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Copy Number and SNP Arrays in Clinical Diagnostics

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Abstract

The ability of chromosome microarray analysis (CMA) to detect submicroscopic genetic abnormalities has revolutionized the clinical diagnostic approach to individuals with intellectual disability, neurobehavioral phenotypes, and congenital malformations. The recognition of the underlying copy number variant (CNV) in respective individuals may allow not only for better counseling and anticipatory guidance but also for more specific therapeutic interventions in some cases. The use of CMA technology in prenatal diagnosis is emerging and promises higher sensitivity for several highly penetrant, clinically severe microdeletion and microduplication syndromes. Genetic counseling complements the diagnostic testing with CMA, given the presence of CNVs of uncertain clinical significance, incomplete penetrance, and variable expressivity in some cases. While oligonucleotide arrays with high-density exonic coverage remain the gold standard for the detection of CNVs, single-nucleotide polymorphism (SNP) arrays allow for detection of consanguinity and most cases of uniparental disomy and provide a higher sensitivity to detect low-level mosaic aneuploidies.

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BACKGROUND AND HISTORY

Since the discovery that trisomy 21 is the cause of Down syndrome, there have been progressive improvements in cytogenetic and molecular methods to identify genetic and epigenetic causes of phenotypic abnormalities. Early chromosome analysis, which was limited to the counting of the total number of chromosomes, progressed to various forms of chromosomal banding to distinguish individual chromosomes. Later, various forms of high-resolution banding, including Giemsa-banded (G-banded) chromosome analysis in prometaphase chromosomes, allowed differentiation of 700–850 bands per haploid genome according to the International System for Human Cytogenetic Nomenclature (ISCN) (6). Reviews of the history of chromosome analysis through the beginning of array methods are available (96, 105). Depending on the banding resolution and characteristics of a region, deletions and duplications of 5–10 megabases (Mb) could be identified with varying degrees of reliability. Fluorescence in situ hybridization (FISH) was developed to score the presence or absence of specific DNA regions of a few kilobases (kb) on a metaphase image, and its primary use was to identify known deletion syndromes such as DiGeorge and Williams syndromes. Although interphase FISH had the potential to detect duplications as well as deletions, it was not widely used, and the discovery of many duplication syndromes awaited the arrival of array technologies described below. By 1996, a complete set of telomere probes was identified (72), and over the next few years, telomere FISH became accepted as a clinically useful tool to identify deletions and translocations involving the tips of chromosome arms containing unique genes and causing intellectual disability (ID) (54). Starting in 2004, reports began to appear describing evidence that array comparative genomic hybridization (CGH) could detect causative deletions and duplications in children with ID, other disabilities, and congenital malformations. Most of the early studies were tabulated in a review published in 2007 (99). Initially, array CGH was performed using DNA from bacterial artificial chromosomes (BACs) spotted on glass slides, but use of oligonucleotide arrays was also reported. Oligonucleotide arrays with or without detection of single-nucleotide polymorphisms (SNPs) had various advantages and disadvantages (as discussed below), but it quickly became evident that chromosome microarray analysis (CMA) could detect causative genetic abnormalities in 5%–15% of patients with serious disabilities who had a previous normal G-banded karyotype analysis. From 2004 to the present, the ability of CMA to detect causative genetic abnormalities associated with various disabilities, congenital malformations, and neurobehavioral phenotypes has experienced meteoric progress, which is unprecedented in the practice of clinical genetics.

TECHNICAL CONSIDERATIONS

The landscape for the use of arrays in clinical genetic diagnosis is somewhat complex, but most arrays were originally designed to detect genomic copy number using array CGH or to perform SNP genotyping. BAC arrays have largely disappeared from use. Two manufacturers of oligonucleotide arrays, Agilent and Nimblegen, designed arrays with longer oligonucleotides of 50–70 base pairs for use in array CGH with the emphasis on quantification of genomic copy number. Two other manufacturers of oligonucleotide arrays, Illumina and Affymetrix, designed arrays that were focused on SNP genotyping but could be used to determine genomic copy number. The Illumina arrays determine SNP genotypes by single-base extension, whereas Affymetrix arrays use single- or multiple-base mismatch hybridization. In array CGH, two DNAs are labeled with fluorescent dyes of different color (usually red and green) and cohybridized to a single array to obtain direct comparison of copy number between a test and a control DNA sample. For SNP arrays, only a single test DNA is labeled and hybridized to the array for comparison to previously run controls. A comparison of array CGH and SNP arrays is provided in **Table 1**.

Table 1 Comparison of array comparative genomic hybridization (CGH) and single-nucleotide polymorphism (SNP) arrays

Array CGH	SNP arrays
Single-sequence oligonucleotides of ~60 bp	Two 20–60-bp oligonucleotides of different sequence
Two labeled DNAs (patient and control) per hybridization	Only patient DNA labeled and hybridized
Resolution down to size of oligonucleotides; exon by exon	Resolution limited by SNP distribution and signal to background
No detection of UPD or consanguinity	Able to detect consanguinity and most UPD
Limited SNP addition possible recently	Detection of most known clinically relevant CNVs but not exon by exon

Abbreviations: CNV, copy number variant; UPD, uniparental disomy.

Array CGH typically provides optimal determination of copy number in part by virtue of comparison to an internal control hybridized to the same array and by use of longer oligonucleotides that can be positioned quite freely across the genome. SNP arrays, in contrast, are limited by the distribution of SNPs in the genome and by the use of shorter oligonucleotides if mismatch hybridization is utilized. All arrays have regions of the genome that are resistant to ideal definition often related to low copy repeat (LCR) sequences and highly repetitive sequences such as long interspersed repetitive elements (LINEs) and short interspersed repetitive elements (SINEs) that complicate the analysis. Highly homologous sequences such as pseudogenes cannot be easily distinguished and genotyped. Although copy number arrays have some advantages, they cannot detect copy number neutral differences associated with loss of heterozygosity (LOH) in tumors or constitutive absence of heterozygosity (AOH) that occur with consanguinity or uniparental disomy (UPD), but LOH and AOH are readily detected by SNP arrays. Various manufacturers have attempted to develop arrays that have the best of both designs by adding nonpolymorphic oligonucleotides to SNP arrays to improve coverage or by adding SNP detection to CGH arrays, but no array fully achieves this goal. A recent modification of Agilent CGH arrays to allow limited screening for blocks of LOH or AOH has advantages in the clinical setting but does not provide robust SNP genotypes (**Figure 1**).

EXON-BY-EXON COVERAGE

Using array CGH, it is feasible to check for copy number of individual exons in the genome, sometimes referred to as exon-by-exon or exon-focused coverage. This can be done by placing 4–6 oligonucleotides within or adjacent to each exon for one or more genes. In one setting, this is typically done when specific gene sequencing is being performed and exonic deletions may be missed by the sequencing strategy. Multiple diagnostic laboratories use this strategy in combination with gene sequencing, but it increases the cost of individual gene sequencing tests significantly. For one or a few genes, MLPA or qPCR can be used to search for exonic deletions at lower cost (40, 116).

Exon-by-exon copy number analysis can also be incorporated into routine array CGH testing with greater emphasis on detecting exonic deletions or duplications in genes where haploinsufficiency is known to cause a phenotype. This approach has been reported using exon-by-exon coverage for ~1,700 genes, and numerous diagnostic deletions were detected (13) (**Figures 2** and **3**). It is now feasible to perform analysis of exon-by-exon copy number for virtually all exons in the genome using an Agilent 1M custom array (P. Celestino-Soper, C. Shaw, S. Sanders, J. Li, M. Murtha, et al., manuscript under review) or Nimblegen 720K catalog or 4M custom arrays. Looking to the future, exome sequencing will likely replace some individual sequencing tests and most sequencing panels. Exome sequencing may be validated to determine exon copy number as well as sequence variants. If exome sequencing does

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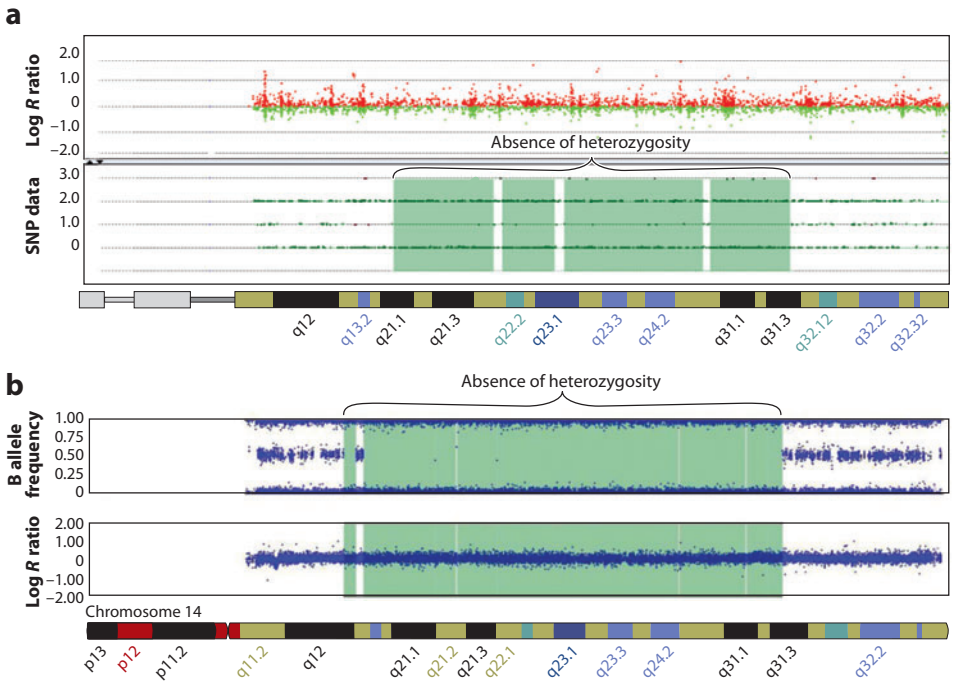

Figure 1

Novel technology of combined comparative genomic hybridization (CGH) and single-nucleotide polymorphism (SNP) array. Uniparental disomy of chromosome 14. (*a*) Agilent Genomic Workbench view of custom 180K CGH+SNP array data for chromosome 14. This new generation of genomic microarray provides high-quality array CGH data for detection of copy number variants plus a limited amount of SNP data to screen for absence of heterozygosity. Top panel shows normal $\log_2$ *R* ratio. Bottom panel shows SNP data. The region of absence of heterozygosity is highlighted in green. (*b*) Illumina GenomeStudio view of 1M HumanOmni1-Quad array data for chromosome 14 on the same sample. This high-resolution SNP array reproduces the findings detected by the combined CGH+SNP array. Normal $\log_2$ *R* ratio is shown in the bottom panel. The B allele frequency plot (*top panel*) with region of absence of heterozygosity is highlighted in green.

not give adequate data for exon copy number, it can be paired with an array to determine copy number for most or all exons in the genome.

DNA METHYLATION

Determination of DNA methylation has clinical utility in detecting cases of Prader–Willi syndrome, Angelman syndrome, Beckwith–Wiedemann syndrome, and other abnormalities of genomic imprinting. Arrays to analyze DNA methylation are available, with an Illumina Infinium platform for analysis of bisulfite-treated DNA being the most readily adapted to the clinical laboratory (7, 104, 111).

