Clinical genetics evaluation in identifying the etiology of autism spectrum disorders

G. Bradley Schaefer, MD1, Nancy J. Mendelsohn, MD2, and the Professional Practice and Guidelines Committee

**Key Words:** pervasive developmental disorders, tiered evaluations, diagnostic yield, Asperger syndrome

Disclaimer: This guideline is designed primarily as an educational resource for medical geneticists and other health care providers to help them provide quality medical genetics services. Adherence to this guideline does not necessarily assure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the geneticist should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen.

The autism spectrum disorders are a collection of conditions, which have, in common, impaired socialization and communication in association with stereotypic behaviors. The reported incidence of autism spectrum disorders has increased markedly over the past decade. In addition, a large amount of attention has been paid to these conditions among lay and professional groups. These influences have resulted in a marked increase in the number of referrals to clinical geneticists for evaluation of persons with autism spectrum disorders. The primary role of the geneticist in this process is to define etiology, if possible, and to provide counseling and contribute to case management based on the results of such investigations. In deciding upon the appropriate evaluation scheme for a particular patient, the geneticist must consider a host of different factors. Such considerations would include (1) Assuring an accurate diagnosis of autism before proceeding with any investigation. (2) Discussing testing options, diagnostic yields, and patient investment before proceeding with an evaluation. (3) Communication and coordination with the patient’s medical home. (4) Assessing the continuously expanding and evolving list of available laboratory testing modalities in light of evidence-based medicine. (5) Recognizing expanded phenotypes of well-described syndromic and metabolic conditions that encompass autism spectrum disorders. (6) Defining an individualized evaluation scheme based on the unique history and clinical features of a given patient. The guidelines in this article have been developed to assist the clinician in the consideration of these factors. *Genet Med* 2008:10(4):301–305.
ies show a concordance of 70% in monozygotic twins; 90% if the broader phenotypic definition is used. This is in contrast to a 3% concordance in dizygotic twins.2–3

As a group, ASDs occur three to four times more commonly in men. Such a sexual dimorphism suggests that X-linked genes play a major role in the etiology of the spectrum. However, whole genome screens have found only four minor linkages to the X chromosome, and X chromosome genes seem to account for only a small portion of the overall genetic contribution. Evidence of linkage has been found to most autosomes, suggesting marked genetic heterogeneity. The most consistently reported linkages have been with chromosomal locations 15q11-13, 7q 22-31 (two loci with parent of origin effect), 13q, 17q 11 (male-specific locus), 2q, and 16p.6–11

Over the past decade, the reported incidence of ASDs has increased markedly with some estimates suggesting a quadrupling in 10 years. The current estimates for autism are now reported to be on the order of 10–60 per 10,000 individuals, if all forms of ASDs are considered. In fact, the Center for Disease Control and Prevention has recently estimated the prevalence of ASDs in the United States at approximately 5.6 per 1000 (1 of 155 to 1 of 160) children.12,13 This rise in the reported prevalence of ASDs is unlikely to represent a true “epidemic” of the condition as has been suggested by some. Rather, it seems that, this reported increase can be attributed to better knowledge of the disease and its variability, broader diagnostic criteria, improved public and professional awareness, and a higher level of acceptance of the diagnosis.

The role of the clinical geneticist is to determine the etiology of the ASDs, if possible, and to provide counseling for the family. In recent years, there has been an explosion of new diagnostic options and tools available to the clinician. Several recent publications have also reported a host of genetic heterogeneity. The most consistently reported linkages have been with chromosomal locations 15q11-13, 7q 22-31 (two loci with parent of origin effect), 13q, 17q 11 (male-specific locus), 2q, and 16p.6–11

The generally reported rate of success for identifying a specific (unifactorial) diagnosis in persons with autism is 6–15%.20–22

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In deciding upon an evaluation plan, the clinical geneticist has the difficult task of balancing an ever-expanding list of available tests and possible diagnoses with the issues of cost, practicality, and expected yield. The guidelines put forth here outline a strategy of a tiered evaluation of the etiology of autism. These recommendations use evidence-based conclusions from the current available literature and cumulative clinical experience.

Rationale for an Evaluation

The rationale for a clinical genetics evaluation for persons with ASDs has been questioned by some. Concerns have been expressed over the high cost of such an evaluation coupled with the fact that the information obtained typically will not change interventions for the patient. The rationale for performing a clinical genetics consultation for a patient with an ASD is clear to the clinical geneticist. Clinical geneticists can contribute to the process by examining and evaluating the patient, the parents, and siblings, as necessary, in establishing the etiology. A definitive diagnosis helps the patient acquire needed services, and is helpful in many other ways for the family. Many families are greatly empowered by knowledge of the underlying cause of a relative’s disorder. Depending on the etiology, associated medical risks may be identified that lead to screening and the potential for prevention of morbidity. Specific recurrence risk counseling—beyond general multifactorial information—can be provided, and targeted testing of at risk family members can be offered. In a limited number of cases (e.g., metabolic disorders) targeted therapies may be or become available. These significant positive benefits strongly justify a medical genetics consultation for all patients with ASDs. One of the best strategies for integrating clinical genetics services into the care of patients with ASDs can be the participation of the geneticist on an interdisciplinary “autism team.” This allows the geneticist to work alongside other professionals involved in the care of persons with ASDs with access to detailed, specific diagnostic information about the patient.

Reported Approaches and Yields

The generally reported rate of success for identifying a specific (unifactorial) diagnosis in persons with autism is 6–15%.20–22 This range is applicable even for evaluations of patients with PDD (NOS), atypical autism, Asperger syndrome, or autistic features, which did not necessarily meet the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, criteria for PDD. Many factors seem to influence the diagnostic yield. The use of newer diagnostic modalities and the aggressiveness of the evaluation seem to be the most critical. Not surprisingly, the skill and experience of the geneticist also factor heavily into the yield.

A critical review of the potential contribution of newer testing techniques suggests that yield can be significantly higher than 15%. Chromosomal studies are consistently reported as giving one of the highest diagnostic yields in persons with ASDs.23–25 Continued improvements in cytogenetic approaches including higher resolution studies have further increased the diagnostic yield.

Numerous submicroscopic deletions and duplications have been reported in association with an autism phenotype. In general, the most commonly reported loci mirror the reported linkage data. Some of the most frequently reported regions with abnormalities in association with ASDs include 15q pericentromeric11–13 region, 17p11, 22q11, 22q13, and 2q37. Most recently, changes in the 16p11.2 region have been reported as occurring in a significantly high frequency in patients with ASDs—prompting the designation of this region as a “hot spot of genetic instability.”26–28

Currently, array comparative genomic hybridization (aCGH) has emerged as a powerful new tool that promises further revolution of clinical genetic testing. The technology of assessing submicroscopic rearrangements is evolving at a mind-bog-
gling rate. New platforms are being developed at rates faster than clinical studies can define their use. The availability of multiple platforms further complicates the ability to compare studies from various sites. Relatively few studies have been published that provide an actual estimate of the diagnostic yield of aCGH in evaluating patients with autism. One study found a 27.5% yield in the study of aCGH in patients with “syndromic” autism. Preliminary data from many sites suggests that the cumulative yield of aCGH will prove to be the highest yield test that is clinically available. If the estimates of the frequency of the most commonly reported anomalies are pooled, current aCGH platforms can be estimated to identify abnormalities on the order of 10%, beyond what would be identified by standard chromosomal testing (G. Schaefer, unpublished data). Until definitive, large-scale studies provide confirmation of the use of aCGH, its role in the evaluation of ASDs may not be fully appreciated. Realistic predictions suggest that the time in which this will occur may be just a few years.

The strong association of autism with Fragile X syndrome has been confirmed in almost every large reported series. Mutations in the Methyl-CPG-Binding Protein 2 (MECP2) gene are reported in a significant number of women with autism. To date, no male with idiopathic autism has been reported with a mutation in the MECP2 gene. Mutations in the phosphatase and tensin homolog (PTEN) gene are reported to occur frequently in the subgroup of patients with autism with a head circumference 2.5 SDs greater than age-appropriate means. Clinically recognizable syndromes and metabolic disorders are other identifiable causes.

A synthesis of the published literature suggests that the following diagnostic yields would be projected in the genetic evaluation of ASDs:

- High-resolution chromosome studies (5%)
- aCGH—beyond what would be detected by chromosomal analysis (10%)
- Fragile X (5%)
- MECP2 (5%—women only)
- PTEN (3%—if head circumference > 2.5 SDs)
- Other (10%)

Thus, using current knowledge and technology, a thorough clinical genetics evaluation of persons with ASDs will result in a positive answer in up to 40% of individuals.

**EVALUATION SCHEME**

The first (and most critical) step in the clinical genetic evaluation of ASDs is the pre-evaluation. Several pieces of critical information need to be obtained before beginning any investigation. An accurate ASD diagnosis is mandatory. The diagnosis of ASDs should be made by appropriately trained professionals using objective criteria. Normal hearing should be documented because children with significant hearing loss tend to have difficulties with socialization and communication that may be misidentified as autism. Recently published guidelines from the American Academy of Pediatrics suggest that primary care providers obtain chromosome and Fragile X studies at the time an ASD is diagnosed. Thus, part of the evaluation may already have been accomplished before referral to the geneticist.

As with all clinical evaluations, an etiologic evaluation must be tailored to the individual patient. The design of the evaluation must take into consideration focused information from the history and physical as well as clinical experience. There is no single approach or algorithm that can be applied to all cases. For practical reasons, a step-wise (tiered) evaluation is considered by many to be the preferred approach. In general, a non-tiered evaluation in which a large battery of tests is ordered as part of the initial testing scheme is poorly tolerated by the patient and family and less acceptable to third-party payers. A stepwise evaluation can be designed such that tests obtained in higher (earlier) tiers have a greater expected diagnostic yield, lower invasiveness of testing, better potential of intervention, and easier overall practicality. A model for such a tiered evaluation is provided in Table 1.

This scheme will evolve with continued advancements in diagnostic testing and improved understanding of the ASD phenotype. Additional conditions have already been reported in association with an ASD phenotype, but to date none of these have been evaluated in a large prospective cohort. Thus, the possibility of a fourth (extended) tier of evaluation is a distinct possibility in the near future. Alternatively, advances in technology may permit bundling of individual tests into an extended, more readily accessible, and less-expensive platform.

**RECOMMENDATIONS**

1. Accurate diagnosis: It is critically important that a firm diagnosis of ASD is made before initiating any genetic evaluation. Although the diagnosis of autism may seem straightforward, many neurodevelopmental disorders have overlapping phenotypes. The diagnosis should be made by a professional trained in the diagnosis of autism. The patient’s parents, siblings, and offspring may also need to be evaluated. Objective criteria with the application of generally accepted tools should be used. All persons with apparent autism should have a formal audiogram to rule out a significant hearing loss.

2. Role of the primary care physician: All persons with autism should have a designated primary care physician (PCP). Often the PCP will be the first professional to raise the question of ASD as a possible diagnosis. Depending on training and comfort level, the PCP may be prepared to make a diagnosis of an ASD. Alternatively, the PCP may make a referral to a school team or mental health professional for diagnostic confirmation. Recent guidelines from the American Academy of Pediatrics suggest that the PCP obtain high-resolution chromosome studies (peripheral karyotype) and Fragile X studies when the diagnosis of an ASD is confirmed. After clinical genetics...
consultation, the PCP and the clinical geneticist should be prepared to partner in ordering, scheduling, and coordination of recommended diagnostic testing.

3. Referral for clinical genetics evaluation: Defining the etiology of an ASD can be of great benefit to the patient and family. Information gained from an identified etiology can help with family counseling, medical management, preventative health strategies, and empowerment of the family. Clinical geneticists have much to offer in this process beyond the initial assessments made by the PCP or mental health professionals working with the individual or family with ASD. As such, a genetic consultation should be offered to all persons and families with ASDs. Evaluations should be considered for any individual along the full autism spectrum. The referring professional should discuss expectations and possible outcomes of such an evaluation before making the referral. The referring professional should be aware of what is involved in such a consultation and the potential diagnostic yields and share this information with the patient and family.

4. Tiered evaluation: The clinical genetic evaluation of an individual with an ASD must be customized to the clinical situation. A patient may be referred to the geneticist with a specific diagnosis that is being considered—seeking confirmation. Alternatively, a syndromic diagnosis may be apparent to the geneticist upon the initial visit. In either case, the diagnosis should be confirmed using accepted clinical criteria and laboratory testing (if available). Many recognizable syndromes have a firmly documented association with autism. For these conditions, further investigation into the etiology of the ASD is unnecessary. There are, however, genetic conditions that have been reported in association with ASDs in which the reported association is not as convincing. For patients with these conditions, it is recommended that an etiologic evaluation for the ASD proceed as an independent condition. Table 2 provides a partial list of these two groups of conditions. If the geneticist does not identify a specific disorder upon the initial evaluation, further testing can be accomplished as outlined in Table 1.

5. Counseling: Upon completion of the clinical genetics evaluation, two groups of individuals will have been identified: those with and those without an identifiable major single etiology. Definitive counseling should be provided to both groups. For those without an identifiable etiology, counseling should be provided for multifactorial inheritance. The best available published empirical recurrence risks for full siblings are 4% if the affected child is a girl and 7% if the affected child is a boy. If a second child has autism, the reported recurrence risk has been from 25% to 50%. A reasonable synthesis of published reports would be around 30%.

6. Follow-up: Clinical geneticists differ greatly in their degree of involvement with patients after completion of diagnostic consultations. Intervening changes in technology and in phenotypes often aid in ultimately obtaining a diagnosis. At a minimum, periodic reevaluations should be considered for patients in whom a definitive etiology is not initially discovered. The timing of interval follow-ups should be a negotiation between the patient and family, the PCP, and the geneticist.
Table 2
Partial list of genetic syndromes with a reported association with autism

<table>
<thead>
<tr>
<th>No work-up indicated</th>
<th>Autism evaluation indicated</th>
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<tbody>
<tr>
<td>Fragile X syndrome</td>
<td>Apert syndrome</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>Williams syndrome</td>
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<tr>
<td>Angelman syndrome</td>
<td>Joubert syndrome</td>
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<tr>
<td>Prader-Willi syndrome</td>
<td>Noonan syndrome</td>
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<tr>
<td>Smith-Lemli-Opitz syndrome</td>
<td>Down syndrome</td>
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<tr>
<td>Smith-Magenis syndrome</td>
<td>Turner syndrome</td>
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<tr>
<td>Tuberous sclerosis</td>
<td>Neurofibromatosis</td>
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<tr>
<td>PTEN associated disorders (Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome)</td>
<td>Myotonic dystrophy, Duchenne dystrophy</td>
</tr>
<tr>
<td>Shprintzen syndrome (22q11 deletions)</td>
<td>Moebius anomalad</td>
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<tr>
<td>Sotos syndrome</td>
<td>Cohen syndrome</td>
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<tr>
<td>CHARGE syndrome</td>
<td>Oculo-auriculo-vertebral spectrum</td>
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<tr>
<td>Hypomelanosis of Ito</td>
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<tr>
<td>Lujan-Frys syndrome</td>
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<tr>
<td>De Lange syndrome</td>
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</table>


RESOURCES
Autism Society of America. Available at: http://www.autism-society.org/site/PageServer

References

M. Schaefer et al.
Phenomic determinants of genomic variation in autism spectrum disorders


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Phenomic determinants of genomic variation in autism spectrum disorders

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ABSTRACT

Background: Autism spectrum disorders (ASDs) are common, heritable neurobiologic conditions of unknown etiology confounded by significant clinical and genetic heterogeneity.

