



Repeat meeting's repeat performance

The Second International Conference on Unstable Microsatellites and Human Disease, Chapel Hill, North Carolina, USA, 17–20 April 1999

Since the discovery of trinucleotide repeat expansion as a novel mechanism of human disease in 1991, the 'repeat disease field' has witnessed incredible growth and now encompasses the study of phenomena ranging from fragile sites to unstable microsatellite repeats to minisatellite expansion mutations¹. Given the 'expanding' nature of this research area, an international meeting was convened two years ago in Sante Fe, New Mexico (USA) to bring together molecular biologists, geneticists, biochemists and biophysicists who were grappling with the two fundamental questions that can be asked of any disease-causing repeat motif: (1) Why does the repeat expansion mutation cause disease? (2) What is the molecular explanation for the repeat's genetic instability? Given the success of the first meeting and the continued exponential growth of the repeat disease field, a Second International Conference was convened in North Carolina, again organized by Jack Griffith (UNC, Chapel Hill, USA), David Nelson (Baylor, Houston, USA) and Robert Wells (Texas A&M, Houston, USA). This second meeting assembled an extremely diverse group of researchers whose work fits under the very large umbrella of the repeat disease field.

Of DNA structure and 'sticky DNA'

Much work has focused on determining whether expanded trinucleotide repeats assume novel DNA structures, once a certain repeat length threshold has been reached. Such structures could interfere with the normal processes of replication or recombination and, thereby, predispose to genetic instability. CAG or CTG repeats probably form hairpins and evidence for formation of hairpin-containing slipped-strand DNA has been presented². The GAA repeat, which expands to cause Friedreich's ataxia (FRDA), is precluded from forming hairpins and instead may form DNA triplexes³. Robert Wells (Texas A&M, Houston, USA) presented data showing that expanded GAA tracts migrate anomalously on agarose gels as retarded bands; they form a novel DNA structure that he has named 'sticky DNA'. Sticky DNA is composed of two purine–purine–pyrimidine triplexes, and its specific formation at expanded GAA-repeat lengths implies that it has a role in repeat instability at the FRDA locus⁴.

SCA8: breaking news that's breaking all the rules...

The month of April introduced yet another dominantly inherited spinocerebellar ataxia (SCA) to the fold of trinucleotide repeat diseases⁵, although, unlike all the other SCAs, SCA8 appears to be caused by a CTG repeat that is transcribed into an RNA lacking an open reading frame. Laura Ranum (Univ. of Minnesota, Minneapolis, USA) presented the latest chapter in this exciting story, suggesting that whereas large repeat expansions (110–250 CTGs) are associated with disease, very large repeat expansions (>250 CTGs) could be innocuous. She speculated that excessively long CTG repeat tracts might not be expressed, thus not

causing disease. The instability of SCA8 is similarly complicated: the CTG repeat is flanked by a stable CTA repeat at its 5' end and occasional CCG interspersions. Inter-generational transmission of the SCA8 repeat can result in alterations in the number of interspersed CCGs, as well as in the length of the longest CTG repeat tract.

So many fragile sites, so little time

'Common' fragile sites exist in the chromosomes of all individuals, whereas 'rare' fragile sites are found in <5% of all chromosomes. Robert Richards (Women's and Children's Hospital, Adelaide, Australia) presented his group's work in understanding the rare AT-rich minisatellite repeats that account for FRA16B and FRA10B. These AT-rich sequences show variation, both during germline transmission and somatically within the same individual. The AT-rich minisatellite and the CG-rich trinucleotide repeat 'rare' fragile sites share the same molecular basis: repeat expansion beyond a particular length threshold. An accumulating body of evidence, including the ascertainment of human families that show multiple fragile sites, was presented. These data suggest that a critical gene product, the function of which is lost or aberrant in *trans*, might contribute to the molecular mechanism that gives rise to repeat expansion.

Confusion over inclusions

Of all the areas in the repeat disease field, the greatest progress has been made in modeling and understanding how polyglutamine tract expansions lead to neurodegeneration. Despite the progress, many questions remain unanswered. In the two years since the First International Conference, Gillian Bates (King's College, London, UK) and colleagues identified inclusions within the nuclei of neurons of transgenic mice that show a Huntington's disease (HD)-like phenotype⁶. Neuronal intranuclear inclusions (NIIs) were subsequently found in most of the CAG/polyglutamine tract diseases. Not all inclusions are restricted to the nucleus, however. Cytoplasmic inclusions have been identified in HD (in which dystrophic neurites were initially reported) and in SCA6 (this meeting). The role that inclusions play in disease pathogenesis and the relevance of a nuclear as opposed to a cytoplasmic location have been the subject of much debate. Recently, an antibody identified by Xiao-Jiang Li (Emory, Atlanta, USA) uncovered a much greater extent of polyglutamine aggregation in the neuropil (extranuclear neuronal domains) in HD postmortem brains⁷ and in HD transgenic mouse brains. Thus, Gillian Bates suggested that neuropil aggregates could actually be more relevant to the degenerative process than NIIs, because they outnumber NIIs in adult-onset HD patients and, in mouse, show a dramatic increase in density with age when compared with NIIs.

