Visualizing Carrier Status: Fragile X Syndrome and Genetic Diagnosis Since the 1940s

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Summary

What does it look like to be the carrier of a genetic disease? Carrier status may be determined through the visual analysis of both genotypic and phenotypic evidence. Over the past 70 years, clinical geneticists have depended upon multiple strategies for identifying disease carriers within a family. This had included pedigree analysis, which was based upon clinical observations of individual family members, and, in recent decades, cytogenetic and molecular methods. Newer techniques have offered novel opportunities to actually see the suspected etiological markers of certain genetic diseases, such as Fragile X syndrome. The visualization of these markers has both clarified and confused previously observed inheritance patterns, in some cases leading to the development of newly distinct diagnostic categories. As a result, what it means to be affected by, or the carrier of, a genetic disease has continuously evolved.

Introduction

Over the past seven decades, human and medical geneticists have utilized three general methodologies of investigation: identified here as pedigree, cytogenetic (or chromosomal), and molecular analysis. These methodological sets are by no means mutually exclusive. Rather each has developed over time by building upon and re-appropriating previously existing concepts and tools. These methodologies incorporate two regimes of visualization, which Nikolas Rose has termed the 'clinical gaze' and 'molecular gaze' [1]. The clinical gaze takes phenotypic factors such as facial and bodily features into consideration, while the molecular gaze involves the analysis of genotypes by making DNA and chromosomes directly or indirectly visible to the eye. By 'visualization' then, I refer to instances in which evidence is physically seen and interpreted.

Medical professionals have developed and applied a variety of methods in recent decades used to look at patients, and to interpret their genetic or disease status. Each methodological set introduced new ways of identifying and conceiving an individual's genetic status and likelihood of passing particular traits on to their children. Pedigree analysis was historically based upon the collection of clinical evidence from genetically related individuals. This data was used to infer the likely genetic status of each person [2]. Those who were seen to have the clinical symptoms of a genetic disease were thereafter categorized as 'affected'. More guesswork went into determining the status of family members who did not show the disorder's symptoms. Those who had affected children were considered disease carriers, if they did not show any symptoms themselves. Beyond this however, pedigree analysis based upon the 'clinical gaze' alone was unable to concretely reveal the genetic carrier status of family members.

A primary goal of cytogenetic diagnosis, and the 'molecular gaze' more broadly, was to overcome this uncertainty. Cytogenetic analysis is based upon the microscopic visualization of chromosomes: the idea being that certain visible chromosomal aberrations can be correlated with particular clinical outcomes. It was hoped that the visualization of chromosomes would directly reveal the etiological cause of genetic diseases. Chromosomal analysis was effective in particular cases, where large aberrations (such as an extra or missing chromosome) were the cause of a disorder [3,4]. However, visual cytogenetic analysis involves significant interpretation and uncertainty. Indeed, cytogenetic results were sometimes difficult to reproduce for diagnostic purposes. In addition to this, the mutations associated with most genetic diseases are often quite small, involving DNA abnormalities far too tiny to be seen microscopically. Molecular techniques, which have been developed more recently, partially circumvent this problem by allowing for the indirect visualization of particular nucleic acids and proteins.

Each of these methodological sets has been applied during the postwar period with the goal of identifying the carriers of particular genetic diseases. Newer techniques have succeeded, in some cases, at more precisely characterizing the mechanisms of inheritance for many genetic disorders. In the course of doing so, visual genetic markers have been developed and associated with particular clinical outcomes. On top of being utilized as diagnostic tools, these markers have also played a central role in the recognition of new 'at risk' and 'affected' individuals. Indeed, this visual genetic evidence has facilitated the clinical expansion of possible disease categories, leading to the development of new types of people and new patient experiences.

The process of identifying individuals who are affected by or carriers of Fragile X syndrome has itself been reshaped by the introduction of new diagnostic methodologies since the 1940s. For decades, women only found out that they were carriers of Fragile X syndrome after giving birth to an affected child. It took until the late-1970s before women could be made aware of their carrier status by cytogenetic analysis. This visual evidence of an individual's Fragile X status however, also began to reshape who could be considered a carrier of the syndrome. Additional complexity arose in the 1990s, when molecular techniques were also applied to determining Fragile X carrier status. While newer methods provided more diagnostic certainty, they also forced clinicians and patients to reconsider the health implications of being a Fragile X carrier. Indeed, throughout this time period, new visualization techniques for identifying carrier status have both clarified and complicated medical understandings of Fragile X syndrome.