Methods: This study evaluated a broad categorisation of phenotypic traits (or phenome) for 100 subjects with Autism Diagnostic Interview-Revised/Autism Diagnostic Observation Schedule-General (ADI-R/ADOS-G) confirmed idiopathic ASD undergoing 1 Mb bacterial artificial chromosome (BAC) array comparative genomic hybridisation (CGH).

Results and conclusions: Array CGH uncovered nine different pathogenic copy number variants (pCNVs) in 9/100 ASD subjects having complex phenotypes (ASD ± intellectual disability (ID; IQ < 70)) and/or physical anomalies, normal karyotype, fragile X analysis, and comprehensive evaluation by a clinical geneticist. Unique pCNVs in our cohort included del(5)(p15.2p15.31) (2.4 Mb), del(3)(p24.3) (0.1 Mb), and dup(18)(p11.3) (0.9 Mb). Five pCNVs were recurrent in our cohort or were previously described in subjects with ASD ± ID: del(7)(q11.23) (1.5 Mb); del(2)(p15p16.1) (6.1 Mb and 7.9 Mb); del(14)(q11.2) (0.7 Mb) and dup(11)(q11q13) (10 Mb), del(3)(q27.3) (470 Kb) in two autistic brothers. Male: female distribution in subjects with pCNVs was reduced to 1.25:1 from 3.2:1 in the original cohort. The authors stratified the study population according to a broad spectrum of clinical features and correlated specific phenotypes with respect to CNV load and pathogenicity. The findings indicate increased prevalence of pCNVs in subjects with microcephaly (<2nd centile; n = 2 of 4 ASD subjects with microcephaly; p = 0.04), and ID (n = 9 of 64 subjects with ASD and ID; p = 0.02). Interestingly, in the absence of ID co-morbidity with an ASD, no pCNVs were found. The relationship between parental ages at delivery and CNV load and pathogenicity was also explored.

Autism spectrum disorders (ASDs) are a group of common neurodevelopmental conditions characterised by impairments in communication, social interaction, and behaviour. At least one of every 166 children is likely to be affected by this spectrum of disorders, with a male: female (M:F) ratio of 4:1,1 and it is the most heritable of all complex neuropsychiatric conditions.2 Since ASDs are considered complex genetic disorders, resulting from the interaction of several genes and environmental factors, the lumping together of all cases of ASD, with no subgrouping based on phenotypic characteristics, makes the identification of contributory genes extremely difficult. The fact that autism is known to be associated with several distinct medical/genetic disorders further highlights its genetic heterogeneity. Thus, comprehensive “whole body” phenotyping and more accurate diagnostic methods are necessary to clarify the underlying comorbidities, causes and symptoms of ASDs—inclusive of neurobehavioral, medical and morphologic traits.

A variety of different approaches to identify genes for ASDs have been undertaken, including cytogenetic assessment for chromosome abnormalities, genome scan linkage studies, and association studies (see Freitag3 for review). The overlap of results among different study methods is limited, largely attributed to the significant clinical and genetic heterogeneity of ASDs based on variable behavioural indices, variations among study populations, and limitation of methods for detecting ASD susceptibility genes of mild to moderate effect.

Most recently, array comparative genomic hybridisation (CGH) technology has been used to screen rapidly the genome for pathogenic copy number variants (pCNVs) associated with ASD.4–8 pCNVs are more prevalent in “complex” syndromic ASDs (27.5%) manifesting intellectual disability (ID; IQ < 70) and/or dysmorphic features4 than in non-syndromic (simple or idiopathic) cases (7.2%)4 manifesting neurobehavioral features alone. Relatively consistent frequencies have been noted in studying simplex (single incidence; SPX) (7–10%)4,6 and multiplex (multiple incidence; MPX) families (2–12%) across different studies.5,8 While current data suggest that pCNVs contribute to ASD pathogenesis, their role within a growing constellation of ASD microdeletion and microduplication syndromes remains poorly understood, due to the absence of consistent, standardised and comprehensive somatic, medical and neurobehavioral phenotyping of ASD subjects.

To address this, we selected 100 subjects with “complex” ASD scores of ≥3 based on criteria modified from de Vries et al4 for array CGH screening for CNVs, and here summarise our systematic categorisation of the broad spectrum of clinical features present in those individuals with and without pCNVs. We stratified our findings with CNV type (putatively pathogenic or benign) and total CNV load and reviewed detailed prenatal, medical, developmental and multi-generation family histories, assigning subjects to specific phenotypic subgroups based on comorbidity with: (1) ID (IQ < 70); (2) presence of prenatal (ultrasound detected) and/or postnatal growth anomalies; (3) micro- or macrocephaly; (4) epilepsy; (5) craniofacial dysmorphisms; (6) congenital physical or systemic
anomalies, (7) pregnancy complications; and (8) neonatal complications. We believe that characterisation of the sum phenomic and genomic determinants of ASD will make the identification of contributory genes possible and the correlation of genetic changes with clinical features more meaningful.

SUBJECTS AND METHODS

Subjects
In the course of systematic medical genetic evaluation of subjects with an ASD recruited through the research registry of the Autism Spectrum Disorders-Canadian American Research Consortium (ASD-CARC; www.AutismResearch.com), 100 subjects with complex features of idiopathic ASD (76 males and 24 females) scoring ≥3 using phenotype criteria modified from de Vries et al5 were randomly selected for array CGH analysis.

All subjects underwent comprehensive screening of medical systemic and morphological features. ASD diagnoses for all subjects were based on standardised Diagnostic and statistical manual, 4th ed (DSM-IV-TR) criteria using Autism Diagnostic Interview-Revised (ADI-R) and/or Autism Diagnostic Observation Schedule-Generic (ADOS-G) standards.10 11 including a variety of measures of cognitive and adaptive function to assess the presence or absence of ID (Leiter International Performance scale-revised; Stanford Binet Intelligence Scale, 4th ed; Weschler Intelligence Scale, 4th ed; Vineland Adaptive Performance scale-revised; Stanford Binet Intelligence Scale, 4th ed; Weschler Intelligence Scale, 4th ed; Vineland Adaptive Behaviour Scales, etc).

A standardised metric of clinical characteristics9 was assigned by the evaluating clinical geneticist (MESL) blinded to the CNV findings. Among the 100 subjects studied, 31 were from SPX families, 45 from MPX-immediate families (MPX-I: sharing an affected family member within the deleted/duplicated genomic regions). Subjects in our study were almost 10 times more likely to present with macrocephaly (OFC, occipitofrontal circumference). Subjects in our study were almost 10 times more likely to present with macrocephaly (OFC, occipitofrontal circumference). Subjects in our study were almost 10 times more likely to present with macrocephaly (OFC, occipitofrontal circumference). Subjects in our study were almost 10 times more likely to present with macrocephaly (OFC, occipitofrontal circumference). Subjects in our study were almost 10 times more likely to present with macrocephaly (OFC, occipitofrontal circumference). Subjects in our study were almost 10 times more likely to present with macrocephaly (OFC, occipitofrontal circumference). Subjects in our study were almost 10 times more likely to present with macrocephaly (OFC, occipitofrontal circumference).

Array CGH
Genomic DNA was extracted from peripheral blood using PUREGENE DNA Isolation Kits (Genta, Minneapolis, Minnesota, USA). A pool of normal male or female control DNAs (Promega, Madison, Wisconsin, USA) was used as reference DNA to match the sex of the samples studied. The 1 Mb bacterial artificial chromosome (BAC) array CGH (Spectral Genomics (SG), Houston, Texas, USA) was performed as previously described.12 High resolution genome-wide human single nucleotide polymorphism (SNP) array 6.0 was performed by Affymetrix for subjects 1 and 2 to refine physical breakpoints further.

Databases cataloguing putatively benign CNVs (Database of Genomic Variants, DGV, http://projects.tcag.ca/variation/) and pathogenic CNVs (Decipher, https://decipher.sanger.ac.uk/) were used to interpret the significance of CNVs. CNVs that were reported in at least two independent studies of healthy control subjects are typically referred to as benign copy number variants (bCNVs).

FISH
FISH analyses were performed using the array CGH identified BAC DNA clones (SG-PerkinElmer: http://www.perkinelmer.com or The Centre for Applied Genomics, TCAG: http://www.tcag.ca/) to confirm the deletions and duplications, as described previously.13 Slides were viewed on a Zeiss Axioplan 2 fluorescence microscope and images captured using MacProbe software (Applied Imaging, Santa Clara, California, USA).

RESULTS

Cohort and clinical demographics
To reduce clinical heterogeneity, we stratified our 100 subjects with ASDs into phenomic subgroups based on a broad spectrum of clinical comorbidities (table 1).

Our cohort had an overall M:F ratio of 3:2:1 with noted comorbidities including epilepsy (21%), ID (64%), minor craniofacial anomalies (36%), and various medical or congenital systemic anomalies (77%) (table 1). Subjects in our study were almost 10 times more likely to present with macrocephaly (occipitofrontal circumference (OFC) >98th centile), as opposed to microcephaly (OFC <2nd centile).

Interestingly, 14% and 21% of subjects had birth weights (BW) ≤5th centile or ≤10th centile, respectively, as compared to the general population (both p = 0.05). However, our results do not confirm an association of low BW with the presence of pCNVs, even when ≤5th and ≤10th centile BW cohorts are combined.

Also of note is a significantly higher percentage of individuals with height measuring ≥98th centile (11%, p = 0.02) or weight measuring ≥98th centile (16%, p = 0.0008). However, no association was found between enlarged growth parameters and pCNV frequency.

A detailed list of these parameters and their respective prevalence in our cohort is shown in table 1.
Characterisation of CNVs in ASD subjects

Among our 100 subjects with idiopathic complex ASDs, we identified 67 non-redundant CNV loci with nine pCNVs in nine cases (9%) and three CNVs of unknown significance in three subjects (table 2).

In concordance with current criteria, we consider the nine pCNVs to be pathogenic because: (1) they were not reported in our control cohorts or in the DGV (http://projects.tcag.ca/variation/); (2) they were either de novo, or maternally inherited X-linked CNVs in male offspring (that is, 470 Kb del (X)(p11.22)); or (3) occurred within reported ASD loci (that is, 1.5 Mb dup(7)(q11.23))

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>No. of cases with the phenotype (M:F ratio)</th>
<th>No. (%) of cases with pCNVs having the phenotype</th>
<th>No. (%) of cases without pCNVs having the phenotype</th>
<th>p Value</th>
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<tr>
<td>Total cases</td>
<td>100</td>
<td>9 (56%)</td>
<td>71 (78%)</td>
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<td>Gender</td>
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</tr>
<tr>
<td>Male</td>
<td>76</td>
<td>5 (56%)</td>
<td>71 (78%)</td>
<td>0.21</td>
</tr>
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<td>Female</td>
<td>24</td>
<td>4 (44%)</td>
<td>20 (22%)</td>
<td>0.21</td>
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<td>ID*</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ID (IQ&lt;70)</td>
<td>64 (2.2:1)</td>
<td>9 (100%)</td>
<td>55 (64%)</td>
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<td>No ID (IQ&gt;70)</td>
<td>31 (6.8:1)</td>
<td>0</td>
<td>31 (34%)</td>
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<tr>
<td>IUGR†</td>
<td>7 (2.5:1)</td>
<td>1(11%)</td>
<td>6 (7%)</td>
<td>0.49</td>
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<td>Postnatal</td>
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<tr>
<td>Small stature*</td>
<td>11 (4.5:1)</td>
<td>1 (11%)</td>
<td>10 (11%)</td>
<td>0.67</td>
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<td>Weight &lt;3rd centile</td>
<td>3 (3:1)</td>
<td>0</td>
<td>3 (3%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Height &lt;3rd centile</td>
<td>8 (3:1)</td>
<td>0</td>
<td>8 (9%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Large stature**</td>
<td>18 (3.5:1)</td>
<td>1 (11%)</td>
<td>17 (19%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Weight &gt;98th centile††</td>
<td>16 (4.3:1)</td>
<td>1 (11%)</td>
<td>15 (16%)</td>
<td>0.61</td>
</tr>
<tr>
<td>Height &gt;98th centile††</td>
<td>11 (4.5:1)</td>
<td>1 (11%)</td>
<td>10 (11%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Microcephaly (OFC &lt;2nd centile)</td>
<td>4 (1:1)</td>
<td>2 (22%)</td>
<td>2 (2%)</td>
<td>0.045</td>
</tr>
<tr>
<td>Macrocephaly (OFC &gt;98th centile)</td>
<td>38 (2.5:1)</td>
<td>1 (11%)</td>
<td>37 (41%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>21 (3.2:1)</td>
<td>3 (33%)</td>
<td>18 (20%)</td>
<td>0.28</td>
</tr>
<tr>
<td>&gt;2 Craniofacial dysmorphisms</td>
<td>86 (2.6:1)</td>
<td>9 (100%)</td>
<td>77 (85%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Systemic congenital anomalies††</td>
<td>77 (3.5:1)</td>
<td>6 (67%)</td>
<td>71 (78%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Pregnancy complications‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>53 (3.4:1)</td>
<td>6 (67%)</td>
<td>47 (52%)</td>
<td>0.31</td>
</tr>
<tr>
<td>&gt;3</td>
<td>14 (2.5:1)</td>
<td>2 (22%)</td>
<td>12 (13%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Neonatal complications§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>36 (4.1:1)</td>
<td>3 (50%)</td>
<td>33 (38%)</td>
<td>0.42</td>
</tr>
<tr>
<td>&gt;2</td>
<td>20 (4:1)</td>
<td>2 (33%)</td>
<td>18 (20%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤3rd centile</td>
<td>8 (3:1)</td>
<td>0</td>
<td>8 (9%)</td>
<td>0.5</td>
</tr>
<tr>
<td>≤5th centile</td>
<td>14 (3.7:1)</td>
<td>2 (22%)</td>
<td>12 (13%)</td>
<td>0.31</td>
</tr>
<tr>
<td>≤10th centile</td>
<td>21 (4.3:1)</td>
<td>2 (22%)</td>
<td>19 (21%)</td>
<td>0.54</td>
</tr>
<tr>
<td>&gt;90th centile</td>
<td>8 (7:1)</td>
<td>0</td>
<td>8 (9%)</td>
<td>0.5</td>
</tr>
<tr>
<td>&gt;95th centile</td>
<td>5 (4:1)</td>
<td>0</td>
<td>5 (5%)</td>
<td>0.65</td>
</tr>
<tr>
<td>&gt;97th centile</td>
<td>4 (3:1)</td>
<td>0</td>
<td>4 (4%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Phanotype score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17 (3.3:1)</td>
<td>1 (11%)</td>
<td>16 (18%)</td>
<td>0.53</td>
</tr>
<tr>
<td>&gt;4</td>
<td>82 (3.2:1)</td>
<td>8 (9%)</td>
<td>75 (82%)</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

