

Perfect endings: a review of subtelomeric probes and their use in clinical diagnosis

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Abstract

Chromosomal rearrangements involving the ends of chromosomes (telomeres) are emerging as an important cause of human genetic diseases. This review describes the development of first and second generation sets of telomere specific clones, together with advances in fluorescence in situ hybridisation (FISH) technology, which have made the prospect of screening for telomeric rearrangements a realistic goal. Initial FISH studies using the telomere specific clones indicate that they will be a valuable diagnostic tool for the investigation of mental retardation, the characterisation of known abnormalities detected by conventional cytogenetic analysis, spontaneous recurrent miscarriages, infertility, haematological malignancies, and preimplantation diagnosis, as well as other fields of clinical interest. In addition, they may help investigate telomere structure and function and can be used in the identification of dosage sensitive genes involved in human genetic disease.

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Cytogenetic analysis at a 400–550 band resolution is the standard investigation for suspected chromosomal rearrangements, yet it cannot routinely detect rearrangements smaller than five megabases (Mb) and much larger abnormalities escape notice if they occur in regions where the banding pattern is not distinctive. Despite numerous attempts to increase reliability and resolution, there is still no practical way to screen the entire genome for rearrangements, regardless of size or chromosomal location.

An alternative to whole genome screening is to focus on specific chromosomal regions, and in this article we review the application of chromosome specific probes for the detection of rearrangements involving the ends of chromosomes or telomeres. Screening telomeres for rearrangements has a number of attractions. First, the majority of translocations involve chromosome ends and therefore an assay that targets telomeres will detect all of

these with 100% sensitivity regardless of size. Second, the regions adjacent to telomeres are gene rich so rearrangements involving telomere adjacent DNA are more likely to have phenotypic consequences than rearrangements in many other parts of the genome.¹ A number of genetic diseases associated with telomeric rearrangements have already been documented and the apparent enrichment for rearrangements suggests that submicroscopic, telomeric anomalies might be responsible for additional human genetic diseases.^{2–7}

Until recently, screening for telomeric rearrangements has been hampered by the complexity of the sequence structure of chromosome ends^{8–13} and the technical problem of designing a highly sensitive and specific assay which would also be cost effective. Both of these issues have now been resolved and an effective assay for screening telomeric rearrangements is in place.¹⁴ In this review, we will first discuss the background to the development of this assay and then consider the clinical findings which have come to light through its implementation.

Telomere structure and function

The ends of all eukaryotic chromosomes are composed of a TG rich repeat, which in all vertebrates is (TTAGGG)_n, ranging from 2 to 15 kb in length.^{15, 16} Without the terminal array, chromosomes are unstable, and prone to end to end fusions and exonucleolytic degradation. In addition, telomeres are required for the complete replication of DNA and play an important part in cell longevity. Immediately adjacent to the (TTAGGG)_n tandem repeats lie complex families of repetitive DNA which may extend for several hundred kilobases (kb). Typically, the repetitive DNA is present on a number of other chromosomes, in each case restricted to the subtelomeric regions.^{8–13} The function, if any, of this subtelomeric repetitive DNA is unknown. Naturally occurring mutations in humans show that chromosomes without subtelomeric repeats can be inherited normally, implying that the sequences have no important biological role.¹⁷ However, sequence analysis of the DNA adjacent to the 4p, 16p, and 22q telomeres suggests that interstitial degenerate (TTAGGG)_n repeats subdivide the subtelomeric regions into distal and proximal

