The fragile x syndrome

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The fragile X syndrome is one of the most prevalent mental retardation problems that are inherited to generations. The clinical features of people with this syndrome are very subtle and hard to diagnose. Recent progress in the field of molecular biology and genetics has outlined the molecular mutation, that causes the syndrome was a triplet repeat mutation. Due to repetitive CGG repeat the respective proteins are not expressed. New molecular methods including direct probe analysis and PCR analysis, have simplified the process of diagnosis. The nature of the gene, their respective gene product and its function has not been yet found clearly. Inheritance may be found due to common ancestral flow of X chromosome at birth.

Introduction

Fragile S syndrome is a genetic disorder that affects the intellectual, physical and mental factors of a human being. It is also known to be martin-bells syndrome and the effects range widely from mild to severe. It is caused by the mutation on the X chromosome of the individual in a single gene called the Fragile X mental retardation gene (FMR1). Psychological problems such as mental retardation may be caused by two main factors, the physical environment or the genetic factor that is hereditary. As we mainly focus on the genetic factor, the FRAXA locus in xq27. 3 is associated in causing fragile X mental retardation. Therefore based on molecular genetic testing of FMR1 gene, fragile X syndrome can be diagnosed. Women are only 50% affected by this syndrome when compared to males due to the fact that they have two X chromosomes where as males have one. Most common symptoms found are seizures, mood instability, attention deficit, sensory over stimulation, aggression, autism, speech disorder and sleep differences. This

syndrome also shows certain physical symptoms such as long narrow face, large ears enlarged testacles in males, flexible joints etc., various researches is being done across the world to find a solution for the disease.

Various genetic techniques has been developed and handled to identify the fragility of the chromosome. There has been so much improvement in research but a definite solution has not been obtained. The treatment for this syndrome is usually a multidisciplinary approach which includes occupational therapy, medical managements, education and linguistics.

The Fragile X Mutation:

Genes are the precursors of specific protein molecules which in turn are specific for various functions of the body. The major cause for the fragile x syndrome is due to the mutation in one single gene called the fragile x mental retardation 1 (FMR1) gene which is the precursor for the fragile x mental retardation protein FMRP. This protein is responsible for the normal development and functioning of the brain. Men have only one x chromosome so presence of mutation in that chromosome will cause the disease whereas in females, they have two x chromosomes and hence full mutation in one copy makes them carriers of the syndrome and they may be affected partially according to the amount of mutation and number of cells expressing the FMR-1 gene copy. The inheritance found on the chromosomes is termed as x linked recessive inheritance which is more complex than the normal x linked genes.

Fig. 1 X chromosome with fragile site [1] Fig. 2 A photograph of X chromosomes showing a fragile site from both a male and a female [2]

The number of CGG repeats on the FMR-1 gene determines the complexity of the syndrome. The repeats are classified as short, medium and long repeats. The short repeat of about six to fifty times which is found very common. These short repeats are mostly unstable and do not definitely cause the syndrome. Yet a genetic counseling along with certain tests is recommended. The medium repeat is about 50 to 200 times and is called permutation.

The fragile x mental retardation protein (FMRP) has lower risk of the syndrome as short sequence repeats. The long repeats are usually more than 200 and are termed as full mutation where the complete FMR-1 gene is altered and production of the FMRP protein is totally stopped. Among the people with full mutation or long repeats, men will have the fragile x syndrome and women will be carriers.

Fragile x mental retardation protein (FMRP)

The FMRP protein is found in the ribo-nucleoprotein complex and is encoded by the FMR1 gene. The FMRP weighs up to 60-70 kD. This protein is associated with the polyribosome or polysomes. Two RNA-binding domains, KH domains or K homology domains are possessed by this protein and it binds to fetal human brain to 4% approximately. It also has the ability to bind to its own mRNA. Even a small amount of mutation in one of the KH domain could stop its interaction with the polysomes leading to the fragile x syndrome.

Inheritance of fragile x syndrome

Males have xy chromosome and hence have only one FMR-1 gene where as females have xx chromosomes and hence they have two FMR-1 genes. On the F1 generation each parent transfers one chromosome each to the offspring where the transfer of the FMR-1 gene is determined. Therefore the possibilities of their offspring's being affected are grouped under two conditions.