EXOME/GENOME SEQUENCING

It is widely speculated that some form of whole-genome or whole-exome sequencing using next-generation or next-next-generation platforms may replace the array methods that are only now becoming firmly entrenched in clinical laboratories. It is clear that this is theoretically possible even now, but when and if this transition might happen as routine clinical method is not clear. Patients will likely have an array analysis as a first step for some time with exome/genome sequencing if the array is normal. High coverage (10–20-fold or more) genomic sequencing can detect copy number and SNP genotyping, but in addition can

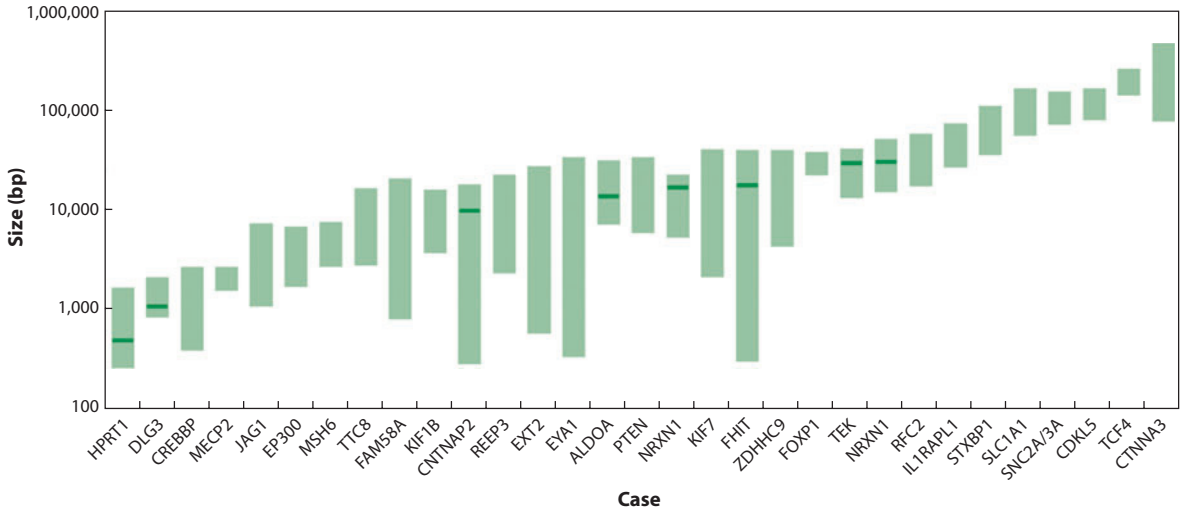

Figure 2

Exon deletions detected by array comparative genomic hybridization (CGH). Exon-by-exon coverage array CGH allows for detection of single-exon and multi-exon deletions and duplications. This image provides a visualization of intragenic copy number variants (CNVs) from $<10^3$ base pairs (bp) to $>10^5$ bp in size that have been detected in our own patient cohort at the Molecular Genetics Laboratories at Baylor College of Medicine. Each position along the abscissa represents a unique case, labeled by the gene containing an intragenic CNV. Green bars span the size range predicted by array CGH. Cases for which DNA sequencing has been performed are marked by a dark green line at the exact size of the rearrangement. Figure taken from Reference 13.

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detect point mutations that may cause a disease phenotype. The major obstacle to be overcome for routine clinical exome or genome sequencing is cost, but there are other challenges, such as interpretation of enormous amounts of data, some of which will be of uncertain significance. Sequencing artifacts and false-positive results are also a concern. However, for identifying genetic causes of congenital disabilities and for genotyping of tumors, whole-genome or whole-exome sequencing can provide data that are not obtainable with array methods. Thus, it is likely that sequencing-based methods will eventually complement if not replace array-based methods.

EVALUATION OF DEVELOPMENTAL DELAY, INTELLECTUAL DISABILITY, OR MULTIPLE CONGENITAL ANOMALIES

ID affects 2%–3% of the general population and represents the most costly cause of lifelong morbidity in the United States (60). The causes of ID are manifold and include monogenic and chromosomal disorders, teratogenic exposures, perinatal asphyxia, infections, and others. Identifying the exact underlying etiology can be challenging and may be frustrating for parents/guardians and physicians alike, as the diagnostic workup may be time consuming, expensive, and often still remains negative. However, establishing an underlying diagnosis can decrease future diagnostic investigations, improve anticipatory guidance for the family, and allow for more specific therapeutic interventions in some learning disabilities. As an example, it has been hypothesized that deletions of *CHRNA7*, coding for the alpha7 nicotinic acetylcholine receptor subunit, could be treated with nicotine patches or cholinergic agonists (94). For type 1 autosomal dominant nocturnal frontal lobe epilepsy, caused by mutations in *CHRNA4*, nicotine patches were reported to have an antiepileptic effect in an *n*-of-1 study (121). Successful treatment of rage outbursts in an adult with 15q13.3 deletion

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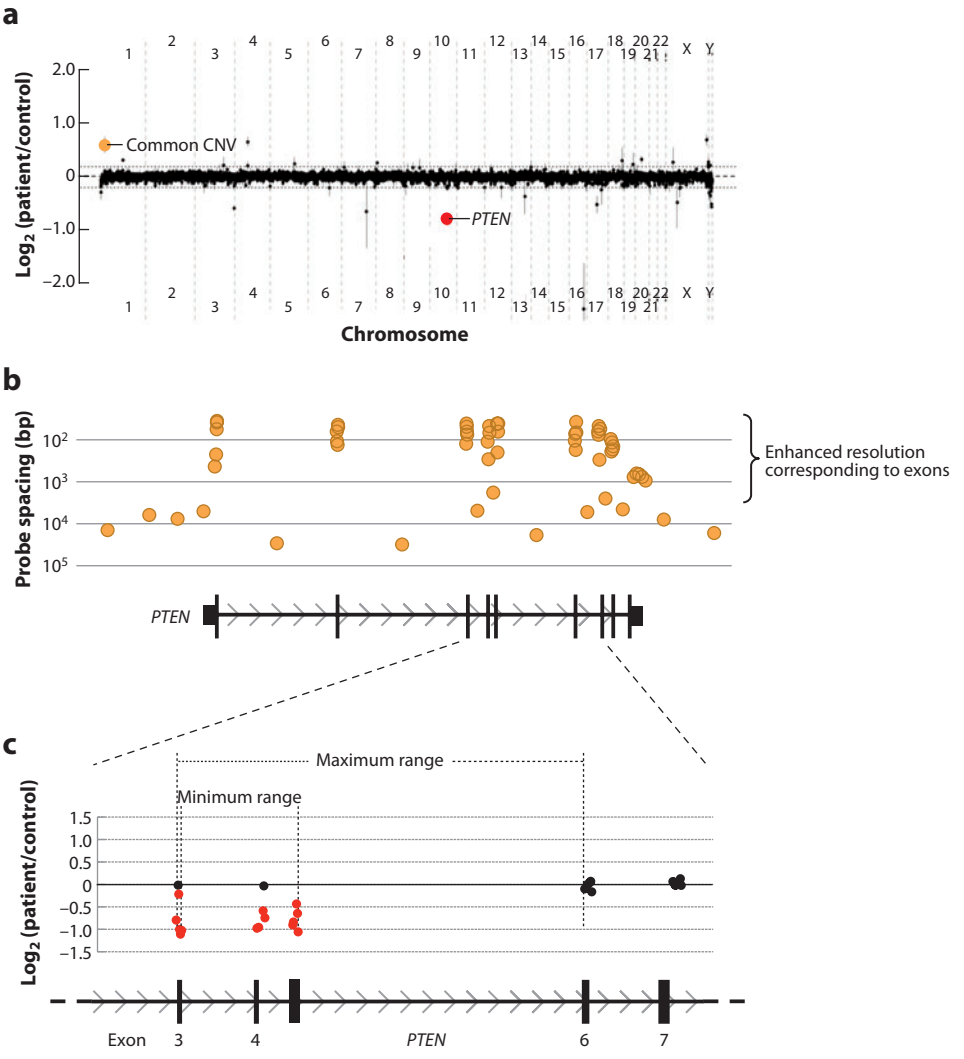

Figure 3

PTEN intragenic deletion detected by array comparative genomic hybridization (CGH). Detection of exons 3–5 in the *PTEN* gene in a patient with clinical presentation of Bannayan–Riley–Ruvalcaba syndrome. (*a*) Genome-wide view of array CGH data. The red point indicates the copy number loss of interest (*PTEN*). The orange point indicates a known benign copy number variant (CNV). (*b*) Array probe density is shown for a portion of chromosome 10, illustrating that probes are disproportionately localized to exons of a gene of interest (*PTEN*). The horizontal coordinate of each orange dot corresponds to the midpoint between the genomic locations of two consecutive array probes; its vertical coordinate is the number of base pairs (bp) between these probes. In this example, exonic resolution is up to 103-fold higher than the genomic baseline. (*c*) Local view of the *PTEN* intragenic deletion and genomic map of this region of *PTEN*. The graphics are aligned with one another. Plot of individual array probes, from which a minimum range and maximum range (defining the minimum and maximum expected boundaries of the deletion, respectively) can be established. The red dots represent single oligonucleotide probes with loss of copy number. The black dots represent oligonucleotide probes for which no copy number change was detected. Panels *a* and *c* taken from Reference 13.

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syndrome with galantamine, an acetylcholine esterase inhibitor, has been reported (24).

Establishing the etiology may also decrease anxiety and allow for proper recurrence risk estimates for future pregnancies when the respective parents plan to have more children in the future. Although the recurrence risk for developmental disabilities of unknown etiology based on empirical datasets would be quoted as 3%–7% (113), a de novo microdeletion or microduplication would have a much lower recurrence risk. In contrast, an ID syndrome of autosomal recessive inheritance would have a 25% chance of recurrence.

Traditional karyotype analysis typically yields relevant findings in approximately 5% of probands with ID (112). With the implication of array CGH and high-density SNP arrays into clinical practice, it has become increasingly clear that the diagnostic yield of these tests in patients with idiopathic developmental delay (DD), ID, or multiple congenital anomalies (MCAs) is higher as compared to G-banded karyotyping and FISH technology. The detection rate depends on microarray platform, coverage, and probe density, as well as the phenotypic spectrum of the patients studied and the prescreening process, but the underlying etiology can be uncovered in at least 10%–20% of cases (101). The more severe and complex the phenotype, the higher the yield, with dysmorphology, congenital anomalies, and more significant ID all correlating with a higher likelihood of identifying a causative underlying CNV.

A 44 kb oligonucleotide array CGH used among 1,499 probands with ID at five academic diagnostic laboratories achieved a diagnostic yield of 7%–14%, and the authors suggested 300 kb as a practical cutoff to be used for further investigations of the clinical relevance of CNVs detected (124). A meta-analysis of 19 studies and 13,926 subjects for array CGH in patients with ID and congenital anomalies estimated the overall diagnostic yield to be 10% (81). The detection of CNVs inherited from unaffected parents was considered a false-positive result, and these were seen in 7% (81) of all cases tested; the authors concluded that caution regarding this technology in clinical practice should be advised (81). However, disease-causing CNVs can be nonpenetrant in healthy parents, and mild cognitive and behavioral phenotypes can easily be overlooked during clinical evaluation that is focused on the condition of the child. In a different meta-analysis based on 36,325 unselected, consecutive individuals ascertained for ID, pathogenic aberrations were detectable in 19% of cases if array platforms with 30–70 kb median probe spacing were used (43). The same study provided important insight into the cases that would be detected by karyotype analysis, but missed by array-based investigations. A minimum of 0.78% of all referrals had a balanced chromosomal rearrangement that would have remained undetected by array analysis. These included familial rearrangements (0.48% of all referrals), de novo reciprocal translocations and inversions (0.23% of all referrals), de novo Robertsonian translocations (0.04% of all referrals), and 69,XXX triploidy (0.03% of all referrals). Given its high diagnostic yield, detecting >99% of all pathogenic chromosomal anomalies in their study, the authors proposed high-resolution array-based genome analysis as the first genetic diagnostic investigation to be performed in cases of DD/ID. They further concluded that karyotyping following an initial array-based investigation would identify a chromosomal abnormality with possible pathogenic consequences for the patient or the family in 0.78% of all DD/ID referrals (43).

Most recently, the International Standards for Cytogenomic Arrays (ISCA) Consortium performed a meta-analysis of 33 studies, including 21,698 patients tested by CMA. The study concluded that CMA offers a much higher diagnostic yield (15%–20%) for genetic testing of individuals with unexplained DD/ID, autism spectrum disorders (ASDs), or multiple congenital anomalies when compared with G-banded karyotype (3%, excluding Down syndrome and other recognizable chromosomal syndromes). A consensus statement was published, recommending chromosome microarray as a first-tier clinical diagnostic test

for individuals with developmental disabilities or congenital anomalies. They state that G-banded karyotype analysis should be reserved for patients with obvious chromosomal syndromes (e.g., Down syndrome), a family history of chromosomal rearrangements, or a history of multiple miscarriages. They also commented on the added value of standard karyotyping after negative CMA testing regarding the potential existence of balanced translocations. It was noted that balanced translocations are identified only in 0.3% of patients with ID, tested by karyotype analysis (69). Balanced translocations are more common in children with ID (7 in 455) than in control individuals (4 in 1,679) (35). However, although cytogenetic events might appear balanced on the microscopic level, many (30%–40%) have a submicroscopic imbalance when tested with high-resolution array technology. In summary, the intent of this statement, as confirmed in discussions at the January 2011 ISCA meeting, is to indicate unanimously that a karyotype is not clinically justifiable based on cost benefit considerations in a patient with DD/ID and a normal CMA result. Although balanced translocations may be present, they are still relatively unlikely to be the cause of the DD/ID. Rarely, disruption of dosage-sensitive genes causing a phenotype will be undetected if no karyotype is performed.

In the presence of multiple congenital anomalies, the combination of clinical features may suggest a specific genetic diagnosis, but if molecular genetic testing remains negative, or if only one pathogenic mutation can be identified in a disorder known to be caused by autosomal recessive mutations, then copy number analysis of the respective gene should be considered. Whole-gene or single-exon deletions have been described for several classic monogenetic disorders, including Rubinstein–Taybi syndrome, Alagille syndrome, Rett syndrome, Townes–Brocks syndrome, Pitt–Hopkins syndrome, Banayan–Riley–Ruvalcaba syndrome, Lesch–Nyhan syndrome, and others (13, 14, 87, 107).