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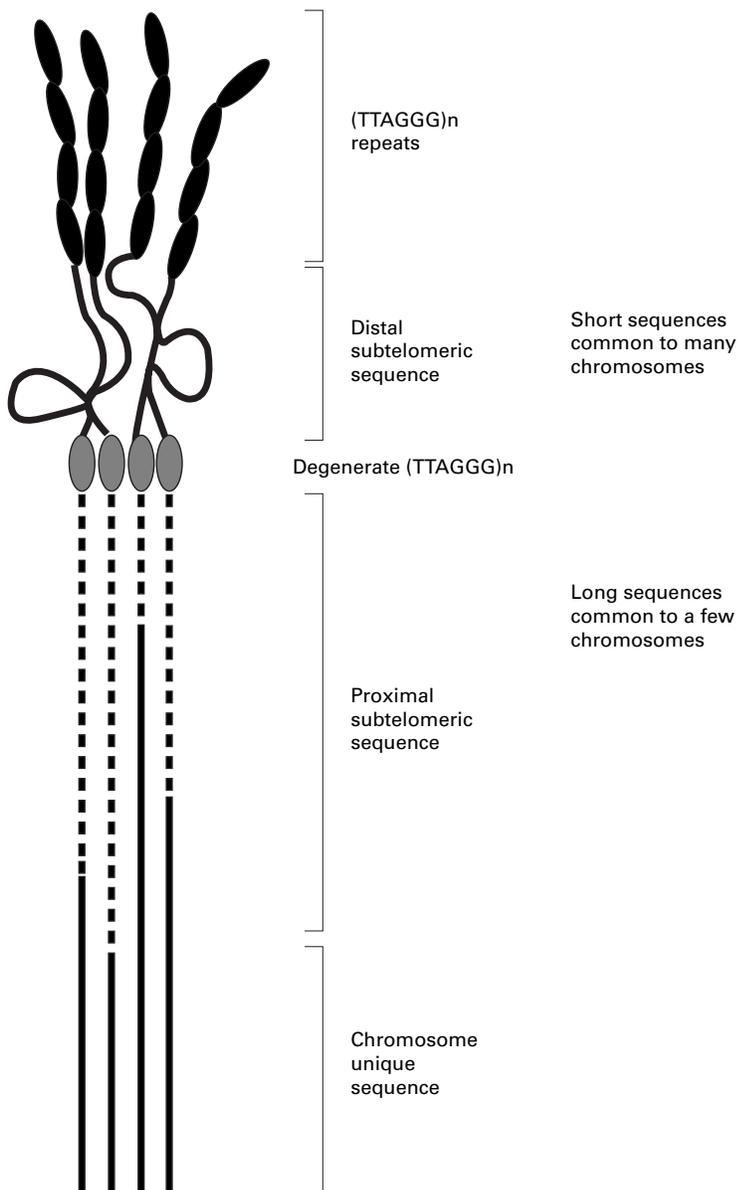


Figure 1 Sequence organisation of telomeres and subtelomeric domains.¹⁸

subdomains with different patterns of homology to other chromosome ends.¹⁸ This view of the sequence organisation of telomeres and subtelomeric domains is shown in fig 1. The patterns of homology between different chromosome ends are thought to reflect the action of different sequence homogenisation processes, whereas the interstitial (TTAGGG)_n may have functional significance, serving to compartmentalise the subtelomeric domains in the nucleus, thereby limiting the nature and extent of sequence interactions.

The size and complexity of the subtelomeric domains has made them extremely difficult to analyse at a molecular level. Subtelomeric sequence from one chromosome is frequently over 95% identical to the sequence from another and homologies often extend over many kilobases.^{11 18 19} For example, the homology between the terminal 30 kb of the p and q arms of human chromosome 4 is 98%.¹⁸ Importantly, some subtelomeric sequences do

include functional genes. At the 4p telomere there is a zinc finger gene, three exons of which are distributed on the 13p, 15p, 21p, and 22p acrocentrics¹⁸; the Xq/Yq subtelomeric region contains the interleukin 9 receptor gene, with partial copies, again with very high sequence conservation, close to the 16p, 9p, and 10q telomeres.^{19 20} The high degree of sequence similarity almost certainly reflects the action of frequent "cross talk" between telomeric regions, a process which may well result in chromosomal rearrangements. One case has been documented at the molecular level; a subtelomeric rearrangement involving the short arm of chromosome 16 has been shown to be the result of a recombination event between two Alu repeat sequences, one of which lay in a subtelomeric repeat.²¹

Assays of telomere integrity

The high degree of sequence similarity, the presence of genes in subtelomeric DNA, and chromosomal rearrangements occurring within subtelomeric repeats have important implications for strategies aimed towards assessing subtelomeric integrity. Given our knowledge of telomere structure (fig 1) and the fact that all current approaches rely on the presence of unique sequence to investigate subtelomeric regions, it follows that any screening assay which can be developed will include a trade off between sensitivity and specificity. If sequences which are chromosome unique (and hence more centromeric) are incorporated in the screening, then rearrangements which are more telomeric will be missed. However, if repetitive subtelomeric DNA sequences are incorporated, then the exact chromosomal origin of the detected DNA will be uncertain. A pragmatic solution is to devise an assay which uses unique sequence probes and to accept that a small proportion of rearrangements may be missed.