Condition-1 [3]

If a male has a mutated gene in his chromosome it can be transferred only to his daughter because only the Y chromosome can be transferred to his son by him. So if he was crossed with a female with normal genes all their sons will be normal and the daughters will have one fragile gene and remain as carriers.

father

áº‹

y

mother

x

xáº‹ (carrier daughter)

Xy (normal son)

x

xáº‹ (carrier daughter)

Xy (normal son)

áº‹ – fragile x gene

Condition-2 [4]

If a female has one mutated gene in her chromosome and is crossed with a normal male then there is 50% chance of all the offspring’s, be it male or female to have the syndrome.

father

x

y

mother

áº‹

xáº‹ (carrier daughter)

áº‹ y (fragile son)

x

xx(normal daughter)

Xy (normal son)

áº‹ – fragile x gene

Symptoms

The symptoms of fragile x syndrome are categorized into :

Physical

Large eyes

Prominent forehead

Large testecles

Seizures

Cognitive development

Social and emotional

hyperactivity

Behavioral

Shyness

Social anxiety

Speech and language

Rapid and repetitive

Inability to adopt words

Over-talkative

Autism

Flapping of hands

Poor eye contact

All these symptoms need not be necessarily seen. A combination of various symptoms may vary from person to person based on the amount of gene altered in their chromosome. Sometimes there may also be no visible symptoms making the chances of early diagnosis even worse.

Molecular diagnosis [5]

The chromosome associated with the syndrome is classified into three major types based on the number of CGG repeats as

Normal allele

the CGG repeat in FMR1 is 6-50

PCR analysis is sufficient to study all normal type genes

Specific point mutations and mosaicism must be studied using specific types of pcr or other molecular methods

Visualization is achieved either by radio-active labeling or auto radiography followed by automated sequencing

Agarose gel electrophoreses may be used for simple separation analysis with stains such as ethidium bromide. Appropriate size markers and size controls are very important.

Controls used for analysis mut approximately contain 50 repeats.

Pre mutation

The CGG repeat in FMR1 is 55-200

PCR analysis is not possible hence southern blot is always preferred.

Since both premutation and full mutation have methylation status, specific methylation sensitive enzymes such as EagI or NreI is used to resolve the size of the fragment.

Methylated alleles are cut only by one enzyme where as non methylated normal alleles are cut by both the enzymes.

Prenatal diagnosis is very important for pre mutation carriers.

Rather than normal PCR a radioactive PCR can be used to test for premutation and then the result can by confirmed using southern analysis.

Full mutation

The CGG repeats in FMR1 ranges from 200 to thousands

This can be analysed only by a southern blot technique.

At complications, if a confirmable result could not be obtained from a southern analysis then a radio active PCR can be run combined with a linkage analysis and the result can be confirmed with southern blotting.

Intermediate alleles

The CGG repeats in FMR1 gene is usually between 45-55

Since they are in the overlapping region between stable normal allele and unstable premutation alleles, diagnosis and interpretation is very difficult

Diagnostic Tools and Methods

With the advancement in technology DNA tests are always effective in diagnosis of fragile x syndrome. With the findings of Sutherland et al. that folic acid deficient cell culture medium was able to induce a fragile site at xg27. 3 cytogenetics was the major way to determine the presence of the syndrome but after cloning of the FMR-1 gene direct methods for identifying the x linked gene has become possible. By using monoclonal antibodies specific to FMRP it is also possible to show the expression of FMR-1.

The most common methods used for diagnosis in the genetic level are

Polymerase Chain Reaction

Southern Blotting

Antibody test

Denaturing gradient gel electrophoresis

Single strand confirmation polymorphism

Non-radioactive molecular diagnosis.

Polymerase chain reaction

Polymerase chain reaction may be defined as a technique where one copy of a DNA is amplified into numerous copies at a rate of 2n where n is the number of cycles. It is achieved under specific conditions of temperature, along with polymerase enzyme.

PCR amplification is one of the preliminary methods in diagnosing fragile x syndrome. Since the syndrome is associated with CGG repeats PCR is not considered as the best method always, since the amplification across C-G composition could be unreliable for PCR technique. However now its very much possible for a PCR to identify CGG repeats in combination with various techniques.

methylation specific PCR of the FMR1 locus

fluorescent methylation – specific PCR

methyl-CpG-binding PCR

Some of the major advantages of PCR are that it is less time consuming, a very small amount of the sample is enough to produce numerous copy and the tri-nucleotide repeat in the FMR-1 gene is accurately sized. There are also various disadvantages of this technique. When there are more than hundreds of tandem repeats it is impossible for the PCR to determine the complete mutation which may give a different result. Due to differential amplification PCR is incapable of detecting mosacism between pre mutation and normal alleles.