Defining Genetic Carrier Status

One is considered to be a carrier of a genetic disease if he or she is thought to possess a particular disease-causing genetic trait, but does not show the standard clinical symptoms associated with the disorder. This may be the case for a variety of reasons. In most instances, it is because the genetic disease has a 'recessive' inheritance pattern, meaning that only people with two copies of the causative allele show clinical expression. Another possibility is that the genetic trait is 'non-penetrant'. This may be because the effects of the genetic disease tend to be seen later in life, as in the case of Huntington disease, or because the mutation itself is not severe enough to bring about clinical effects. One's carrier status therefore, may have important implications for his or her future health. However, for most patients, it is the risk of passing on the genetic trait, and potentially its clinical effects, to offspring that is of primary concern [1,3,4].

When determining carrier status, it is just as important to define what a particular genetic disease does *not* look like, as what it does. For the most part, being considered a carrier of a genetic disease is about being classified as *clinically invisible* for that disorder. One might still

be considered a carrier if he or she shows only mild physiological abnormalities associated with a disease (such as a subtle facial features), but otherwise to be a carrier is to be clinically unaffected. This does not imply that one must otherwise be healthy in order to be a carrier. It is possible to be considered an unaffected carrier of one syndrome, while also being impacted by another disorder: even one that is associated with the very same gene [5,6]. Indeed, while carrier status is primarily based on the absence of specific clinical expression, the association of a visual genetic marker with a disease can facilitate the identification of newly distinct diagnostic groupings, and lead to the recognition of previously overlooked clinical outcomes.

Fragile X Syndrome and Clinical Pedigree Analysis

Fragile X syndrome is the most common cause of genetically inherited, moderate to severe intellectual disability. Historically, it was difficult to distinguish Fragile X syndrome from other forms of intellectual disability. This is because outward bodily manifestations of Fragile X syndrome are quite mild, the most common being prominent ears, abnormally large testicles, and hyper-extensible finger joints. Figure 1 shows the characteristically large and protruding ears in two patients. As its name suggests, Fragile X syndrome is associated with the X chromosome, of which normal females possess two, and normal males have one. As a result, if a male inherits an X chromosome with a mutant Fragile X allele, he will likely be severely affected by the disorder. Females who inherit one mutant Fragile X allele however, usually also possess a second unaffected X chromosome, which acts to mitigate, but may not fully overcome, the negative clinical effects. Since the one mutant allele may partially override the one normal allele, Fragile X syndrome is considered an 'X-linked dominant' genetic disease. This classification however, has not always been applied historically. Indeed, the etiology of Fragile X syndrome is complex, and has only been well understood for about 20 years.[1]

The history of Fragile X syndrome is many decades older than its current name. Fragile X syndrome almost always occurs in families, the first of which was identified in the early-1940s. By performing pedigree analysis on an extended family affected by thirteen cases of intellectual disability over two generations, clinical geneticists J. Purdon Martin and Julia Bell, of the National Hospital in London, England, were able to identify the apparent inheritance pattern of the Fragile X phenotype (Figure 2). Eleven of the thirteen family members affected were male and, as the pedigree shows, the two impacted females showed milder intellectual disability. Martin and Bell inferred from this sex distribution, that the intellectual disability was most likely an X-linked recessive trait: namely one that is passed down through unaffected females to approximately half of their sons, who are clinically affected. The researchers acknowledged however, that the inheritance pattern suggested did not fully account for the pedigree they had constructed. X-linked recessive diseases are not supposed to affect females, and yet two showed a mild form of intellectual disability. Even more perplexing, since the trait had been passed down from a common ancestor to multiple branches of the family, as seen in Figure 2, it must have been transmitted through two brothers in an earlier generation, who had no clinical effects. Hence, looking at the larger family pedigree suggested that certain males too had been carriers of the genetic trait, without themselves showing signs of intellectual disability.[2]

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[1] Hagerman, R.J. and A. Cronister, eds. 1996. *Fragile X Syndrome: Diagnosis Treatment and Research, Second Edition*. Baltimore, MD: Johns Hopkins University Press.