The discovery that telomeres from human chromosomes can be cloned by functional complementation in yeast^{22 23 24 25} enabled the molecular characterisation of subtelomeric repetitive and chromosome unique DNA. When yeast artificial chromosome (YAC) vectors with only one functional telomere are ligated to human genomic DNA, clones survive in the yeast host cells if stabilised by the presence of a second telomere derived from the human genomic DNA ("half YACs"). Characterisation of half YAC libraries produced in this way has resulted in the development of a valuable set of molecular resources for analysing most, if not all, chromosome ends.^{26 27}

Two methods for detecting telomere rearrangements have been developed. One method relies on detecting deviation from Mendelian inheritance of alleles at polymorphic loci close to telomeres.^{28 29} The other exploits fluorescence in situ hybridisation (FISH) and uses telomeric probes to assess directly whether there is a loss or gain of telomere copy number.¹⁴ The first method, the use of DNA polymorphisms, requires DNA samples from the child and both parents. When both parents are heterozygous and share no alleles, a

rearrangement in the child can be inferred from the presence of only a single allele (a deletion) or the presence of three alleles (a trisomy). This technique has the advantage of being able to detect isodisomy (the inheritance of two chromosome homologues from one parent), but it is limited by the degree of polymorphism of the marker and by the need to have access to samples from both parents. Indeed, marker informativity must be very high for this technique to be efficient.^{28,30} Even when a marker has a heterozygosity of 80%, only 70% of monosomies and even fewer trisomies (50%) will be detected. The problem can be circumvented partly by using more than one polymorphic marker for each telomere, but in this case many of the markers will be placed quite far from the chromosome ends and so small distal deletions will not be detected.

The second strategy involves FISH of telomere specific probes to metaphase chromosomes.¹⁴ Using this approach, deletions are detected by the absence of signal from the end of the cognate chromosome and trisomies by the presence of three signals. The chromosomal location of the signals is immediately apparent from cytogenetic characterisation of the chromosomes and thus both balanced and unbalanced translocations can be instantly detected and accurately described. In contrast to the DNA techniques, the FISH approach is unable to detect isodisomy, but as isodisomy appears to be very rare, this is not a great disadvantage. More problematical has been the isolation of probes specific for each telomere and finding an efficient way of analysing all chromosome ends using only a single assay per patient.

A major advance came in 1996 when a collaboration between the National Institute of Health and The Institute of Molecular Medicine reported the isolation and characterisation of a complete set of specific FISH probes representing each telomere.²⁷ This first generation set of clones were cosmids, P1s, and PACs (P1 derived artificial chromosomes) mainly derived by exploiting half YACs, which possess intact terminal fragments of human chromosomes, and thus most clones were located within 300 kb of the chromosome end. Half YACs were not available for seven telomeres (1p, 5p, 6p, 9p, 12p, 15q, and 20q) and with the exception of 5p, clones for these ends were derived by screening PAC libraries with the most telomeric markers on the human radiation hybrid map. Consequently, the physical distance between these clones and their cognate telomeres was unknown.

Once the first generation set of telomere specific clones had been isolated, the next difficulty was to develop a FISH based technique capable of analysing all telomeres of a person simultaneously. One possibility was to label telomere specific clones so that each telomere could be detected in a different pseudocolour, thus requiring the analysis of only a single metaphase chromosome spread per patient. Another approach currently being developed involves FISH based comparative genome hybridisation to a "telomere chip" containing

Table 1 Current uses of subtelomeric probes in the UK²²

Detection of cryptic rearrangements in idiopathic mental retardation
Investigation of recurrent spontaneous miscarriages and infertility
Characterising known abnormalities
Characterising haematological malignancies
Preimplantation diagnosis (in selected cases)
Interphase diagnosis (early CVS, amniocentesis)

BAC (bacterial artificial chromosome), PAC, or P1 clones unique to every human telomere.³¹ However, to date, only one technique has been both devised and implemented for the detection of chromosomal rearrangements in clinically relevant populations. Already commercially available, this is the Cytocell Ltd Multiprobe^T technique developed by Knight *et al.*,³³ a FISH based assay which allows every telomere to be investigated using only a single microscope slide per patient.

