FISH, flexible joints and panic: are anxiety disorders really expressions of instability in the human genome?

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With only modest evidence for a genetic component, it is all the more surprising that the major cause of panic, agoraphobia and related anxiety disorders could lie almost entirely within the human genome (Gratacos et al, 2001). This appears to be because of a bizarre twist in our DNA: an unprecedented common duplication of genetic material on chromosome 15, leading to three instead of two copies of about 60 genes in most cells. This is particularly interesting as panic and social phobias have been regarded as some of the least heritable psychiatric disorders, and molecular genetics has until now been unsuccessful in unravelling the aetiology. This unexpected and novel cause of a psychiatric disorder could have fundamental implications for the causes of mental illness – are some psychiatric disorders really 'genomic disorders', and how will this change our perceptions of mental illness? And why did no one notice this before?

GENOMIC DISORDERS AND DUPLICATION

The human genome is a mess. Not only is it full of apparently redundant, highly repetitive and unstable DNA sequences, but also as much as 5–10% of the genome is duplicated (Bailey et al, 2002; Stankiewicz & Lupski, 2002). The evolution of the genome cannot have occurred without duplication events; most genes, such as those for the many serotonin receptors, are part of families that arose from ancestral gene duplication and 'exon shuffling' events (Thornton & DeSalle, 2000). The realisation that as well as aiding human evolution, large-scale genome rearrangement could also cause disease led to the coining of the term 'genomic disorders' (Lupski, 1998). Aberrant recombination between or within chromosomes can cause problems by deleting, disrupting or duplicating genes, leading to changes in gene dosage. Normally this does not matter too much, but for certain genes two copies are required for normal function, and the loss of one copy (haplo-insufficiency) as seen in velo-cardio-facial syndrome (Shaikh et al, 2000; Shprintzen, 2000), is enough to cause disease. The converse of this is duplication, where aberrant recombination or disjunction results in extra copies of sections of the genome (Ji et al, 2000a; Emanuel & Shaikh, 2001). The most striking example is the whole chromosome duplication seen in Down's syndrome, but duplication on a smaller scale occurs in genetic disorders such as Charoct–Marie–Tooth disease.

CLUES FROM RHEUMATOLOGY AND CYTOGENETICS

But why did three genome-wide linkage scans of panic disorder in families fail to point to chromosome 15? For schizophrenia, it took many decades for linkage to unequivocally identify genetic loci (Riley & McGuffin, 2000), so most observers would not have been surprised when these scans for linkage in panic/agoraphobia families failed to turn up a major genetic locus (Gelernter et al, 2001). Two important clues previously lacking led to a breakthrough. First was the observation in a series of family studies of a curious co-morbidity between panic disorder and benign joint laxity with hypermobility (Martin-Santos et al, 1998). In this syndrome, the joints, muscles, tendons and ligaments are laxer and more fragile, presumably as a result of differences in connective tissue proteins. The result is joint laxity with hypermobility, and with it comes vulnerability to the effects of injury (see http://www.hypermobility.org/). Despite the fact that patients with panic disorder are up to 16-fold more likely to have joint laxity, this finding was ignored largely as a curiosity, perhaps because a previous association with panic disorder (i.e. mitral valve prolapse) turned out to be spurious.

Second, the same group of geneticists noticed something curious about the chromosomes of patients with panic disorder; some of these patients had a slightly longer arm of chromosome 15 (Gratacos et al, 2001). These patients came from families with multiple incidences of panic disorder and joint laxity that were intended for a genome scan for linkage, and the cytogenetic examination was a prelude to this. The genome scan was postponed and 6 years of careful investigation followed.

The first stage was to look for linkage in the families. Four phenotypes were segregating: panic/agoraphobia; social phobia; simple phobia; and joint laxity (Gratacos et al, 2001). The findings were interesting; separate anxiety phenotypes failed to provide significant evidence for linkage in this region. Pooling diagnoses into a broader psychiatric phenotype helped, as a combination of agoraphobia, panic and social phobia produced a LOD (logarithm of the odds) score of above 3, but the highest LOD scores were reserved for phenotypes including joint laxity. These LOD scores might have been observed in a genome scan for panic that included a broad phenotype; however, two of the three genome-wide scans used panic disorder only and one used panic disorder and agoraphobia. None of these found any evidence for linkage between chromosome 15 markers.

FISHING FOR THE DUPLICATION

These LOD scores clearly indicated that the increase in size of chromosome 15 might well be relevant to the disease, so this was examined in more detail using fluorescence in situ hybridisation (FISH), which provided evidence for a genomic duplication (termed DUP25). Not only did the duplication segregate with panic–hypermobility spectrum disorders in the families, but also unrelated patients with panic disorder were much more likely to have the duplication. Indeed, about 7% of the normal population were found to have the duplication compared with 68 of 70 patients with panic disorder, meaning that DUP25 could be the cause of panic disorder in the overwhelming majority of cases.

Surprisingly, the duplication was complex, occurring in more than one form; either direct or inverted and lying either towards the centre or end of the chromosome. Inheritance of the duplication is also non-Mendelian (i.e. following an irregular pattern). It appeared to sometimes be transmitted from parent to offspring intact, sometimes arise *de novo*, sometimes convert back to a normal chromosome, or even swap between types of duplication, indicating that the duplication is highly unstable. Finally, the duplication is mosaic, which is a very unusual finding. Mosaicism occurs when different cells of the body contain functionally different DNA, usually because of DNA sequence changes occurring during normal mitotic cell division. In any one person, the DUP25 duplication is found in only about 60% of cells, whereas the remaining cells appear normal. The most remarkable thing is that there are on average about 2.6 copies of the genes in the DUP25 region, a seemingly small increase compared with the two copies seen in normal cells, meaning that some of the genes there must be very sensitive to dosage. However, mosaicism can vary between tissues and it is possible that the proportion of cells in the brain which carry the duplication is higher.