OFC, occipitofrontal circumference.
*ID, intellectual disability; IQ<70. Unknown for 5 cases.
†IUGR, intrauterine growth retardation; confirmed via standardised ultrasound mediated fetal biometry indicating fetal weight below the 10th centile for gestational age and abdominal circumference below the 2.5th centile.
‡(1) amniotic fluid anomalies (poly-, oligohydramnios); (2) morphologic fetal anomalies detected on ultrasound (club feet, cleft lip, etc); (3) placental abruption; (4) spontaneous premature labour (~37 weeks); (5) very-small-for-gestational-age (birthweight <3rd centile); (6) vaginal bleeding at any time point; (7) decreased fetal movements; (8) eclampsia/hypertension.
†(1) Respiratory distress requiring oxygen; (2) feeding difficulties (including lactose and gluten intolerance); (3) failure to thrive; (4) neonatal seizure.
*Small stature: weight, height and OFC all ≤3rd centile.
**Large stature: weight, height and OFC all ≥98th centile.
††Including major and minor non-craniofacial congenital anomalies (for example, visual, hearing, neuromotor, thoracic, cardiac, abdominal, urogenital, limb, hand, feet, skin, musculoskeletal, spinal, central nervous system structural) guided by morphologic indices coded in the London Dysmorphology Database (LDDDB) (London Medical Databases Ltd, London, UK), similarly adopted for the evaluation of all craniofacial features.
‡‡Significantly different from normal curve (p = 0.05 for both centile groups).
§Indicates the p value rendering significance (p<0.05).
**Significantly above normal height and weight growth curves (height p = 0.02, weight p = 0.0008).
Abnormal copy number variants (CNVs) detected in 100 subjects with autism spectrum disorders (ASDs) using array comparative genomic hybridisation (CGH)

<table>
<thead>
<tr>
<th>Group</th>
<th>CNV and cytoband</th>
<th>Subject</th>
<th>Origin</th>
<th>Size (Mb)</th>
<th>Minimal genomic region (bp)</th>
<th>Secondary validation</th>
<th>No. of genes</th>
<th>Candidate gene and function*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic CNVs</td>
<td>del 2p15-16.1</td>
<td>1</td>
<td>De novo</td>
<td>6.1</td>
<td>56,773,497/62,885,689</td>
<td>FISH; RT-qPCR; Affymetrix 6.0 SNP array</td>
<td>18</td>
<td>XPD: protein transporter activity. REL: transcription factor activity</td>
</tr>
<tr>
<td></td>
<td>del 2p15-16.1</td>
<td>2</td>
<td>De novo</td>
<td>7.9</td>
<td>55,481,143/63,372,980</td>
<td>FISH; RT-qPCR; Affymetrix 6.0 SNP array</td>
<td>30</td>
<td>OXY1: brain development. CDCDC88A: microtubule binding</td>
</tr>
<tr>
<td></td>
<td>del 3p24.3-25</td>
<td>3</td>
<td>De novo</td>
<td>0.2</td>
<td>15,780,358/15,940,642</td>
<td>FISH</td>
<td>1</td>
<td>ANKRDB2: protein binding</td>
</tr>
<tr>
<td></td>
<td>del 5p15.2-15.31</td>
<td>3</td>
<td>De novo</td>
<td>2.4</td>
<td>9,334,790/11,738,791</td>
<td></td>
<td>1</td>
<td>CTNN2: neuron adhesion. SEMA5A: neuron adhesion</td>
</tr>
<tr>
<td></td>
<td>dup 7q11.23</td>
<td>4</td>
<td>Unknown</td>
<td>1.5</td>
<td>72,200,000/73,767,523</td>
<td>RT-qPCR</td>
<td>27</td>
<td>GTF2I: signal transduction. STX1A: synaptic transmission</td>
</tr>
<tr>
<td></td>
<td>del 14q11.2</td>
<td>5 and 6</td>
<td>De novo†</td>
<td>0.8</td>
<td>19,570,792/20,341,734</td>
<td>FISH; RT-qPCR</td>
<td>27</td>
<td>TEP1: telomerase activity. APEX1: protein binding</td>
</tr>
<tr>
<td></td>
<td>dup 15q11-13</td>
<td>5 and 6</td>
<td>De novo†</td>
<td>−10</td>
<td>20,538,416/30,830,821</td>
<td>FISH; RT-qPCR</td>
<td>75</td>
<td>UBE3A: brain development. NIPA1: neuronal development</td>
</tr>
<tr>
<td></td>
<td>dup 18p11.3</td>
<td>7</td>
<td>De novo</td>
<td>0.9</td>
<td>5,910,725/6,063,460</td>
<td>RT-qPCR</td>
<td>1</td>
<td>L3MBTL4: cell adhesion</td>
</tr>
<tr>
<td></td>
<td>del Xp11.22</td>
<td>8 and 9</td>
<td>Maternal</td>
<td>0.5</td>
<td>53,970,960/54,326,640</td>
<td>RT-qPCR</td>
<td>3</td>
<td>PHF8: midline formation. WNK3: neuronal excitability</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CNVs of unknown significance</th>
<th>Group</th>
<th>Subject</th>
<th>Origin</th>
<th>Size (Mb)</th>
<th>Minimal genomic region (bp)</th>
<th>Secondary validation</th>
<th>No. of genes</th>
<th>Candidate gene and function*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>del 4q32.3</td>
<td>10</td>
<td>Maternal</td>
<td>0.2</td>
<td>165,338,176/165,541,022</td>
<td>RT-qPCR</td>
<td>1</td>
<td>ANP32C: protein binding</td>
</tr>
<tr>
<td></td>
<td>del 13q22.2-33.1</td>
<td>11</td>
<td>Paternal</td>
<td>0.2</td>
<td>100,477,931/100,637,276</td>
<td>RT-qPCR</td>
<td>1</td>
<td>NALCN: neuronal excitability</td>
</tr>
<tr>
<td></td>
<td>dup Yp11.22</td>
<td>12</td>
<td>Unknown</td>
<td>0.2</td>
<td>16,849,553/17,076,671</td>
<td>RT-qPCR</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridisation; RT-qPCR, real-time quantitative polymerase chain reaction; SNP, single nucleotide polymorphism.

*Indicates the top two most likely candidate genes identified by Endeavour (http://www.bits.vib.be/endeavour/).

†Indicates a familial change, maternally transmitted in an unbalanced fashion to each proband of an aunt-niece relationship as a consequence of their mothers being balanced carriers of a cryptic translocation t(14;15)(q11.2;q13.3).

*Indicates genes previously implicated in ASD (see text for references).

Correlation of pCNVs with specific phenotypes

We compared the presence of pCNVs in a study of 100 ASD subjects with standardised phenotypic subtypes utilising a scoring metric modified from de Vries et al. to compare cases with and without pCNVs. Of all clinical features interrogated, microcephaly (OFC < 2nd centile) and ID (IQ < 70) severity were found to be most significantly associated with the identification of pCNVs (fig 1, table 1). Two of the nine cases with pCNVs (22.2%) had microcephaly versus only 2/91 (2.2%) cases without pCNVs (p = 0.04) (fig 1). All subjects with pCNVs had comorbidity with ID (9/9; 64%) subjects without pCNVs had ID (p = 0.02), whereas no pCNVs were found in the absence of ID comorbidity (n = 31/86) (fig 1, table 1). Seven of nine cases with pCNVs (78%) were found to have moderate to severe ID (IQ < 50) versus 35/86 (41%) for patients without pCNVs (p = 0.04; data not shown). A relative risk (RR) for harbouring pCNVs in patients with ID versus those without could not be computed, since no pCNVs were identified in the 31 subjects without ID (thus rendering an RR equal to infinity). Microcephaly was not a sensitive clinical parameter listed in table 1 and the presence or absence of ID revealed low specificity for pCNVs (36%, 95% CI 4% to 60%), but was highly specific (98%, 95% CI 92% to 100%). The presence of ID revealed low specificity for pCNVs (56%, 95% CI 26% to 47%) but was highly sensitive (100%, 95% CI 63% to 100%). All cases with microcephaly also presented with ID. No other clinical features were found to be more prevalent in subjects with pCNVs.

Correlation of total CNV load with clinical phenotypes

Total CNV load, including benign, pathogenic and CNVs of unknown significance, was determined for each ASD proband and correlated with clinical phenotypes. Subjects with a clinical phenotype score ≥4 had a significantly higher tendency to harbour at least one CNV (76/83, 91.6%) compared to those with cut-off scores of 3 (12/17, 70.6%) (p = 0.03), as originally described by de Vries.3 There were no significant differences between any clinical parameter listed in table 1 and the presence or absence of CNVs. No significant difference in the number of CNVs was found in groups of ASD subjects divided by family type (SPX or MPX), and we did not find any correlation between the number of CNVs/person and the presence of pCNVs.
### Table 3  Clinical features of subjects with abnormal copy number variants (CNVs)

<table>
<thead>
<tr>
<th>Subject</th>
<th>With pathogenic CNVs</th>
<th>With CNVs of unknown significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Abnormal CNV(s) detected</td>
<td>del(2)(p15.1p16.1)</td>
<td>del(2)(p15.1p16.1)</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Family type</td>
<td>SPX</td>
<td>SPX</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Autistic disorder</td>
<td>Autistic disorder</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>White</td>
<td>Mixed bi-racial</td>
</tr>
<tr>
<td>Microcephaly (&lt;2nd centile)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Macrocephaly (&gt;98th centile)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Prenatal GR</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Postnatal GR (&lt;5%)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Postnatal large stature (&gt;98%)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>≥2 CDs</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>≥2 SCAs</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenotype Score</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>ID severity§</td>
<td>Severe</td>
<td>Severe</td>
</tr>
</tbody>
</table>

CDs, craniofacial dysmorphisms; F, female; GR, growth retardation; ID, intellectual disability (IQ < 70); M, male; SCAs, systemic congenital anomalies; + presence of the phenotype; – absence of the phenotype.

*In years at time of clinical evaluation; age ranges have been provided rather than specific ages for anonymisation purposes.
†Aunt of subject 6
‡Brother of subject 9
§Definition of ID severity according to IQ level: severe = IQ scores between 20 and 35; moderate = IQ scores between 35 and 50; mild = IQ scores between 50 and 70.
Correlation of CNVs with gender
A decreased M:F ratio was found in pCNV cases compared to the initial sample set (1.25:1 versus 3.2:1) (table 2). The nine pCNVs were found in five of 76 male (6.6%) and four of 24 (16.7%) female ASD subjects (p = 0.21). No significant association was found between gender and phenotype scores (p = 0.25). However, there was a higher percentage of cases with >2 craniofacial dysmorphisms (100%) in females, compared to 62/76 (81.6%) males (p = 0.04). We examined whether gender was associated with co-occurrence of ID and found an increased M:F sex ratio in 31 ASD subjects without ID (6.75:1), but only a trend towards association between ID and female gender (p = 0.06). No other major somatic phenotype was associated with one specific gender. Finally, no significant difference in the total number of CNVs was found between males and females (p = 0.28).

Correlation of CNVs with parental age
We applied different statistical methods to delineate if there was any association between the presence of pCNVs in autistic offspring according to parental age at the time of birth. Surprisingly, lower paternal age was associated with increased pCNV frequency in children of fathers under 30 years of age (n = 20) (RR 2.66, 95% CI 1.17 to 6.04; p = 0.02). The same was true for children born to fathers under age 35 years, but the relationship was weaker (n = 52) (RR 1.65, 95% CI 1.19 to 2.30). Although lower maternal age was not as strongly associated with pCNVs in their offspring, the relative risk was significantly above 1 for mothers under age 30 years (n = 40) (RR 1.92, 95% CI 1.19 to 3.10). Next we examined total CNV load in relation to parental ages and found that offspring of both fathers and mothers under the age of 30 and 35 years, respectively, had significantly more CNVs (benign and pathogenic) than children of older parents (p = 0.02 and p = 0.05, respectively).

DISCUSSION
To our knowledge, this is the first report describing detailed correlation analyses between standardised ‘‘whole body’’ phenotypes and CNVs in ASD subjects. Of the indices examined, only microcephaly and ID were significantly associated with the presence of pCNVs. Furthermore, we also found that subjects with pCNVs have a much higher percentage of cases with moderate to severe ID (IQs between 20–50) than subjects without pCNVs. A recent study of ID subjects found that those with moderate to severe ID were twice as likely to harbour pCNVs as those with borderline to mild ID.21 Our data agree with this determination as 7/42 (16.7%) of our ASD probands with moderate to severe ID had detectable pCNVs in comparison to 2/22 (9%) of subjects with mild ID (IQs between 50–70). Our results thus suggest that microcephaly and IDs may represent a strong phenomic predictor of pCNV risk in persons with ASDs.

It is interesting to note that macrocephaly or seizures, both commonly described with ASDs, did not associate with the presence of pCNVs, even if subjects in our cohort were almost 10 times more likely to manifest macrocephaly as compared to microcephaly. Other reports have also showed that macrocephaly and seizures are not good aetiological indicators of ASDs.22 23 Extremely low (<3rd centile) or high (>98th centile) birth weights did not associate with significant pCNV risk, although smaller babies (<5th and <10th centile birth weight for gestational age) were overrepresented in our cohort. Prenatal and postnatal growth abnormalities, craniofacial dysmorphisms, systemic anomalies, pregnancy and neonatal complications also did not associate with significant pCNV risk.