With the advent of the first generation set of telomeric FISH probes and the availability of the Multiprobe^T technique, many diagnostic laboratories in the UK have initiated preliminary studies to evaluate both the use of the probes and the technique for the detection of submicroscopic, subtelomeric rearrangements. Some laboratories are preparing their own probes and are using the Cytocell Ltd slides and devices, whereas others are using the Cytocell Ltd "Chromoprobe-T" telomere probe kits. A few laboratories are using individual probes supplied by Vysis and Oncor. Table 1 lists the main purposes for which subtelomeric probes are currently being used in the UK.

Clinical applications

SUBTELOMERIC REARRANGEMENTS IN IDIOPATHIC MENTAL RETARDATION

Mental retardation is a common disorder, yet its origins are poorly understood and there are few therapeutic options. In moderate to severe mental retardation (IQ<50) as many as 34% cases currently remain undiagnosed and in mild mental handicap the cause is unknown in up to 80% of cases even after investigation. Such poor understanding of the aetiology prompted Wilkie²⁸ to put forward a strategy which involves the use of hypervariable DNA polymorphisms for the detection of subtelomeric abnormalities and uniparental disomy in patients with unexplained mental retardation. Flint *et al.*²⁹ used this strategy to perform a pilot study to establish whether submicroscopic, subtelomeric rearrangements might be a significant cause of idiopathic mental retardation in children. In this study, polymorphic loci were selected to study the subtelomeric regions of 28 chromosome ends in 99 mentally retarded subjects. All karyotypes had been reported as normal by routine cytogenetic analysis. The study identified three cases of monosomy, one arising from a terminal deletion and two from the "de novo" inheritance of derivative translocation chromosomes. The results suggested that at least 6% of idiopathic mental retardation might be explained by submicroscopic rearrangements involving

Table 2 Subtelomeric studies of patients with idiopathic mental retardation

Reference	No of patients	Selection criteria	No of subtelomeric rearrangements detected		
			High resolution studies	Subtelomeric FISH	Prevalence (%)
38	17	Mental retardation, dysmorphic features, and minor criteria, eg family history suggestive of inherited disorder, convulsive and behavioural disorders	ND	4	23.0
37	209	Mild and severe mental retardation and congenital abnormalities	ND	8	3.8
34	44	27 with severe mental retardation, 31 with dysmorphic features, 14 with positive family history	ND	6	13.0
35	93	Idiopathic mental retardation	2	0	2.2
36	43	Mental retardation and dysmorphic features	0	1	2.3
		High resolution banding normal			
33	284	Moderate to severe idiopathic mental retardation	ND	21	7.4
	182	Idiopathic mild mental handicap	ND	1	0.5

ND = not done.

telomeres. If true, then subtelomeric rearrangements could be the second most common cause of mental retardation after Down's syndrome. Therefore, it was important to extend these studies to include all possible telomeres and a larger sample set.

The largest study to date has been that of Knight *et al.*³³ Using the Multiprobe^T approach, 284 children with unexplained moderate to severe mental retardation and 182 children with unexplained mild retardation were analysed for the integrity of the chromosome ends. The results indicated that subtle chromosomal abnormalities occurred in 7.4% children with moderate to severe mental retardation compared to 0.5% in the mild mental handicap group. Importantly, almost half of the positive cases examined (10 out of 21) were familial and this resulted in informative tests being carried out in a further 49 family members.

