Abstract

The North West Thames Regional Genetics Service (NWTRGS) provides cytogenetic, molecular and clinical genetics services for a population of 3.6 million in metropolitan London and the adjacent counties. The NWTRGS offers CGH microarray analysis (aCGH) as the first line investigation for all children presenting with congenital anomalies, dysmorphic features and developmental delay/learning difficulties. However, an increasing number of requests are being received from local paediatricians with autism/autism spectrum disorder as the main indication for testing. Given the cost/resource implications of aCGH, we decided to audit the array service to determine the diagnostic yield for unselected patients referred with isolated autism, but no syndromic features. A total of 285 requests for aCGH were received over a 10 month period in 2012 for ‘autism’/‘autism spectrum disorder (ASD)’ only and ‘autism’/‘ASD’ + learning difficulties. The overall aCGH abnormality rate in this patient cohort was 13.9%, with 3.35% assessed as pathogenic or likely pathogenic, which is lower than previously reported. We outline the challenges arising from application of aCGH technology in this patient cohort and discuss future genetic work up strategies.

1. Introduction

Autism Spectrum Disorder (ASD) is a very heterogeneous, multifactorial developmental disorder with a strong genetic component. Studies have consistently shown a high monozygotic twin concordance, and it is widely accepted that autism/autism spectrum disorders (ASD) often cluster in families. In a smaller number of cases autism can be part of a wider genetic syndrome (‘syndromic autism’). Overall, a specific genetic cause is found in 6-20% of patients (Fombonne et al, 2003). The advent of comparative genomic hybridisation techniques (CGH) in the late 1990’s has generated an ever increasing interest in the significance of copy number variants (CNVs) identified in this patient group. (Sebat et al 2007; Pinto D et al 2010). CNVs are segments of DNA ranging from 1 kilobase (kb) to several megabases (Mb), for which copy number differences (e.g. deletions, amplifications) have been revealed by comparison of 2 or more genomes. Initially, the application of aCGH technology to autism research cohorts appeared to yield promising results, subsequently amplified by the popular press. (Independent on Sunday, June 2010). Extensive media coverage of genetic research into autism has raised patient and parent expectation of imminent breakthrough discoveries, however, few CNVs are specific for autism/ASD. CNVs are involved in the cause of intellectual disability and a wide range of neurodevelopmental and neuropsychiatric disorders including ASD. (deVries et al 2005, Cook EH Jr and Scherer S W 2008).

Some of the more common causative genetic variants are summarised in the table below. Many of the associated genes encode proteins thought to be important in neuronal development and synaptic function, and can be associated with other neurodevelopmental disorders as well as schizophrenia and epilepsy (Glessner et al, 2009).
Copy number variants | Single-gene associations
---|---
15q24del | Cell-adhesion molecules: NLGN1, ASTN
16p11.2del/dup | Ubiquitin pathway proteins: UBE3A, PARK2, RFWD2, FBXO40
1q21.1dup | Neurexin/neuroligin/PSD-95/SAPAP/Shank pathway
15q13.3del | FOXP1, GRIN2B, SCN1A, LAMC3, SHANK, CNTNAP2
16p13.11del/dup | and many more (>500)
17q12dup | 15q11-q13 duplication is the most common
22q11dup | chromosomal abnormality in individuals with ASD
22q13del |

**Table: Genetic variants thought to be associated with autism spectrum disorders**

Array CGH (aCGH) has now become a widely used diagnostic technique. The NWTRGS introduced aCGH as the first line investigation for all children with congenital anomalies, dysmorphism and developmental delay/learning difficulties in April 2011. The platform used is the Agilent 8x60k oligonucleotide array, 250kb resolution. Array analysis of this patient group has so far revealed 3.5 times more abnormalities than found with conventional karyotyping. The yield of array CGH in patients presenting with autism/ASD is known to be lower when dysmorphic features or intellectual disability are absent, and NICE currently do not recommend performing aCGH unless “specific dysmorphic features, congenital anomalies and/or evidence of intellectual disability” are present (NICE, 2011).

Many study groups started to apply array technology to large autism cohorts, frequently linked in together with learning disability, epilepsy and developmental delay. Studies focusing more specifically on autism were carried out by Shen et al and Rosenfeld et al in 2010: in a cohort study of 933 patients with autism spectrum disorders, aCGH detected variants in 204/848 patients (24.1%). These were of definite or possible significance in 7.0% of patients [95% CI 5.5-8.5] (Shen et al, 2010). Rosenfeld et al analysed 1.461 samples referred with an ASD indication. 180/1.461 (12.3%) were reported as abnormal. 113 of these were considered to be potentially causative, yielding a detection rate of 7.7% (Rosenfeld et al 2010). The large number of samples included in the Rosenfeld survey spanned a period of >5 years, during which 4 different types of aCGH were used.

Most requests for aCGH at the North West Thames Regional Genetic Service are received from local paediatricians, and many referral forms specify ‘autism’ and/or “ASD” as the sole indication for aCGH. We therefore decided to audit the array service to determine our diagnostic yield for unselected patients referred with isolated autism/ASD and no features suggesting a specific syndrome.

