



Carolyn's genetics cookbook



Imagine, if you will, a set of cookbooks. The recipes have been in your family for generations. You have two copies of each of the 23 volumes (46 books total). These are enormous books that contain a total of about 80-100,000 recipes needed to make millions of complex menus (a half set has about 3,300,000,000 letters). Your parents each gave you a copy of each volume and you, in turn, will dutifully copy them for your children. To copy them for your kids, you send them to the printer, who chooses one copy of each chapter to put into the set. Thus, one of your children may get your grandmother's recipe for biscuits, while another gets the one from your grandfather's side of the family. Inevitably, there are some mistakes in the recipes in these books- some have been there for generations- some don't matter because you seldom make it anyway or because there is another copy of the recipe that works well.

Occasionally, there are mistakes in the same recipe in both copies, which may be insignificant (your fruitcake is always dry, but really, no one knows the difference) while others are in things that you really like to make, but don't seem to turn out like you want them to. Each time the books are copied, there will be some new mistakes made, but you don't get to proofread and test the final copies of each of the recipes- so there is no way to catch them.

The first 22 pairs of books vary slightly between the two copies of each volume, but each pair contains the same set of recipes in the same order. But, one pair of books in the series is clearly different from the rest. Volume X is a great big book, chock full of recipes (a few thousand in fact) while its counterpart, Volume Y, is quite abbreviated and only contains a few recipes. Females have two copies of volume X while males have a mixed pair with Volume X and Volume Y. Mother's have two copies of Volume X so they give a copy to each of their children- while father's give a copy of Volume X to their daughters and Volume Y to their sons. To keep things fair, girls only refer to one copy of their two Volume X's each time they make a recipe from it, while boys always use the copy of the recipes in Volume X that their mom's gave them.

What is this, a cooking show? I thought we were talking about genetics!

To use this analogy to understand basic human genetic principles, our genes are like the recipes in these books; they are grouped together in structures called chromosomes that are like the different volumes in the series. When you or your child has a chromosome test or karyotype, it is simply looking to see whether all the copies of the books are there. The content of the books is not checked for accuracy and only if something is missing or rearranged, would a chromosome test identify mistake.

In every cell in the body, the entire set of genes is present, although any given cell only uses some of the genes. The genes or recipes have been ordered seemingly at random, making the index critical for the ability to find the different recipes for desserts, entrees etc. In addition, the recipes run into one another with no page breaks and are often interspersed with pages of repeated letters that don't spell anything that you can understand. The pairs of nearly identical books are like the *autosomes*; we use both copies of the recipes each time we make things from them, so many mistakes are corrected or correctable after the fact- we only know about mistakes if both copies carry mistakes in the same recipe (comparable to autosomal recessive inheritance).

Volume X and Y are the sex chromosomes; females have two copies of the X chromosome, while males have a copy of the X and the Y. Genes that are found on the X chromosome are different, because of the process of *X-chromosome inactivation*. Although females carry two copies of genes that are on the X chromosome, in a particular cell, only one copy is readable because the other has been silenced early during development (described in more detail below).

Okay, so what is a gene? a mutation?

The analogy of genes to recipes holds too- each recipe is listed in the index, has a list of ingredients and the directions that need to be followed. In the case of genes, the gene has a specific position on the chromosome (index), coding regions that specify how the protein is made (ingredient list) and regulatory regions that determine when and where it will be active the directions for its use (directions). There can be minor differences in genes that do not change the final product significantly but can be useful for following a particular copy of a gene through a family. There can also be differences that are equivalent to typographical errors in any part of the gene –these are mutations that affect the final product. For a given gene, the type of mutation may vary widely- just as you can imagine in the cookbooks- the index may list it on the wrong page, the page can be missing, there could be too much salt, or the oven temperature could be wrong.

How do you find a gene that causes a particular disorder?

To identify genes that cause genetic disorders, the first and critically important step is to find families that are affected by the disorder. For most inherited disorders, you then track particular positions on chromosomes and see what parts of which chromosomes are found in people who have the disorder and not found in other people in the family. This works well when the disorder is caused by an inherited mutation (like a typo that has been transmitted from parent to child for several generations). So, let's go back to the cookbook analogy- we find a family that has some people whose bread does not rise. We then look at the 23 copies of the books and see which pages are shared by the people with flat bread and different from the people in the same

family whose bread rises. Once we narrow in on the pages where the typo must be- we can either search those pages letter by letter for the typo (sequencing the genes), or test out the recipes and see which one makes bread that does not rise. In Rett syndrome, this was how the gene was originally identified. We know that most cases of Rett syndrome occur because of a new error in the copying of the *MECP2* gene, but in a very small number of families, Rett syndrome occurred in more than one child in the family and in more than one generation. Using these rare families, we were able to narrow down the region on the X-chromosome to search for the gene that causes Rett syndrome because we knew that it had to lie on the part of the chromosome that was shared by the affected individuals. Once the "candidate region" was narrowed down to the end of the long arm of the X-chromosome, genes in that region were searched letter by letter for "typographical errors" or mutations in girls with Rett syndrome that were not members of the families that had been used to localize the gene. Using that approach, Dr. Zoghbi and her colleagues found evidence for many types of errors in the *MECP2* gene in unrelated cases, confirming that this was the gene that caused this condition.

What is mosaicism?

