OOPS script

Slide 1

Most of us, at some time or other, have forgotten dates for birthdays and other events; however there is a significant proportion of the male population in which this occurs more frequently. A number of previous studies have shown that this is a result of a mutation in the OOPS gene present on the male-specific Y chromosome. The consequences of this can be severe, both for the personal relationships of the sufferer, and also commercially for the birthday-card industry.

What I would like to do in today's talk is to give you more information on the OOPS gene and to present data which give an indication of the possible function of OOPS in human biology.

To put things in context – the full DNA sequence of the human Y chromosome was obtained in 2003. This allowed us to explore at the molecular level the genes that had already been mapped by Gitschier and others and to gain insight into their function.

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Although the Y chromosome has a small number of genes, a significant proportion appear to be associated with male behavioural traits, as illustrated here. For example, the GOT1 locus is involved in the ability to remember jokes, while the RIF gene is associated with playing air guitar, or violin in older individuals. The OOPS gene, associated with forgetfulness in males, is positioned on the long arm of the Y chromosome and I will now discuss this in more detail.

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The OOPS gene lies approximately thirty megabases into the Y chromosome sequence and has been mapped using the DNA contigs illustrated here. The flanking genes do not give any evidence of gene duplication or homology around this area, and there are no SNPs that have yet been identified within the OOPS gene itself.

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In this talk, I'd like to deal with these questions: what is the OOPS gene and where is it expressed?

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We used the following experimental approach to address these questions:

Firstly, the nucleotide sequence of the OOPS gene was analysed using bioinformatics to look for intron–exon structure and to identify putative functional domains by homology to known genes.

We then used RT-PCR to determine OOPS mRNA expression in a number of human tissues.

The next step involved the production of recombinant OOPS protein, which we have named forgettin. The purified protein was then used as an immunogen to produce specific antibodies for analysis of human tissues using western blotting and immunohistochemistry.

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The genomic organisation of the OOPS gene is shown on this slide. There are eighteen exons spread over approximately five kilobases of DNA. Two of the exons, shown here in orange, are homologous to transmembrane regions, so the expressed protein is likely to be membrane associated. I'll discuss the domain structure of the protein later on in the talk.

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We isolated mRNA from testis and probed with OOPS cDNA on Northern blots. The bulk of the message was 4.1kb in length, but there was some evidence of a minor splice variant as shown here, which has not been investigated further.

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Reverse transcription PCR was used to determine the expression of OOPS mRNA in different tissues. cDNA was produced from total RNA using reverse transcriptase and subjected to PCR using primers designed to amplify only mRNA-derived sequences and not genomic DNA. After twenty-five cycles, amplification products were absent in the negative control and present in brain, testis and spleen, but absent in liver. Further experiments are underway to confirm these results using quantitative PCR.

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Having shown expression of the OOPS gene in a number of tissues, I am now going to turn my attention to the protein encoded by this gene. In keeping with the behavioural defects associated with the OOPS gene, we have called the protein forgettin. We have already seen the exon structure of the OOPS gene and this translates into a series of protein domains as shown on this slide. The predicted domain structure indicates that this large protein is membrane associated, with two C terminal tyrosine phosphatase domains and two extracellular collagen domains. The N terminal domains I and II do not correspond to any known sequence. Taken together, these data indicate that the forgettin protein is similar to the CD45 family involved in cell-to-cell communication and signalling in the immune system.

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In order to determine the function of the forgettin protein, we need to obtain the material in purified form for biochemical assays and to find out where it is expressed. We have begun a series of experiments to address the localisation question and this will be the subject of the remainder of this talk.

We decided to create antibodies to the extracellular portion of the forgettin for use in immunoassays like western blotting and immunohistochemistry. To do this, we amplified cDNA corresponding to residues 1–520 of the forgettin protein from a brain cDNA library and cloned it into a mammalian expression vector containing a C terminal hexahistidine tag. This allowed us to purify the resulting fusion protein using metal chelate affinity chromatography. The results of this are shown on the next slide.

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The 64 kilodalton protein was recovered in the eluate of the affinity column and used for the production of polyclonal antibodies.

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The antibody production scheme is shown here. After immunization, forgettin antibody titres were determined in serum samples by ELISA. High-titre sera were then passed over immobilised forgettin and the specific IgG eluted at low pH. This antibody was then used for western blotting and immunohistochemistry as follows.

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Tissue extracts of liver, spleen prefrontal lobe, brain and testis were blotted onto membranes and probed with forgettin antibody. Total protein levels in each sample were equalised, as confirmed using tubulin antibodies in separate blots, which I haven't shown here. Although there was some protein expression in the liver, most protein expression occurred in the testis, brain and specifically, the prefrontal lobe of the brain. This prompted us to explore this tissue using immunohistochemistry.

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Brain sections were prepared by paraffin embedding and used for probing with purified forgettin antibody and immunoperoxidase detection. You can see from this slide that there is intense antibody staining in the prefrontal lobe area, but little elsewhere in the brain. There is no background staining in the control here using normal IgG. We haven't yet looked at other tissues such as spleen or testis, but these experiments are planned for the near future.

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So to summarise our findings so far:

The OOPS gene on chromosome Y encodes a 135 kD membrane protein (forgettin) with possible PTPase activity

mRNA and protein are expressed in the spleen, testis and brain

Forgettin is highly expressed in the prefrontal lobe.

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So we conclude from this that forgettin may be a cell-associated signalling protein and our expression data adds further support to the role of the prefrontal lobe in memory storage.

Thank you for your attention, I'd be happy to take questions.

Question 1:

How do you explain the discrepancy between the positive expression of forgettin protein in the liver and the negative results you found with the RT-PCR?

Answer:

The question was: why was there a discrepancy between the expression of OOPS mRNA and forgettin protein in the liver?

That's a fair question and I think it is due to the technical limitations of the RT-PCR in that this technique is not really quantitative. As I said in the talk, we are repeating these experiments using a quantitative technique, so this should resolve this discrepancy.

Question 2:

My lab has extensively studied the genetics of forgetfulness in males and has found no association with the OOPS locus. This would appear to invalidate your study.

Answer:

The questioner has stated that his lab has not shown any connection between OOPS and forgetfulness in males.

Thank you for that comment. Clearly there is a discrepancy between our findings that needs to be investigated further. We have based our study on the original genetic work of Johnson *et al.* who studied over 5000 individuals. Furthermore, the location of the OOPS gene product in the prefrontal lobe adds further support to the memory hypothesis. We should discuss this after the meeting.