An important question is whether subtelomeric rearrangements cause mental retardation or any other phenotype in the patients in whom they are found. There is a possibility that small rearrangements may have no phenotype as has been discovered in some cases, discussed in more detail later. In our study, there was little doubt that the rearrangements segregated with the phenotypes in the 10 familial cases detected.³³ However, 12 of the 22 cases with subtelomeric rearrangements were *de novo*. Of these, seven cases were monosomic for regions already known to be implicated in mental retardation, and in the remaining five cases the sizes of the rearrangements were so large that they were unlikely to be without phenotypic effect.

A summary of the findings of other studies is given in table 2, from which it can be seen that the reported prevalence of subtelomeric rearrangements varies from less than 1% to as high as 23%.³³⁻³⁸ Variation in the number of patients entered into the studies and differences in the selection criteria explain the different estimates. It is clear from our large study that the degree of developmental delay or learning disability is a major predictor of the likelihood of finding a subtelomeric rearrangement as the cause of the child's disability.³³ However, not all studies have selected cases by the degree of disability. Furthermore, all positive cases reported to date have physical anomalies, in addition to mental retardation. We suspect that

samples enriched for cases with physical anomalies will therefore find higher prevalence rates for chromosomal rearrangements.

In addition to the use of subtelomeric probes to analyse all telomeres of patients with mental retardation, there have also been studies in which subtelomeric FISH probes have been used to focus on the evaluation of particular telomeric regions, 22q for example. A potential partial monosomy 22q syndrome was first described by Watt *et al.*³⁹ Since this time, a number of cases of monosomy 22q13 have been reported and these patients share several clinical features. Many present with developmental delay, absence of expressive language, and hypotonia. Less frequent traits, but nevertheless observed in several patients, include epicanthic folds, dysplastic ears, and dolichocephaly.²⁹⁻⁴⁰⁻⁴⁴ With the advent of the 22q subtelomeric FISH probes it is becoming clear that there may be more patients who are monosomic for the 22q subtelomeric region than previously realised. A number of patients reported as being cytogenetically normal have now been shown to be monosomic using the 22q subtelomeric FISH probes and at least one patient has been shown to carry an unbalanced translocation arising through the missegregation of a parental balanced cryptic translocation.^{7 45-47}

WHO TO SCREEN?

Who, of the large number of cases of idiopathic developmental delay that pass through most genetics and paediatrics clinics, should be investigated for small chromosomal deletions? Detection rates are higher among those with moderate to severe mental retardation, but screening all subjects in this group may not be practical, simply because of the cost of the investigation.

We have argued that the high rate of familial occurrence does justify blanket screening of all children (and adults) with idiopathic mental retardation on the grounds that in familial cases, all results are informative so the ratio of informative tests to the total screened is increased to 25 per 100 screened, rather than 7 per 100. The cost per informative test is reduced threefold, from \$5700 (assuming a cost of \$400 per test) to \$1600. These figures need confirmation from other surveys.

A more cost effective strategy would be to identify clinically a subgroup in which small deletions occurred at a much higher frequency.

The clinical features identified to date are very diverse, but in our own work, a combination of facial dysmorphism, minor physical abnormalities of the hands or feet, small stature, and microcephaly were consistent findings among those with moderate or severe mental retardation and a small chromosomal anomaly. Additionally, the finding of either a similar, or dissimilar, phenotype in a relative should increase the chances of detecting a small chromosomal rearrangement. Unfortunately, this constellation of findings is common. In a survey of 115 patients with moderate to severe idiopathic mental retardation Fryns *et al*⁴⁸ identified 60 with either dysmorphic features or a family history of mental retardation, or a combination of the two. If the latter group contains all those with small chromosomal deletions, it should be possible to increase the detection rate to 15% with a concomitant halving in the number screened. We need further surveys to confirm this point and to determine if yet more focused clinical screening is possible.

A recent case report⁴⁹ of a family with inherited mental retardation provides a cautionary tale. Mental retardation was found to show autosomal dominant segregation in a five generation pedigree. Molecular-cytogenetic investigations, including multiplex-FISH⁵⁰ (a technique which uses flow sorted whole chromosome painting probes labelled in combination to detect cryptic rearrangements) did not detect any anomalies. As a result, a genome wide linkage search was undertaken and linkage to the terminal region of 16p was found, where microsatellite markers were hemizygous. Subsequent FISH analyses showed that patients inherited a duplication of terminal 3q in addition to confirming the subtelomeric deletion of 16p. The authors concluded that there is a need for fast and reliable screening sets for the subtelomeric regions.