[2] Martin, J. Purdon and Julia Bell. 1943. "A Pedigree of Mental Defect Showing Sex-Linkage." *Journal of Neurological Psychiatry.* 6 (3-4):154-157.

Genetic pedigree analysis based upon the clinical (and molecular) gaze continues to be an important tool for assessing the inheritance of genetic traits and predicting possible outcomes [2]. Martin and Bell's pedigree analysis (Figure 2) led to the assignment of a likely inheritance pattern for intellectual disability in a family, and allowed for the identification of family members who, by virtue of the fact that they had affected children, were definite (commonly referred to as 'obligate') carriers. In pedigree analysis, the risk of being a carrier is estimated based upon the known clinical status of direct relatives. An individual is only represented as a carrier on a pedigree chart in retrospect, after one of their descendants is clinically seen to be affected. In this analytic system, clinical expression in the next generation is necessary for the genotype of a disease carrier to be determined. One can be shown to be a carrier, or at risk for being one, but no individual known to be at risk can be ruled out as a potential carrier.

The Cytogenetic Era

By the early-1960s, with the explosion of cytogenetic analysis, new options were on the horizon. In 1956, the exact number of human chromosomes was determined, facilitating the recognition of aberrations, such as extra, missing, or abnormally sized chromosomes. Specific clinical outcomes could thereafter be associated with microscopically visible chromosomal abnormalities. In 1969, Yale physician Herbert Lubs reported on one particular microscopically visible chromosomal abnormality that he had found to be present in the case of a young boy affected by severe intellectual disability. Lubs described a chromosome that had visible 'satellites' at the end of one of its arms. Upon follow-up, as shown in Figure 3, the satellites were also found in the boy's brother (who was similarly affected) and his mother (who appeared clinically normal). Since it seemed possible to express the chromosomal abnormality in question without showing any clinical effects, Lubs looked for the satellites in additional family members, to determine if they were causative or benign. Other males in the family, who were similarly disabled, also expressed the chromosomal satellites. Some females in the family also possessed this chromosomal aberration, but none showed any sign of intellectual disability.[3]

This inheritance pattern for intellectual disability, in which only the males of a family were affected, suggested to Lubs that he was working with a recessive X-linked disorder. From this, he could infer that the chromosome expressing satellites was most likely the X-chromosome. In order to demonstrate this, Lubs included a pedigree of the extended family in his report. As seen in Figure 4, the pedigree divided family members into three types: normal, female carrier, and affected male. This specific categorization of individuals was based upon the assumption that this was a straightforward recessive X-linked trait that only affected males. A year later, other chromosomal aberrations similar to what Lubs had described, were given the name 'fragile sites'. By the early-1980s, 'Fragile X' syndrome had become a common term for naming the inherited form of intellectual disability that Lubs had linked to this X chromosome fragile site. Around this time, the 'Fragile X' chromosome was also found to be associated with the form of inherited intellectual disability previously described by Martin and Bell in 1943, thus providing additional visual evidence that the inheritance pattern they had suggested was correct.

Visual Shortcomings of Cytogenetic Analysis

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[3] Lubs, Herbert A. 1969. "A Marker X Chromosome." *American Journal of Human Genetics.* 21 (3):231-244.

Cytogenetic analysis offered an etiological link to a visible chromosomal aberration in certain families affected by inherited intellectual disability. Those families in which the 'Fragile X' was the cause of this clinical outcome could be visually identified cytogenetically by the early-1980s. Fragile X syndrome also provided an opportunity for cytogenetic analysis to be used for providing carrier screening. The Fragile X chromosomal marker however, proved to be unreliable, because it was not consistently seen under the microscope. As a result, almost a decade passed between Lubs' identification of the X chromosome satellites and their use by clinical cytogeneticists as a visual diagnostic marker.

This delay occurred largely because Lubs' results proved difficult to reproduce. An ongoing problem with visualizing the Fragile X marker cytogenetically was that it rarely was seen in more than 40% of an affected patient's cells. This didn't pose much of a problem when it came to diagnosing males, who almost always had at least 10% Fragile X expressing cells. However, female carriers of Fragile X rarely showed the fragile site in 10% of their cells, and often it could not be seen at all, even in obligate carriers.