PSYCHIATRIC DISORDERS: AUTISM, SCHIZOPHRENIA, AND OTHER NEUROPSYCHIATRIC DISORDERS

High heritability has been suggested for multiple psychiatric disorders, especially autism, schizophrenia, bipolar disorder, and Tourette syndrome (32, 64, 65, 100). However, the practice of psychiatry has long suffered from the limited information available on the biological basis of mental disorders and the absence of actual genetic alterations being identified in causal association to psychiatric disorders (4).

Autism

In a landmark paper in 2007, Sebat et al. (88) revealed that there is strong association of autism and de novo CNVs. Using an 85 kb oligonucleotide array, they identified de novo CNVs in 10% of patients with sporadic autism. Interestingly, in this study, de novo CNVs were more strongly associated with simplex autism compared with multiplex families. Several other studies followed within a short time, replicating the association of CNVs and ASDs. Marshall et al. (62) used a 500K genome-wide SNP array and found de novo CNVs in 7% of simplex and 2% of multiplex families with ASDs.

Since 2007, array technology has proven the most fruitful approach to identify autism susceptibility loci (e.g., del 16p11.2, del 15q13.3, del 17q12, dup 7q11.23) (56, 57, 70, 93, 94, 102). The respective data, and some preliminary genotype-phenotype association studies, have caused a paradigm shift in the community of geneticists and neuropsychologists, making CMA a standard test in the evaluation of individuals with ASDs. The identification of specific underlying etiologies has enabled affected families to find family support groups and networks based on the respective condition and its associated subphenotypes.

Many CNVs associated with ASDs encompass multiple genes, and it has proven challenging to pinpoint the actual culprit genes responsible for autistic behaviors in the affected

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individuals (e.g., del and dup16p11.2). An exception to the rule is 15q13.3, in which the larger 1.5 Mb deletion/duplication between breakpoints 4 and 5 affects a total of six genes, but subsequently smaller deletions were described that only remove the entire *CHRNA7* gene and the first exon of *OTUD7A*. Encoding for a neuronal acetylcholine receptor subunit, *CHRNA7* has been determined as the most likely culprit for the associated neuropsychiatric phenotypes (94). Reciprocal *CHRNA7* duplications are particularly common and will be extremely important clinically if they prove to be associated with neurobehavioral phenotypes (102). These cases might provide valuable insight into the pathophysiology of neuropsychiatric disorders, as they allow for follow-up studies in mouse and other model systems, focusing on a single susceptibility gene. Although one may argue that those studies are based on findings in a very small minority of autism cases overall, the studies might still provide insight into the underlying pathways that play a role in the pathogenesis of ASDs in general.

Using current array technology, a genetic cause can currently be identified in 10% to 25% of all children with ASDs. The underlying genetic cause is more likely to be identified in cases of lower functioning, syndromic (complex) ASDs, and less likely in the high-functioning and nonsyndromic (essential) cases of ASDs (68). With increasing density of clinically available arrays, the resolution continues to increase, and the detection rate of CNVs will continue to increase accordingly. Some groups have argued that custom-designed, exon-targeted arrays would increase the detection of small, intragenic deletions and duplications (13). In fact, intragenic deletions of several genes (e.g., *DPYD*, *NRXN1*, *IL1RAPL1*, and *FOXP1*) have been identified in individuals with ASDs (17, 18, 76, 122).

Schizophrenia

Several large studies have revealed that numerous, individually rare CNVs are associated with schizophrenia (46, 101, 109, 117). The overall burden of large, rare CNVs is increased among probands with schizophrenia as compared to the general population, and these CNVs are more likely to be associated with genes and may more frequently involve genes with roles in neurodevelopment (117). There are several overlapping, recurrent CNVs associated to schizophrenia, and interestingly, all of these have also been associated to other neurodevelopmental and neuropsychiatric disorders, including ID and autism (e.g., 1q21.2, 15q13.3, 16p11.2, and 22q11.2) (63, 101). The same applies to deletion of exons or the entire gene of *NRXN1*, which have been associated to schizophrenia, autism, and ID (33, 50, 52, 62, 80, 103). One study of 235 patients with both schizophrenia and epilepsy found likely causative abnormalities in 5.1% of cases (L. Stewart, A.L. Hall, S.H. Kang, C.A. Shaw & A.L. Beaudet, manuscript under review).

Sebat et al. (89) provided a very insightful review that discusses not only the genetic heterogeneity of schizophrenia but also the phenotypic heterogeneity as a characteristic of all schizophrenia-associated mutations. Rare mutations at many loci throughout the genome contribute to the etiology of schizophrenia, and the authors suggested considering a model of multiple rare variants and polygenic models as complementary in the etiology of schizophrenia. The actual phenotype in a patient may be influenced by a large effect in one of more mutations, but also by common genetic variants (modifiers) and other factors, including epigenetic variations and environmental exposures.

While every astute geneticist and psychiatrist familiar with the current literature realizes that autism, schizophrenia, and other neuropsychiatric disorders can each be caused by many different mutations at many different genetic loci, it is also true that a single mutation (or CNV) can lead to multiple disorders. There is high phenotypic variability even for mutations with high penetrance. Even within the same family, a CNV may cosegregate with neuropsychiatric phenotypes, but the individual mutation carriers may present with large clinical variability. In probands

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ascertained for schizophrenia and found to carry microduplications of 16p11.2, other mutation carriers within the same family had diagnoses of schizophrenia, bipolar disorder, psychosis not otherwise specified (NOS), and major depression (63). The same holds true for individuals with microduplications of *CHRNA7* in 15q13.3, carriers of which manifest with bipolar disorder, ADHD, major depressive disorder, and ID, all within one family (102). It is intriguing that some of the same mutations are associated with pediatric neurodevelopmental disorders in one study and adult neuropsychiatric disorders in another, suggesting that the phenotype of the respective individuals might be dependent, in part, on the age of ascertainment. While individuals with velocardiofacial syndrome caused by deletion 22q11.2 may manifest with schizophrenia in adulthood, this is frequently preceded by learning deficits and abnormal behaviors in childhood and adolescence. Some of these children meet diagnostic criteria for ASDs (15, 78). In fact, most CNVs that are statistically overrepresented in schizophrenia have also been associated with other disease phenotypes and neuropsychiatric disorders. Especially prominent is the overlap between schizophrenia and autism. One may argue that autism and schizophrenia represent different clinical manifestations of the same actual disorder, and that there is age-dependent expressivity, with children more likely to manifest with ASDs and adults more likely to manifest schizophrenia. Alternatively, autism and schizophrenia could be regarded as clinically distinct disorders for which the same genetic variant may represent a predisposition, whereas modifier genes and environmental factors determine the actual clinical phenotype. From a clinical perspective, the overlap between schizophrenia and autism is not without basis, as the broader phenotypes of these disorders do intersect (51).

CONCEPTS OF CAUSALITY

CNVs of uncertain clinical significance are frequently detected by SNP arrays or array CGH and represent a major challenge to the clinical provider and the affected family. Counseling symptomatic and asymptomatic individuals with CNVs of uncertain significance is challenging when it comes to deciding if the diagnostic evaluation needs to be continued, and they can hold significant potential for stigmatization. In a prenatal setting, findings of uncertain clinical significance can increase anxiety for parents, may cause uncertainty about their reproductive decisions, and can lead to the question of whether to continue or terminate a pregnancy (34).

The ISCA Consortium published criteria to aid the assessment of pathogenicity of CNVs in their consensus statement of 2010 (69). They defined primary criteria indicating that the CNV is probably pathogenic as the following: (*a*) identical CNV inherited from an affected parent; (*b*) expanded or altered CNV inherited from a parent; (*c*) similar CNV in an affected relative; (*d*) CNV overlapping a genomic imbalance defined by a high-resolution technology in a CNV database for patients with ID/DD, ASDs, or MCA; (*e*) the CNV overlapping genomic coordinates for a known genomic imbalance syndrome (i.e., previously published or well-recognized deletion or duplication syndrome); and (*f*) CNV containing morbid MIM genes or the CNV being gene rich. Primary criteria indicating that the CNV is probably benign were the following: (*a*) inheritance from a healthy parent, (*b*) similar CNV in a healthy relative, (*c*) CNV completely contained within genomic imbalance defined by a high-resolution CNV database of healthy individuals, and (*d*) the CNV being gene poor. Aside from primary criteria, the consortium also commented on general findings that may indicate if a CNV was more likely benign or pathogenic. Generally, a CNV is more likely to be pathogenic if (*a*) it is a deletion, (*b*) it is a homozygous deletion, or (*c*) if it is an amplification (with greater than one copy gain). It is generally more likely benign when (*a*) it is a duplication (with no dosage-sensitive genes within) and (*b*) it is devoid of known regulatory elements.

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Girirajan et al. (37) proposed that the co-occurrence of two CNVs increases the severity of developmental delay in the affected infants. They suggested that deletion 16p12.1 is a significant, independent risk factor for ID, which acts in concert with other factors to modify neurological phenotypes. A secondary insult during development will produce a more severe clinical manifestation, and they suggested that their two-hit model may help explain the significant comorbidity that exists among cognitive impairment, schizophrenia, and autism or the underlying phenotypic variability reported for several recurrent microdeletions. They proposed that the second hit could potentially be another CNV, a disruptive single base pair mutation in a pathway- or phenotypically related gene, or an environmental event influencing the phenotype. Notably, the proportion of second hit CNVs was higher in microdeletion and microduplication syndromes for which variable expressivity had been reported (including genetic loci 15q13.3, 16p11.2, and 22q11.2). They found inverse correlation between the proportion of de novo cases and the presence of a second hit. Although most cases of 16p11.2 deletions and duplications, as well as 15q13.3 duplications, are inherited, the vast majority of cases of Williams, Smith–Magenis, and deletion Angelman syndromes arise de novo. A low level of second hits was reported among these canonical syndromes (37). Following the same concept, sequencing studies on individuals with autism support a multihit model for disease risk (75), and a model of oligogenic heterozygosity has been proposed, especially for individuals with high-functioning ASDs (85).

Interestingly, there are multiple examples, especially in the field of neurodevelopmental disorders, for which both the microdeletion syndromes and their reciprocal duplication syndromes cause a clinical phenotype. In addition, there may be quite significant overlap in the clinical presentation associated with the deletion and the duplication, so that one wonders if deletions truly cause loss of function and duplications gain of function, or whether the CNV at that respective locus simply disrupts neuronal homeostasis in general.

There are a few examples for which deletion and duplication of the same genomic locus truly cause what could be considered opposing phenotypes. For example, Williams syndrome, caused by deletion 7q11.23, manifests with a typical cognitive profile and unique personality characteristics that include hypersociability, empathy, and overfriendliness (67). To the contrary, duplication of 7q11.2 causes language deficits and decreased social interaction, with many affected individuals meeting diagnostic criteria for ASDs (9). Maternal duplication of 11p15 or paternal deletion of 11p15 causes decreased expression of *IGF2*, manifesting with impaired growth or Silver–Russell syndrome (26, 27, 38). Paternal duplication of 11p15, paternal uniparental disomy (UPD), or maternal imprinting mutations of 11p15 lead to increased expression of *IGF2*, manifesting with overgrowth and Beckwith–Wiedemann syndrome (119, 125).

However, there exist multiple examples for which both the deletion and duplication cause overlapping, if not similar, clinical phenotypes. Both Charcot-Marie-Tooth Neuropathy type 1A (caused by duplications of *PMP22* on 17p11.2) and hereditary neuropathy with liability to pressure palsies (HNPP, caused by deletions of *PMP22*) represent demyelinating neuropathies, yet with a distinct clinical presentation (91). Smith–Magenis syndrome (caused by deletion 17p11.2, involving the *RAI1* gene) and Potocki–Lupski syndrome (caused by the reciprocal duplication) manifest with hypotonia, ID, behavioral problems, autistic behaviors, and abnormal sleep patterns (97, 106). Both deletions and duplications of 16p11.2 have been associated with ID, ASDs, motor delays, ADHD, and seizure disorder (31, 56, 84, 92, 93, 118). And even for some monogenic disorders, loss and gain of function may cause significant overlap in clinical presentation. Rett syndrome (caused by loss-of-function mutations in *MECP2*) and *MECP2* duplication syndrome share several clinical features, including ID, behavioral problems,

autistic behaviors, epilepsy, anxiety, abnormal breathing, and poor motor control (79).