CHARACTERISATION OF KNOWN ABNORMALITIES

Subtelomeric FISH probes have been used to characterise known abnormalities. Often, diagnostic laboratories have patient samples for which standard cytogenetic studies have shown an abnormal karyotype and yet further submicroscopic rearrangements cannot be ruled out because the resolution of standard karyotyping is insufficient for precise genotyping. For example, Horsley *et al*⁵¹ showed that a patient reported to have an 18p deletion by conventional G banding was also trisomic for the 2p subtelomeric region with the additional copy of 2p being translocated on to the del(18). Another example is in the characterisation of the 18q- syndrome. Brkanac *et al*⁵² used telomeric FISH probes to analyse 35 patients previously reported to have de novo terminal deletions of the q arm of chromosome 18. They found that five patients possessed more complex rearrangements and that three retained the most distal portion of 18q, consistent with an interstitial rather than a terminal deletion. Similarly, Joyce *et al*⁵³ found the use of subtelomeric FISH probes an invaluable approach for confirming and characterising semicryptic de

novo telomeric rearrangements at the limit of cytogenetic resolution. A de novo del(13) was shown to be an unbalanced 13q;19q translocation and a mosaic del(18q) was shown to be an add(18)t(4p;18q). In addition, the FISH probes enabled abnormalities which could not be fully characterised by conventional G banding alone to be resolved. In this way, an add(5p) clearly visible at the 550 band level was shown to have both a deletion of the cri du chat critical region and a cryptic 5p;6p subtelomeric translocation and an add(18q) was shown to have an 18p subtelomeric signal at both ends.

As a direct result of the availability of the first generation set of telomere clones, many more patients who are monosomic for the terminal region of 1p have now been identified and their concordant phenotypes have led to a new mental retardation syndrome being defined.⁵³⁻⁵⁷ Chromosome 1p36 deletion syndrome is a contiguous gene syndrome characterised by a wide range of clinical features including variable degrees of mental retardation, growth delay, seizures/abnormal EEGs, hypotonia, developmental delay, early puberty, orofacial clefting or palate anomalies, characteristic dysmorphic features, hearing deficits, and cardiomyopathy. Molecular studies have shown that the prevalence of certain phenotypic features appears to correlate with deletion size. Specific features may occur when certain subregions of 1p36 are deleted resulting in hemizygosity for critical dosage sensitive genes or unmasking of recessive mutations in the non-deleted allele.⁵⁵

PREIMPLANTATION DIAGNOSIS

Preimplantation screening of cleavage stage embryos with appropriate subtelomeric probes can detect unbalanced embryos and thus increase the chances of proceeding with a successful pregnancy.⁵⁸ The strategy can also be extended to allow the rapid screening of amniotic fluid or CVS samples in established pregnancies or where one partner carries a known chromosome rearrangement.

HAEMATOLOGICAL CANCERS

Chromosomal rearrangements in haematological cancers can be complex and the timely arrival of subtelomeric FISH probes has provided a useful tool for their evaluation. Monosomy 7 and deletion of the long arm of chromosome 7 are frequent findings in myelodysplastic syndromes and acute myeloid leukaemia, particularly associated with therapy related disease. Tosi *et al*⁵⁹ used a 7q telomeric specific probe to refine the characterisation of leukaemic cells from 26 patients with various haematological malignancies and known abnormalities of 7q. Of these cases, seven were confirmed as pure deletions, four were found to be interstitial, and two were terminal. The remaining 10 cases were translocations and complex rearrangements. In two cases in which del(7q) was reported as the sole cytogenetic abnormality by G banding, FISH showed cryptic translocations involving 7q.