Carrier identification in females therefore, could be successful in identifying women who were certainly carriers of the Fragile X genetic trait, and therefore at risk of having an affected son. However, because of the not infrequent problem of being unable to visually identify the Fragile X chromosomal marker, even in women who already had affected sons, cytogenetic testing could never be used to absolutely rule out carrier status. As a result, cytogenetic analysis offered no new reassurance for a woman that she was not at risk for having an affected child. Rather, it could only verify that she did possess the mutant trait.

Reclassifying Certain Female Heterozygotes

Cytogenetic findings, though of limited diagnostic value, did offer researchers an opportunity to define two visually distinct categories of female carriers: those who could reliably be seen to express the Fragile X marker in their cells, and those who could not. With this basic cytogenetic means of differentiation in place, researchers became more open to the idea that the clinical effects of Fragile X syndrome could also impact females. Gillian Turner, a physician at the Prince of Wales Hospital in Sydney, Australia and one of the world's most prominent Fragile X researchers since the 1970s, has said that early in her research, she noticed that mothers of Fragile X boys "occasionally seemed slower than their non-carrier sisters". She however, assumed that this resulted from the stresses of raising an affected son. This assumption continued until Turner met the sister of a Fragile X affected boy, who herself showed moderate intellectual disability. Turner suggested that visual chromosomal analysis be performed, expecting that the girl's clinical condition might result from possessing an additional X chromosome. To Turner's surprise, it was seen that the girl instead showed Fragile X marker expression in her cells.[4]

Was it possible that females could also be clinically affected by possessing the Fragile X marker chromosome? To address this query, Turner looked to a larger population of young girls diagnosed with mild intellectual disabilities. Her study found that some of these girls (5 out of

[4] Turner, Gillian. 1983. "Historical Overview of X-Linked Mental Retardation." Pp. 1-16 in *The Fragile X Syndrome: Diagnosis, Biochemistry, and Intervention*, edited by Randi Jenssen Hagerman and Pamela McKenzie McBogg. Dillon, CO: Spectra Publishing Company, pp. 13.

128) were indeed from Fragile X families and that their cells visually possessed the X-marker.[5] Was it then possible that certain female carriers of the Fragile X genetic trait were not carriers at all, but instead 'mildly affected heterozygotes'? Additional studies demonstrated that this was indeed the case in about one-third of all female heterozygotes. While the visual appearance of the Fragile X marker in a female's cells could not be used to predict her phenotypic outcome, it was clear that almost all females who had mild Fragile X symptoms also possessed a visible X chromosome marker. Having a visible cytogenetic marker for Fragile X syndrome meant that a woman was heterozygous for the trait. As Figure 5 shows however, in many cases, due to clinical effects, certain heterozygotes were no longer diagnosed as Fragile X carriers.

The idea that female heterozygotes might show the clinical effects of Fragile X syndrome dates back to the inheritance pattern mapped by Martin and Bell in 1943. The concept was later categorically discounted in Lubs' pedigree (no symbol was provided for an affected female, see: Figure 4), and generally overlooked by prominent researchers, such as Gillian Turner, throughout the 1970s. Ultimately, it was the differences, made visible by cytogenetic analysis, among women with affected sons that paved the way for the recognition and clinical diagnosis of mildly affected female heterozygotes. Chromosomal analysis had provided visual evidence that was used to divide obligate carrier females into two categories (based on the microscopically visible presence of the Fragile X marker), and as a result had created two distinct populations for comparison. Once 'Fragile X marker-possessing obligate carrier' was considered a visually distinct diagnostic grouping, it became possible for certain 'carrier' females to be clinically re-categorized, and to understand themselves as 'affected heterozygotes'.

