

The Molecular Basis of Human Disease

Duchene Muscular Dystrophy

Recessive X-linked mutation that causes a progressive muscle wasting disease

Trickily, around 1/3 of affected males have new mutations, which arose in their mother's germ line

They found a boy suffering from DMD who was found to carry a deletion of a cytologically visible part of the X-chromosome

The idea was that by comparing normal X chromosomes with the X from the deletion patient, one could isolate DNA present on the normal X but absent from the mutant X and get to the region containing the gene

DNA from a cell containing 4 X chromosomes was cut in a restriction enzyme (lots of sticky ends - can be cloned)

This was denatured and mixed with a 200-fold excess of denatured DNA extracted from the deletion boy's cell

This DNA had been **sheared** - no sticky ends, can't be cloned

The idea was that as the DNA re-associates, since the deletion DNA is in excess, all the sequences from the normal X will form hybrids with this, won't have sticky ends since one comes from sheared DNA and won't be able to be cloned

This used a technique called **subtractive cloning**

However, the bits in the normal X that have no counterpart would be and would have clonable sticky ends

No PCR - was very hard!!

They found a gene that was absent in DMD sufferers

Lots of zoo blots and northern identified it

It spans about 2.5 Mb (0.1% of human genome), 79 exons

Responsible for encoding the protein that links the actin cytoskeleton of muscle cells to the extracellular matrix

The huge size probably explains the high *de novo* mutation rate

Exon deletion

Missense amino acid changes prevent the protein from folding properly

Splicing defects that generate aberrant proteins

Could be

The complexity of the gene makes it very hard to diagnose

With 79 exons, it has taken almost 20 years to being to develop reliable genetic tests - for examples, PCR assays for every exon (followed by blots to see which are missing)

However, this cannot detect point mutations or splicing defects - only deletions (see the size of the exon!)

Cystic fibrosis

Caucasians carry a mutation in the **cystic fibrosis (CF)** gene and die young if untreated, primarily from accumulation of mucus in the lungs

Using RFLP analysis to find a polymorphism that segregates with the disease

Using the **hybrid cell technique** to identify the chromosome

Fuse human tissue cells with mouse tumour cells to form a **heterokaryon**

These loses human chromosomes and after a few passages are left with only a single one

These can be identified using the banding patterns

Doing this several times and seeing where the RFLP went allowed to map CF to the long arm of chromosome 7

A clone for a genomic library mapping to the appropriate region of chromosome 7 was used to initiate a chromosome walk

The initial walk contained over 2Mb of DNA

Further RFLP mapping reduced this to ~500kb

Fragments from throughout this region were hybridised to genomic DNA from other species (a **zoo blot**)

The idea was that genes are likely to be conserved in sequence, whereas junk is not

Fragments were also hybridised to Northern Blots to look for fragments of RNA expressed in particular tissues (e.g.: lungs)

It was then found!

It's big! Spans over 250kb of DNA and contains 24 exons

The 14kb mRNA encodes a 1500 amino acid protein that acts as a transmembrane chloride channel

The inability to regulate sodium transport across epithelial cell membranes is the cause of the phenotype

66% of the worldwide CF occurrences are due to a single mutation

The Phenylalanine at position 508 is missing

There is a precise 3 base deletion in the gene

This is within the critical ATP-binding domain

Other mutations are associated with specific populations

E.g.: in Ashkenazi Jews, a premature stop codon (Trp1282 - Stop) causes a truncated protein

1525 different mutations have been identified in the CF gene

While ~70% have common mutations, around 30% have rarer alleles

This makes diagnostic screening very difficult - over 1500 different assays are expensive

However, if it can be diagnosed, there are therapeutic possibilities - e.g.: delivering normal CFTR protein to affected lungs is being assessed

Mapping genes by linkage

Everything we've done so far is **linkage analysis** - we find polymorphic markers which associate with disease segregating in family pedigrees