Our study indicates that the standardised phenotype scoring system of de Vries et al24 does not help better predict the presence of pCNVs in persons with ASDs, as there was no correlation between increased phenotype scores and pCNV presence. Our
results may be confounded by our population cohort, which focused on the analysis of persons with more complex forms of idiopathic ASD. However, when considering CNV load we found that the group with greater phenotypic complexity had a greater chance of harbouring at least one CNV. Increased CNV burden may be relevant in contributing to a “network” effect of CNVs, and/or “additive aneuploidy effect” where the sum of genes that are lost or gained contributes to a more severe clinical phenotype.19 These findings were obtained using the 1 Mb resolution SG array and warrant further studies using higher resolution arrays and a different template for phenotypic evaluations incorporating ID and microcephaly as key components.

Interestingly, we observed that children born to younger fathers had a higher prevalence of pCNVs. A similar relationship for maternal age was present, albeit weaker. Consistent with this is our finding that children of younger parents (fathers under age 30 and mothers under age 35 years) also have a significantly higher total CNV load than children born to older parents. A number of epidemiological studies have shown different results for the distribution of parental ages in ASD cohorts in comparison with cohorts of typically developing individuals.20–26 While some studies have found higher maternal and/or paternal ages in ASD cohorts,25 other studies have showed a “U shaped” distribution of parental ages relative to the offspring’s risk for ASDs or other genetic conditions.26 These findings are counterintuitive to the expected paradigm underscoring increased age with markers of genomic instability including point mutations and altered DNA methylation.27–29 Increased pCNV risk to offspring of younger fathers could possibly result from the immaturity of spermatids or low activity of DNA repair or antioxidant enzymes in younger parents.29 Our findings compare individuals with and without pCNVs from a population cohort of persons with phenotypically complex ASDs and thus should be interpreted with caution until larger ASD and control cohorts can be similarly studied.

In concordance with other reported array based pCNVs studies in ASDs, we found a similar pCNV detection rate (9%) and a decreased M:F sex ratio among subjects with pCNVs from the original sample cohort (1.25:1 vs the original cohort 3.2:1) (table 4).4–6 The observed rates of pCNVs in SPX (6.5%) and MPX-I (2.2%) families are in general agreement with those previously reported (~7–10% and 2–3% in respective family types).4–6 Probands from MPX-E families had a higher pCNV frequency (12.5%) than observed in our own or previously reported SPX or MPX-I families.4–6 pCNV frequency in probands from MPX-E families have not been previously reported, since most studies have focused on affected members (that is, sibling pairs) from the immediate family. Failure to record information about extended family histories could lead to the misclassification of MPX-E cases as SPX individuals.

Although female gender did not directly associate with the presence of detectable pCNVs, we did find a decreased M:F ratio in subjects with pCNVs (1.25:1) versus those without pCNVs (3.6:1), a trend that has been reported in every other large scale CNV study of ASDs (table 4).4–6 This strongly suggests that pCNVs may play a substantial role in determining the ASD phenotype and more equally contribute to ASD risk in both genders, due to increased penetrance.6 These pCNV changes could also encompass genes for one or more sex limited ASD traits within the heterogeneous ASD population. Furthermore, our findings of an increased M:F ratio in subjects without ID (6.75:1), and of females with an ASD more commonly manifesting two or more craniofacial dysmorphisms, are consistent with clinical morphology research that shows that more simple forms of ASD have a significantly higher male-to-female ratio than complex subgroups with abnormal physical phenotypes.30

It is reasonable to assume that the genes within or close to the pCNVs are the most likely ASD related candidate genes. Close to 200 genes are contained within the pCNV loci identified in our study (table 2). We applied the computational candidate gene prioritisation software, Endeavor, to assist with further pinpointing potential ASD related genes that share similarity to selected autism related reference genes.13 Different from other gene prioritisation tools, the publicly available Endeavor software can access many more data sources (currently up to 20 for Homo sapiens) including categories of functional annotations, protein interactions, expression profiles,
regulatory information, sequence based and text mining data\textsuperscript{15} with all referenced information validated by in vivo experiments.\textsuperscript{15} However, the rank of prioritised genes in a specific locus is mainly dependent on the training set of genes and the selection of models in the database. We used a list of 19 autism related genes\textsuperscript{16} as our training set and selected all models in our analysis. Among the top two prioritised genes in each loci in table 2, most were involved in either mammalian nervous system development and/or neuronal excitability—for example, \textit{WNK3} and \textit{NACL1}—or have been reported to be associated with neurodevelopmental disorders including \textit{NIPA1} (involved in hereditary spastic paraplegia),\textsuperscript{34} and \textit{CTNNDD2} (severe ID in cri-du-chat syndrome).\textsuperscript{35} Some have been reported in ASD related studies, such as \textit{PHF8},\textsuperscript{37} \textit{WNK3},\textsuperscript{38} \textit{SEMA4A},\textsuperscript{39} \textit{GTF2I},\textsuperscript{40} \textit{STX4},\textsuperscript{41} \textit{NIPA4}\textsuperscript{42} and \textit{UBES3}.\textsuperscript{2}

Three pCNV loci (del(14)(q11.2), dup(15)(q11q13) and del (X)(p11.22)) were recurrent in being detected in multiple individuals within their respective families. One recurrent locus (del (2)(p15p16.1)) was identified in two unrelated probands with overlapping but non-identical deletions,\textsuperscript{36} with additional recurrences described in two other reports confirming striking concordance in clinical phenotype and refinement of the genes involved\textsuperscript{16} (DECIPHER. http://decipher.sanger.ac.uk/). The genotype–phenotype analysis of these collective cases will help us further understand this newly recognised syndrome and its relationship to ASD/ID pathophysiology and facilitate the identification of the disease genes in the syndrome.

This study is, to our knowledge, the first to attempt standardised, comprehensive phenomic analyses in correlation with genomic CNV findings. The advantages of identifying "whole body" phenomic and genomic biomarkers as diagnostic tools for ASD include the potential for better standardisation of behaviour based diagnoses, evaluations of treatment response, and earlier identification allowing earlier treatment. Moreover, the pursuit of aetiologic clues that define ASDs physiologically and causally pathways leading to autism, while enriching the evidence base for genetic counselling that awaits refinement from the growing spectrum of genetic and }

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Minor physical anomalies in autism: a meta-analysis

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Autism is a complex neurodevelopmental disorder in which the interactions of genetic, epigenetic and environmental influences play a causal role. Despite the compelling evidence for a strong heritability, the etiology and molecular mechanisms underlying autism remain unclear. High phenotypic variability and genetic heterogeneity confounds the identification of susceptibility genes. The lack of robust indicators to tackle this complexity in autism has led researchers to seek for novel diagnostic tools to create homogenous subgroups. Several studies have indicated that patients with autism have higher rates of minor physical anomalies (MPAs) and that MPAs may serve as a diagnostic tool; however, the results have been inconsistent. Using the cumulative data from seven studies on MPAs in autism, this meta-analysis seeks to examine whether the aggregate data provide evidence of a large mean effect size and statistical significance for MPAs in autism. It covers the studies using multiple research methods till June 2007. The current results from seven studies suggested a significant association of MPAs in autism with a robust pooled effect size ($d=0.84$), and thereby provide the strongest evidence to date about the close association between MPAs and autism. Our results emphasize the importance of MPAs in the identification of heterogeneity in autism and suggest that the success of future autism genetics research will be exploited by the use of MPAs. Implications for the design of future studies on MPAs in autism are discussed and suggestions for further investigation of these important markers are proposed. Clarifying this relation might improve understanding of risk factors and molecular mechanisms in autism.

*Original Article*

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**Keywords:** autistic disorder; heterogeneity; etiology; genotype; phenotype; biological marker

Introduction

Autism (OMIM 209850) is a severe neurodevelopmental disorder, characterized by qualitative impairments in social interaction and communication, accompanied by repetitive and stereotyped behaviors and interests. These symptoms manifest in the first 3 years of age with a lifelong persistence.1 The prevalence is estimated to be approximately 1 in 150, making it one of the most prevalent medical conditions of childhood.2,3 Boys are affected approximately four times more than girls, with an even higher ratio in milder forms of the broad spectrum.4

The vulnerability for developing autism is highly genetically determined. Twin studies indicated substantially greater concordance for autism in monozygotic than in dizygotic twins.5–10 Moreover, family studies revealed a recurrence risk of 5–6% among siblings of affected individuals, which is much higher than the prevalence rate in the general population.11,12 Together, these data show that autism is a strongly genetically influenced multifactorial childhood psychiatric disorder.13–15

The prevailing view is that its etiology involves a complex interaction between multiple genes and possibly environmental insults, leading to an aberrant neurodevelopment.16–18 Researchers have attempted to overcome the challenges posed by such a complexity with reliable diagnostic tools, including the study of head circumference and other morphological characteristics.19–22 Excessive head growth found in the first year of life, in children later diagnosed with autism, has been one of the most promising quantitative traits.23–27

As to other morphological characteristics, an excess of minor physical anomalies (MPAs) in autistic individuals received specific attention. MPAs are defined as slight morphological deviations that have no serious medical or cosmetic significance to an individual. However, they are of great value to the clinician because they can be utilized as indicators of underlying disease susceptibility or disturbed development (for example, they are found to be more common in individuals with an obvious major
The presence of such MPAs in autism has been suggested to be related to the shared genetic risk of developing autism.\textsuperscript{19,20} Overall, the presence of congenital anomalies or minor malformations in autistic individuals has been suggested to be related to the shared genetic risk of developing autism.\textsuperscript{19,20} Although the psychiatric literature ‘MPAs’ is generally accepted, many different terms are used to describe them, including dysmorphic features, minor congenital anomalies or minor malformations. The majority of the studies that have assessed the incidence of MPAs in autism used the Waldrop scale (vide infra), with occasional modifications and omissions of items.\textsuperscript{21–30} MPAs in that list originated from an unpublished study by Goldfarb and Botstein\textsuperscript{31} to classify schizophrenic patients. However, although the Waldrop scale is able to dissociate patients from controls, it has been criticized for inherent limitations regarding both content and form, its restricted range of 18 items, low sensitivity, subjective nature, ethnic and gender differences. There is substantial variation in MPA scores across studies, for autistics as well as for the control groups. Moreover, effect sizes in the individual studies have not been quantitatively reviewed and integrated in a meta-analytical way.

The aim of the present meta-analysis was to produce a synthesized effect-size estimate that has considerably more power than the individual studies.\textsuperscript{32} In addition, in order to identify the sources of variation across the studies, effects of the study characteristics on the findings were analyzed.

**Materials and methods**

**Search strategy**

An extensive bibliographic search was conducted to identify relevant articles that examined the incidence of MPAs in autism. Pubmed, Cinahl, PsycINFO and the Cochrane library were searched from inception to June 2007. For the query translation, Medical Subject Headings terms were used where they were available. The thesaurus for index terms was also checked to identify possible synonyms. The keywords used in the computerized search were ‘clinical morphology’, ‘minor physical anomalies’, ‘dysmorphology’ and ‘autism’. The reference lists cited in these studies and published reviews were examined to identify additional studies. In addition, individuals with expertise in the area of dysmorphology were asked for studies which were in press or any other papers of particular interest. Abstracts of studies identified by the search strategies were then scrutinized to determine whether they could be included or not. This search returned 78 potential hits of which the abstracts were evaluated and 16 articles were found to be relevant to the study.

**Study selection**

Studies were considered eligible for inclusion if: (1) they were designed as a case–control study where the controls were healthy children; (2) they had used the Waldrop scale or some variant of it in the MPA assessment; (3) they had presented sufficient data to compute effect size in the form of a standardized difference between means (that is, means, standard deviations, exact $P$, $t$ or $F$ values); (4) they were written in English. As the primary focus of this study was on MPAs that are listed on a standard scale (Waldrop),\textsuperscript{35,36} reports that examined only head circumference or major abnormalities were all excluded. Studies that reported previously published data were also excluded. Selection of the studies for inclusion was completed by two authors (HMO and JWH). Authors of the identified studies were contacted if there were queries regarding their studies.

**Data extraction**

Data were independently extracted by two authors (HMO and JWH), using a structured proforma. For each eligible study, recorded data variables were authors, year and country of publication; demographic variables (mean age, male/female ratio and ethnicity); diagnostic criteria (if applicable); study size (number of participants and controls) and rating methodology (whether raters were trained to recognize dysmorphic features, blinding of the authors and inter-rater reliability). The assessment scale used to identify and quantify MPAs was classified as ‘Waldrop scale’ or ‘Waldrop scale modified’. The number of MPA scale items used in each study was also recorded. Any discrepancy between ratings was discussed and resolved by consensus.

**Statistical analysis**

The key to meta-analysis is defining an effect size capable of representing the quantitative findings of a set of research studies in a standardized form that permits meaningful comparison and analyses across the studies.\textsuperscript{33} Therefore, for each individual study, an unbiased standardized mean difference ($d$) was calculated. This effect size was computed as the difference between the mean of the autistic group and that of the control group, divided by the pooled standard deviation. The resulting effect size was corrected for upwardly biased estimation due to small sample size by using Hedges’ formula.\textsuperscript{34,35} When means and standard deviations were not available, $d$ values were calculated from the reported $t$ or $F$ values. After computing individual effect sizes for each study, a weighted mean effect size ($g$) was obtained which indicated the magnitude of the association across all studies.\textsuperscript{36} Each effect size was weighted by the inverse of its sampling variance when calculating the pooled effect size, to account for the different sample sizes on which each effect size was based.\textsuperscript{37} An effect size between 0.2 and 0.5 is considered to be weak, between 0.4 and 0.6 moderate,
and greater than 0.8 is considered to be a large effect size.46

To investigate the significance level of the effect, a 95% confidence interval (CI) and z-value was calculated. Once the effect size for each study was obtained, the variance across effect sizes was assessed. The homogeneity statistic Q was calculated to test whether the observed variability in the distribution of effect size estimates is greater than that expected from sampling error.43 As published research findings suggest a number of variables that may influence effect size, a weighted regression analysis was performed to determine the extent to which selected study characteristics might explain between-study variations in effect size. Potential moderator variables for analysis were year of publication, the number of Waldrop scale items used and the use of siblings as control group. Other variables with a potential influence on effect size such as IQ, case–control sex ratio, autistic symptoms and types of autism could not be analyzed because of the small number of studies reporting results for these parameters. Data analyses were performed using random-effects framework. A random-effects model assumes that each observed effect size differs from the population mean by differences in sample sizes plus a value that represents other sources of variability assumed to be randomly distributed. To obtain a good estimate of the random effects variance component, we chose the noniterative method based on the method of moments.43 Subsequently, a sensitivity analysis was performed to explore the influence of each study’s effect size on the overall effect size, by deleting each study sequentially. The pooled effect size for the remaining studies is recomputed each time with the removal of each study, along with their 95% CI. This analysis allowed testing the overall robustness of the meta-analysis as well as detection of the most influential studies.