### 2. Aims and audit standards

The aim of the audit was to answer the following questions:

- What proportion of patients referred with isolated autism/ASD, i.e. no features to suggest a specific syndrome diagnosis, have an abnormal array CGH result?
- What proportion of these abnormalities are thought to be pathogenic?
- How do these diagnostic yields compare to published data?
Based on previous studies, we would expect the following:

- Proportion of results flagged as abnormal: \( \geq 15\% \)
- Proportion of results that are likely pathogenic: \( \geq 7\% \)

### 3. Data collection (sample, methodology, data source)

Appropriate referrals were identified from our database covering a 10 month period, from February to December 2012. We included referrals coded as ‘autism’ or ‘autism spectrum disorder’ in the ‘clinical details’ section of the referral form. 285 records were obtained. We excluded all requests referring to features suggesting a syndromic diagnosis (pulmonary stenosis, ash-leaf macules, macro- or microcephaly etc.) resulting in a final figure of 209 reports.

Reports were sub-divided into those with learning disability (LD) mentioned on the request form, and those without. Patients’ results were then classed as ‘abnormal’ or ‘apparently normal’. Abnormal results were further stratified into one of the following categories as per the NWTRGS protocol (Burbridge & Bewes, 2012 – based on the American College Guidelines, Kearney HM et al (2011) Genet Med;13(7):680-685):

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic (PATH)</td>
<td>Strongly suspected to be the cause of the patient’s clinical condition</td>
</tr>
<tr>
<td>Variant of unknown significance, likely pathogenic (VOUSP)</td>
<td>Either the CNV has been described in a single report or a gene within the CNV has a function which is relevant to the patient’s phenotype.</td>
</tr>
<tr>
<td>Variant of unknown significance (VOUS)</td>
<td>The genetic content of the imbalance is not well documented or there are multiple contradictory reports regarding the imbalance.</td>
</tr>
<tr>
<td>Variant of unknown significance, likely benign (VOUSB)</td>
<td>The CNV has been reported in a small number of cases in databases of variation in the normal population but does not fulfill the laboratory’s criteria as a benign variant.</td>
</tr>
<tr>
<td>Benign (BEN)</td>
<td>A CNV is classified as benign and is not reported when the variant is recorded on DGV (Database of Genomic Variants) or equivalent in at least 3 separate studies, from 3 separate populations. The imbalance should be the same type (i.e. deletion or amplification), and for sex chromosome abnormalities, in controls of the same sex. CNVs occurring with high frequency ((&gt;1%)) in control populations are not generally reported.</td>
</tr>
</tbody>
</table>

### 4. Results

**Patient characteristics**

82.9% of patients were male (M:F ratio 4.8:1), with a mean age at testing of 5.1 years (SD 3.6 years).

Learning disability (intellectual disability) is relatively commonly associated with autism, and is likely to increase the likelihood of finding an array abnormality (NICE, 2011). In view of this, the referrals were stratified by the presence/absence of ‘learning disability’ (LD) in the clinical information. 16.3% (34/209) of referrals mentioned LD. For pathogenic abnormalities (PATH or VOUSP), the overall yield was higher amongst patients with LD: 5.88% (2/34), versus 3.43% (6/175) for those without it. However, it is not possible to say from these small numbers whether LD significantly increases the chance of an abnormal array finding being present.
**Array CGH results**

86.1% (180/209) patients had a normal aCGH result. 13.9% (29/209) of patients had an array CGH result that was reported as ‘abnormal’. Combining the categories above allows the results to be summarised as either ‘likely pathogenic’ (PATH or VOUSP) or ‘uncertain significance’ (VOUS):

These are summarised in Fig. 1, below:

![Fig. 1- aCGH abnormalities by significance, and by presence/absence of LD](image)

**Pathogenic CNVs**

Five CNVs were considered to be pathogenic - four with relevance to autism and one incidental finding. 3/5 were inherited from an apparently normal parent:

<table>
<thead>
<tr>
<th>Copy Number Variant (pathogenic)</th>
<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4 Mb amplification 16p13.11</td>
<td>Yes</td>
</tr>
<tr>
<td>1.3 Mb deletion 17p12 (incidental finding)</td>
<td>No</td>
</tr>
<tr>
<td>1.3 Mb amplification 1q21.1</td>
<td>Yes</td>
</tr>
<tr>
<td>2 Mb amplification in 1q21.1</td>
<td>No</td>
</tr>
<tr>
<td>1.4 Mb amplification 17q12 102kb deletion 8q24</td>
<td>No</td>
</tr>
</tbody>
</table>
We found 3 likely pathogenic abnormalities, 2/3 inherited from an apparently normal parent:

<table>
<thead>
<tr>
<th>Copy Number variant (likely pathogenic)</th>
<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6 Mb amplification 2p24</td>
<td>No</td>
</tr>
<tr>
<td>5.3 Mb deletion 8p23.2</td>
<td>No</td>
</tr>
<tr>
<td>152 kb amplification 13q34</td>
<td>No</td>
</tr>
<tr>
<td>445 kb amplification 15q13.3</td>
<td>No</td>
</tr>
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**Discussion**

**Demographics**
The results showed a 4:1 male: female ratio, as would be expected given the male predominance of autism. The majority of referrals came from community paediatricians working in the NWTRGS catchment area, with a much smaller proportion from district general hospitals; only 5.74% of referrals originated from clinical geneticists at the NWTRGS, reflecting the practice that individuals with an autism/ ASD diagnosis and no associated syndromic features are rarely referred to Clinical Geneticists. Most aCGH referrals for this indication are from community paediatricians as expected.

