptly.

Genetic studies may affect future medication management of children or adults with 22q11 deletion syndrome and psychiatric problems. The genes *catechol-O-methyl-transferase (COMT)* and proline dehydrogenase both reside within the commonly deleted region of 22q11.2. It has been hypothesized that in patients with the *COMT* gene missing as part of their deletion, the *COMT* insufficiency leads to elevated serum proline levels and abnormal brain function. By studying over fifty children with 22q11 deletions and comparing to healthy controls, researchers confirmed a significant interaction between a *COMT* deficient genotype, increased proline, and behavioral problems or psychiatric features.¹⁴ The conclusion was that in some patients with 22q11 deletion syndrome, elevated proline negatively affects brain function by an increase in dopamine in the prefrontal cortex (elevated dopamine is more likely due to the reduction of *COMT*, which metabolizes dopamine, which should be true wherever dopamine is secreted). In the future, one can envision using medications that reduce proline levels in patients with 22q11 deletion syndrome and psychiatric disorders. The future may hold more promising pharmacological interventions targeted for this particular genetic syndrome. The hope for other microdeletion or duplication syndromes is that, like in the case of 22q11 deletion syndrome, genes involved in particular pathology will be further characterized and targeted for pharmacogenetic interventions.

RESOURCES FOR FAMILIES WITH MICRODELETION OR DUPLICATION SYNDROMES

When families are faced with a new diagnosis, it is naturally overwhelming but, in some ways, a relief to end their diagnostic odyssey. Genetic counseling helps families learn more about the diagnosis and, in turn, empowers them to advocate for their child's medical needs. Support groups are especially helpful for families and patients who have rare microdeletion or duplication syndrome diagnoses. These groups invite all families with new diagnoses to contribute information about their child's medical problems and outcomes and encourage

families to connect to those with the same or similar diagnoses via e-mail, phone, and even in person.¹⁵

CONCLUSION

In recent years, with the widespread use of aCGH in the work-up of patients with global developmental delay, the diagnostic yield of genetic testing has increased considerably. We have found that even well-characterized genetic syndromes have a wide range of expressivity, thus we have cast our net wider in considering which children may have syndromes. Patients may receive commonly reported diagnoses such as 22q11 deletion syndrome or diagnoses of more rare microdeletion or duplication syndromes. For all these patients and families, we are optimistic that the future holds more genotype-phenotype correlations, understanding of particular medical problems, and even pharmacogenetic interventions.

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