An Additional Complication: Normal Transmitting Males

Similar uncertainty also eventually developed concerning the status of males who possessed the microscopically visible Fragile X genetic trait. As Figure 4 shows, in his pedigree, Lubs had assumed that all males possessing the Fragile X marker would be affected. Martin and Bell, on the other hand, had identified two males, seen in Figure 2, from an earlier, unaffected generation who were obligate carriers of the disabling genetic trait, but did not show its clinical effects. Throughout the 1980s, pedigree analysis continued to be the only means for identifying unaffected male 'transmitters' of the Fragile X trait. Statistical analysis, performed using a large number of Fragile X pedigrees, revealed that families had about 20% less affected males than would be anticipated for a recessive X-linked trait. It was also clear that clinically affected boys had grandfathers who must have passed the Fragile X genetic trait down to them through their mothers. However, the grandfathers in question showed neither the symptomatic effects of Fragile X nor its cytogenetic marker.[6]

These 'transmitting' grandfathers were, by definition, male carriers of Fragile X syndrome. But, how could a hemizygous male possess an X-linked genetic trait without it being clinically expressed? For a variety of reasons, genetic traits are sometimes 'non-penetrant', meaning that they are visibly present genetically, but not clinically apparent. This situation is not unheard of, or even necessarily unusual in genetics. However, what made this case

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5 Turner, G., R. Brookwell, A. Daniel, M. Selikowitz, and M. Zilibowitz. 1980. "Heterozygous Expression of X-linked Mental Retardation and X-Chromosome Marker fra (X)(q27)." *New England Journal of Medicine.* 303 (12):662-664.

6 Sherman, S. L. et al. 1985. "Further Segregation Analysis of the Fragile X Syndrome with Special Reference to Transmitting Males." *Human genetics.* 69 (4):289-299.

particularly perplexing was the known presence of affected female heterozygotes. How was it possible that the Fragile X aberration could be both strong enough to override a normal X chromosome in a heterozygous female, but at the same time go unexpressed in a male who's only X chromosome possessed the disease-causing allele? Ultimately, a new method of visually identifying genetic carriers would prove necessary to resolve these questions.

In retrospect, while it could not explain all of Fragile X syndrome's various complexities, cytogenetic analysis did succeed in making carriers, and affected individuals, newly visible, as demonstrated in Figure 3. Indeed, the diagnosis of carrier status for Fragile X was no longer exclusively retroactive in the era of chromosomal analysis. Unfortunately, the Fragile X cytogenetic marker remained invisible in many women who were in fact carriers of the disease-causing genetic trait. Cytogenetic analysis had however, led to the formation of a new cohort of heterozygotes, and provided a means for recognizing and diagnosing clinically affected females.

Fragile X Syndrome in the Molecular Era

The application of molecular techniques to the identification of Fragile X syndrome began in the 1980s. Molecular methods are perhaps best distinguished from cytogenetic techniques by the size of the biological entities employed for analysis. Cytogenetics depends on the direct microscopic visualization of cells and chromosomes. Molecular tools are much too small to be individually seen under a microscope, and therefore must be visualized en masse. As shown in Figure 5, while one small piece of DNA or protein cannot be seen on its own, millions of copies of the same piece, all present together in the same small location, can be visualized and interpreted when properly treated and stained.

In 1991, the gene associated with Fragile X syndrome was definitively located and sequenced. Knowledge of the DNA sequence associated with Fragile X, and the ability to compare and contrast genetic data from affected and unaffected family members, led to entirely new ways of visualizing and understanding carrier status and inheritance risk. The Fragile X mutation turns out to be more dynamic than most other disease-related genetic aberrations. Rather than a single nucleotide mutation or the deletion of a DNA segment, Fragile X syndrome is almost always caused by an expansion of the tri-nucleotide repeat CGG. Researchers found that individuals who show the distinct clinical effects of Fragile X syndrome usually appear to possess at least 200 CGG repeats in the Fragile X 'critical region' of the X chromosome. Those individuals known by pedigree analysis to be obligate carriers of Fragile X almost always have between 50 and 200 CGG repeats. Family members who did not inherit the Fragile X trait at all, and thereby who have no risk of passing it on, generally have 40 CGG repeats of fewer.

CGG repeat length is determined via the visual analysis of an electrophoresis gel. Numerous copies of an individual's Fragile X allele(s) (women have two) are produced via the DNA replication technique known as the 'polymerase chain reaction' (PCR), and made to pass through an electrophoresis gel by the force of an applied electric current. Due to the resistance of the gel, DNA segments with more CGG repeats move more slowly, and therefore less far through the gel, than those having fewer repeats. The separation of DNA fragments by length using gel electrophoresis however, is not an exact process. Analysis of these gels is highly visual and interpretive. The size of a fragment is determined by its relative location in the gel compared to standards of known length. As Figure 5 demonstrates, the exact lengths of DNA fragments, and therefore the number of CGG repeats, can often only be approximated. Also, due to technical problems associated with PCR, the lengths of the fragments that are reproduced and visualized may vary over a significant range, creating a smear.