Usually, the mutation happens in either the egg or the sperm that make up a single child. In this situation, every cell in that child's body carries a copy of the gene that has the error in it and almost always- the child has symptoms of the syndrome. But, what if the mistake was made in the copying of the gene at *some early point in development of one of the parents*. Cells that descend from the cell where the mutation occurred all carry the mutation, but some cells in the body carry only normal copies of the gene. If the mutation occurs in a cell that develops into the egg or sperm (germ cells) but not into cells in the brain, the person could have several germ cells that have the abnormal copy of the gene and thus they could transmit it to more than one child without having symptoms of the disorder themselves. Mosaicism has been seen in some autosomal dominant disorders and X-linked dominant disorders and can occur in males or females. The reason this is important in Rett syndrome is that mosaicism can occur in the "germline" in either parent so that if we test the blood of the parent for the mutation in *MECP2*, the test is normal. However, it is possible that additional egg or sperm cells carry the error in the gene, which could lead to another affected child. The risk of this is low, but not zero, thus, we suggest prenatal counseling for parents considering having additional children if they have a daughter with Rett syndrome. Counseling will provide them the information to make an informed decision about genetic testing of the pregnancy.

Okay, so what about X-chromosome inactivation?

While there are still just a thousand or so cells, *in females (or really, in cells with two X chromosomes)*, each cell turns off one copy of the X chromosome because having two "active" copies of all those genes on the X chromosome has severe effects on

development. This is usually a random event; meaning that some cells turn off (inactivate) the maternal X chromosome, while other cells inactivate the paternal X chromosome. When these cells then divide, they keep the same X chromosome active from then on. So, a cell that contains an active maternal X chromosome gives rise only to cells with the maternal X active. They don't turn the other copy on again.

The easiest way to understand it is to think about a calico or tortoiseshell cat. Tortoiseshell cats have inherited a gene for yellow coat color from one parent and a gene for black coat color from the other. These genes are on the X chromosome, so you can look at the color of the hair to see which X copy is active in the cells that made up that hair. Although the color is apparent only in the skin, the mixture of X-inactivation is occurring in all cells throughout the body.



Fig 1. Tortoiseshell cat showing random X-chromosome inactivation. Roughly half of the colored hairs are black and half are yellow. If you were to take a sample from one of the “spots” and test activity of the X chromosomes, you might see a bias toward yellow or black but overall, the pattern is fairly random. Importantly, this process is occurring in every cell, not just the skin cells.

In any single cell, only one copy of the X chromosome (and the genes on it) is active. Most times, the choice of which chromosome to inactivate is entirely random- a cell is just as likely to turn off the X chromosome from the mother as the one that came from the father. But, because the process happens early in development, occasionally a tissue is made from cells that inactivated the same X chromosome each time, which is



Fig 2. Tortoiseshell cat showing skewed X-chromosome inactivation. Most of the active X chromosomes are the ones that generate the yellow coat color. If the “black” gene were the one with the Rett syndrome mutation, she might not have symptoms because only a small percentage of cells have activated the gene with the mutation.

called “skewing”.

Sometimes, skewing happens because one of the X chromosomes contains a gene that has a mutation that interferes with cellular growth, so cells with the *other* X chromosome active have an advantage and may be the only cells detectable. In less than 5% of women, there is skewing of inactivation in most cells in the body seemingly just by chance.

When we test the blood of girls with Rett syndrome, we typically find that they have random X-chromosome inactivation. This test can be useful for cases that are extremely mild, because it may show us that there is skewing of inactivation that is protecting the girl/woman from the effects of the mutation in the gene. For most girls with Rett syndrome, the X-inactivation test is not helpful for providing information on severity.

An important concept in thinking about X-chromosome inactivation in Rett syndrome is that the brain is a network of cells that work together forming millions of circuits. In each of these circuits, there are likely to be cells that have the normal X chromosome active and cells that have the mutant X-chromosome active. While the overall percentage of cells that are activating the normal X-chromosome is likely to play an important role in determining how well a girl functions, even a small number of cells in critical parts of circuits could have widespread effects in brain and the interaction between the two groups of cells could cause more widespread or localized effects.

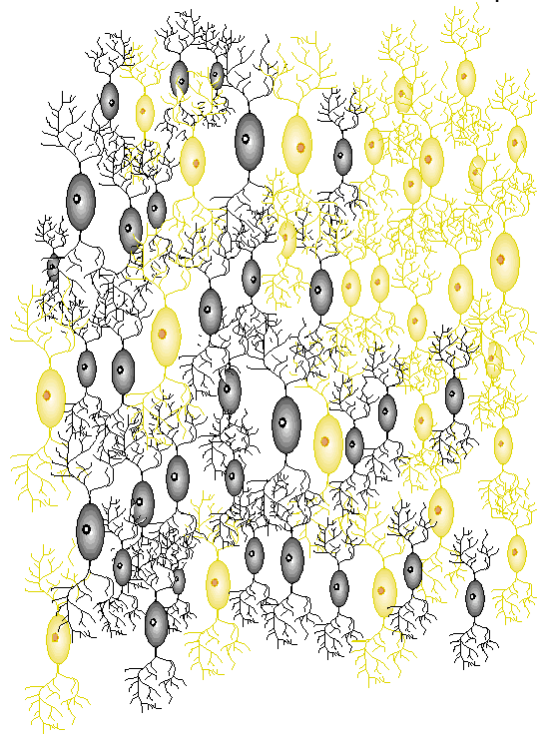


Fig 3. X-inactivation patterns in networks of neurons may affect expression of symptoms.