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The future of Prenatal Cytogenetic Diagnostics: A Personal Perspective

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Few cytogeneticists are unable to recall T.C. Hsu's seminal contribution to chromosome analysis – namely the rediscovery of the hypotonic solution pretreatment for metaphase chromosome preparation. In his historical perspective of human and mammalian cytogenetics (Hsu 1979), T.C. Hsu surmises, "From the way human and mammalian cytogenetics has developed... it is not difficult to predict that the growth rate of this field will remain exponential for a long time to come...". I would have to agree with him on this matter. Indeed over the past two decades, I have witnessed a phenomenal technological and conceptual evolution in cytogenetics, that has dramatically transformed its use in current day prenatal genetic testing with over a million such tests having been now been performed world-wide (Randolph 1999). Since the late 1980s and early 1990s, application of fluorescence *in situ* hybridization (FISH) has permitted the rapid detection (within 24 hours) of the common chromosome aneuploidies (i.e., those involving chromosomes 13, 18, 21, X and Y) in amniocytes and chorionic villi samples. More recently, array-based comparative genomic hybridization (array CGH) is being applied to prenatal samples (including blastomeres in preimplantation genetic diagnosis – Le Caignec *et al.* 2006; Vanneste *et al.* 2009) to detect gains and losses on a genome-wide level at an unprecedented resolution (e.g., Larrabee *et al.* 2004; Rickman *et al.* 2006; Sahoo *et al.* 2006; Kleeman *et al.* 2009). Hence, reminiscing on T.C. Hsu's thoughts, I, too, remain optimistic that the next ten years of cytogenetics will continue to evolve rapidly, to further enhance future prenatal cytogenetic testing.

Array-based comparative genomic hybridization

Array CGH technology has actually been available for over ten years (Solinas-Toldo *et al.* 1997, Pinkel *et al.* 1998) but has really only gained wide-spread use in the diagnoses of constitutional abnormalities during the last 5–6 years. At the moment, array CGH is being used in prenatal diagnoses in a limited number of laboratories around the world. My impression is that the lack of wide spread adoption of array CGH in prenatal diagnoses is primarily due to the ongoing apprehension of being able to correctly interpret pathogenic genomic imbalances from the numerous clinically-insignificant copy number variants that are now being detected in all human genomes (Sebat *et al.* 2004; Iafrate *et al.* 2004; Lee *et al.* 2007).

It is interesting to note that interpretation of array CGH technologies needs to be made in the context of chromosome structure. For example, Figure 1A shows an array CGH result with a deletion from 9q24.2 -> 9pter and a duplication from 9p21.3 -> 9p24.2 (Hulick *et al.* 2009). It is not enough to indicate that there is a deletion and a duplication on the short arm of chromosome 9 in this patient. Rather, one must interpret this finding in the context of chromosome structure, being suspicious of an inverted duplication on the short arm of one abnormal chromosome 9 (Figure 1B).

The American College of Obstetricians and Gynecologists recently released a position paper (American College of Obstetrics and Gynecologists, 2009) indicating that the "... use of array CGH technology in prenatal diagnosis is currently limited by several factors, including the inability to detect balanced chromosomal rearrangements, the detection of copy number variations of uncertain clinical significance, and significantly higher costs than conventional karyotype analysis. Although array CGH has distinct advantages over classic cytogenetics in certain applications, the technology is not currently a replacement for classic cytogenetics in prenatal diagnosis." I would agree with this assessment (with the exception of their economic assessment – as the cost for performing array CGH is now similar or lower than that of conventional karyotyping), but advocate that since all technologies have limitations, they should first be considered as adjunct/complementary technologies before being considered as a replacement technology. Indeed, in the past, many speculated that FISH would replace conventional chromosome banding, but 20 years later, both methods continue to be actively used in clinical chromosome analysis. If array CGH will one day replace conventional chromosome banding analysis, it will likely only be after we are more comfortable with the interpretation of copy number variants and only if it is also determined that pathogenic balanced chromosomal rearrangements occur at a negligible frequency – which I am not yet sure is truly the case.

SNP detecting platforms

Over the next 10 years, I suspect that more laboratories will begin to adopt array CGH for prenatal diagnostic testing. At the same time, more laboratories will employ technologies that simultaneously detect single nucleotide polymorphisms (SNPs) as well as provide copy number information. Current SNP-detecting platforms are already capable of detecting both SNPs and copy number changes, at a resolution better than the 100 kb that is used by some as a clinically-informative threshold for non-targeted regions of the genome. Some of the latest SNP detecting platforms include the Affymetrix 6.0 and 2.7 M chips (www.affymetrix.com) and the Illumina HumanOmni-1 Quad and Human CytoSNP-12 Beadchip (www.illumina.com). Analysis of SNP data (with copy number data) provides copy neutral loss-of-heterozygosity (LOH) information that can be indicative of regions of uniparental disomy (UPD), mitotic recombination, gene conversion events, or simply regions that are identical by descent - which cannot be obtained from array CGH platforms (Figure 2). As the use of SNP-detecting platforms continues to increase for constitutional cytogenetic cases, it is not too difficult to imagine that more regions of pathogenic UPD will be uncovered that can in turn be diagnostically informative in a prenatal setting. Some studies have observed LOH segments as large as 5 Mb in healthy individuals and therefore a germline threshold parameter for pathogenicity could be at this size range or larger for non-targeted regions of the genome (McQuillan *et al.* 2008). Furthermore, it can be predicted that our understanding of epistasis in human disorders will continue to grow. We will begin to decipher which combinations of genetic variants leads to varying degrees of pathogenicity. For example, one can speculate that a given copy number change that is highly penetrant and pathogenic, has a reduced clinical phenotype in the presence of another CNV/SNP. Likewise, a copy number variant (CNV) may be clinically insignificant most of the time, but when in the presence of a rare CNV/SNP in certain individuals, could lead to a