Molecular analysis of Fragile X alleles suggests that those with more than 50 CGG repeats often become unstable during reproduction, and may expand in size from one generation to the next. As a result, individuals with between 50 and 200 CGG repeats are now classified as possessing 'pre-mutations'. Fragile X syndrome is known to affect more individuals, with greater severity, over the generations of a family. This is because pre-mutations, which cause no noticeable symptoms in earlier generations, suddenly expand into full mutations in children and grandchildren. Therefore, those found to possess pre-mutation alleles, which by the visual analysis of an electrophoresis gel appear to contain between 50 and 200 CGG repeats, are now informed that they are carriers of Fragile X, and at risk for having affected descendents.

What does a carrier of Fragile X syndrome look like in the age of molecular diagnosis? DNA sequence analysis has, greatly reduced uncertainty over who is a Fragile X carrier in affected families. However, in terms of clinical effects, the difference between having 199 and 200 CGG repeats is not concretely fixed or visually distinguishable molecularly. A band on an electrophoresis gel may appear to be in the range of having 200 CGG repeats, but this does not absolutely imply a full mutation. In practice, the difference between a pre- and full mutation is about gene expression. When an individual has a pre-mutation, the FMR-1 gene (Fragile X-related Mental Retardation), it is still expressed, whereas, in the case of a full mutation, it is silenced. It is the lack of gene product that leads to the Fragile X clinical phenotype. This is why affects are usually milder in females: they still have one active FMR-1 gene.

Associating Phenotypic Effects with 'Pre-mutation' Carriers

As the history of Fragile X syndrome demonstrates, the development of a visible marker for identifying a genetic disease trait can at once improve diagnosis and complicate perceptions of who is at risk. Genetic markers of disease may facilitate the formation of new populations of potentially affected individuals. In the late-1970s, obligate carriers who expressed the Fragile X marker site became a new, visually distinct cohort of individuals, leading to the recognition that females may sometimes be clinically affected by Fragile X syndrome. Likewise, the development of 'pre-mutation' carrier status has also led to the formation of a diagnostically distinct and clinically relevant population. In recent years, this cohort has also been identified as being at risk for having certain clinical effects resulting from their Fragile X genotype.

Around 2000, researchers began to notice that clinically 'normal' grandfathers of Fragile X affected children were frequently experiencing Parkinsonian tremors late in life. While it is not all that rare for men in their 70s and 80s to develop such tremors, Randi Hagerman, of the University of California at Davis, began to wonder if these symptoms were occurring more often in older men with Fragile X affected grandchildren. A population analysis of Fragile X families verified that this was indeed the case. Men who are pre-mutation carriers for Fragile X, develop tremors and ataxia, both earlier and much more often than most other men. This phenotypic outcome is now known as Fragile X-associated Tremor and Ataxia Syndrome (FXTAS).[7]

The increased incidence of tremors and ataxia in pre-mutation carrier males was only recognized once this diagnostically distinct population had been brought to the attention of clinicians by the molecular analysis of the Fragile X trait. Before they became pre-mutation

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[7] Hagerman, RJ et al. 2001. "Intention tremor, Parkinsonism, and Generalized Brain Atrophy in Male Carriers of Fragile X." *Neurology.* 57 (1):127-130.; Jacquemont, S. et al. 2004. "Penetrance of the Fragile X–Associated Tremor/Ataxia Syndrome in a Premutation Carrier Population." *JAMA.* 291 (4):460-469.

carriers, these men essentially had nothing in common, except that some of their grandchildren had Fragile X syndrome. However, the creation of this population, based upon the technique of CGG tri-nucleotide repeat analysis, opened the door for researchers familiar with Fragile X affected families to observe and diagnose a distinct set of clinical outcomes in these men.