The overlap of neurodevelopmental and neuropsychiatric phenotypes that results from either loss or gain of function of the same proteins (or in some cases RNA molecules) supports the idea that normal cognition and behavior depend on tight neuronal homeostatic control mechanisms (79). Neurons are required to be highly flexible regarding their level of excitability, depending on developmental time points and in response to constantly changing environmental factors. Considering a model of neuronal homeostasis, both loss- and gain-of-function mutations can lead to an overall dysfunctional neuronal network that has hindered synaptic flexibility, leading to impaired function of the overall system. Researchers proposed that impaired synaptic flexibility potentially explains the overlapping clinical phenotypes caused by microdeletions and -duplications of the same genomic region. The impaired responsiveness of neuronal networks to internal and external stimuli may also explain the fluctuating nature of many psychiatric disorders.

MOSAICISM, CHIMERISM, AND UNIPARENTAL DISOMY

Genome-wide SNP arrays have the ability to detect absence of heterozygosity, UPD, and CNVs. But they have also been shown to be quite sensitive for detecting low-level mosaic aneuploidies and chimerism (21). Chromosomal mosaicism is defined as the presence of two or more different chromosome complements within an individual who developed from a single zygote, while chimerism is based on fusion of two different zygotes within a single embryo.

Mosaicism

Mosaicism has been reported for many types of chromosome abnormalities, including trisomy, monosomy, triploidy, deletions, duplications, rings, and other types of structural rearrangements. Mosaic aneuploidy is the most common type of mosaicism (42). Studies on early human embryos have demonstrated that mosaicism for chromosome anomalies is seen in more than 50% of all embryos generated by in vitro fertilization (11). More recently, it was uncovered that chromosomal rearrangements occur at high frequency in early IVF embryogenesis, but not in the preceding premeiotic or meiotic cell cycles. Segmental imbalances were detected in as much as 70% of all embryos analyzed. Of the 23 embryos studied (all at 3- or 4-day-stage), 35% were mosaic, consisting of normal blastomeres as well as blastomeres with either whole-chromosome or segmental aneuploidies or both (114). While chromosomal mosaicism can generally be identified cytogenetically, the sensitivity is directly correlated to the percentage of mosaic cells present in the sample analyzed. Identification of lower levels of mosaicism can be challenging, as many cells have to be counted. Standard routine chromosome analysis, counting and analyzing 20 cells, will detect 14% mosaicism with 95% confidence (45). Lower level mosaicism can only be detected if more cells are counted; however, this would not be done unless there is an actual suspicion for chromosomal mosaicism. As routine chromosome analysis will use cultured lymphocytes (or amniocytes for prenatal samples) and chromosomally abnormal cells may not grow and divide as well in culture, this could introduce an additional bias, and the low-level mosaicism may be missed for that reason (77).

It is well established that some mosaic chromosome abnormalities are not found in metaphase preparations from cultured peripheral blood, causing the respective conditions to be missed by routine karyotype analysis. For example, Pallister–Killian syndrome, caused by mosaic tetrasomy 12p, is routinely detected in skin fibroblasts, although the karyotype of cultured lymphocytes appears normal (58). The identification of mosaic tetrasomy 12p by array CGH suggested that the use of genomic DNA extracted from uncultured peripheral blood may more accurately identify the true level of mosaicism in a given peripheral blood specimen

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when compared with stimulated lymphocytes and routine karyotype analysis (5).

Array analysis by comparative genomic hybridization and SNP array analysis offer several advantages for detection of mosaicism when compared with standard chromosome analysis. First, a large number of cells are surveyed at once because DNA is extracted from a pool of many cells. Second, both interphase and metaphase cells are analyzed, thereby reducing the culture bias that is introduced by analysis of metaphase cells only (21). Using dilution series of known chromosomal abnormalities on array CGH or SNP array platforms, earlier studies estimated the minimal detection rate of mosaicism at 10%–20% (5, 23, 123). More recently, Conlin et al. (21) showed how SNP-array technology can detect chromosomal mosaicism down to a level of 5%, as demonstrated by serial dilutions and a report of actual patient data. In particular, they commented that B allele frequency was more sensitive to the subtle loss or gain of a haplotype when compared with $\log_2 R$ ratio and its ability to detect subtle shifts in intensity levels. Mosaicism that introduces a new haplotype in the abnormal cell would be exquisitely sensitive to detection, and mosaicism levels of even lower than 5% may be detectable in that particular scenario.

In an early report using a BAC microarray with 969 clones and 3,600 routine clinical samples, a mosaic aneuploidy discovery rate of 0.2% was reported (5). Among 2,019 patients referred for clinical diagnostic testing using a genome-wide SNP array, mosaic aneuploidy accounted for 1% of all patients (21). UPD (0.4%) and chimerism (0.05%) were less frequent findings.

Chimerism

Chimerism only used to be recognized in individuals with disorders of sex development (DSD), i.e., in ovotesticular DSD, formerly known as true hermaphroditism. The affected individuals are chimeras of 46,XX and 46,XY cell lines. Those cases are readily discernable cytogenetically. However, in the absence of a difference in sex chromosomes, chimerism would not be detectable by standard cytogenetic technology, and additional molecular analyses would be required if chimerism was suspected. The use of a genome-wide SNP array makes the differentiation between mosaicism and chimerism possible, even in the absence of sex chromosome differences in the two coexisting cell lines, as the presence of additional genotypes is readily detectable in the B-allele frequency of SNP-arrays from chimeric individuals (21).

Uniparental Disomy

UPD arises when an individual inherits two copies of a chromosome pair from one parent and no copy from the other parent. There are at least three primary mechanisms by which UPD can occur for whole chromosomes: (*a*) trisomy rescue, i.e., mitotic loss of one of the three copies of the trisomic chromosome; (*b*) monosomy rescue, i.e., duplication of the single copy of a chromosome pair via nondisjunction; and (*c*) gamete complementation, whereby a gamete missing one chromosome pair unites with a gamete containing two copies of the same chromosome pair by chance (28). Each of these mechanisms has been reported, but trisomy rescue seems to be the most common mechanism causing UPD, followed by monosomy rescue then gamete complementation. UPD can occur with certain translocations, but it cannot be documented by standard cytogenetic techniques and requires testing by analysis of allelic inheritance or in the case of regions subject to genomic imprinting by methylation patterns of the respective chromosome or chromosomal region of interest. All cases of UPD caused by monosomy rescue would be detectable by genome-wide SNP array of the patient only, as those mechanisms would lead to isodisomy of the respective chromosome. In the case of trisomy rescue or gamete complementation, pure heterodisomy would not be detectable by SNP-array, but all cases of isodisomy and cases with crossover events, which lead to blocks of isodisomy and heterodisomy, would be detectable (48) (**Figure 4**). Of

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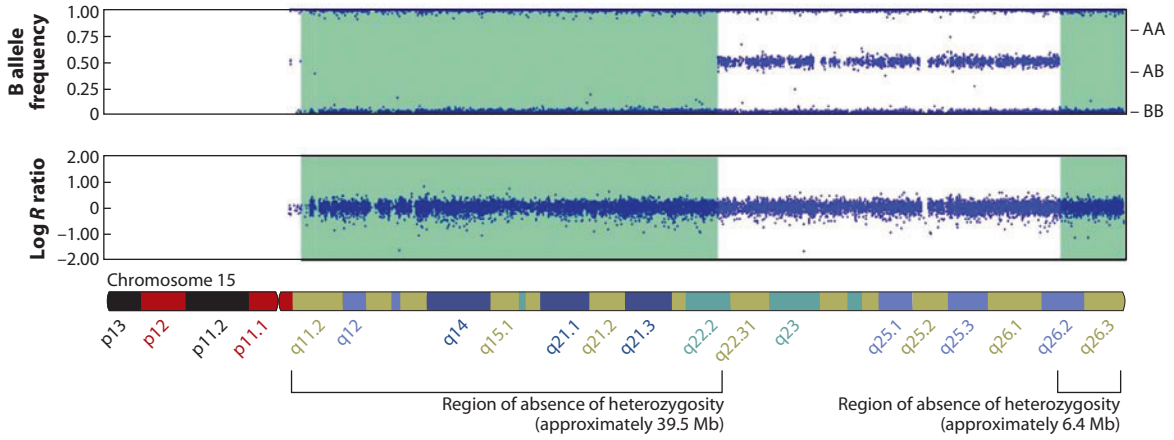

Figure 4

Isodisomy and heterodisomy on one chromosome. Maternal uniparental disomy of chromosome 15 detected in a patient with Prader–Willi syndrome using Illumina Human610-Quad BeadChip. The B allele frequency plot (*top panel*) shows two regions of absence of heterozygosity of approximately 39.5 Mb and 6.4 Mb interrupted by a region of heterozygosity indicating a recombination event. The $\log_2$ *R* ratio (*bottom panel*) indicates the presence of two copies of chromosome 15.

course all cases of UPD can be documented by performing SNP arrays of both parents and the child and analyzing patterns of allelic transmission.

Consanguinity and Incest

High-density SNP arrays have been used in the research arena for homozygosity mapping in consanguineous families to map disease loci for autosomal recessive disorders (36). The same approach can be taken in the clinical setting, especially for disorders with significant locus heterogeneity. In cases where the family history is suggestive of consanguinity or identity of descent (e.g., both families coming from the same town or rural area), the identification of blocks of AOH may help narrow down those genomic regions in which causative recessive disease loci may be found (**Figure 5**).

Although SNP-based microarrays are generally ordered to identify CNVs, UPD, or AOH in specific disease-associated genomic loci, they will also uncover large regions of AOH on multiple chromosomes if present. Several cases have been reported where these regions of AOH accounted for as much as one fourth of the genome—a finding most consistent with the patient having been conceived by first-degree relatives (**Figure 6**).

The identification of incestuous parental relationships by SNP-based DNA microarray can have significant ethical implications and legal consequences, especially if the mother is a minor. It has therefore been suggested to establish institutional committees to discuss these legal and ethical implications of SNP-based microarrays with the purpose of drafting practice guidelines dealing with issues of consent, results disclosure, and reporting (86). Finally, one could also imagine a scenario where the use of SNP array may uncover parental identity of descent that was not known to them, e.g., if both were conceived by artificial insemination by the same donor or in cases where children were given for adoption, grew up separately, and without knowledge of being related met and had children together.

CARRIER TESTING

Population-based carrier testing has a long history in medical genetics, including testing for Tay–Sachs disease and other diseases frequent in the Ashkenazi population, thalassemias, sickle cell anemia, cystic fibrosis,

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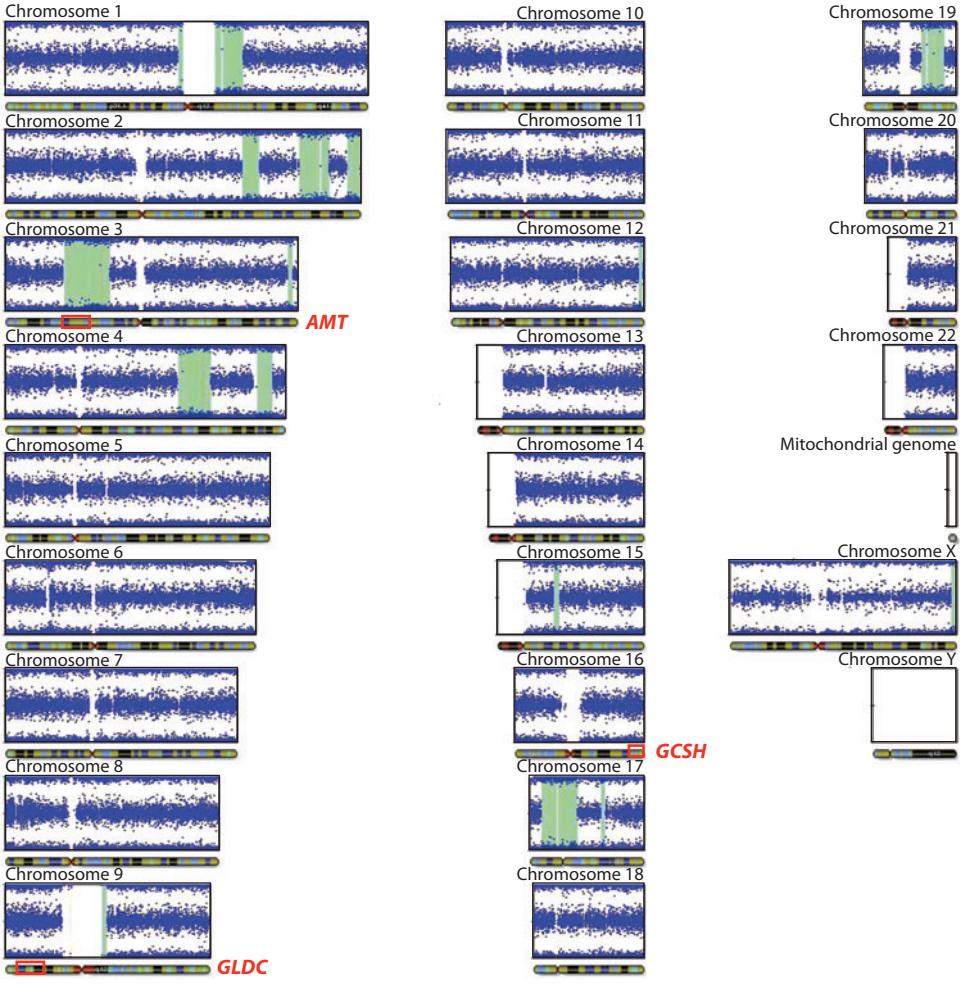