For computations of the mean effect size and the meta-regression, SPSS macros developed by Wilson43 were used. All other analyses were carried out using the Meta.Win 2.0 statistical package.47

To investigate the possibility of publication bias, Rosenthal’s fail-safe N statistic was computed.48 Publication bias implies that studies with no effect may not be published, posing a threat to the stability of the obtained effect size. This method determines the number of unpublished studies with null results that would be required to reduce the overall effect size to a nonsignificant level. A large fail-safe number makes the ‘file-drawer’ problem negligible. Furthermore, publication bias was also assessed using Egger’s test.49

Results

Description of studies

The combined literature search yielded 78 references. After eliminating overlapping references and those that clearly did not meet the criteria, 16 studies were identified and retrieved for further scrutiny. Of those, one was excluded because it was investigating major congenital anomalies, rather than MPAs,50 two because controls were not included33,51 and two because the Waldrop scale or a variant of it in their assessment was not used.52,53 Two more studies were excluded because of absence of sufficient data to compute a mean effect size even after contacting the investigators.54,55 Two final studies were excluded because of the absence of relevant data in the published articles and no response from the investigators.56,57

In the end, a total of seven studies, published between 1975 and 2005, met our inclusion criteria and contributed to the meta-analysis.5,58–63 Each sample was included independently into analysis. These studies included 330 patients with autism (mean age: 9.75, 80% male) and 382 healthy controls (mean age: 10.3, 70% male). In two studies only boys were included60,62 and three studies had mixed ethnicities.58–60 Three studies used siblings as their control group.5,58,60 Four of the seven selected studies were conducted in the United States58,59,62,63 and two were carried out in Canada60,61 and one in the United Kingdom.5 The main characteristics of the studies are listed in Table 1.

Meta-analysis

Effect sizes were calculated of all studies that provided mean MPA scores on the basis of the Waldrop scale. As graphically presented in Figure 1, the results of our meta-analysis indicate that mean MPA scores in patients with autism differ from those of healthy controls.

In all seven studies the direction for the effect size indicates that autism patients show higher MPA scores than controls. None of the seven studies had a negative effect size or included the value zero, indicating a statistically significant effect for each study. Finally, a single pooled effect size of 0.84 (P<0.001) was found, with 95% CI ranging from 0.47 to 1.21. The results of our meta-analysis indicate a significant difference in the mean number of MPA scores between patients with autism and healthy controls. The pooled effect size is in the range of a robust effect.43,46 There was considerable heterogeneity across studies (Q = 36.90, d.f. = 6, P<0.01) and distributional analysis of effect size estimates indicated one positive outlier. Without this potential outlying case, the effect size distribution was no longer heterogeneous. However, the new combined effect size was still statistically significant. We were reluctant to remove this study from the analysis because removal may lead to an underestimation of the estimated mean effect size. In addition, the study completely fit our inclusion criteria, and closer examination revealed nothing unusual about the outlying study. Results of the sensitivity analysis revealed that removing any single
The study failed to result in a significant shift in the pooled effect size estimate (Figure 2). The largest negative shift occurred following removal of the study by Gualtieri, which was expected based on the large effect size reported in this study. The largest positive shift occurred following removal of Soper et al. In both cases, however, there was a negligible net effect on the overall pooled estimate. This indicates that all seven studies were similarly influential and that the meta-analysis is generally robust. Because of the large CI (0.47–1.21; see Table 1) and the small number of studies, there was sufficient variability to warrant further analysis. Therefore, we performed weighted regression analysis where the relationship between quantitative study characteristics and effect size was explored. Neither the number of MPA scale items nor the use of siblings as controls, or gender rates accounted for a significant proportion

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Country</th>
<th>Dominant ethnicity</th>
<th>Diagnosis</th>
<th>Case N</th>
<th>Control n</th>
<th>% Male</th>
<th>MPA scale items</th>
<th>Interrater reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steg and Rapoport</td>
<td>1975</td>
<td>USA</td>
<td>Caucasian</td>
<td>Clinical charts examined</td>
<td>28</td>
<td>31</td>
<td>100</td>
<td>18</td>
<td>0.95</td>
</tr>
<tr>
<td>Walker</td>
<td>1977</td>
<td>USA</td>
<td>Caucasian</td>
<td>Clinical diagnosis + behavioral criteria</td>
<td>74</td>
<td>74</td>
<td>81</td>
<td>16</td>
<td>One examiner</td>
</tr>
<tr>
<td>Campbell et al.</td>
<td>1978</td>
<td>USA</td>
<td>Mixed</td>
<td>Kanner + Rutter criteria</td>
<td>52</td>
<td>29</td>
<td>80</td>
<td>18</td>
<td>0.73</td>
</tr>
<tr>
<td>Links et al.</td>
<td>1980</td>
<td>Canada</td>
<td>Mixed</td>
<td>National society of autism definitions</td>
<td>45</td>
<td>52</td>
<td>71</td>
<td>18</td>
<td>0.92</td>
</tr>
<tr>
<td>Gualtieri et al.</td>
<td>1982</td>
<td>USA</td>
<td>Mixed</td>
<td>DSM-III</td>
<td>39</td>
<td>76</td>
<td>71</td>
<td>12</td>
<td>0.71</td>
</tr>
<tr>
<td>Bailey et al.</td>
<td>1995</td>
<td>UK</td>
<td>Caucasian</td>
<td>ICD-10, ADI, ADOS</td>
<td>20</td>
<td>20</td>
<td>77</td>
<td>19</td>
<td>NA</td>
</tr>
<tr>
<td>Soper et al.</td>
<td>2005</td>
<td>Canada</td>
<td>Caucasian</td>
<td>DSM-IV</td>
<td>72</td>
<td>100</td>
<td>75</td>
<td>17</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: MPA, minor physical anomaly; N, number of subjects with autism; n, number of healthy controls; NA, not available.

Here the main characteristics of the seven studies included in this meta-analysis are listed.

![Figure 1](Molecular_Psychiatry_303.png)

**Figure 1** Meta-analyses of case–control studies investigating the relationship between MPAs and autism. Meta-analysis pooling results across studies. The black square and horizontal line correspond to weighted mean effect size and 95% CI for each study. The summary diamond bar [at the bottom of the figure] represents the pooled effect size estimate and 95% CI. Meta-analysis indicates significant association between MPAs and autism ($P < 0.001$). CI indicates confidence interval; MPAs, minor physical anomalies.
Included. The meta-analysis supports the conclusion by sensitivity analysis, regardless of the dataset. A compelling, robust effect showed strong consistency which is considered to be a large effect. This effect was calculated from all seven studies was 0.84 (95% CI 0.71–1.0), with a small range. Moreover, the pooled effect size of the between-study variance in effect size. None of the regression models were statistically significant (P > 0.5). However, the failure to find a moderator is not surprising given the small number of studies included.

Publication bias
Publication-bias analysis indicated a fail-safe number of 43 that means that at least 43 studies reporting no effect need to be found before the mean results are no more significant, large enough to credence to our findings. The estimated bias with the Egger’s test was 0.30 (95% CI 0.21–0.49), P = 0.97, which also indicates an absence of publication bias.

Discussion
This meta-analysis integrated the results of seven studies that compared MPAs of 330 patients with autism with those of 388 healthy controls. Each original study consisted of a relatively small sample of autistic cases ranging from 20 to 74. The results indicate that the mean total MPA scores in children with autism differ from those of healthy controls. In each of the seven studies the autistic sample had significantly higher rates of MPAs than those of the controls. In none of the individual studies, the magnitude of the case–control difference was in the small range. Moreover, the pooled effect size calculated from all seven studies was 0.84 (P < 0.001), which is considered to be a large effect. This compelling, robust effect showed strong consistency by sensitivity analysis, regardless of the dataset removed. The meta-analysis supports the conclusion that patients with autism have significantly more MPAs than those of normal controls; this finding is consistent with the findings of the individual studies.

Despite this finding, a high degree of variability in the magnitude of the effect size magnitude was observed among these seven studies. In order to identify potential factors that could be responsible for constraining this variation, we performed regression analyses. None of the earlier mentioned moderator factors (that is, year of publication, number of Waldrop scale items used and the use of siblings as control group) were able to account for a statistically significant relationship to the observed between-study variation in effect sizes (P > 0.05). This failure to identify a moderating factor is not surprising given the small number of studies included in this meta-analysis. The effect of other potentially important moderator variables (for example, sex, IQ, familiarity) could not be analyzed because of a lack of information on these characteristics in most of the studies as well as the small number of studies.

Although this meta-analysis clearly shows a robust effect for significant excess of MPAs in the autistic population, the results are complicated by a number of methodological issues. Although having the advantage of being short, there have been concerns about Waldrop scale’s limitations. A major methodological issue is the unblended nature of the measurement. Although a good inter-rater reliability can be achieved, one should be cautious that patients with autism might be different during even the minimal interaction needed to measure some of the Waldrop scale items (for example, head circumference). Although few studies have been carried out blinded, complete blinding is very difficult given the close personal interaction involved when assessing MPAs and facial measurements. In addition, one should keep in mind the subjectivity regarding the validity of certain Waldrop items (for example, hair quality, hypertelorism, clinodactyly). In addition, we believe that other methodological variability among the studies may explain some of the variation in MPA scores: inconsistencies in sample size and composition, differences in MPA items, lack of consensus in the terminology, and ethnic diversity. For instance, the majority of cases were Caucasian, however, three studies used mixed ethnicities in their population samples.

As we confirmed the higher rate of MPAs in autistic patients as compared to controls, we are faced with new challenging questions. First of all, why do autistic patients have higher rates of MPAs? Apparently, a common genetic vulnerability for developing autism is reflected in MPAs. Several developmental genes have recently been identified that play a paramount role in shaping body structures. Moreover, new insights into craniofacial morphogenesis have indicated that a rapidly increasing number of genes are known to regulate cerebro-craniofacial development. It can be speculated that the genes that determine the craniofacial morphology overlap...
with candidate genes for autistic disorders. MPAs could also be related to prenatal infection, or other environmental exposures which are associated with autism. Prenatal or postnatal exposure to infections, such as rubella, herpes simplex virus and cytomegalovirus, has been reported in several patients with autism. Thalidomide exposure during pregnancy is consensually associated with autism.\(^7\) In addition, both de novo and familial cytogenetic abnormalities may be associated with an increased number of MPAs.\(^{68}\) Copy number variation (CNV), including deletion and duplication, translocation, inversion of chromosomes, has been identified in some individuals with autism.\(^{14,69}\) In fact, Engels et al.\(^{70}\) showed a direct association between the severity of physical anomalies and the chance to find mutant CNVs. The results of this meta-analysis suggest that MPAs in autism are, at least in part, related to the risk of developing the disease and that these MPAs may therefore precede the clinical onset of the disorder.

Another critical question is whether these physical anomalies in autism are broad population characteristic of all patients in the spectrum, or whether patient–control differences derive from overrepresentation of those abnormalities among only a specific subgroup of patients. Some evidence for the latter comes from Miles et al.\(^{33}\) who hypothesized that autistic patients with high MPA scores represent ‘nonfamilial or sporadic’ autism due to single environmental insults or nontransmitted genetic events, whereas autistic patients with low MPA scores represent ‘familial’ autism (where genetic clustering of psychiatric disturbance reflects variable expression of the underlying genotype).\(^{33,42}\) These findings were confirmed by Links et al.\(^{60}\) and replicated in a later study by Miles et al.\(^{33}\)

Another fundamental issue to be addressed is whether sets of certain physical anomalies are related to specific phenotypic behavioral characteristics in children with autism, and whether clustering of certain anomalies to groups of patients would yield homogenous subgroups. Except one early study by Walker,\(^{65}\) no other study in the literature dealt with clustering. However, he found random association and heterogeneity in distribution with few exceptions (for example, high palate and low setting ears).

A final, more speculative, question is about the specificity of MPAs. Are the MPAs seen in autism different from those in other disorders? In a recent meta-analysis, a higher prevalence of MPAs was also established in schizophrenia.\(^{31}\) Do MPAs seen in autism have a different etiology than those in schizophrenia, or do disorders associated with MPAs share a common etiological basis with schizophrenia and autism? Findings indicating overlapping markers could provide important clues regarding the underlying genetic bases of these disorders. Some evidence for such an overlap comes from the observation that individuals with autism spectrum disorders may also be at greater risk for schizophrenia.\(^{71}\) And, recent findings indicate that most complex disorders are probably rooted in genetic variation that is significantly shared by multiple disease phenotypes.\(^{72}\)

Robust diagnostic specificity is often lacking for several other disease markers as well as MPAs and reflects the fact that different disorders may share genes, and also share partially overlapping neural substrate dysfunction and clinical features.\(^{73}\)

**Limitations and strengths of this meta-analysis**

There are certain limitations that should be borne in mind when interpreting the results.

First, as with all meta-analysis studies, the results depend on the quality of the individual studies. Although we used well-defined inclusion criteria, we had to accept some methodological diversity among the studies in order to compare a sufficient number of studies. We should also mention that the inclusion or exclusion of a given study in this analysis was not based on the scientific value of the publication. We had to exclude some valuable publications, as they did not meet the specific goal of the present study.

Second, the diagnosis of autism was occasionally problematic in the early studies, written before the introduction of the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) classification. Nevertheless, those studies were included because they met all our inclusion criteria. Sensitivity analysis confirms that the change in the type of criteria used for autism diagnosis does not appear to influence the effect size. Moreover, perhaps, in part, due to a true increase in the prevalence rate or, in part, due to the introduction of DSM 4 or greater awareness of the syndrome, the prevalence of autism has significantly increased over the last decades. Therefore, we cannot exclude that the effect size of the association today may somewhat differ from that of the earlier studies included in the meta-analysis. However, the effect size reported by the most recent study (Soper et al.\(^{60}\)) is not different from that of the older studies. And again, sensitivity analyses show that the publication year does not influence the effect size.

Third, three studies were included that measured MPAs in sibling controls, which may introduce a confounding factor. However, because of the small number of studies in the meta-analysis, these studies, which met all criteria, were included. Interestingly, there were no significant differences in MPA scores when compared to either a sibling or nonfamily control group.\(^{5,58,60}\)

Fourth, we were unable to examine topography of dysmorphic features in autism, because we had too little information comparing these across studies. Yet, such information is fundamental to understanding the timing and nature of dysmorphic events. Interestingly, increased head circumference, which is a well-documented finding in autistic children, was not consistently reported in these seven MPA studies.