recognizable clinical phenotype. Already, preliminary data for such a phenomena are arising for combinations of copy number changes (e.g., Girirajan et al. 2010). Strategic designs of future SNP-detecting platforms (and adoption of certain DNA sequencing approaches) should make detection of such epistatic conditions more efficient.

Latest generation of DNA sequencing

Personally, I have lost track of what is considered next generation sequencing, third generation sequencing, etc. The bottom line is that DNA sequencing is getting faster and cheaper, such that companies are now claiming that they can sequence a given human genome within a day and for less than \$6,000 (recently reviewed in Venter 2010). Hence, DNA sequencing will undoubtedly be increasingly used in clinical genetic diagnostics and will eventually supercede use of SNP-detecting platforms. However, many of the latest DNA sequencing platforms employ relatively short DNA reads (100 bp -> ~400 bp) and while able to detect single base pair changes and small indels (i.e., insertions and deletions less than 100 bp in length), have more difficulty in detecting many larger structural variants (genomic gains, losses, translocations, inversions, etc). Therefore, to obtain a more comprehensive and accurate annotation of human genomes, recent studies are combining whole genome DNA sequencing with other technologies (e.g., array CGH) (e.g., Kim et al. 2009; Lupski et al. 2010). This may become unnecessary as DNA sequencing reads become longer (for example, Pacific Biosciences is now touting reads of 3 kb -> 10 kb in length – www.pacificbiosciences.com).

We are now beginning to appreciate that different tissues in a given individual can have different genetic variants (i.e., with respect to SNPs and CNVs – Piotrowski *et al.* 2008; Gottlieb *et al.* 2009). As tissue-specific variants become associated with specific phenotypes, there could be an increased desire to sample multiple fetal tissues for genetic characterization. Therefore, as methodologies arise to safely sample multiple fetal tissues for genetic analysis, one can envision the application of DNA sequencing strategies (and epigenetic tests) in such scenarios.

One goal that DNA sequencing should try to achieve is accurate annotation of the entire genome from a single cell. One major advantage of cytogenetic analysis is its capability of analyzing single cells – which makes detection of mosaicism easier and reliable. Current array-based, PCR-based, and sequencing-based methodologies make use of genomic DNA from a “pool” of cells and therefore lack cell-to-cell analytical capabilities or attempt to amplify genomic DNA from a single cell which is always accompanied by concerns of non-uniform amplifications or amplification of technical artifacts. If DNA sequencing technology could obtain the entire genome sequence of a single cell, with accurate annotation for SNPs and structural variants, one could expect its increased use on blastomeres in preimplantation genetic diagnoses.

Concluding comments

The past 20 years in cytogenetics has been extremely exciting for me. I have had the pleasure of witnessing technological advances that occur years before I would have imagined and if my errors in timing continue, it is possible that much of what is written here may actually come to fruition well before the anticipated target date of 2020! In my opinion, the major challenge for us, as a scientific and medical community, will not be whether we will be able to implement these technologies to prenatal diagnoses, but rather how we are going to accurately interpret the data being generated. We need to be fully supportive of basic and translational research, especially by those that are also involved in clinical practice to begin to narrow the gap between technological advances/data collection and biological

understanding. T.C. Hsu concluded in his book that “Cytogenetics has come a long, long way, baby. ... (and) it still has a long, long way to go.” I, for one, would agree and anxiously look forward to an exciting 10 years!

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FIGURE 1.

(A) Array CGH results for chromosome 9 in a patient with large orbits, hypertelorism, bulbous nose, thin vermillion, small appearing mouth and subtle micrognathia, and clinodactyly. The array CGH was performed in a dye-swap fashion, showing deletion of proximal 9p and duplication from nucleotide 3,761,025 at 9p24.2 to nucleotide 20,111,633 at 9p21.3. (B) Partial karyotype of the patient showing the normal (left) and the abnormal (right) chromosome 9 after GTG banding.

FIGURE 2.

(A) GTG-banded karyotype of an 8 week product of conception (POC) shows a normal result. (B) Array CGH results using an Agilent genome-wide 244k array also shows a normal result. Three clinically-insignificant copy number variants are not shown. (C) SNP array heterozygosity for the entire chromosome 19 – which is likely pathogenic. The top and lower panels reveal the LOH for this chromosome.