Figure 5

Single-nucleotide polymorphism (SNP) array to guide the molecular diagnosis of nonketotic hyperglycinemia (NKH). SNP array data for a female patient with NKH. Analysis was performed using Illumina Human610-Quad BeadChip. B allele frequency plots for each chromosome are shown. The array detected approximately 192 Mb of absence of heterozygosity (AOH, *green blocks*), consistent with family history of consanguinity as parents are first cousins (coefficient of inbreeding F = 1/16; expected absence of heterozygosity 179 Mb). The positions of the three genes associated with NKH (*AMT*, *GLDC*, and *GCSH*) are marked in red. Sequencing analysis identified a homozygous pathogenic mutation in the *AMT* gene, the only one of the three genes that resides within the AOH block. Figure courtesy of Drs. Lorraine Potocki, Fernando Scaglia, and Michael Wangler.

fragile X syndrome, and spinal muscular atrophy, as reviewed elsewhere (25, 73). Most of these disorders are caused by recessive mutations at autosomal loci, but screening can be important for X-linked disorders, as exemplified by fragile X syndrome. Some of these programs have led to a dramatic reduction of the number of infants born with the relevant conditions, for example, Tay–Sachs disease (49) and β-thalassemia (20). Until recently, this form of testing was carried out using various genotyping platforms, although hematologic screening can still be used for hemoglobinopathies. Carrier testing for

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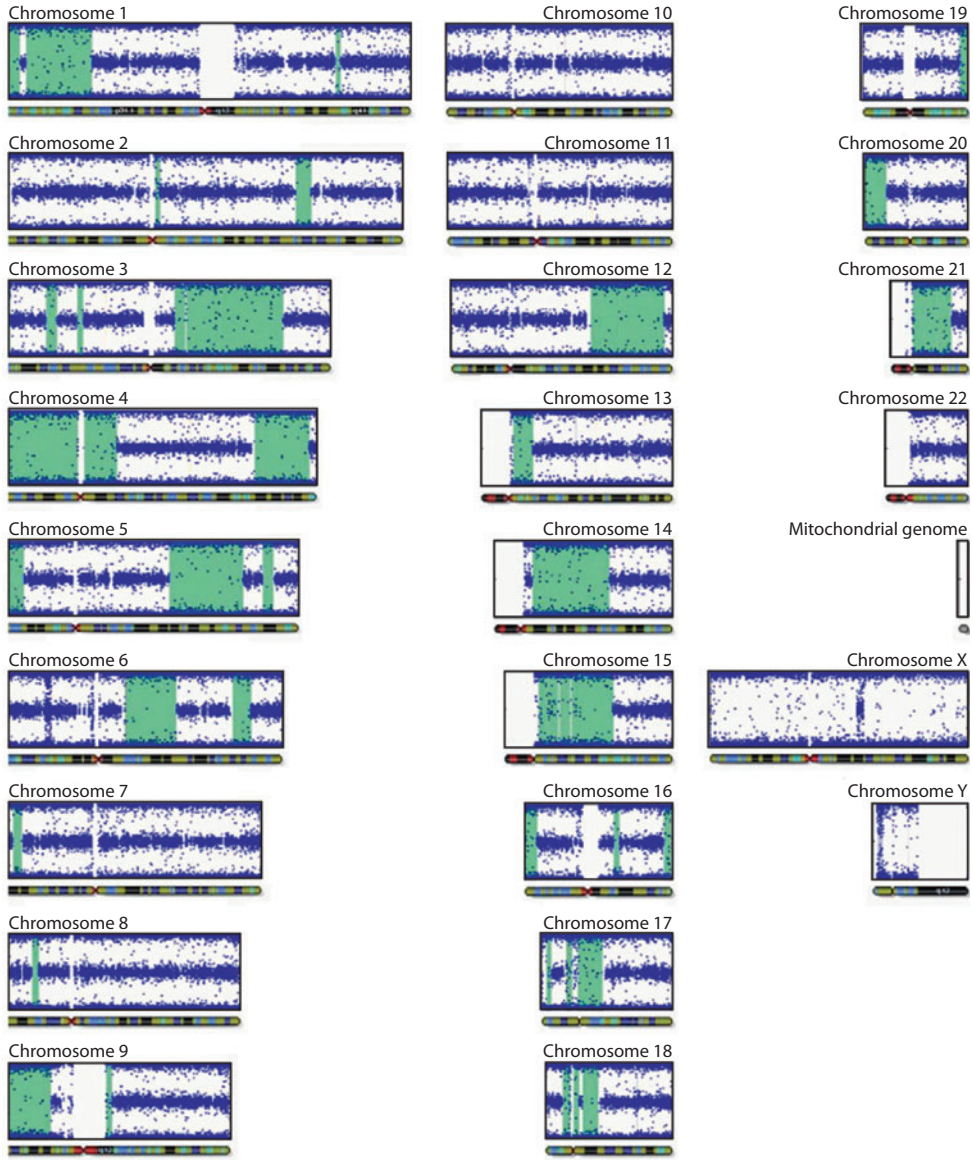

Figure 6

Incestuous parental relationship uncovered by single-nucleotide polymorphism (SNP) array. SNP-based microarray (using 620,901 markers with mean and median marker spacing of 4.7 kb and 2.7 kb, respectively) on a three-year-old boy with multiple medical problems, indicating 668 Mb of absence of heterozygosity (*green blocks*). This finding is consistent with the patient being conceived as the product of a mating between first-degree relatives (coefficient of inbreeding F = 1/4; expected absence of heterozygosity 716 Mb). For matings between second-degree relatives (e.g., uncle-niece, double first cousins), the inbreeding coefficient would be 1/8 and expected absence of heterozygosity would be 358 Mb. Figure taken from Reference 86.

fragile X syndrome and spinal muscular atrophy is technically more challenging because of the triplet repeat expansion in the former and the interfering pseudogenes in the latter. Although carrier screening has focused on recessive mutations in the context of reproductive screening, the same strategies can be applied for dominant mutations, as exemplified by *BRCA1*, *BRCA2*, and the loci causing hereditary nonpolyposis colon cancer.

As the spectrum of disorders and of mutations at a single locus (e.g., cystic fibrosis) has expanded, there has been a push to implement higher throughput methods. One approach has been to try to test for hundreds of mutations at many loci using a molecular inversion probe methodology (98). However, currently, the most attractive methodology appears to be the use of next-generation sequencing technology to sequence the coding exons of relevant genes. Bell et al. (8) reported the use this approach to test for 448 severe recessive childhood disorders. These authors compared microdroplet PCR with hybrid capture for target enrichment and compared an Infinium Omni1-Quad array for genotyping with the SOLiD3 sequencing-by-ligation and Illumina GAIIx sequencing-by-synthesis for sequencing. This publication marks the launch of a new era in carrier testing that is likely to be relatively universal and sequencing based.

PRENATAL DIAGNOSIS

All of the array analyses that can be performed on blood and other postnatal samples can be performed on prenatal samples. The published experience with prenatal use of CMA is very limited by comparison to the tens to hundreds of thousands of pediatric samples that have been studied. Reports of experience studying 25 to a few hundred cases are available (30, 53, 59, 90, 110, 115). Depending on the characteristics of the pregnancies studied, these reports found abnormalities in 5%–10%, including some findings that were not detectable by karyotype. It is feasible to perform direct analysis of uncultured amniotic fluid cells or chorionic villus cells with results in 5–6 days (10). Whole-genome amplification (WGA) can be used reliably for a minority of samples where the yield of DNA is limiting. It is desirable to check all DNA preparations for maternal cell contamination. An NIH study to compare karyotype with CMA on more than 4,000 prenatal samples is nearing the completion of enrollment, and reports at meetings and for publication should be available soon (83).

One might ask why the utilization of prenatal analysis has lagged so dramatically behind pediatric utilization. For the most part, the disorders detected on prenatal analysis are the same as those found postnatally, and some would argue that CMA should be the first-tier test for prenatal samples just as it is for postnatal samples. This is largely true for highly penetrant deletions with severe clinical phenotypes, such as those causing Angelman syndrome (*UBE3A* deletion), Smith–Magenis syndrome (17p11.2 deletion), Phelan–McDermid syndrome (*SHANK3* deletion), deletion of 1p36, deletion of 9qter, and others. Parents choosing to have an invasive prenatal test should be offered the best and most sensitive diagnostic test available. As several well-characterized disorders are detectable by array but not karyotype analysis, CMA should be offered to the parents and it should be up to their disposition which test to choose once they have been counseled about the advantages and disadvantages of each technology.

What are the arguments against widespread use of prenatal CMA rather than karyotype, and how might the circumstances be different from postnatal practice? The answers to these questions will be much clearer upon completion of the NIH study comparing CMA and karyotype, but some aspects are already evident. Most laboratories, including the NIH study, strongly recommend that parental blood samples be submitted simultaneously with the prenatal sample. This allows for determination of whether a CNV is de novo or inherited before issuing a final report or undertaking genetic counseling. For some CNVs, the penetrance is incomplete and/or the phenotypes may be relatively mild.

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This is not new and is exemplified by karyotype results of 45X, 47 XYY, marker chromosomes, and de novo apparently balanced translocations. There are CNVs with lack of penetrance, such as deletion 15q13.3, and others of very uncertain significance, such as deletion 15q11.2, which is quite common among the general population. We believe that families should be informed prior to testing that results associated with substantial uncertain clinical significance occur in approximately 1% of cases (22, 90, 110), but we believe that with proper counseling, most couples are able to work through such uncertainties. In cases where a small CNV of unknown significance is present in a fetus and in a healthy parent, these are usually treated as low-risk circumstances and readily accepted as such by parents. In the case of de novo CNVs, all of the criteria discussed above for interpretation of likely pathological significance of postnatal findings apply in the prenatal setting. Just as large, cytogenetically visible deletions and duplications have traditionally been accepted as having high risk for phenotypic abnormality, the same is true for large CNVs containing many genes. There is certainly a continuum of size and gene content for de novo events. Many de novo events are recurrent and involve CNVs seen commonly in normal controls, and these are of low risk. Other single exonic deletions may be very high risk in the case of genes, such as *SHANK3*, *NRXN1*, *MECP2*, etc., but genetics is certainly complex. This is demonstrated by a recent report of an exonic deletion of *SHANK3* with a normal phenotype that was explained by the fact that an exon designated in the then-current genome browser is not regularly incorporated into the transcript (55).

Another clinical circumstance can arise where a highly penetrant but not 100% penetrant CNV, such as deletion of 15q13.3, may be detected in a fetus. Study of the parents may reveal the deletion in a parent. This parent may have mild phenotypic features such as learning disabilities largely overcome or idiopathic epilepsy, or the parent may be completely normal. This raises additional considerations in the counseling regarding feelings of guilt or stigmatization of a parent. One might try to minimize these concerns. If there is any history of learning disabilities, epilepsy, or major psychiatric diagnosis in either parent, one option is to perform array analysis on both parents prior to undertaking prenatal diagnosis. Another theoretical option, not in use to our knowledge, might be to offer the parents the option of only being informed about de novo CNVs in the fetus, assuming both parents are viewed as healthy. The great majority of the most severe phenotypes will be of de novo origin.

Ethical concerns have been raised regarding expanded prenatal diagnosis, for example, when one commentator described microarrays as a "roadblock for life" and argued that "[o]nce fetal DNA can be noninvasively obtained, screening practices will be able to generate a massive amount of information of uncertain importance. And, if we screen for 1000 genetic variations in this population, we will have 1,000,000 false-positive results—i.e., every fetus will be identified as abnormal" (95). These concerns are wildly exaggerated and do not reflect the reality of the ongoing NIH study. There is a question of whether invasive prenatal diagnosis should be offered to all women. Even prior to the availability of CMA, there was evidence that invasive prenatal diagnosis can be cost effective at any age or risk level (41). In 2007, the American College of Obstetrics and Gynecology (ACOG) recommended that "invasive diagnostic testing for aneuploidy should be available to all women, regardless of maternal age" (2). In 2009, ACOG concluded that CMA "technology is not currently a replacement for classic cytogenetics in prenatal diagnosis" (3), but it is widely anticipated that this position will be reversed after publication of the ongoing NIH study.