Harry Orr (Univ. of Minnesota, Minneapolis, USA) and Huda Zoghbi (Baylor, Houston, USA) presented a significant advance in understanding whether aggregate formation is pathogenic or protective. Their studies on SCA1

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transgenic mice that overexpress expanded ataxin-1 with 82 glutamines showed that, for the mutant protein to be toxic, it must localize to the nucleus but does not have to form aggregates. Deletion of ataxin-1's self-association domain, which is necessary for the protein to form aggregates, did not prevent neuronal dysfunction and the ataxic phenotype, despite preventing aggregates⁸. Furthermore, ongoing studies are yielding hints that, without the formation of aggregates, more mutant protein is available to wreak havoc in the nucleus, leading to neuronal dysfunction and demise.

Conclusions

The wealth of ideas represented at this meeting, as well as the varied backgrounds and perspectives of the investigators, certainly bode well for further advances in the repeat disease field. There can be little doubt that the lessons to be learned from these most basic elements will have relevance to many fundamental processes and disease states. The complexity of the problems raised by the repeats, and the conundrums yet to be solved, certainly belie their designation as 'simple sequences'.

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Genomics and psychiatry

Genes and the Neurobiology of Susceptibility: the Society of Biological Psychiatry 54th Annual Scientific Convention, Mayflower Renaissance Hotel, Washington DC, USA, 13–15 May 1999

Approximately 18% of the human genome has already been sequenced, and Francis Collins (NHGRI, Bethesda, MA, USA) announced at this meeting the first 'working draft' (90% complete) by the end of 2001. An entire representative sequence is expected two years ahead of time around mid-2002. The sequences of all the common polymorphisms will also eventually become known. Expectations that this new knowledge ('structural' and 'functional' genomics) can be applied to improve human mental health are high.

Finding the genes

Genetic epidemiology has confirmed that genetic variations in vulnerability contribute to psychiatric disorders. These common diseases are complex disorders, likely resulting from the interaction of relatively common alleles at multiple (perhaps a dozen or so) loci. Environmental factors are also involved. Furthermore, as no single locus is required, different combinations of alleles in different families probably produce the deleterious phenotype (locus heterogeneity)¹. These observations may explain the failure to replicate earlier findings derived from large multiplex affected families studied with parametric linkage analysis in search of single major loci (including results obtained using candidate genes strategies)². Identification of the relevant alleles in the absence of knowledge concerning physiopathology appears a daunting endeavor.

Genome scans have nonetheless provided a number of mildly positive findings (two- to threefold increase in relative risk) in schizophrenia, including evidence for linkage on chromosomes 6p, 8p and 13q32. In the case of bipolar mood disorder, conflicting reports of linkage to a variety of loci on chromosome 18 have attracted much attention. Wade Berrettini (Univ. of Pennsylvania, Philadelphia) presented data showing linkage with markers D18S37 and

D18S53 at 18p11. The effect in these pedigrees is small but significant, with evidence for paternal imprinting. Furthermore, some of the 25 or so loci in the region correspond to promising candidate genes (such as Golf).

The most complex of complex traits

Many investigators argue that these dismal results reflect the poor specificity of psychiatric phenotypes as currently defined. These nearly always include symptoms of two or more psychopathologic syndromes (comorbidity). Alternative ('intermediate phenotype') approaches have been proposed for mapping purposes. Phenotype identification through candidate symptoms among affected subjects and sub-clinical traits among non-affected relatives (endophenotypes) may have better genetic validity. Phenotyping using non-clinical variables is another strategy. Thus, Robert Freedman (Univ. of Colorado, Denver) used a quantitative electrophysiological assessment of attentional dysfunction that is associated with schizophrenia (diminished inhibition of the P50 auditory evoked potential) and showed linkage to a dinucleotide polymorphism at 15q13-q14.

New tools for complex disease studies

Innovations to facilitate mapping of complex traits will also facilitate finding genes for these disorders. New association paradigms (haplotype relative risk, transmission/disequilibrium) and non-parametric linkage methods based on allele sharing (affected sib pair, affected pedigree member) have been introduced. High-throughput sequencers allow the assessment of all relevant alleles in many individuals. Analysis of allele frequency distributions in DNA samples pooled prior to PCR amplification has been proposed as a simple screen for large numbers of markers when these sequencers are not available. Marta Blumenfeld (Genset, Evry) discussed the power of single



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