In the 1980s, the acknowledgement that female obligate carriers could indeed show a milder form of the Fragile X phenotype led to their clinical diagnosis as 'affected heterozygotes'. Should FXTAS-affected men therefore also be re-categorized from 'pre-mutation carriers' to 'affected hemizygotes' (a term used to describe males because they have only one X chromosome) based on their Fragile X related clinical outcomes? Researchers have not chosen to pursue such a reclassification, in part because the allele that these carriers possess cannot actually cause Fragile X syndrome in its pre-mutation form, and also because FXTAS has not been observed in individuals who are affected by Fragile X syndrome. Therefore, pre-mutation males are still diagnosed as 'carriers' of Fragile X syndrome, but as a result, are informed that they are at a significant risk for expressing serious FXTAS symptoms later in life.

Comparing Carrier Status for Fragile X Syndrome and Cystic Fibrosis

The complicated situation described above, in which an individual is considered a carrier for one disorder, with no chance of being affected by it and, due to the same allele, at risk for developing another, is not unique to Fragile X syndrome. Perhaps the best known other example involves cystic fibrosis (CF), which is an autosomal recessive genetic disease. This means that if an individual carrier possesses one mutant allele for CF, they are expected to be unaffected clinically, but those with two copies of the same mutant allele will likely express the disorder. The gene associated with CF however, is subject to hundreds of unique mutations. As a result, it is possible for an individual to possess two CF alleles, each containing a different mutation. Could this person, as a heterozygote, be at risk for being affected by CF? Is it possible to have two different mutant alleles for this disease and remain a carrier? The CF status of such individuals has been a matter of debate, negotiation, and compromise for decades [5,6].

When working through these genetic complications, researchers must begin by agreeing upon what CF looks like clinically. Should CF be defined by a narrow set of symptoms, or is it a 'spectrum' disease: meaning that in some cases it may be expressed in milder forms? Most men affected with CF are infertile, though this is not considered to be among the definitive CF clinical phenotypes (involving the lungs, skin, and pancreas). The form of male infertility associated with CF is known as 'congenital bilateral absence of the vas deferens' (CBAVD), and is considered by many researchers to be a mild variant of CF. This is because, in many cases, CBAVD has been associated with the possession of at least one mutant CF allele. Should CBAVD be considered a mild form of CF if it occurs in the presence of a mutant CF allele, but independent of the more 'classic' CF symptoms? Is a male with one or more CF gene mutations and the symptoms of CBAVD a carrier of CF, or clinically affected by it? More broadly, if an allele or gene has become associated with one particular disease, are all other clinical outcomes etiologically linked to that genetic location therefore components of that disorder [5,6]?

Case studies of CF are valuable for comparison when considering the nature of Fragile X syndrome. Should Fragile X be thought of as occurring over a spectrum that includes FXTAS, or is FXTAS a separate syndrome all together? To what extent does the association of FXTAS with the Fragile X allele influence its clinical relationship with Fragile X syndrome? Should one still be considered an unaffected carrier of Fragile X syndrome when they show the clinical effects of FXTAS? For Fragile X researchers, FXTAS is considered a separate but related

syndrome, meaning that showing its symptoms does not alter one's status as an unaffected carrier of Fragile X syndrome. At the same time, if a female is heterozygous for a Fragile X full mutation and appears to have mild intellectual disabilities, she is be referred to as a 'carrier' of Fragile X syndrome, but rather is diagnosed as a clinically 'affected heterozygote'.[8]

What is the decisive difference between a 60 year-old man with a pre-mutation allele who has tremors, and a 10 year-old girl with a heterozygous full mutation who suffers from mild intellectual disabilities? The answer to this depends largely upon what medical professionals and affected families decide Fragile X syndrome should clinically look like, and who can be thought of as having the disorder. Fragile X syndrome has always been a disorder defined by inborn and life-long intellectual disability. Certain factors set Fragile X syndrome apart from other forms of inherited intellectual disability, including its unique inheritance pattern and certain physical abnormalities such as having prominent ears or oversized testicles. The clinical expression of these minor irregularities however, is not sufficient for a Fragile X syndrome diagnosis: some form of inborn intellectual disability must also be present. FXTAS is also a serious and debilitating syndrome, which can itself lead to cognitive impairment later in life. Nonetheless, FXTAS does not fit the clinical mold of Fragile X syndrome. As a result, FXTAS continues to be considered a related, but distinct disorder from Fragile X syndrome.