Fifth, although the age of the participants has been thought to facilitate differences in effect size among the studies, the results of moderator variable analysis,
possibly, in part, due to the small number of studies failed to confirm this hypothesis. Additionally, although gender is known to affect the incidence of autism, the studies included in this meta-analysis did not provide enough data to examine gender effects. Thus the possibility that some of the effects found in this meta-analysis study were caused by confounding factors such as age and sex cannot be ruled out.

Despite these limitations, the present results offer several methodological advantages for future inquiry. This is the first report studying the association between MPAs and autism in a meta-analytic way. This study provides evidence that MPAs are significantly increased in the autistic population and that some, unknown biological mechanism is likely responsible for producing these anomalies which may yield further knowledge about the developmental origins of the disease.

**Recommendations for future research**

It is obvious that the assessment of MPAs in autism require further study. With the aforementioned caveats in mind, we have the following recommendations.

Although MPA measurement is considered simple, noninvasive and inexpensive, we should caution that their genetic architecture may be as complex as that of autism itself. This does not mean there is no advantage to use them for genetic studies. More and larger studies in ethnically homogenous populations are needed to search for a possible correlation between MPAs and family history as well as to achieve sufficient power to search the potential role of moderating variables such as gender, autism symptoms and IQ.

Our results provide strong support for the association between MPAs and autism. This meta-analysis emphasizes the importance of MPAs in the identification of heterogeneity in autism and suggests that the success of future autism genetics research will be exploited by the use of MPAs. Although these findings reflect a vulnerability to developing autism, it is still unclear how and to what extent genes and/or environment are involved. Future studies should focus on the search for susceptibility genes, chromosomal alterations (for example, mutations, duplications, deletions or CNVs) as well as different environmental factors in relation to morphological characteristics by using detailed definitions of the phenotype and an internationally accepted classifying list to enable comparison of the results. MPAs might serve as a helpful instrument in autism research, delineating subgroups which provide a more homogenous basis for unraveling the etiology and predicting prognosis.

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Essential Versus Complex Autism: Definition of Fundamental Prognostic Subtypes


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Heterogeneity within the autism diagnosis obscures the genetic basis of the disorder and impedes our ability to develop effective treatments. We found that by using two readily available tests, autism can be divided into two subgroups, “essential autism” and “complex autism,” with different outcomes and recurrence risks. Complex autism consists of individuals in whom there is evidence of some abnormality of early morphogenesis, manifested by either significant dysmorphology or microcephaly. The remainder have “essential autism.” From 1995 to 2001, 260 individuals who met DSM-IV criteria for autistic disorder were examined. Five percent (13/260) were microcephalic and 16% (41/260) had significant physical anomalies. Individually, each trait predicted a poorer outcome. Together they define the “complex autism” subgroup, comprising 20% (46/233) of the total autism population. Individuals with complex autism have lower IQs (P = 0.006), more seizures (P = 0.0008), more abnormal EEGs (48% vs. 30%), more brain abnormalities by MRI (28% vs. 13%). Everyone with an identifiable syndrome was in the complex group. Essential autism defines the more heritable group with higher sib recurrence (4% vs. 0%), more relatives with autism (20% vs. 9%), and higher male to female ratio (6.5:1 vs. 3.2:1). Their outcome was better with higher IQs (P < 0.02) and fewer seizures (P = 0.0008). They were more apt to develop autism with a regressive onset (43% vs. 23%, P = 0.02). Analysis of the features predictive of poor outcome (IQ < 55, functionally non-verbal) showed that microcephaly was 100% specific but only 14% sensitive; the presence of physical anomalies was 86% specific and 34% sensitive. The two tests combined yielded 87% specificity, 47% sensitivity, and an odds ratio of 4.81 for poor outcome. Separating essential from complex autism should be the first diagnostic step for children with autism spectrum disorders as it allows better prognostication and counseling. Definition of more homogeneous populations should increase power of research analyses.

KEY WORDS: autism; essential; dysmorphology; head circumference; outcome

INTRODUCTION

Autism is a neuropsychiatric disorder of early childhood, defined exclusively on the basis of impairments in social interactions and communication, and repetitive or stereotypic behaviors, delineated in the DSM-IV [American Psychiatric Association, 1994]. Clinically, the autism diagnosis is variable with some children having more or fewer symptoms in each of the three domains, very low to above average IQs, and outcomes that range from entering regular school and functioning well in society to requiring lifelong care [Bailey et al., 1998; Spence, 2001; Silverman et al., 2002].

There have long been concerns that autism research is hampered by a lack of diagnostic uniformity, leading to mixed populations which make inter-study comparisons difficult [Link et al., 1998]. In the late 1990s, the adoption of standardized, replicable diagnostic instruments and diagnostic criteria (DSM-IV, ADI-R, ADOS-G, ICD-10, CARS) [Schopler et al., 1986; World Health Organization, 1992; American Psychiatric Association, 1994; Lord et al., 1994, 1998] largely insured that major research studies were conducted with children who satisfy similar neurobehavioral criteria. The constancy of the core manifestations has stood up to intensive scrutiny and clearly separates autism from other behavioral diagnoses such as mental retardation and ADHD. It bears repeating that future autism studies must be grounded on a firm behavioral diagnosis.

Despite this meticulous attention to diagnosis, there remains enormous clinical variability within autism [Folstein et al., 1998; Spiker, 1999; Beglinger and Smith, 2001; Spiker et al., 2002]. Children with autism vary in their presentation, course and outcomes, in the quality and intensity of their core behaviors, delineated in the DSM-IV [American Psychiatric Association, 1994] largely insured that major research studies were conducted with children who satisfy similar neurobehavioral criteria. The constancy of the core manifestations has stood up to intensive scrutiny and clearly separates autism from other behavioral diagnoses such as mental retardation and ADHD. It bears repeating that future autism studies must be grounded on a firm behavioral diagnosis.

Despite this meticulous attention to diagnosis, there remains enormous clinical variability within autism [Folstein et al., 1998; Spiker, 1999; Beglinger and Smith, 2001; Spiker et al., 2002]. Children with autism vary in their presentation, course and outcomes, in the quality and intensity of their core autism symptoms, their adaptive and cognitive levels and responses to therapy [Asperger, 1944; Wing and Gould, 1979; Wing, 1981a; Prizant and Schuler, 1987; Wing and Atwood, 1987; DeLong and Dywer, 1988; Rutter and Schopler, 1988; Volkmar et al., 1989; Coleman, 1990; Szatmari, 1992; Castelloe and Dawson, 1993; Roux et al., 1997; Bailey et al., 1998; Fein et al., 1999; Spence, 2001; Silverman et al., 2002]. In 1994, the diagnostic term “infantile autism” was replaced by “autistic disorder” [American Psychiatric Association, 1994] implying that the autism phenotype comprises a spectrum of disorders varying in severity, associated symptoms and causality.

Unraveling the genetic heterogeneity in autism and dissecting its correspondence, or lack thereof, with clinical characteristics has proven much more difficult than for the prototypic single gene disorders [reviewed in Fein et al., 1999; Spence, 2001]. Reports of sibs discordant for their autism spectrum disorders led to the disheartening expectation that we would not be able to understand the heterogeneity until we found specific autism genes and we could not find the autism genes...
until we understand the heterogeneity [DeLong and Dwyer, 1988; Silverman et al., 2002].

Most attempts to discover the fundamental genetic bases of autism have focused on cognition and the core diagnostic symptoms. Variance in IQ has garnered the most attention, since between half and three quarters of autistic children have IQ scores below 70 [Lotter, 1986; Rutter, 1983; Steffenburg and Gillberg, 1986; Lincoln et al., 1995; Chakrabarti and Fombonne, 2001] and because cognitive levels measured in early childhood have been a strong predictor of outcome [Cohen et al., 1987; Lord and Schopler, 1989a,b; Kobayashi et al., 1992; Venter et al., 1992; Volkmar, 1992; Fein et al., 1999]. Stevens et al. [2000] reported that children who tested during preschool as low functioning (based on non-verbal IQ, receptive vocabulary and socialization) either remained low functioning or dropped significantly in their level of functioning when retested at school age. They concluded that early normal or near-normal non-verbal IQ was the best predictor of adequate functioning by grade school. In multiplex sibships, non-verbal IQ scores correlated positively [Szaftari et al., 1996; Spiker et al., 2002], indicating that cognitive abilities in autism, as in typically developing populations, were largely genetically determined. Moreover, IQ levels appeared to correlate with a fundamental genetic variable, the male to female ratio; the more severely retarded the population, the lower the male to female ratio [Wing, 1981a,b; Gillberg, 1989; Szaftari et al., 1989]. The greatest male predominance occurred in Asperger syndrome as did the highest IQ scores.

In the young autistic child or in individuals with pockets of intelligence, IQ assessment is difficult [reviewed in Bailey et al., 1996], and IQ and DQ scores measured in early childhood may change over time and with therapy. Lord and Schopler [1989b] reported mean differences greater than 23 points comparing test scores prior to age 4 with those at age 8 and older, findings which have been replicated in other populations [Sigman et al., 1999]. Even without special treatment, children first assessed in early preschool years are likely to show marked increases in IQ score by school age [Lord and Schopler, 1989b]. It is unclear if this reflects limitations of assessment methods in younger children, the natural history of the disorder, or, since none of these studies are longitudinal, some bias of ascertainment [Freeman et al., 1991]. Evidence that IQ is not the primary genetic variable in autism, was first presented by Le Couteur et al. [1996] who found that IQ scores can vary widely in identical twins. Jorde et al. [1990] found that dividing the Utah cohort by IQ revealed no significant differences in recurrence risks and gave no indications of inheritance patterns. This is consistent with our data, which show that though individuals with complex autism as a group have lower IQ scores than those with essential autism, it is the essential/complex autism distinction that provides the more fundamental separation. IQ scores did not correlate with sex ratios, recurrence risks, family histories of autism, or type of onset [Miles et al., 2001; Miles, unpublished data].

Numerous studies have focused on language variability to create more homogeneous autism subgroups. Language competence, like cognitive ability, is under significant genetic control, demonstrated by the robust language correlations reported within sibships, twins, and/or more distant relatives [Fombonne et al., 1997; Piven and Palmer, 1997; Bailey et al., 1998; Hughes et al., 1999; MacLean et al., 1999; Pickles et al., 2000; Spiker et al., 2002; Silverman et al., 2002]. Silverman et al. [2002] found reduced variance within sibships for speech delays and age at phrase speech. Likewise, in a study of 171 autism sibships, Spiker et al. [2002] found a highly significant association within autism sib pairs for language delay and absence of phrase speech. Pickles et al. [2000] studied over 5,000 relatives from 149 autism families and found that the percent of relatives with an autism phenotype correlated positively with the severity of autism for the verbal probands, but not for probands lacking speech, suggesting a genetic difference between verbal and non-verbal autism probands. The utility of using language to identify genetically distinct subgroups, was demonstrated by Hutcheson et al. [2003], who found higher LOD scores for linkage to the AUT1 region on chromosome 7q in the more language-impaired group. This was the first time functional phenotypes were successfully correlated with genetic linkage. Nevertheless, language development does not appear to be the primary autism genetic determinant, since many non-verbal autistic children have parents and/or siblings with high functioning autism or Asperger syndrome [Eisenmajer et al., 1996; Volkmar et al., 1998; Gillberg, 1999; Gillberg and Wing, 1999]. Recently, Miller and Ozonoff [2000] compared individuals with high-functioning autism with impaired language to individuals with Asperger’s; with IQ differences controlled, they found no significant group differences in motor, visual spatial, or executive functions, suggesting language development is primarily IQ dependent.

Some investigators have tried to correlate genetic indicators with behavioral symptoms. In a well executed study of 212 multiplex sibships, Silverman et al. [2002] found evidence for familiality for the ADI items which describe preoccupations, compulsive routines, and rituals and deficits in non-verbal communication. We found that autism without repetitive motoric behaviors correlated with a family history of obsessive compulsive disorder [Miles et al., 2000b]. Studying 171 multiplex families, Spiker et al. [2002] found the autistic behavioral symptoms did not identify distinct behavioral phenotypes, but clustered along a continuous severity dimension such that children with the lowest non-verbal IQ scores were more apt to be non-verbal and have more severe impairment in ADI-R scores. Sib comparisons did, however, reveal positive correlations for IQ scores, verbal status, total non-verbal language and rituals scores. Recent efforts by Joseph et al. [2002] to distinguish an autism subtype based on different cognitive profiles found that children with poor verbal skills had more social impairment and this correlated with macrocephaly [Deutsch and Joseph, 2003]. They hypothesized that this could be an autism subtype associated with a greater disturbance in brain development. Taken in total, most studies have shown that in autism, IQ and language differences are clearly heritable, while social features exhibit a lesser degree of heritability. They do not, however, allow us to determine the primary genetic basis of these associations.

In the course of our studies, we noted that a significant subset of children with autism have congenital anomalies which clearly indicate some insult to early embryologic development has occurred [Miles and Hillman, 2000]. We hypothesized that the distinction between the autistic children who suffered an abnormality of morphogenesis and those who did not, would allow us to define fundamentally different subgroups whose autism was due to different causes. This paper describes the subdivision of the autism spectrum disorders into two subgroups, complex and essential autism. Complex autism is defined by the presence of a significant number of physical anomalies and/or microcephaly. The remainder, in whom we find no evidence of abnormal morphogenesis, we have labeled as having essential autism. The essential and complex subgroups are relatively easy to identify clinically, and appear to differ in their outcomes, recurrence risks, sex ratios, and family histories.

MATERIALS AND METHODS

Subjects

The study sample consisted of 260 unrelated patients diagnosed with autistic disorder or Asperger syndrome at the Autism Center at the University of Missouri-Columbia.
Hospitals and Clinics. Of 412 consecutive patients referred to the Autism Center between 1994 and 2001, 77% (316/412) met DSM-IV [American Psychiatric Association, 1994] and CARS (Childhood Autism Rating Scale) [Schopler et al., 1986] criteria for the diagnosis of a pervasive developmental disorder. The autism spectrum diagnoses included 244 with autistic disorder, 16 with Asperger syndrome and 56 with PDD-NOS. The 260 individuals with autistic disorder or Asperger syndrome are included in this study.

Because this was the first dedicated autism clinic in Missouri and was supported by the Missouri Department of Mental Health, patients with a suspected diagnosis of autism were drawn from the entire state for diagnosis, medical management, and guidance on behavioral issues and school placement. There was no recognizable ascertainment bias toward more or less phenotypically abnormal, mentally retarded or multiplex subjects and no exclusion of individuals who met autism diagnostic criteria specified by DSM-IV and CARS criteria. Each patient was evaluated by the Autism Center directors using a center-based version of the ADI scoring protocol and only those meeting criteria were included in the study. Independent diagnostic evaluations were conducted by a child psychiatrist and a neuropsychologist. The results were compared and in any case where there was a disparity, the individual was discussed jointly to reach a consensus. One third of the patients were evaluated with the complete ADI-R [Lord et al., 1994]; in all cases the ADI-R confirmed the previous diagnosis.

