How to Conquer a Chromosome Abnormality—What is microarray analysis?

Microarray analysis allows us to detect very small chromosome copy number changes and to assess the entire genome in one experiment. This is how it works. Two DNA samples are compared for differences. One sample is the normal sample, in this case it is labeled with a green fluorescent dye. The patient DNA sample is labeled with a red fluorescent dye. These two samples are then mixed together and placed on a microscope slide. This slide has been specially prepared with the application of tens of thousands of micro spots of DNA from known locations across the genome. The DNA in these spots is only 60 base pairs in length. (This would be 1/350th of an inch on our football field.) The DNA in this mix of normal and patient DNA then compete with each other to attach to the spots on the slide. The slide is scanned by a laser reader that measures the level of red and green fluorescence attached to each of the micro spots and compares their ratios.

In the example shown here, at every place at the end of the chromosome 18p arm there are two green for every red. More green will attach to those spots from the end of the p arm and the spots in the slide will be more green than red. However, for the remainder of the chromosome the red and green labeled DNA compete evenly to attach to the spots on the slide.

The data from this experiment is shown to the right. The data points are shown at the position along chromosome 18 corresponding to the location of the DNA in the spot on the slide. When the normal and patient DNA samples have an even red/green ratio, the data points are aligned at or near the zero axis. Where there is a deletion and therefore more green than red the data points are closer to the –1 axis. If there were a duplication, the data points would show more red than green and the data points would cluster closer to the +1 axis.
Agilent Technologies system

In our laboratory we use the Agilent Technologies system. This is a slide from them showing actual data from a cancer tumor sample. In this example you can see that there are two regions with deletions—green dots. And one region with a duplication, the red dots. Their statistical analysis program labels the regions it considers abnormal by using a colored bar. In this case the bar is blue.

Cytogenetics vs. Microarrays

Here is the comparison of the resolution of this technique relative to the other chromosome analysis techniques that we have discussed. The left half of this picture shows the same diagram you have seen before. It depicts the end of the p arm of chromosome 18. The yellow box indicates the smallest detectable change using cytogenetics. The white box on the right is actual data from a microarray experiment from someone without a deletion of this region of the chromosome. All the data points cluster around the zero axis. The red arrows point to the same genes in each diagram, this way you can see they are aligned. You can easily see the number of data points that could detect a deletion or duplication and how small such a change could be in comparison to a cytogenetic karyotype. The microarray data shown here is from a low resolution array. Arrays can be produced with a density of data points that are at a density of more than 20 times what is shown here. This means that we can detect VERY small changes in chromosome copy number.
CGH Analytics

This is the software display of the microarray data. We can see if there are copy number changes anywhere in the genome from the panel on the left. Changes are indicated by the pink bar. We can zoom in on any single chromosome, shown in the center panel. We can zoom in on any region of an individual chromosome, shown in the panel on the right.

Array CGH

Although this is an amazing technology that can help us move research forward at a much faster pace, it too has its limitations—illustrated in this example.

The individual whose DNA is pictured here appears to have an interstitial deletion of chromosome 18. However, the microarray really only tells us about copy number changes. We only know that this person is missing one copy of a section of chromosome 18 from the middle of the long arm.

FISH

However, now that we know what region of the chromosome we need to test, we can use FISH to determine the location of that chromosome segment.

In the picture shown here we used a green FISH probe for the chromosome 18 centromere in order to identify chromosome 18. We used a red FISH probe to identify the end of 18q. In this case, the end of 18q is on chromosome 18, so the deletion is indeed interstitial.

So in this case both techniques, microarray and FISH, were necessary in order to fully characterize this individual’s chromosome change.

“...in some cases both microarray & FISH are necessary to fully characterize an individual’s chromosome change.”
A Child with dysmyelination

The ability to detect small deletions and duplications anywhere in the genome in a single experiment has had some unexpected consequences.

Here is an example. We were using the microarray technology to search for very small previously undetectable 18q deletions. We recruited children with dysmyelination of the brain and no other known syndromes. This child was found to have a small duplication of chromosome 3p—shown in the top panel. As a control of this experiment we also performed the same microarray on this child’s parents. —to correlate the dysmyelination in the child with a unique chromosome change in the child.

The parents’ results are in the bottom panels. We can see that the child’s father also has the 3p duplication. In this case, the father is a healthy, well-educated, normally functioning adult. Thus, this chromosome 3p duplication is most likely not associated with dysmyelination and is considered a normal variant.

I hope you have concluded that some genes do not have abnormal consequences when they exist in some copy number other than 2. But you also can conclude that some genes have major consequences when they have an abnormal copy number and some genes have minor consequences when they have an abnormal copy number. Here is an example of two individuals with deletions of 18q. The individual on the left has a deletion that includes a single gene, yet this person is significantly developmentally delayed. The individual on the right has a deletion 60 times bigger, including 15 genes, yet has an IQ that is above average.

This points to our fundamental challenge—to identify the key genes that cause a functional difference when they are present in an abnormal copy number.

Copy Number Variation

Here is a region of chromosome 8p that does include genes and is commonly found to be in single copies in typically developing individuals.

The implication for us is very significant. Not all chromosome copy number changes cause abnormal development of function.

Not all deletions are equal

In a large study of children with development disabilities and karyotypically normal chromosomes, microarray analysis was used to look for small deletions or duplications. Out of 100 children in the study, 97 of them had some sort of copy number change. If fact, some children had more than one copy number change because a total of 258 copy numbers changes were detected in those 97 children. Only 10 children had new copy number changes not found in their parents. Of those, 7 were deletions and 3 were duplications.

So the conclusion is that almost everyone has copy number differences. However, most are not associated with disease even though genes are included.
For more information, you may contact the authors and principal investigators of the Chromosome 18 Clinical Research Center at the phone numbers or email shown to the left.

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Our Motto
To provide individuals and families affected by chromosome 18 abnormalities with comprehensive medical and educational information with a focus on treatment options.

We are on the web!
http://pediatrics.uthscsa.edu/centers/chromosome 18/

Information provided by The Chromosome 18 Clinical Research Center to:

http://www.chromosome18.org/
210-657-4968