Noninvasive Prenatal Diagnosis

Noninvasive prenatal diagnosis (NIPD) refers to the ability to analyze the genome of the fetus starting with a maternal blood sample or (less likely to be utilized perhaps) with a cervical sample collected using methods similar to

those for Pap smears. NIPD has been the holy grail of prenatal diagnosticians for more than two disappointing decades, but it appears that success may be imminent. There is evidence that CMA can be performed using a single cell, most often with WGA and array CGH but also with SNP arrays. This has been applied for preimplantation genetic diagnosis (39, 114), and there is evidence that single-cell CMA using lymphoblasts of known genotypes can successfully detect deletions and duplications of 1 Mb or more, which would include most of the common deleterious CNVs (W. Bi, A.M. Breman, C.A. Shaw, P. Stankiewicz, T. Gambin, et al., manuscript under review). However, the ability to reliably recover single fetal cells from the maternal blood or cervix remains elusive, although evolving technologies developed for the recovery of circulating tumor cells may help to overcome this obstacle.

A second source of fetal DNA is found in maternal plasma. All people have DNA of short half-life in the circulation in a nucleosomal configuration (44). In pregnant women, from 2% to 11% of this DNA is of fetal origin, and it is present in both the first and second trimester. It has been demonstrated using up to 65-fold coverage with next-generation sequencing technology that the fetal genome can be characterized quite thoroughly (61). The analysis takes advantage of knowing SNP genotypes for both parents. The fetal DNA is of smaller size and slightly different nucleosomal configuration, and can be enriched modestly relative to maternal DNA based on these differences. Enrichment reduces the level of sequencing coverage needed for analysis of the fetal genome. If fetal DNA could be enriched to be 50%–90% purity, CMA rather than next-generation sequencing might be feasible. Using the strategy of sequencing plasma DNA, NIPD is possible but prohibitively expensive today. Given the plummeting costs of DNA sequencing, it appears that NIPD will be available soon whether using plasma DNA or single cells in combination with DNA sequencing or CMA. DNA sequencing has the advantage of being able to detect de novo point mutations, such as those causing achondroplasia and thanatophoric dysplasia.

DIRECT-TO-CONSUMER TESTING

An increasing number of private companies are marketing genetic tests directly to consumers over the Internet. While several of these companies offer tests based on sequencing to identify mutations in individual genes, there are also more comprehensive tests offered, often referred to as "complete genomic profiling," that provide information about disease risk of some dozens or hundreds of disorders, for which SNP array technology is commonly used. While most of these SNPs are not necessarily pathogenic per se, they modify the individual's lifetime risk of being or becoming affected with the respective disorder.

In some cases, consumers can order and pay for the tests online and typically receive results within two to three months. Consultation with a health care professional (e.g., a physician, geneticist, or genetic counselor) is not a prerequisite at any point in the process (82). However, in 2010, the FDA announced that it was planning to regulate genetic tests sold directly to consumers and considering requiring counseling for direct-to-consumer (DTC) tests for high-risk mutations (16). The health-related genetic tests available in a DTC setting fall into three general categories. The first category includes tests evaluating the carrier status of autosomal recessive disorders. These tests are marketed mostly to couples to determine the risk for certain autosomal recessive (or X-linked) disorders in their offspring prior to conception. The variants detected here would typically have a high penetrance and strong effect on disease phenotypes when present in homozygous or compound heterozygous state. However, missense and other variants of uncertain significance are also discovered. The second group encompasses SNPs that have been associated to an increase (or decrease) in risk of having (or developing) complex multigenic conditions in the tested individual, such as diabetes mellitus, cardiovascular disease,

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obesity, and some cancers. The respective variants identified might be fairly common among the population and their effect size is typically modest (with odds ratios typically below 2–3, and for most psychiatric disorders below 1.5). The third category of variants tested relate to a specific clinically relevant/actionable genotype (e.g., factor V Leiden) or pharmacogenetics. These tests determine variants that alter an individual's response to certain drugs—both efficacy and toxicity.

In 2004, the American College of Medical Genetics published a statement expressing concerns about DTC genetic testing and recommending that a health care professional should be responsible for both ordering and interpreting genetic tests (1). The European Society of Human Genetics provides a guideline to DTC genetic testing in the form of a policy statement, recommending some level of pretest counseling, the offer of genetic counseling appropriate to the type of test and disease, and the availability of some psychosocial follow-up when necessary (29).

Concerns regarding DTC testing are threefold: concerns about (*a*) the quality and validity of the data, (*b*) the ordering individual's ability to interpret them, and (*c*) psychological stress the information about increased disease susceptibility may cause in the affected individual. A recent review of the genetic tests currently marketed online found that all but two of the gene-disease associations tested for were fairly small, with odds ratios ranging from 1.04 to 3.2 for elevated risk and 0.54 to 0.88 for protective effects (47). This has led critics to argue that the results of personal genome testing based on SNP technology provide virtually no clinically useful information about the risk of most disorders. It has been reported for some specific conditions and disorders that the same test on the same individual revealed both above- and below-average risks depending on the company administering it (82). One of the underlying causes may be the selective use of data from specific studies and the lack of bona fide references for calculation of risk associated to the respective SNPs.

A population-based public survey among adults ascertained through random digit dialing found that there is strong interest in predictive genetic testing for a reported susceptibility to depression. Once the benefits and disadvantages of such testing had been considered, there was significantly greater interest in seeking such a test through a physician (63%) versus DTC (40%). Personal history of mental illness, self-estimation of being at higher than average risk for depression, belief that a genetic component would increase rather than decrease stigma, and endorsement of benefits of genetic testing significantly predicted interest in having such a test (120).

Skeptics of DTC genetic testing assert that such testing has the potential to cause harm, including anxiety and increased use of unnecessary and expensive screening and medical procedures. However, a recent study of 2,037 individuals who completed follow-up after undergoing genome-wide testing showed that such testing did not result in any measurable short-term changes in psychological health, diet or exercise behavior, or use of screening tests (12).

PERSONALIZED GENOMIC PROFILING

An alternative approach to DTC genetic and genomic testing has been coined as personalized genomic profiling (PGM). This generally refers to large-scale sequencing or SNP array–based clinical tests that allow screening for multiple disease-associated variants and mutations, carrier status for certain mutations, and variants of pharmacogenomic relevance. These PGM tests are offered by private and academic laboratories and generally require pretest counseling by a physician or counselor with documentation of such on the request and consent forms. The approach taken by most institutions is to emphasize extensive screening for rare disease-causing mutations rather than testing common risk-modifying variants of small effect size (108). The boundary between risk-modifying SNPs and actionable disease

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genotypes is not sharp. For all disease-causing mutations that are tested for in PGM screening tests, it should be recognized that these have been identified and validated in the clinical setting (meaning in the presence of certain clinical symptoms or a positive family history) but they have not been validated as screening tests for the general population. The penetrance may be quite different when looking at the effect of a genetic variant detected as part of a screening test in the general population when compared with its penetrance among individuals that were evaluated because of a personal history or a strong family history of the respective disorder.

THE IMPORTANCE OF GENETIC COUNSELING

Both oligonucleotide- and SNP-based microarrays generate a complex amount of information, and unanticipated findings may be uncovered. Therefore, the importance of genetic counseling cannot be underestimated. Pretest counseling, especially when done prenatally, should include a generalized discussion of chromosomes and genes, a description of the test, and possible outcomes. Pretest counseling should also briefly review potential benefits and limitations of the test, and highlight the abnormalities it can and cannot detect. While a signed informed consent document for the testing would be desirable, it is recognized that this may not always be feasible in a clinical setting.

Ideally, abnormal test results should be communicated to the family by a genetics provider. Findings should be explained in lay terms, and one should discuss current knowledge of the association of the variant with a known genetic disorder and review the phenotype of the detected disorder. In some situations, further laboratory testing may be necessary to determine the extent and the clinical consequence of the abnormality, e.g., parental testing for variants of uncertain significance. The role of these tests should be explained to the family.

Clinical chromosome microarray testing does not interrogate every part of the genome, and a normal result does not imply that the respective individual does not have a genetic disorder. As some exon-targeted arrays target genes of known Mendelian disorders, the nongeneticist ordering provider may be under the wrong impression that the array would actually be an appropriate test to rule out all the respective disorders. Copy number arrays are only designed to detect large deletions or duplications, and small indels will not be detected. They are not intended to replace complete gene sequencing or very high-resolution targeted array analysis for individual genes linked to the sequencing tests.

As mentioned above, array technology may detect CNVs of unknown significance, resulting in the need to communicate some degree of uncertainty in clinical interpretation to the family. While variants of uncertain significance are not new to the medical community per se, they can impose a burden on clinicians, laboratories, and families alike. Other medical screening tests, especially radiological imaging techniques (ultrasound, MRI, etc.), frequently uncover unanticipated findings, as well as findings of uncertain significance (19, 66, 71, 74). It has been argued that regarding genetic array technology, concerns about identification of variants of uncertain significance can be adequately addressed through (*a*) choosing a level of resolution that balances sensitivity and specificity, (*b*) increased data sharing through common databases, and (*c*) parental studies to determine whether CNVs are de novo or inherited (69). As more data are collected and clinical presentations are correlated with molecular findings over time, the number of variants of unknown significance should decrease. Various efforts are currently underway to catalog CNVs and their respective clinical phenotypes, and these databases provide a valuable resource whenever a CNV of uncertain significance is reported. Two examples of such databases include the Database of Genomic Variants (DGV) and the Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources (DECIPHER).

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DISCLOSURE STATEMENT

C.P.S., J.W., and A.L.B. are faculty members in the Department of Molecular and Human Genetics at Baylor College of Medicine, Houston, Texas, which offers extensive genetic laboratory testing, including the use of copy number and SNP arrays, and the department derives revenue from this activity. The authors receive no remuneration from for-profit corporations.

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LITERATURE CITED

1. Am. Coll. Med. Genet. Board Dir. 2004. ACMG statement on direct-to-consumer genetic testing. *Genet. Med.* 6:60
2. Am. Coll. Obstet. Gynecol. 2007. ACOG Practice Bulletin No. 88, December 2007: Invasive prenatal testing for aneuploidy. *Obstet. Gynecol.* 110:1459–67
3. Am. Coll. Obstet. Gynecol. 2009. ACOG Committee Opinion No. 446: Array comparative genomic hybridization in prenatal diagnosis. *Obstet. Gynecol.* 114:1161–63
4. Avramopoulos D. 2010. Genetics of psychiatric disorders methods: molecular approaches. *Psychiatr. Clin. North Am.* 33:1–13
5. Ballif BC, Rorem EA, Sundin K, Lincicum M, Gaskin S, et al. 2006. Detection of low-level mosaicism by array CGH in routine diagnostic specimens. *Am. J. Med. Genet. A* 140:2757–67
6. Bayani J, Squire JA. 2004. Traditional banding of chromosomes for cytogenetic analysis. *Curr. Protoc. Cell Biol.* Chapter 22:Unit 22.3
7. Bell CG, Teschendorff AE, Rakyan VK, Maxwell AP, Beck S, Savage DA. 2010. Genome-wide DNA methylation analysis for diabetic nephropathy in type 1 diabetes mellitus. *BMC Med. Genomics* 3:33
8. Bell CJ, Dinwiddie DL, Miller NA, Hateley SL, Ganusova EE, et al. 2011. Carrier testing for severe childhood recessive diseases by next-generation sequencing. *Sci. Transl. Med.* 3:65ra4
9. Berg JS, Brunetti-Pierri N, Peters SU, Kang SH, Fong CT, et al. 2007. Speech delay and autism spectrum behaviors are frequently associated with duplication of the 7q11.23 Williams-Beuren syndrome region. *Genet. Med.* 9:427–41
10. Bi W, Breman AM, Venable SF, Eng PA, Sahoo T, et al. 2008. Rapid prenatal diagnosis using uncultured amniocytes and oligonucleotide array CGH. *Prenat. Diagn.* 28:943–49
11. Bielanska M, Tan SL, Ao A. 2002. Chromosomal mosaicism throughout human preimplantation development in vitro: incidence, type, and relevance to embryo outcome. *Hum. Reprod.* 17:413–19
12. Bloss CS, Schork NJ, Topol EJ. 2011. Effect of direct-to-consumer genomewide profiling to assess disease risk. *N. Engl. J. Med.* 364:524–34
13. Boone PM, Bacino CA, Shaw CA, Eng PA, Hixson PM, et al. 2010. Detection of clinically relevant exonic copy-number changes by array CGH. *Hum. Mutat.* 31:1326–42
14. Borozdin W, Steinmann K, Albrecht B, Bottani A, Devriendt K, et al. 2006. Detection of heterozygous SALL1 deletions by quantitative real time PCR proves the contribution of a SALL1 dosage effect in the pathogenesis of Townes-Brocks syndrome. *Hum. Mutat.* 27:211–12
15. Briegel W, Schneider M, Schwab KO. 2008. 22q11.2 deletion syndrome: behaviour problems of children and adolescents and parental stress. *Child Care Health Dev.* 34:795–800
16. Brower V. 2010. FDA to regulate direct-to-consumer genetic tests. *J. Natl. Cancer Inst.* 102:1610–12, 17
17. Carter M, Nikkel S, Fernandez B, Marshall C, Noor A, et al. 2011. Hemizygous deletions on chromosome 1p21.3 involving the DPYD gene in individuals with autism spectrum disorder. *Clin. Genet.* 79. In press
18. Ching MS, Shen Y, Tan WH, Jeste SS, Morrow EM, et al. 2010. Deletions of NRXN1 (neurexin-1) predispose to a wide spectrum of developmental disorders. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 153B:937–47