**Clinical Evaluation**

The Autism Center evaluation utilized a standard data set for the collection of historical information including prenatal, perinatal, development, language, behavior, neurologic, dietary, health and family history. All pertinent records including school, therapy, and IEP reports as well as psychological, developmental, and medical testing were reviewed. A detailed history of the onset of autistic symptoms was obtained including the age of onset of each symptom and whether delays and or losses occurred in language, gross motor, fine motor, activities, or social interactions. Laboratory tests included G banded chromosomes, DNA for fragile X, urine metabolic screen, organic acids, urine amino acids, short chain fatty acids, thyroid profile, CMP, heme profile, and lead level. Brain MRIs were obtained in 65% of the subjects and EEGs in 58%.

Physical examinations were performed including standard morphologic measurements of the head, face, hands, feet, body proportions, and dermatoglyphic analysis [Hall et al., 1989; Aase, 1990; Jones, 1997]. The skin was examined with a Woods lamp. Parents and other available relatives were examined and family photographs were reviewed. All study data were entered into a fully searchable relational database.

**Morphology Classification**

The method used for phenotypic classification has been described previously [Miles and Hillman, 2000]. In this study, we classified children as “dysmorphic” if they had more than six abnormal physical features including minor anomalies, measurement abnormalities, and descriptive features not present in their non-autistic parents. Individuals with less than three features were defined as “non-dysmorphic.” Those with between three and six features were placed into an equivocal group. Each of the study individuals were examined by one of two medical geneticists (JHM/REH). Interrater reliability studies were carried out on 100 children and provided a reliability score of 0.88. The interrater reliability for the 30 patients examined independently by four dysmorphologists was 0.83 [Miles et al., 2003]. Of the 260 individuals examined 41 (18%) were dysmorphic, 191 (74%) were non-dysmorphic, and for 27 (10%) individuals the examination was equivocal.

**IQ/DQ Assessment**

Each patient was assigned an IQ/DQ score based on the most recent and comprehensive neuropsychological evaluation. Children were evaluated by the Autism Center’s neuropsychology team (53%) or recent results from the schools or other psychologists were used (47%). When more than one set of test results were available, non-verbal IQ scores were used with the order of preferred testing being the Leiter-R [Roid, 1997], the WISC-III [Wechsler, 1991], and the Stanford Binet [Thorndike et al., 1986]. For younger children, developmental quotients, specifically daily living skills scores from the Vineland [Sparrow et al., 1984] were used. Scores reported in Table II were from the Leiter [76], Vineland [61], Wechsler [30], Stanford Binet [5], Slossen [1], other [2]. To be certain that the use of Vineland developmental quotient scores did not distort the data, the IQ score comparisons between essential and complex autism were calculated for the 112 individuals who had available IQ scores. No significant differences were noted. The mean IQ in the essential group was 79 (SD 26.5) and in the complex group was 55.8 (SD 24.1); P value = 0.0006. In the essential group, 62% had an IQ > 70; 19% an IQ < 55. In the complex group, 31.5% had an IQ > 70; 47.4% an IQ < 55. Differences between the essential and complex group scores were both significant at P = 0.01. Though the use of the Vineland DQ scores did not affect the comparisons, the combined the IQ/DQ scores were slightly lower than the IQ scores alone.

**Language Assessment**

Children were defined as functionally verbal when they used sentences to convey their wants and needs and produced a range of flexible sentence types. This corresponds to fluent speech defined in the Autism Diagnostic Observation Schedule [Lord et al., 1998, 1999, 2000]. Each individual was designated as either functionally verbal or not based on either formal testing using the ADOS, ADI-R, VABS [Sparrow et al., 1984], or CELF-III [Semel et al., 1995] or by a combination of parental report and clinical observation. Of the 103 subjects who were at least 8 years old, 49/103 (48%) were defined as verbal.

**Brain MRI**

Brain MRIs were obtained on 65% (170/260) of the subjects using a Siemens 1.5T scanner following our autism brain protocol and interpreted by a CAQ (Certificate of Added Qualifications) neuroradiologist. Patients were anesthetized by a board-certified anesthesiologist using our autism protocol (both protocols available on request). Brains were classified as abnormal if the neuroradiologist determined that the brain structure fell outside the normally accepted range. We recognize that this determination is inexact. For instance, individuals with a mega cisterna magna or an Arnold-Chiari malformation may or may not have developmental concerns. This inexactness, however, reflects the current state of the science and is accepted as such. MRIs that were equivocal were reviewed by a pediatric neurologist and the neuroradiologist and the consensus determination was accepted.
Outcome Assessment

Outcomes were specified for individuals age 8 years or older using an outcome classification based on IQ/DQ scores and verbal ability. Excellent outcome was defined as having an IQ/DQ score over 70 and being functionally verbal. Good outcome included those individuals with IQ/DQ scores between 55 and 70 who had functional verbal abilities. Poor outcome was defined as an IQ/DQ score < 55 or being functionally non-verbal by the age of 8 years. Of the 74 individuals included in the analysis, only seven were tested by the Vineland. A similar comparison, limited to individuals with IQ scores produced similar results (data not presented).

Family History

Family histories were obtained by direct semi-structured interviews using the family history method [Orvaschel et al., 1982; Thompson et al., 1982; Andreasen et al., 1986; Rice et al., 1995; Yuan et al., 1996; Davies et al., 1997]. Using our investigator-based family history interview form, an in-depth interview was conducted. The informants were asked about each first, second, and third degree relative individually, inquiring about any medical, psychological/psychiatric, or behavioral problems. Disease specific questions were then used to clarify the diagnosis. A family history of autism was rated as significant if the proband had either (1) an affected first degree relative or (2) a second degree relative affected plus at least two additional affected individuals in the same family branch in a pattern suggesting mendelian inheritance.

Statistical Analysis

Tables I and II give summary data comparing cases of essential and complex autism. Continuous random variables were summarized by their mean, standard deviation, and range. For categorical random variables, separately for the essential and complex cases, the proportion of cases in each category are given. For the categorical random variables, univariate comparisons of essential and complex autism were made using $\chi^2$ tests. For age and sex ratio, comparisons were made using Students $t$-test. Because the distribution of IQ is skewed, comparisons for IQ were made using the Kolmogorov–Smirnov test and the $t$-test after log transforming IQ.

We constructed a logistic regression model in order to determine which features best predicted poor outcomes. The following variables were used in the regression model: gender, dysmorphology classification, microcephaly, macrocephaly, seizures, regressive onset. Abnormal MRI, abnormal EEG were excluded from the analysis because of the large number of missing values. In addition to modeling outcome for all cases, outcome was modeled separately for complex and essential cases.

RESULTS

Two hundred sixty consecutive patients evaluated at the University of Missouri Autism Center between 1994 and 2001 met DSM-IV diagnostic criteria for autism (94%) or Aspergers syndrome (6%). The study population was primarily children with a mean age of 9 years; 86% were Caucasian, 7% biracial, 5% African-American, and 1.5% Asian (Table I). No significant differences emerged between individuals with essential and complex autism.

Phenotypic features which we considered potentially useful for the separation of the behavioral autism diagnosis into more homogeneous subgroups were identified. We sought features that were consistently present in a substantial percentage of autistic children, and were discrete, measurable and "replicable." The crucial requirement was that the feature be pathophysiologically relevant with potential to become a genetic marker. Because we wanted to identify features that manifest prior to the onset of autistic symptoms, we looked for features that were stable from birth. The two features identified as the most informative were "dysmorphology," which occurred in 16% of the population, and microcephaly which occurred in 5%.

The presence of significant "dysmorphology" and microcephaly were used to define the complex autism subgroup, providing physical evidence of an insult to early embryological development. The remainder, who were physically non-dysmorphic and non-microcephalic were designated "essential autism." The two groups differ in their genetic features (sex ratio, sibling recurrence risk, family history of autism), measures of outcome (IQ, seizures) and type of onset (regressive). All subjects identified with an autism related syndrome were in the complex group (Table II).

Forty-eight percent of those with essential autism have IQ/DQ scores > 70, compared with only 22% of those with complex autism (see Table II). Correspondingly, only 25% of those with essential autism have IQ/DQ scores < 55, compared with 52% of those with complex autism. Because the distribution of IQ/DQ scores was found to be highly skewed, the IQs of those with complex autism were compared to those with essential autism using the Kolmogorov–Smirnov test ($P = 0.003$). Comparisons using a log transformation of the IQ scores gave similar results ($P < 0.003$).

To further elucidate the differences between complex and essential autism we compared outcomes for subjects age 8 years or older, using an outcome classification based on IQ/DQ scores and verbal ability (Table III). Of the 15 individuals with excellent outcomes, 93% had essential autism and only 7% had complex autism. In the good outcome group, 79% had essential autism and 21% complex. However, individuals with a poor outcome were almost evenly divided between those with essential and complex autism, demonstrating that complex autism is a more specific indicator of poor outcome than essential autism is an indicator of an excellent outcome.

Because many features might be associated with outcome, we tested each feature individually to determine which were most predictive of a poor outcome (Table IV) and an excellent outcome (Table V). For poor outcome, microcephaly was most predictive with a positive predictive value of 100%. The dysmorphology classification was the next most predictive single feature. The complex autism designation provided the highest sensitivity with a positive predictive value of 86%. Seizures and a regressive onset appeared somewhat predictive. Gender, abnormal brain MRI, abnormal EEG, and macrocephaly were not. Excellent outcomes were less predictable with only essential autism having a positive predictive value above 50% (Table V).

| TABLE I. Demographic Characteristics of Essential Versus Complex Autism* |
|-----------------------------|-----------------------------|
| **Essential autism** (N = 187) | **Complex autism** (N = 46) |
| Mean age (SD) | 8.1 (7.2) | 12.6 (9.5) |
| Range | 1.4–55.9 | 1.0–41.2 |
| Ethnicity | | |
| White | 81% | 96% |
| Black | 7% | 2% |
| Asian | 2% | 0% |
| Bi-racial/multi-racial | 10% | 2% |
| Socio-economic status | | |
| Group I and II | 45% | 34% |
| Group III | 32% | 21% |
| Group IV and V | 23% | 45% |

*Socio-economic status of birth family was unknown for 56 individuals.
TABLE II. Essential Versus Complex Autism (Total = 233)*

<table>
<thead>
<tr>
<th></th>
<th>Essential autism = 187 (80%)</th>
<th>Complex autism = 46 (20%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic indicators</td>
<td>162:25</td>
<td>33:11</td>
<td>0.08</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>6.5:1 (162:25)</td>
<td>3.2:1 (33:11)</td>
<td></td>
</tr>
<tr>
<td>Sib recurrence risk (true autism)</td>
<td>4% (9/231)</td>
<td>0% (0/52)</td>
<td>ns</td>
</tr>
<tr>
<td>Sib recurrence (true + traits)</td>
<td>12% (27/231)</td>
<td>6% (3/52)</td>
<td>ns</td>
</tr>
<tr>
<td>Latter sib recurrence (true autism)</td>
<td>6% (6/97)</td>
<td>0% (0/18)</td>
<td>ns</td>
</tr>
<tr>
<td>Latter sib recurrence (true + traits)</td>
<td>13% (13/97)</td>
<td>5% (1/18)</td>
<td>ns</td>
</tr>
<tr>
<td>Family history of autism*</td>
<td>20% (32/159)</td>
<td>9% (3/35)</td>
<td>ns</td>
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</tbody>
</table>

Outcome indicators
Mean IQ/DQ (SD)
70.4 (25.4) 53.1 (22.8) 0.0009
Range
20–160 20–91 0.003
Score >70
48% (71/145) 22% (6/27) 0.01
Score <55
25% (37/145) 52% (14/27) 0.006
Verbal language (≥8 years)
47% (27/58) 80% (24/30) 0.003
Seizures
17% (31/187) 39% (18/46) 0.0008
Clinical course indicator
Regressive onset*
43% (79/185) 24% (11/45) 0.02
Other associations
Abnormal EEG*
30% (32/107) 46% (12/26) ns
Abnormal brain MRI*
13% (15/122) 28% (8/29) 0.08
Syndrome diagnosis
0% (0/187) 24% (11/46) <0.0005

*Twenty-seven subjects were not included because their dysmorphology status was equivocal.

To further determine the predictive value of the factors, a logistic regression model was built which compared the physical features, laboratory results, and gender with outcome. When all cases were considered together, only macrocephaly was a significant predictor of poor outcome \( P < 0.02 \), (score test). When complex cases were considered alone, macrocephaly remained significant \( P = 0.03 \), (score test). However, when essential cases were analyzed separately, regression and (not macrocephaly) was significant \( P = 0.03 \).

To see if it would be possible to detect additional outcome related variables, similar analyses were carried out within the more homogeneous essential autism subgroup (Table VI). Regressive onset correlated most strongly with a poor outcome \( P < 0.05 \). Male sex and seizures, also emerged as significant predictive variables of poor outcome. Within the essential subgroup, no strong predictors for an excellent outcome emerged (Table VII).

Eleven individuals, comprising 4.2% of the total population, were identified with either a chromosomal, single gene or teratogenic syndrome which was considered to be the cause of their autistic disorder (Table VIII). All 11 individuals were classified as complex autism and comprised 24% of that group. Both subjects with tuberous sclerosis were identified by hypopigmented macules and shagreen patches identified during the physical examination and therefore were included in the complex group. Exclusion of the individuals with identified syndromes yielded no significant changes in the data (not shown).

**DISCUSSION**

There has been a renewed interest in physical phenotypic features in autism, with the hope that they might function as biological markers to separate subsets of subjects for genetic studies. Kanner [1943] originally described children with autism as well formed, beautiful, and free of obvious defects. Although this image is entrenched in the autism literature, it is obvious to the dysmorphologist that a significant number of children with autism have multiple physical anomalies. In the 1970s and early 1980s a number of studies documented that taken as a group, autistic children had physical features outside the norm [Mukhin and Isaev, 1975; Steg and Rapoport, 1975; Walker, 1977; Campbell et al., 1978; Links, 1980; Links et al., 1980; Gualtieri et al., 1982]. Walker, using the Waldrop weighted scoring scale [Waldrop and Halverson, 1971] for 16 anomalies, studied 74 autistic and non-autistic children matched for age, sex, socioeconomic group and geographic domicile, and found that the mean minor anomaly score of 5.76 for the autistic children was significantly higher than the control group score of 3.53. He concluded that this shift to a greater number of anomalies in the autistic subjects proved organicity in autism. Links et al. [1980] recognized that autistic children had more anomalies than their sibs, and that the autistic children with the higher anomaly scores had lower IQs, spent more time in the hospital, had less frequent family histories of psychotic illness, drug, or alcohol abuse. They concluded that the anomalies were the result of some unknown organic factor that played a role in the etiology of autism. Smalley et al. [1988] declared that MPAs result from insults, either genetic or environmental, that occur in the first trimester, and represent an indirect marker of some unknown factor that played a role in the etiology of autism. Smalley et al. [1988] declared that MPAs result from insults, either genetic or environmental, that occur in the first trimester, and represent an indirect marker of some unknown factor that played a role in the etiology of autism.