THE SOURCE OF INSTABILITY

DUP25 is also unique, because unlike all other known duplications causing human disease, it does not arise during cell division for the generation of gametes (meiosis). Instead it appears to arise during normal cell division (mitosis). This is probably the reason the duplication is mosaic – even if it is passed on by a sperm or egg containing the duplication, it appears to revert back to normal in many cells. The probable cause of this is the presence of large repeat sequences, called low copy repeats, which are typically 10–400 kilobases in length. The duplication arises because of recombination between these repeats, termed HERC2 (Ji *et al*, 2000*b*), which are specific to chromosome 15 and actively transcribed, making them much more prone to recombination. In some individuals the level of transcription, orientation or size of these repeats could mean that the whole region becomes highly unstable and prone to recombination. In other people the region

is much more stable and duplications might rarely occur.

The duplicated region on the chromosome is big, probably at least 15 million base pairs. So why was it missed? At the standard 100 × magnification used in cytogenetics, DUP25 is only just visible under a light microscope and cannot be reliably scored. Being mosaic, it is not present in all cells. With a few exceptions, cytogenetics is aimed at genetic changes present in all cells, so that a standard translocation would need to be checked in only a few cells of any patient. In contrast, for a mosaic change dozens of cells would need to be examined to have a clear picture of what is happening over the steady background of chromosome artefacts usually seen. This cytogenetic abnormality was very difficult to spot as it occurred in only about half the cells of these patients, and there are several such chromosome polymorphisms.

WHICH GENES ARE INVOLVED?

It is highly unlikely that all 60 or so genes in the duplicated region have an effect on the panic–hypermobility spectrum of disorders seen in these patients. It is far more likely that duplication of the majority of these genes simply has no effect, either because overexpression has a negligible physiological effect, or the regulatory feedback loops in place overcome excess expression. This is clearly not the case for all proteins but some must be exquisitely sensitive to gene dosage. The most likely suspects can be identified simply by examining the genes present in this region; for example, there are three nicotinic acid receptors and the neurotrophin-3 receptor gene (NTRK3) present, all of which are reasonable candidate genes for panic disorder. NTRK3 is particularly interesting, as it is the only neurotrophin receptor expressed in the locus coeruleus, a region of the brain thought to be involved in responses to stressful or novel stimuli. For joint laxity, lysyl oxidase, a protein involved in generating cross-links in connective tissue (Csiszar, 2001), is a prime candidate. It is probable that several genes in this region are involved in the panic–hypermobility spectrum. The best way to discover which of the 60 or so genes are important for the development of panic disorder or joint laxity could be to generate transgenic mice overexpressing specific

genes from the region. This should establish which genes give rise to a panic/anxiety phenotype in the mouse when duplicated, and by analogy, in humans. This is the strategy that is currently being adopted to delineate velo-cardio-facial syndrome and related 22q11 duplication/deletion syndromes (Funke *et al*, 2001).

WHY IS DUP25 SO COMMON IN THE POPULATION?

An interesting idea is that DUP25 has been selected for during human evolution because it provides some selective advantage. Fear responses are under genetic control as, for example, animals can be bred to be more or less defensive (Marks, 1986). Although panic disorder can be debilitating, it is also not difficult to see that a more active fear system could have survival advantages (as could joint hypermobility), for example in warfare or other threatening or confrontational situations. Lelliott *et al* (1989) postulated an evolutionary factor to explain why most first-panics occur outside the home and mainly in public places. So in the same way that human chromosomes have evolved over time through duplication and recombination, DUP25 could have established itself as a common variant in humans because of small survival advantages it confers.

The immediate implication of the finding of a chromosome 15 duplication in panic and joint laxity, if confirmed, is that a genetic test could soon become available for panic disorder and related phenotypes. This might involve FISH analysis, or more likely a test-tube 'gene dosage' assay that could identify the presence of extra copies of the genes in this region. This could have some advantages for diagnosis, because patients with anxiety disorders could be classified on the basis of their genome. Importantly, it will necessitate a re-evaluation of the definition of anxiety disorders as it is now evident that panic, agoraphobia, social phobia and hypermobility can be manifestations of the same underlying genomic event. Genetic testing might also make patients feel better about their condition once they know that it has a biological origin. These, like all genetic tests, will also have implications for other family members, and of course would need to be performed in the context of established clinical genetics.

More interesting is the prospect for improved treatment for panic disorder. This might occur, for example, by targeting antagonists to some of the receptors that are duplicated in the region, in order to damp down their activity. Of course if overexpression of the key genes involved had a 'hard wired' effect on neuro-development (such as an aberrant effect of NTRK3 during neuronal differentiation), then this will not be so easy. It should be remembered, however, that the best treatment for genetic or genomic diseases may still be cognitive; as Marks pointed out (1986), the fact that some phobias appear to be highly genetic is not incon-sistent with successful treatment using cognitive strategies.

Finally, the question of whether this new genetic mechanism is a cause of other psychiatric disorders has to be asked. The identification of triplet-repeat instability as a new mechanism of human disease led to the rapid identification of DNA expansion in many rare disorders, such as fragile-X (Bowater & Wells, 2001), and the same is of course possible for genome instability in complex psychiatric disorders. It will take some time before the answer to this is known, but you can be sure that many psychiatric geneticists are now examining chromosomes much more carefully.

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