

## **BIOMOLECULAR APPROACHES TO FRAGILE X SYNDROME THERAPY**

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This session will feature an overview of potential therapies for Fragile X Syndrome (FXS), as well as additional details regarding gene therapy, presented by this speaker (Dr. Robert Bauchwitz). This will be followed by two talks on potential therapies for FXS that fall outside of classical gene therapy. Dr. Pietro Chiurazzi will present studies designed to determine how the inactive FMR1 genes with full-expanded repeats might be chemically reactivated. Dr. Alan Tartakoff will then provide an overview of a relatively new technique, which could allow the FMRP protein itself to be used as a drug to directly restore biochemical function to neurons, which lack it in those, affected by FXS.

By using the term “biomolecular approaches” I mean to expand the range of possible therapeutic avenues considered beyond classical gene therapy. Fragile X Syndrome is in most cases the result of an inactivating gene mutation, which results in the absence of the FX protein, FMRP, in an affected person’s cells. In more than 95% of FXS cases, the FMR1 gene is enlarged. The FMR1 gene enlargement is known as a triplet repeat expansion. The FMR1 expansion causes a secondary chemical alteration of FMR1 known as methylation. Methylation leads to a shut down of gene activity.

Figure 1 outlines several of the avenues by which current research into FXS might lead to a treatment or cure. The list presented is not meant to be exhaustive, but rather to highlight that there are multiple potential approaches. At this point it is not possible to state with any certainty which approach will eventually prove to be the first or best to be applied. Rather, this represents a research investment portfolio as it might appear at this time to a funding agency such as FRAXA Research Foundation.

Gene therapy involves placing a functioning copy of the FMR1 gene into the cells of the brain. The purpose of the gene, or transgene as it is known, used in gene therapy is to produce the right form of FMRP in the right place at the right time and in the right amounts. This is not a trivial task for several reasons, one of which is that the FMR1 gene is large (38kb) compared to the space in viral vectors, which might carry the gene. Second, there are several forms of FMRP which are produced (by alternative splicing of exons). If the large genomic fragment has to be reduced in size, then it will be necessary to determine which forms of FMRP are required for treatment; Fragile X animal models are being used to determine this. In order to express the right amount of FMRP at the right time and place, its expression must be properly regulated. My laboratory has taken the approach that the best way to achieve this will be to identify the regulatory elements that in FMR1 naturally accomplish this and then try to incorporate such regulatory elements into a gene therapy vector. The regulatory elements required actually have an additional role, which might not be found near FMR1 itself: they must be able to resist the repressive effects of heterochromatin should the gene therapy virus integrate into such a region of the patient’s genome. Thus, the vector will require elements to keep it active even should it land in regions which would otherwise extinguish transcription of the FMR1 transgene. My laboratory has never observed a lowly expressing or silenced human FMR1 transgene in mice, suggesting that our construct carries sequence elements capable of preventing repression by heterochromatin.

Upon assembling the appropriate form of FMR1 and required regulatory elements, the transgene

must then be introduced into a sufficient number of the brain's neurons. Several viruses might be used to accomplish this. Herpes simplex virus (HSV) could carry the entire FMR1 gene and considerable surrounding sequences. So far, however, expression of experimental transgenes carried by HSV into the mouse brain has been extinguished fairly quickly (~2 weeks), apparently due to silencing and immune responses. Adenoviruses cannot carry the entire FMR1 gene, nor is its use risk free (as we have recently seen). Expression of adenovirus transgenes in the brain has been reported for as long as 8 weeks before being lost. Adeno-associated viruses (AAV) are a single stranded DNA parvovirus (in contrast to the double stranded DNA HSV and adenovirus). It has the advantage that it can integrate in cells in a site specific manner, in the case of the whole virus. AAV vectors do not produce a strong immune response either, which allows more stable transgene expression than observed for HSV or adenovirus. However, AAV has a low efficiency of infection and requires a helper virus. Finally, there is a form of retrovirus known as lentivirus which can integrate into cells without the need for cell division, such as would be required to treat nondividing neurons. The most well known lentivirus is HIV, which is responsible for AIDS. Lentiviruses also may not provoke inactivating immune responses. However, lentivirus vectors can currently only hold an 8-10 kb transgene. This would provide only enough space for an FMR1 cDNA and a very well trimmed set of regulatory elements.

In addition to gene therapy, it may be possible to bypass the lost activity of FMRP (Figure 1). To do this, we must determine what critical role FMRP has in cognition and behavior at the molecular level and then search for a drug, which can replace FMRP's function or act downstream of it. It may also be possible to repair the mutant form of FMR1 such that the repeats are reduced and FMR1 transcription might occur. Pietro Chiurazzi will provide evidence that FMR1 might be chemically reactivated by reversing chemical changes caused by the expanded repeats. Finally, Alan Tartakoff will describe a fascinating new technique by which the FMRP protein could be modified such that it could be used as a drug to directly enter the neurons of the brain (and perhaps all other cells of the body). If this technique is validated for FMRP, then it would allow a protein replacement which could be compared to the use of insulin for diabetics. However, it should be noted that all of our approaches depend on the ability of a FX brain to recover at different stages of development. We do not yet know what the potential for such recovery will be; however, new mouse models of FXS are being made, including in my laboratory, which should answer this critical question.

**Figure 1**  
**Approaches to Fragile X Treatment**

1. Gene therapy  
Place a functioning copy of the **FMR1** gene into the cells of the brain.
2. Bypass  
Find out what critical role FMRP has. Then find a drug which can act in its place or downstream of it.
3. Gene repair  
Reduce the enlarged portion of the **FMRI** gene.
4. Reactivation  
Find drugs to reverse the methylation or associated chemical changes which keep the enlarged **FMRI** gene inactive.
5. Protein Replacement  
Provide the missing protein as a drug. Insulin for diabetes is an example.