19. Cioni G, Di Paco MC, Bertuccelli B, Paolicelli PB, Canapicchi R. 1997. MRI findings and sensorimotor development in infants with bilateral spastic cerebral palsy. *Brain Dev.* 19:245–53
20. Colah R, Gorakshakar A, Nadkarni A. 2010. Global burden, distribution and prevention of beta-thalassemias and hemoglobin E disorders. *Expert Rev. Hematol.* 3:103–17
21. Conlin LK, Thiel BD, Bonnemann CG, Medne L, Ernst LM, et al. 2010. Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. *Hum. Mol. Genet.* 19:1263–75
22. Coppinger J, Alliman S, Lamb AN, Torchia BS, Bejjani BA, Shaffer LG. 2009. Whole-genome microarray analysis in prenatal specimens identifies clinically significant chromosome alterations without increase in results of unclear significance compared to targeted microarray. *Prenat. Diagn.* 29:1156–66
23. Cross J, Peters G, Wu Z, Brohede J, Hannan GN. 2007. Resolution of trisomic mosaicism in prenatal diagnosis: estimated performance of a 50K SNP microarray. *Prenat. Diagn.* 27:1197–204
24. Cubells JF, Deoreo EH, Harvey PD, Garlow SJ, Garber K, et al. 2011. Pharmaco-genetically guided treatment of recurrent rage outbursts in an adult male with 15q13.3 deletion syndrome. *Am. J. Med. Genet. A* 155:805–10
25. Cunniff C, Hudgins L. 2010. Prenatal genetic screening and diagnosis for pediatricians. *Curr. Opin. Pediatr.* 22:809–13
26. Eggermann T. 2010. Russell-Silver syndrome. *Am. J. Med. Genet. C Semin. Med. Genet.* 154C:355–64
27. Eggermann T, Meyer E, Obermann C, Heil I, Schuler H, et al. 2005. Is maternal duplication of 11p15 associated with Silver-Russell syndrome? *J. Med. Genet.* 42:e26
28. Engel E. 2006. A fascination with chromosome rescue in uniparental disomy: Mendelian recessive outlaws and imprinting copyrights infringements. *Eur. J. Hum. Genet.* 14:1158–69
29. Eur. Soc. Hum. Genet. 2010. Statement of the ESHG on direct-to-consumer genetic testing for health-related purposes. *Eur. J. Hum. Genet.* 18:1271–73
30. Evangelidou P, Sismani C, Ioannides M, Christodoulou C, Koumbaris G, et al. 2010. Clinical application of whole-genome array CGH during prenatal diagnosis: study of 25 selected pregnancies with abnormal ultrasound findings or apparently balanced structural aberrations. *Mol. Cytogenet.* 3:24
31. Fernandez BA, Roberts W, Chung B, Weksberg R, Meyn S, et al. 2009. Phenotypic spectrum associated with de novo and inherited deletions and duplications at 16p11.2 in individuals ascertained for diagnosis of autism spectrum disorder. *J. Med. Genet.* 47:195–203
32. Folstein S, Rutter M. 1977. Genetic influences and infantile autism. *Nature* 265:726–28
33. Friedman JM, Baross A, Delaney AD, Ally A, Arbour L, et al. 2006. Oligonucleotide microarray analysis of genomic imbalance in children with mental retardation. *Am. J. Hum. Genet.* 79:500–13
34. Fruhman G, Van den Veyver IB. 2010. Applications of array comparative genomic hybridization in obstetrics. *Obstet. Gynecol. Clin. North Am.* 37:71–85
35. Funderburk SJ, Spence MA, Sparkes RS. 1977. Mental retardation associated with "balanced" chromosome rearrangements. *Am. J. Hum. Genet.* 29:136–41
36. Garshasbi M, Motazacker MM, Kahrizi K, Behjati F, Abedini SS, et al. 2006. SNP array-based homozygosity mapping reveals MCPH1 deletion in family with autosomal recessive mental retardation and mild microcephaly. *Hum. Genet.* 118:708–15
37. Girirajan S, Rosenfeld JA, Cooper GM, Antonacci F, Siswara P, et al. 2010. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. *Nat. Genet.* 42:203–9
38. Grønskov K, Poole RL, Hahnemann JM, Thomson J, Tümer Z, et al. 2011. Deletions and rearrangements of the H19/IGF2 enhancer region in patients with Silver-Russell syndrome and growth retardation. *J. Med. Genet.* 48:308–11
39. Gutiérrez-Mateo C, Colls P, Sánchez-García J, Escudero T, Prates R, et al. 2010. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. *Fertil. Steril.* 95:953–58
40. Hantash FM, Redman JB, Starn K, Anderson B, Buller A, et al. 2006. Novel and recurrent rearrangements in the CFTR gene: clinical and laboratory implications for cystic fibrosis screening. *Hum. Genet.* 119:126–36
41. Harris RA, Washington AE, Nease RF Jr, Kuppermann M. 2004. Cost utility of prenatal diagnosis and the risk-based threshold. *Lancet* 363:276–82

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42. Hassold TJ, Jacobs PA. 1984. Trisomy in man. *Annu. Rev. Genet.* 18:69–97
43. Hochstenbach R, van Binsbergen E, Engelen J, Nieuwint A, Polstra A, et al. 2009. Array analysis and karyotyping: workflow consequences based on a retrospective study of 36,325 patients with idiopathic developmental delay in the Netherlands. *Eur. J. Med. Genet.* 52:161–69
44. Holdenrieder S, Stieber P, Bodenmüller H, Busch M, Von Pawel J, et al. 2001. Circulating nucleosomes in serum. *Ann. N. Y. Acad. Sci.* 945:93–102
45. Hook EB. 1977. Exclusion of chromosomal mosaicism: tables of 90%, 95% and 99% confidence limits and comments on use. *Am. J. Hum. Genet.* 29:94–97
46. Int. Schizophr. Consort. 2008. Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature* 455:237–41
47. Janssens AC, Gwinn M, Bradley LA, Oostra BA, van Duijn CM, Khoury MJ. 2008. A critical appraisal of the scientific basis of commercial genomic profiles used to assess health risks and personalize health interventions. *Am. J. Hum. Genet.* 82:593–99
48. Jinawath N, Zambrano R, Wohler E, Palmquist MK, Hoover-Fong J, et al. 2010. Mosaic trisomy 13: understanding origin using SNP array. *J. Med. Genet.* 48:323–26
49. Kaback MM. 2001. Screening and prevention in Tay-Sachs disease: origins, update, and impact. *Adv. Genet.* 44:253–65
50. Kim HG, Kishikawa S, Higgins AW, Seong IS, Donovan DJ, et al. 2008. Disruption of neurexin 1 associated with autism spectrum disorder. *Am. J. Hum. Genet.* 82:199–207
51. King BH, Lord C. 2010. Is schizophrenia on the autism spectrum? *Brain Res.* 1380:34–41
52. Kirov G, Rujescu D, Ingason A, Collier DA, O'Donovan MC, Owen MJ. 2009. Neurexin 1 (NRXN1) deletions in schizophrenia. *Schizophr. Bull.* 35:851–54
53. Kleeman L, Bianchi DW, Shaffer LG, Rorem E, Cowan J, et al. 2009. Use of array comparative genomic hybridization for prenatal diagnosis of fetuses with sonographic anomalies and normal metaphase karyotype. *Prenat. Diagn.* 29:1213–17
54. Knight SJ, Horsley SW, Regan R, Lawrie NM, Maher EJ, et al. 1997. Development and clinical application of an innovative fluorescence in situ hybridization technique which detects submicroscopic rearrangements involving telomeres. *Eur. J. Hum. Genet.* 5:1–8
55. Kolevzon A, Cai G, Soorya L, Takahashi N, Grodberg D, et al. 2010. Analysis of a purported SHANK3 mutation in a boy with autism: clinical impact of rare variant research in neurodevelopmental disabilities. *Brain Res.* 1380:98–105
56. Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, et al. 2008. Recurrent 16p11.2 microdeletions in autism. *Hum. Mol. Genet.* 17:628–38
57. Kumar RA, Marshall CR, Badner JA, Babatz TD, Mukamel Z, et al. 2009. Association and mutation analyses of 16p11.2 autism candidate genes. *PLoS ONE* 4:e4582
58. Larramendy M, Heiskanen M, Wessman M, Ritvanen A, Peltomaki P, et al. 1993. Molecular cytogenetic study of patients with Pallister-Killian syndrome. *Hum. Genet.* 91:121–27
59. Le Caignec C, Boceno M, Saugier-Veber P, Jacquemont S, Joubert M, et al. 2005. Detection of genomic imbalances by array based comparative genomic hybridisation in fetuses with multiple malformations. *J. Med. Genet.* 42:121–28
60. Leonard H, Wen X. 2002. The epidemiology of mental retardation: challenges and opportunities in the new millennium. *Ment. Retard. Dev. Disabil. Res. Rev.* 8:117–34
61. Lo YM, Chan KC, Sun H, Chen EZ, Jiang P, et al. 2010. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. *Sci. Transl. Med.* 2:61ra91
62. Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, et al. 2008. Structural variation of chromosomes in autism spectrum disorder. *Am. J. Hum. Genet.* 82:477–88
63. McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, et al. 2009. Microduplications of 16p11.2 are associated with schizophrenia. *Nat. Genet.* 41:1223–27
64. McGuffin P, Farmer AE, Gottesman, II, Murray RM, Reveley AM. 1984. Twin concordance for operationally defined schizophrenia. Confirmation of familiality and heritability. *Arch. Gen. Psychiatry* 41:541–45
65. McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A. 2003. The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. *Arch. Gen. Psychiatry* 60:497–502

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66. McKenna DA, Laxpati M, Colletti PM. 2008. The prevalence of incidental findings at cardiac MRI. *Open. Cardiovasc. Med. J.* 2:20–25
67. Mervis CB, Robinson BF, Bertrand J, Morris CA, Klein-Tasman BP, Armstrong SC. 2000. The Williams syndrome cognitive profile. *Brain Cogn.* 44:604–28
68. Miles JH, Takahashi TN, Bagby S, Sahota PK, Vaslow DF, et al. 2005. Essential versus complex autism: definition of fundamental prognostic subtypes. *Am. J. Med. Genet. A* 135:171–80
69. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, et al. 2010. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am. J. Hum. Genet.* 86:749–64
70. Moreno-De-Luca D, Mulle JG, Kaminsky EB, Sanders SJ, Myers SM, et al. 2010. Deletion 17q12 is a recurrent copy number variant that confers high risk of autism and schizophrenia. *Am. J. Hum. Genet.* 87:618–30
71. Moses P, Courchesne E, Stiles J, Trauner D, Egaas B, Edwards E. 2000. Regional size reduction in the human corpus callosum following pre- and perinatal brain injury. *Cereb. Cortex* 10:1200–10
72. Ning Y, Roschke A, Smith ACM, Macha M, Precht K, et al. 1996. A complete set of human telomeric probes and their clinical application. National Institutes of Health and Institute of Molecular Medicine collaboration. *Nat. Genet.* 14:86–89
73. Norton ME. 2008. Genetic screening and counseling. *Curr. Opin. Obstet. Gynecol.* 20:157–63
74. Nosarti C, Rushe TM, Woodruff PW, Stewart AL, Rifkin L, Murray RM. 2004. Corpus callosum size and very preterm birth: relationship to neuropsychological outcome. *Brain* 127:2080–89
75. O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, et al. 2011. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nat. Genet.* 43:585–89
76. Piton A, Michaud JL, Peng H, Aradhya S, Gauthier J, et al. 2008. Mutations in the calcium-related gene IL1RAPL1 are associated with autism. *Hum. Mol. Genet.* 17:3965–74
77. Priest JH, Rust JM, Fernhoff PM. 1992. Tissue specificity and stability of mosaicism in Pallister-Killian +i(12p) syndrome: relevance for prenatal diagnosis. *Am. J. Med. Genet.* 42:820–24
78. Ramelli GP, Silacci C, Ferrarini A, Cattaneo C, Visconti P, Pescia G. 2008. Microduplication 22q11.2 in a child with autism spectrum disorder: clinical and genetic study. *Dev. Med. Child Neurol.* 50:953–55
79. Ramocki MB, Zoghbi HY. 2008. Failure of neuronal homeostasis results in common neuropsychiatric phenotypes. *Nature* 455:912–18
80. Rujescu D, Ingason A, Cichon S, Pietilainen OP, Barnes MR, et al. 2009. Disruption of the neurexin 1 gene is associated with schizophrenia. *Hum. Mol. Genet.* 18:988–96
81. Sagoo GS, Butterworth AS, Sanderson S, Shaw-Smith C, Higgins JP, Burton H. 2009. Array CGH in patients with learning disability (mental retardation) and congenital anomalies: updated systematic review and meta-analysis of 19 studies and 13,926 subjects. *Genet. Med.* 11:139–46
82. Samuel GN, Jordens CF, Kerridge I. 2010. Direct-to-consumer personal genome testing: ethical and regulatory issues that arise from wanting to 'know' your DNA. *Intern. Med. J.* 40:220–24
83. Savage MS, Mourad MJ, Wapner RJ. 2011. Evolving applications of microarray analysis in prenatal diagnosis. *Curr. Opin. Obstet. Gynecol.* 23:103–8
84. Schaaf CP, Goin-Kochel RP, Nowell KP, Hunter JV, Aleck KA, et al. 2011. Expanding the clinical spectrum of the 16p11.2 chromosomal rearrangements: three patients with syringomyelia. *Eur. J. Hum. Genet.* 19:152–56
85. Schaaf CP, Sabo A, Sakai Y, Crosby J, Muzny D, et al. 2011. Oligogenic heterozygosity in individuals with high-functioning autism spectrum disorder. *Hum. Mol. Genet.* In press
86. Schaaf CP, Scott D, Wiszniewska J, Beaudet A. 2011. Identification of incestuous parental relationships by SNP-based DNA microarrays. *Lancet* 377:555–56
87. Schollen E, Smeets E, Deflem E, Fryns JP, Matthijs G. 2003. Gross rearrangements in the MECP2 gene in three patients with Rett syndrome: implications for routine diagnosis of Rett syndrome. *Hum. Mutat.* 22:116–20
88. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, et al. 2007. Strong association of de novo copy number mutations with autism. *Science* 316:445–49
89. Sebat J, Levy DL, McCarthy SE. 2009. Rare structural variants in schizophrenia: one disorder, multiple mutations; one mutation, multiple disorders. *Trends Genet.* 25:528–35