Concluding Remarks

A historical look at the diagnosis of Fragile X syndrome reveals researchers' flexible and evolving understandings of what it means to be a carrier of a genetic disease. As new methods for analyzing Fragile X syndrome have been introduced, and new visible markers for the disorder developed, researchers have continually reassessed who should be considered a carrier of this disorder, and what such an individual might look like. Over time, this has involved the creation, re-conceptualization, and expansion of various categorizations of individuals.

Carrier status involves both clinical and genetic distinctions. Since carriers are defined as *clinically invisible*, what the expression of a genetic disease *does* look like must be determined first, in order to define what a carrier of the disorder will not. Carrier status is also defined by the possession of a specific genetic trait. Clinical pedigree analysis, cytogenetics, and molecular techniques have each been used throughout the postwar period to provide visual evidence for the detection of this genetic trait in potential carriers. In the process, these genetic markers have also become a new means of identifying new categories of affected and at-risk individuals.

The introduction of visual genetic markers for Fragile X syndrome played a central role in reshaping what it means to be a Fragile X carrier. Beyond improving the mechanistic understanding and diagnosis of Fragile X syndrome, these cytogenetic and molecular markers provided a visual and scientific basis for new groupings of individuals. Once new populations, such as 'female heterozygotes cytogenetically expressing the Fragile X marker' or 'male pre-mutation carriers', were recognized, there existed a new basis for explaining certain clinical characteristics that they shared in common. Indeed, visible genetic markers provided both new pieces of evidence and a new sense of etiological distinctiveness, which helped give credence to the secondary clinical trends that Fragile X researchers sometimes observed.

The identification of Fragile X syndrome carriers requires agreement about the disorder's defining clinical characteristics. Researchers have always maintained that the most central and necessary signifier of Fragile X syndrome is lifelong intellectual disability. This distinction

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[8] For more on this: http://www.fragilex.org/html/carriers.htm

underlies why mildly affected full mutation heterozygotes, once recognized, were diagnosed as 'affected' by Fragile X syndrome, whereas older men with pre-mutations, who experienced tremors and cognitive impairment, continued to be told they were 'carriers'. While there are real differences in the symptomatic outcomes experienced by individuals possessing a pre-mutation versus a full mutation, this molecular distinction does not objectively exclude FXTAS from being considered a component or variant of Fragile X syndrome. Indeed, the isolation of FXTAS from the Fragile X spectrum is a socially negotiated decision that directly reflects conceptions of what it means, and what it looks like, to be affected by Fragile X syndrome.

Fragile X syndrome is a unique disorder in terms of its pattern and mechanism of inheritance. Nonetheless, its diagnostic history is demonstrative of how the analysis and understandings of genetic diseases have continuously evolved since the 1940s. What it 'looks like' to be the carrier of a genetic disease has expanded significantly over this time, in large part due to the development of new cytogenetic and molecular visualization techniques. These novel diagnostic methods have both clarified and confused the Fragile X carrier status of individuals. New genetic evidence, unexpectedly, provided unique visual markers around which novel clinical populations were formed. In some instances, these newly distinct groupings have led to the recognition that a particular genotype, once assumed to benign, has its own clinical implications. More broadly, researchers have been led to reassess what it looks like, and indeed what it means to be a carrier of a particular genetic disease.

References:

[1] Rose, Nikolas. 2007. *The Politics of Life Itself: Biomedicine, Power, and Subjectivity in the Twenty-First Century*. Princeton, NJ: Princeton University Press.

[2] Nukaga, Y. and A. Cambrosio. 1997. "Medical Pedigrees and the Visual Production of Family Disease in Canadian and Japanese Genetic Counselling Practice." *Sociology of Health & Illness.* 19 (19B):29-55

[3] .Cowan, Ruth Schwartz. 2008. *Heredity and Hope: The Case for Genetic Screening*. Cambridge, MA: Harvard University Press.

[4] Lindee, Susan. 2005. *Moments of Truth in Genetic Medicine*. Baltimore, MD: Johns Hopkins University Press.

[5] Kerr, Anne. 2000. "(Re)Constructing Genetic Disease." *Social Studies of Science.* 30 (6): 847-894.

[6] Hedgecoe, Adam M. 2003. "Expansion and Uncertainty: cystic fibrosis, classification and genetics." *Sociology of Health & Illness.* 25 (1):50-70.