**Abnormal reports**
The diagnostic yield for aCGH in individuals referred with autism or ASD was 3.35% (7/209) for pathogenic and likely pathogenic CNVs. This is lower than has been previously reported. In a large cohort study of aCGH results in autistic children, 24.1% for all VOUS and 7.0% for pathogenic CNVs were observed (Shen et al, 2010). Similar numbers were obtained by Rosenfeld et al. In a broader meta-analysis and systematic review of 19 studies and nearly 14000 patients with learning disability and congenital anomalies, the overall diagnostic yield was 10% (Sagoo et al, 2009). Miller et al summarise 33 studies with 21,698 subjects. The diagnostic yield ranges from 5.1% to 35%. However, the cohorts differ vastly in subject number, indications and array type used, most of them focussing on patients with developmental delay, intellectual disability and dysmorphism and are therefore difficult to compare. (Miller et al, 2010).

Why is the yield lower at the NWTRGS? Several factors may contribute:

1. **Different setting.** Most published work applies aCGH on autism research cohorts. Individuals included in autism research are usually worked up using gold standard autism diagnostic instruments and psychometric testing. Our audit looked at children mainly referred from community paediatricians who likely use less stringent and more diverse sets of diagnostic tools for autism/ ASD. Access to psychometric testing tends to be very limited in the community setting and state of the art confirmation/ exclusion of learning disability/ intellectual disability is patchy. This is likely to lead to an element of ascertainment bias in our study.

2. **Different population of children.** Studies that include children with dysmorphism, learning difficulties and epilepsy will give a higher diagnostic yield, as these features increase the likelihood of an abnormal aCGH result (Jacquemont et al, 2006). In the study by Shen et al, 3.5% of children had dysmorphic features, and the
aCGH yield amongst these patients was 63%. Half of their cohort were children with pervasive developmental disorder not otherwise specified (PDDNOS); these children were not separately identified in the NWTRGS referral population.

3. **Different definitions of autism.** In our audit, relevant clinical notes were not available due to the wide geographical spread of referrers, and therefore we had to rely on the array request form, giving potentially incomplete clinical information. It could also not be determined from the referral form whether autism had been formally diagnosed, which diagnostic criteria had been used (and how expertly applied), or how high the clinical suspicion of an autism spectrum disorder was.

4. **Different assignment of significance to abnormalities.** Interpreting the clinical significance of CNVs is a notoriously difficult area, for which the NWTRGS has its own guidelines (Burbridge & Bewes, 2012). Shen et al did not specify the protocols they used to assign significance. Inheritance of a CNV from phenotypically normal parents is sometimes used as evidence of a benign variant: however, some CNVs can be inherited from parents who are phenotypically normal, but are still thought to be pathogenic in the autistic child – for example, 1q21.1 deletions (Mefford et al, 2012). Additionally, for some CNVs it has been suggested that family members who are ‘normal’ carriers actually display subtle autistic traits when examined carefully (Ullmann et al, 2007).

**Future work up strategies**

Challenges ahead for the community paediatrician and the clinical geneticist alike are changing commissioning arrangements for genetic testing through NHS England, commonly referred to as “mainstreaming of genetics”. Paediatric services will have to consider cost/benefit ratios when requesting aCGH and other genetic testing in the future.

DSM and ICD diagnostic criteria are currently under revision (DSM-V already published) and might re-group diagnostic categories. Changing conceptualisation of autism/ASD and heightened awareness of the ASD phenotype increase referral rates and might broaden the fraction of individuals with ASD and no intellectual disability in the referral population for aCGH.

Subtle autistic traits in parents are well recognised, but these individuals are rarely formally identified and usually not reported as ‘affected’ to the cytogenetic laboratory. If those parents and their offspring diagnosed with ASD share a previously unreported CNV, the molecular cytogeneticist is more likely to interpret this finding as a benign familial variant. The availability of more robust clinical information on specifically designed aCGH referral forms should aid array data interpretation in the future.
Conclusions

- At 3.35%, the diagnostic yield of array CGH for patients referred to the NWTRGS with autism/ASD is lower than would be expected from previous studies. This discrepancy may reflect our unselected referral population.
- In contrast to other countries, NICE does not recommend aCGH as a first line investigation for all individuals with autism/ASD and no associated features. (NICE 2011)
- The use of a variety of array platforms with different resolutions makes it very difficult to compare diagnostic yield data.
- CNV interpretation is a fluent process and current protocols informing interpretation are likely to change with clearer phenotype delineation.
- Detailed and accurate referral information is always vital.

References


Independent on Sunday June 2010: “Autism and genetics: A breakthrough that sheds light on a medical mystery.”