Annu. Rev. Genom. Hum. Genet. 2011.12:25-51. Downloaded from www.annualreviews.org
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90. Shaffer LG, Coppinger J, Alliman S, Torchia BA, Theisen A, et al. 2008. Comparison of microarray-based detection rates for cytogenetic abnormalities in prenatal and neonatal specimens. *Prenat. Diagn.* 28:789–95
91. Shchelochkov OA, Cheung SW, Lupski JR. 2010. Genomic and clinical characteristics of microduplications in chromosome 17. *Am. J. Med. Genet. A* 152A:1101–10
92. Shimojima K, Inoue T, Fujii Y, Ohno K, Yamamoto T. 2009. A familial 593-kb microdeletion of 16p11.2 associated with mental retardation and hemivertebrae. *Eur. J. Med. Genet.* 52:433–35
93. Shinawi M, Liu P, Kang SH, Shen J, Belmont JW, et al. 2010. Recurrent reciprocal 16p11.2 rearrangements associated with global developmental delay, behavioral problems, dysmorphism, epilepsy, and abnormal head size. *J. Med. Genet.* 47:332–41
94. Shinawi M, Schaaf CP, Bhatt SS, Xia Z, Patel A, et al. 2009. A small recurrent deletion within 15q13.3 is associated with a range of neurodevelopmental phenotypes. *Nat. Genet.* 41:1269–71
95. Shuster E. 2007. Microarray genetic screening: a prenatal roadblock for life? *Lancet* 369:526–29
96. Smeets DF. 2004. Historical prospective of human cytogenetics: from microscope to microarray. *Clin. Biochem.* 37:439–46
97. Smith AC, Dykens E, Greenberg F. 1998. Behavioral phenotype of Smith-Magenis syndrome (del 17p11.2). *Am. J. Med. Genet.* 81:179–85
98. Srinivasan BS, Evans EA, Flannick J, Patterson AS, Chang CC, et al. 2010. A universal carrier test for the long tail of Mendelian disease. *Reprod. Biomed. Online* 21:537–51
99. Stankiewicz P, Beaudet AL. 2007. Use of array CGH in the evaluation of dysmorphology, malformations, developmental delay, and idiopathic mental retardation. *Curr. Opin. Genet. Dev.* 17:182–92
100. State MW. 2010. The genetics of child psychiatric disorders: focus on autism and Tourette syndrome. *Neuron* 68:254–69
101. Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, et al. 2008. Large recurrent microdeletions associated with schizophrenia. *Nature* 455:232–36
102. Szafranski P, Schaaf CP, Person RE, Gibson IB, Xia Z, et al. 2010. Structures and molecular mechanisms for common 15q13.3 microduplications involving CHRNA7: benign or pathological? *Hum. Mutat.* 31:840–50
103. Szatmari P, Paterson AD, Zwaigenbaum L, Roberts W, Brian J, et al. 2007. Mapping autism risk loci using genetic linkage and chromosomal rearrangements. *Nat. Genet.* 39:319–28
104. Thirlwell C, Eymard M, Feber A, Teschendorff A, Pearce K, et al. 2010. Genome-wide DNA methylation analysis of archival formalin-fixed paraffin-embedded tissue using the Illumina Infinium HumanMethylation27 BeadChip. *Methods* 52:248–54
105. Trask BJ. 2002. Human cytogenetics: 46 chromosomes, 46 years and counting. *Nat. Rev. Genet.* 3:769–78
106. Treadwell-Deering DE, Powell MP, Potocki L. 2010. Cognitive and behavioral characterization of the Potocki-Lupski syndrome (duplication 17p11.2). *J. Dev. Behav. Pediatr.* 31:137–43
107. Tsai AC, Dossett CJ, Walton CS, A EC, Eng PA, et al. 2011. Exon deletions of the EP300 and CREBBP genes in two children with Rubinstein-Taybi syndrome detected by aCGH. *Eur. J. Hum. Genet.* 19:43–9
108. Tweardy DJ, Belmont JW. 2009. "Personalizing" academic medicine: opportunities and challenges in implementing genomic profiling. *Transl. Res.* 154:288–94
109. Vacic V, McCarthy S, Malhotra D, Murray F, Chou HH, et al. 2011. Duplications of the neuropeptide receptor gene VIPR2 confer significant risk for schizophrenia. *Nature* 471:499–503
110. Van den Veyver IB, Patel A, Shaw CA, Pursley AN, Kang SH, et al. 2009. Clinical use of array comparative genomic hybridization (aCGH) for prenatal diagnosis in 300 cases. *Prenat. Diagn.* 29:29–39
111. Van der Auwera I, Yu W, Suo L, Van Neste L, van Dam P, et al. 2010. Array-based DNA methylation profiling for breast cancer subtype discrimination. *PLoS ONE* 5:e12616
112. Van Karnebeek CD, Jansweijer MC, Leenders AG, Offringa M, Hennekam RC. 2005. Diagnostic investigations in individuals with mental retardation: a systematic literature review of their usefulness. *Eur. J. Hum. Genet.* 13:6–25
113. Van Naarden Braun K, Autry A, Boyle C. 2005. A population-based study of the recurrence of developmental disabilities—Metropolitan Atlanta Developmental Disabilities Surveillance Program, 1991–94. *Paediatr. Perinat. Epidemiol.* 19:69–79

Annu. Rev. Genom. Hum. Genet. 2011.12:25-51. Downloaded from www.annualreviews.org
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114. Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, et al. 2009. Chromosome instability is common in human cleavage-stage embryos. *Nat. Med.* 15:577–83
115. Vialard F, Molina Gomes D, Leroy B, Quarello E, Escalona A, et al. 2009. Array comparative genomic hybridization in prenatal diagnosis: another experience. *Fetal. Diagn. Ther.* 25:277–84
116. Volikos E, Robinson J, Aittomaki K, Mecklin JP, Jarvinen H, et al. 2006. LKB1 exonic and whole gene deletions are a common cause of Peutz-Jeghers syndrome. *J. Med. Genet.* 43:e18
117. Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, et al. 2008. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science* 320:539–43
118. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, et al. 2008. Association between microdeletion and microduplication at 16p11.2 and autism. *N. Engl. J. Med.* 358:667–75
119. Weksberg R, Shuman C, Beckwith JB. 2010. Beckwith-Wiedemann syndrome. *Eur. J. Hum. Genet.* 18:8–14
120. Wilde A, Meiser B, Mitchell PB, Hadzi-Pavlovic D, Schofield PR. 2010. Community interest in predictive genetic testing for susceptibility to major depressive disorder in a large national sample. *Psychol. Med.* 15:1–9
121. Willoughby JO, Pope KJ, Eaton V. 2003. Nicotine as an antiepileptic agent in ADNFLE: an N-of-one study. *Epilepsia* 44:1238–40
122. Wisniowiecka-Kowalnik B, Nesteruk M, Peters SU, Xia Z, Cooper ML, et al. 2010. Intragenic rearrangements in NRXN1 in three families with autism spectrum disorder, developmental delay, and speech delay. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 153B:983–93
123. Xiang B, Li A, Valentin D, Nowak NJ, Zhao H, Li P. 2008. Analytical and clinical validity of whole-genome oligonucleotide array comparative genomic hybridization for pediatric patients with mental retardation and developmental delay. *Am. J. Med. Genet. A* 146A:1942–54
124. Xiang B, Zhu H, Shen Y, Miller DT, Lu K, et al. 2010. Genome-wide oligonucleotide array comparative genomic hybridization for etiological diagnosis of mental retardation: a multicenter experience of 1499 clinical cases. *J. Mol. Diagn.* 12:204–12
125. Zollino M, Orteschi D, Marangi G, De Crescenzo A, Pecile V, et al. 2010. A case of Beckwith-Wiedemann syndrome caused by a cryptic 11p15 deletion encompassing the centromeric imprinted domain of the BWS locus. *J. Med. Genet.* 47:429–32

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Contents

Putting Medical Genetics into Practice
Malcolm A. Ferguson-Smith 1

Copy Number and SNP Arrays in Clinical Diagnostics
Christian P. Schaaf, Joanna Wiszniewska, and Arthur L. Beaudet 25

Copy-Number Variations, Noncoding Sequences, and Human Phenotypes
Eva Klopocki and Stefan Mundlos 53

The Genetics of Atrial Fibrillation: From the Bench to the Bedside
Junjie Xiao, Dandan Liang, and Yi-Han Chen 73

The Genetics of Innocence: Analysis of 194 U.S. DNA Exonerations
Greg Hampikian, Emily West, and Olga Akselrod 97

Genetics of Schizophrenia: New Findings and Challenges
Pablo V. Gejman, Alan R. Sanders, and Kenneth S. Kendler 121

Genetics of Speech and Language Disorders
Changsoo Kang and Dennis Drayna 145

Genomic Approaches to Deconstruct Pluripotency
Yuin-Han Loh, Lin Yang, Jimmy Chen Yang, Hu Li, James J. Collins, and George Q. Daley 165

LINE-1 Elements in Structural Variation and Disease
Christine R. Beck, José Luis Garcia-Perez, Richard M. Badge, and John V. Moran 187

Personalized Medicine: Progress and Promise
Isaac S. Chan and Geoffrey S. Ginsburg 217

Perspectives on Human Population Structure at the Cusp of the Sequencing Era
John Novembre and Sohini Ramachandran 245

Rapid Turnover of Functional Sequence in Human and Other Genomes
Chris P. Ponting, Christoffer Nellåker, and Stephen Meader 275

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Recent Advances in the Genetics of Parkinson's Disease
Ian Martin, Valina L. Dawson, and Ted M. Dawson 301

Regulatory Variation Within and Between Species
Wei Zheng, Tara A. Gianoulis, Konrad J. Karczewski, Hongyu Zhao, and Michael Snyder 327

The Repatterning of Eukaryotic Genomes by Random Genetic Drift
Michael Lynch, Louis-Marie Bobay, Francesco Catania, Jean-François Gout, and Mina Rho 347

RNA-Mediated Epigenetic Programming of Genome Rearrangements
Mariusz Nowacki, Keerthi Shetty, and Laura F. Landweber 367

Transitions Between Sex-Determining Systems in Reptiles and Amphibians
Stephen D. Sarre, Tariq Ezaz, and Arthur Georges 391

Unraveling the Genetics of Cancer: Genome Sequencing and Beyond
Kit Man Wong, Thomas J. Hudson, and John D. McPherson 407

Indexes

Cumulative Index of Contributing Authors, Volumes 3–12 431

Cumulative Index of Chapter Titles, Volumes 3–12 435

Errata

An online log of corrections to *Annual Review of Genomics and Human Genetics* articles may be found at http://genom.annualreviews.org/errata.shtml

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