Fig 3: fragile x analysis using PCR [6]

Southern Blotting

Southern blotting is one of the best methods of diagnosing fragile x syndrome. It is modtly used as the confirmatory test after PCR. The variations between the mutations and permutations along with the amount

of methylation occurred can be clearly obtained by the southern blotting technique. The process can be summed up in two simple steps:

Step 1: the patients DNA is digested using restriction enzymes.

Step 2: southern hybridization is carried out along with specific radioactive probes after separation of FMRI region.

Using southern Blotting, the differences in full mutation and pre mutation can be easily identified. Full mutations usually cause smearing of the band and are always unstable. The only advantage of this technique is that its accuracy whereas its labor intensive, time consuming. The major drawback of this method is its inability to determine the exact number of tandem repeats of the CGG nucleotides which is very much necessary in determining whether the patient is completely affected or a carrier.

Fig 4: fragile x analysis by southern blot [7]

N refers to normal

Specific tools for analysis

DNA probe

A DNA probe can be defined as a single strand of DNA which act as a template to identify the target DNA molecule. To identify the fragility of chromosome on the DNA specific probes were designed which increases the accuracy rate of the diagnosis. Chemicon (Millipore) [8] has designed a special probe named “ The CHEMI” probe which is labeled with dioxegenin to detect the CGG repeats in the FMR-1 gene.

Markers

There were special markers called the microsatelite markers used in linkage analysis. This came to an end with the advent of modern techniques. However these markers are now being used under special circumstances like prenatal diagnosis where southern blotting has failed. Some of the markers used are DXS548, FRAXAC1 and FRAXAC2 combined with PCR. They are considerably accurate and they undergo low recombination mechanism with CGG repeats.

Treatments

There are no gene therapies or genetic treatments available for fragile x syndrome though a lot of other therapies are available which include speech-language therapy, occupational therapy, physiotherapy and behavioral therapy.

There are also a large number of medications available as listed in the table below:

Symptoms

Medications

Seizures

Mood instability

- Carbamazepine (Tegretol)

- Valproic acid or divalproex (Depakote)

- Lithium carbonate

- Gabapentin (Neurontin)

- Lamotrigine (Lamictal)

- Topiramate (Topomax), tiagabine (Gabitril), and vigabatrin (Sabril)

- Phenobarbital and primidone (Mysoline)

- Phenytoin (Dilantin)

Attention deficit (With or without hyperactivity)

- Methylphenidate (Ritalin, Concerta) and dexamethamphetamine (Adderall, Dexedrine)

- L-acetylcarnitine

- Venlafaxine (Effexor) and nefazodone (Serzone)

- Amantadine (Symmetrel)

- Folic acid

Hyperarousal

Sensory over-stimulation (Often occurs with ADD/ADHD)

- Clonidine (Catapres TTS patches)

- Guanfacine (Tenex)

Aggression

Intermittent explosive disorder

Obsessive-compulsive disorder

(Often occurs with anxiety and/or depression)

- Fluoxetine (Prozac)

- Sertraline (Zoloft) and citalopram (Celexa)

- Paroxetine (Paxil)

- Fluvoxamine (Luvox)

- Risperidone (Risperdal)

- Quetiapine (Seroquel)

- Olanzepine (Zyprexa)

Sleep disturbances

- Trazadone - Melatonin

Table 2: symptoms and medications for FRAXA [9]

(*these prescriptions have serious effects. DO NOT INTAKE ANY OF THESE WITHOUT CONSULTING A MEDICAL PRACTITIONER)

Current Research

Gene Therapy: studies are carried out on the recombination strategy of the target gene, whether removal or replacement of the defective gene with a recombinant gene would eliminate the syndrome.

Protein Replacement Therapy: research is being carried out on the possibility of producing FMR protein and supplying to the patients through external sources like food or tablets.

Psychopharmacology: research is being carried out in finding medications for all the symptoms of fragile x syndrome.

Conclusion

Fragile x syndrome is one of the genetic diseases that causes psychological problems due to the lack of FMR protein responsible for the mental behavior of the person. The protein is not expressed in the individual due to fragility of the FMR1 gene in the x chromosome. Though PCR and southern blotting are the only tools available for diagnosis they are considerably accurate and research is being carried out on various re-combinative tools for diagnosis. A complete cure has not been still devised for the syndrome though various behavioral and physical therapies help the patients gain psychological strength.