TABLE III. Outcome Levels for Individuals With Essential Versus Complex Autism at ≥8 Years of Age

<table>
<thead>
<tr>
<th></th>
<th>Excellent (N = 15)</th>
<th>Good (N = 14)</th>
<th>Poor (N = 52)</th>
<th>Total (N = 81)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential autism</td>
<td>93% (14/15)</td>
<td>79% (11/14)</td>
<td>54% (28/52)</td>
<td>53</td>
<td>0.009</td>
</tr>
<tr>
<td>Complex autism</td>
<td>7% (1/15)</td>
<td>21% (3/14)</td>
<td>46% (24/52)</td>
<td>28</td>
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</table>
The clinical and genetic differences that we identify between complex and essential autism validate our hypothesis that the groups are etiologically distinctive and also provide practical prognostic information. For all outcome measures, individuals with complex autism do less well. They are twice as likely to have IQ/DQ scores less than 55 (52% vs. 25%) and less than half as likely to have IQ/DQ scores in the normal range (22% vs. 46%). They are twice as likely to develop seizures (39% vs. 17%), and twice as likely to have abnormal brain structure (28% vs 13%). Our outcome analyses, based on both the acquisition of functional language and IQ/DQ scores, show that though many features correlate with poor outcomes, the complex designation is the most sensitive test, yielding an 86% positive predictive value of poor outcome. These results are consistent with our retrospective study of 19 children with autism who completed one year of 22 hr/week 1:1 early intensive behavioral intervention [Stoelb et al., 2004]. The most significant predictor of change in performance scores over the year of therapy was the dysmorphology designation (P = 0.009); the presence or absence of dysmorphology predicted language acquisition for 90% of the non-verbal participants.

The genetic consequences of the distinction between complex and essential autism are equally informative. Individuals with essential autism are twice as likely to be male (6.5:1 vs. 3.2:1), are more than twice as likely to have a family history of autism (20% vs. 9%) and the sib recurrence risk is 4%–6% compared with no recurrences in the 46 families of children with complex autism. Finding lesser autistic traits in sibs of children with essential autism was twice that for complex autism families (12% vs. 6%). The small number of sibs limits the power of the calculations; however, the very similar magnitude of differences for all sibs, latter born sibs and for classical autism and milder autistic traits illustrates a consistent pattern. The fact we have not observed any recurrence in sibs in the complex

### TABLE IV. Autism*: Predictors of Poor Outcomes (IQ < 55 and Non-Verbal at ≥8 Years) (N = 62)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcephaly</td>
<td>14</td>
<td>100</td>
<td>100</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>Dysmorphism status</td>
<td>34</td>
<td>86</td>
<td>81</td>
<td>5.6</td>
<td>1.5–20.5</td>
</tr>
<tr>
<td>Complex autism</td>
<td>47</td>
<td>87</td>
<td>86</td>
<td>4.2</td>
<td>1.4–12.5</td>
</tr>
<tr>
<td>Seizures</td>
<td>37</td>
<td>81</td>
<td>77</td>
<td>2.4</td>
<td>0.9–6.5</td>
</tr>
<tr>
<td>Regressive onset</td>
<td>40</td>
<td>74</td>
<td>72</td>
<td>1.9</td>
<td>0.8–4.7</td>
</tr>
<tr>
<td>Male sex</td>
<td>81</td>
<td>22</td>
<td>64</td>
<td>1.2</td>
<td>0.4–3.3</td>
</tr>
<tr>
<td>Female sex</td>
<td>19</td>
<td>78</td>
<td>60</td>
<td>0.8</td>
<td>0.3–2.3</td>
</tr>
<tr>
<td>Abnormal brain MRI</td>
<td>21</td>
<td>72</td>
<td>58</td>
<td>0.7</td>
<td>0.2–2.5</td>
</tr>
<tr>
<td>Abnormal EEG</td>
<td>35</td>
<td>50</td>
<td>59</td>
<td>0.5</td>
<td>0.2–1.7</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>29</td>
<td>44</td>
<td>47</td>
<td>0.3</td>
<td>0.1–0.8</td>
</tr>
</tbody>
</table>

*All autism, includes individuals with essential and complex subtypes.

### TABLE V. Autism*: Predictors of Excellent Outcome (IQ > 70 and Verbal at ≥8 Years) (N = 18)*

<table>
<thead>
<tr>
<th>Feature</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not microcephaly</td>
<td>100</td>
<td>11</td>
<td>20</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>Essential autism</td>
<td>98</td>
<td>41</td>
<td>58</td>
<td>9.7</td>
<td>1.2–78.1</td>
</tr>
<tr>
<td>Non-dysmorphic</td>
<td>77</td>
<td>46</td>
<td>25</td>
<td>6.9</td>
<td>0.9–54.6</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>56</td>
<td>65</td>
<td>26</td>
<td>2.3</td>
<td>0.8–6.5</td>
</tr>
<tr>
<td>Male sex</td>
<td>83</td>
<td>21</td>
<td>19</td>
<td>1.3</td>
<td>0.3–5.2</td>
</tr>
<tr>
<td>Normocephaly</td>
<td>44</td>
<td>46</td>
<td>16</td>
<td>0.8</td>
<td>0.3–2.4</td>
</tr>
<tr>
<td>Female sex</td>
<td>17</td>
<td>79</td>
<td>15</td>
<td>0.7</td>
<td>0.2–2.9</td>
</tr>
<tr>
<td>Normal EEG</td>
<td>57</td>
<td>40</td>
<td>12</td>
<td>0.5</td>
<td>0.1–2.5</td>
</tr>
</tbody>
</table>

*All autism, includes individuals with essential and complex subtypes.
group is undoubtedly a statistical aberration since children with complex autism may have disorders such as fragile X syndrome and familial chromosome disorders which confer explicit recurrence risks. Notwithstanding, we feel that once chromosome disorders, fragile X syndrome, Sotos syndrome, and tuberous sclerosis have been ruled out, parents of children with complex autism can be counseled that their recurrence risk is lower than the 4%–6% observed with essential autism. This is consistent with the observation Szatmari [1999] who noted that relatives of probands with higher IQs were at greater risk than those of probands with lower IQ.

Taken together the different sex ratios, family histories, and recurrence risks establish that complex autism is genetically distinct from essential autism. The relationship of complex to essential autism resembles the genetic consequences of another complex genetic disorder, cleft lip ± cleft palate (CL ± P). Originally considered a relatively homogeneous, multifactorial disorder [Carter, 1976; Fraser, 1976], CL ± P is now recognized to have many etiologies. About 5% of cases are caused by various single gene mutations and chromosome disorders; the rest are caused by various genes and/or environmental effects [Marazita et al., 2002]. Like autism, about 30% of the children with CL ± P have other minor and major anomalies and those children have a lower sib recurrence risk [Gorlin et al., 1990].

The assumption is that for those children, the etiology was a sporadic environmental insult. This theory is hard to prove since most environmental insults to morphogenesis are difficult to diagnose. Nevertheless, it does provide a rational hypothesis to explain the lower risk of recurrence for families whose child has the complex autism phenotype.

An additional practical consequence of the separation is that all of the individuals with recognizable syndromes fall in the complex autism diagnosis, or in the case of tuberous sclerosis can almost always be recognized by the dysmorphology examination [Reach et al., 1999]. As more children are being diagnosed with autism, and as the demand for diagnostic services increases, our data indicate that the use of extensive laboratory studies can be limited to children with complex autism. We have not identified any children with chromosome disorders, including the 15q duplications who have not declared themselves by the morphology examination.

For research, ramifications of this distinction come from defining the more genetically homogeneous essential subgroup. By analyzing essential autism separately, we have already made a number of significant observations. First, within essential autism, a regressive onset and macrocephaly have emerged as stronger predictors of poor outcomes. A history of regression in language at the onset of the autistic symptoms predicted a poor outcome with 46% sensitivity, 73% specificity, and a positive predictive value of 84%. Recognizing these outcome predictors is a first step toward designing treatments to improve outcomes. Furthermore, since essential autism is the more heritable subgroup, removing complex autism probands from analyses should improve the power of linkage and sib pair analyses.

The proportion of patients with essential versus complex autism in a population is a function of ascertainment. Patients ascertained from clinics that serve children with other developmental disabilities in addition to autism are likely to be weighted toward patients with complex autism while dedicated autism clinics are weighted toward essential autism. Study populations that select for sib pairs are weighted toward essential autism. The sex ratio of a population will provide a rough estimate of the proportion of essential to complex autism patients, since the male to female ratio is higher in essential autism.

The distinction between complex and essential autism represents the first pass at dissecting the etiologic heterogeneity within the autism diagnosis. The tools we have available to render this classification are neither entirely precise or completely accurate. Though the morphology examination has allowed geneticists to delineate hundreds of discrete genetic disorders, it is still impossible to determine for every individual whether certain physical features which straddle the line between normal and abnormal are the consequence of a significant insult to early morphogenesis or rather result from a benign familial predisposition. Ten percent of autistic individuals could not be unequivocally assigned as either dysmorphic or non-dysmorphic. And macrocephaly, though a potent predictor of poor developmental outcomes, is defined arbitrarily based on a head circumference measured at 2 SD. We attempted to assess brain dysmorphology as a third factor.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Sensitivity for poor outcome (%)</th>
<th>Specificity for poor outcome (%)</th>
<th>Positive predictive value (%)</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regressive onset</td>
<td>61</td>
<td>43</td>
<td>41</td>
<td>4.6</td>
<td>1.4–15.3</td>
</tr>
<tr>
<td>Male sex</td>
<td>86</td>
<td>24</td>
<td>56</td>
<td>1.9</td>
<td>0.5–7.7</td>
</tr>
<tr>
<td>Seizures</td>
<td>29</td>
<td>80</td>
<td>62</td>
<td>1.6</td>
<td>0.4–5.7</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>50</td>
<td>52</td>
<td>54</td>
<td>1.1</td>
<td>0.4–3.2</td>
</tr>
<tr>
<td>Abnormal MRI</td>
<td>17</td>
<td>75</td>
<td>50</td>
<td>0.6</td>
<td>0.1–3.6</td>
</tr>
<tr>
<td>Female sex</td>
<td>14</td>
<td>76</td>
<td>40</td>
<td>0.5</td>
<td>0.1–2.1</td>
</tr>
<tr>
<td>Abnormal EEG</td>
<td>33</td>
<td>50</td>
<td>53</td>
<td>0.5</td>
<td>0.1–1.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feature</th>
<th>Sensitivity for excellent outcome (%)</th>
<th>Specificity for excellent outcome (%)</th>
<th>Positive predictive value (%)</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal brain MRI</td>
<td>87</td>
<td>23</td>
<td>29</td>
<td>2.1</td>
<td>0.2–21.0</td>
</tr>
<tr>
<td>Male sex</td>
<td>86</td>
<td>21</td>
<td>28</td>
<td>1.5</td>
<td>0.3–8.3</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>50</td>
<td>51</td>
<td>27</td>
<td>1.0</td>
<td>0.3–3.6</td>
</tr>
<tr>
<td>Normocephaly</td>
<td>50</td>
<td>49</td>
<td>26</td>
<td>0.9</td>
<td>0.3–3.2</td>
</tr>
<tr>
<td>Female sex</td>
<td>15</td>
<td>79</td>
<td>20</td>
<td>0.6</td>
<td>0.1–3.5</td>
</tr>
<tr>
<td>Normal EEG</td>
<td>50</td>
<td>37</td>
<td>13</td>
<td>0.6</td>
<td>0.1–3.5</td>
</tr>
</tbody>
</table>
in the complex/essential distinction. Though dysmorphic individuals are more than twice as likely to have abnormalities on their brain MRI, the range of abnormalities was too broad to be useful. We believe this is due to our relative lack of experience in interpreting brain structure variations by MRI. Once we have accumulated more experience interpreting findings like asymmetry of the ventricles and a giant cisterna magna, we suspect that morphogenesis of the brain will be incorporated into the diagnostic algorithm for complex versus essential autism. DeLong [1999] formulated an intriguing hypothesis that two autism subgroups should be distinguishable on the basis of brain damage. The first type is characterized by bilateral brain damage in early life and the second, more common, idiopathic form is not associated with brain damage. His hypothetical idiopathic form closely resembles essential autism. For now, the separation of complex and essential autism can make more of a contribution to studies of brain morphology in autism than vice versa. Limiting studies of brain morphology to individuals with the essential autism should decrease the background noise of structural variation associated with generalized insults to brain morphogenesis and allow analysis of the more uniform population.

REFERENCES


Beglinger LJ, Smith TH. 2001. A review of subtyping in autism and proposed applications in interpreting findings by MRI. Once we have accumulated more experience interpreting findings like asymmetry of the ventricles and a giant cisterna magna, we suspect that morphogenesis of the brain will be incorporated into the diagnostic algorithm for complex versus essential autism. DeLong [1999] formulated an intriguing hypothesis that two autism subgroups should be distinguishable on the basis of brain damage. The first type is characterized by bilateral brain damage in early life and the second, more common, idiopathic form is not associated with brain damage. His hypothetical idiopathic form closely resembles essential autism. For now, the separation of complex and essential autism can make more of a contribution to studies of brain morphology in autism than vice versa. Limiting studies of brain morphology to individuals with the essential autism should decrease the background noise of structural variation associated with generalized insults to brain morphogenesis and allow analysis of the more uniform population.


TABLE VIII. Syndromes Identified in the 260 Autism Probands

<table>
<thead>
<tr>
<th>Chromosomal</th>
<th>Single gene disorders</th>
<th>Teratogen</th>
<th>Total syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>46,XX,r(8), 46,XY,del(8)(p22.2), isodicentric 15q, der 15t(4;15) (p16;q13) mat, 47, XY, +21, 47,XXY</td>
<td>Tuberous sclerosis (2), Sotos syndrome, Sotos like syndrome</td>
<td>Fetal valproate syndrome</td>
<td>6 (2.3)</td>
</tr>
<tr>
<td>4 (1.5)</td>
<td>1 (0.4)</td>
<td>11 (4.2)</td>
<td></td>
</tr>
</tbody>
</table>