The use of subtelomeric probes for the characterisation of haematological malignancies has also been reported by Foot *et al*,⁶⁰ who used

the subtelomeric probes to characterise cytogenetic abnormalities in two patients. The first patient was diagnosed with chronic myeloid leukaemia and G banding showed only a t(15;16). However, further analysis using whole chromosome paints (WCP) and subtelomeric probes showed that a translocation involving chromosomes 16 and 22 was also involved giving the karyotype t(15;16)(q24;q24)t(16;22)(q12;p11.1). Patient 2 had secondary leukaemia with obvious evidence of an 11q23 rearrangement and abnormalities affecting chromosomes 4 and 12 on G banding. Using FISH probes, the karyotype showed a t(11;16) and an insertion of chromosome 4 into the short arm of chromosome 12. Studies such as these underline the potential of subtelomeric FISH probes for the refined characterisation of subtle rearrangements which could not be detected by conventional cytogenetics or WCP alone.

Experiences using the first generation set of telomere specific clones

The feedback from laboratories using the first generation set of telomere clones has clearly highlighted clone characteristics, such as polymorphisms and cross hybridisation, which can complicate interpretation, and has also shown common technical difficulties which arise during the FISH analyses.

POLYMORPHISMS AND CROSS HYBRIDISATION

A summary of the reported polymorphisms and cross hybridisations encountered using the first generation set of telomere clones is given in table 3. Polymorphisms and cross hybridisation arise because of the inherent structure of human telomeres outlined in fig 1. The polymorphisms encountered are the result of the variable length of the target sequence to which a probe hybridises. The more target sequence that is present the stronger the signal, but, conversely, the smaller the target sequence the fainter the signal. In the case of the 2q subtelomeric clone, this signal may be so faint that a deletion may be suspected. In the case of the Xp and Yp subtelomeric clone, the target sequence may not be present at all, again suggesting a deletion. Such situations emphasise the need to investigate parental samples when an abnormality is suspected. The phenomenon of a polymorphism is different from that of cross hybridisation. Cross hybridisation occurs because of the sequence similarity which is shared between telomeres. As mentioned earlier, it is believed that interstitial degenerate (TTAGGG)_n repeats subdivide the subtelomeric regions into distal and proximal subdomains with different patterns of homology to

other chromosome ends (fig 1). If a clone is derived from the distal telomeric region containing blocks of short sequences then it may hybridise to many chromosomes, whereas if it is derived from the proximal telomeric region containing long blocks of sequences it may only cross hybridise to the few other chromosomes that share homology. Thus, in producing the first generation set of telomere clones, the need to be close enough to the telomere to detect rearrangements was balanced with the need for chromosome specificity.

As more and more laboratories use the subtelomeric probes, the characteristics will become better defined. Already Joyce *et al*⁵⁵ have reported two subtelomeric anomalies in 40 controls. In one case a der(Y) chromosome containing both a Yp and a 17q subtelomere was identified and in the other case the person was monosomic for the 4q subtelomeric region. If the same observation had been made in the parental samples, then it would be assumed that these represented rare polymorphisms. However, both cases were de novo events occurring in normal relatives or parents chosen from a group of patients referred for structural abnormalities and for whom the results were normal. The diagnostic implications of this remain unknown, but reiterate the need for caution when interpreting de novo events in patient samples.

Diagnostic limitations of telomeric FISH clones

The first generation set of telomere specific clones identify 41 out of the 46 human telomeres. The five telomeres which are not represented are the p arm telomeres of the acrocentric chromosomes, chromosomes 13, 14, 15, 21, and 22. The satellited regions of these chromosomes are made up primarily of ribosomal DNA and their duplication or deletion is not generally thought to be phenotypically significant. However, there is some evidence to suggest that translocated ribosomal genes can have a position effect, particularly in some cases of satellited chromosome 4 where recurrent miscarriages and variable reproductive outcomes have been reported.⁶¹ Therefore, when performing subtelomeric studies of patients it is important to bear in mind the possibility of acrocentric p arm involvement.

Knight *et al* (unpublished data) investigated a family in which both the affected proband and the unaffected mother were thought by conventional cytogenetic analyses to have an anomaly involving one of the 9q telomeric regions. Investigation of this region using the 9q subtelomeric FISH probe showed that the patient was monosomic for 9q whereas the mother actually had a translocation of the 9q telomeric region onto the p arm of the acrocentric chromosome 13. Further analysis using a bacteriophage derived from chromosome 4p which cross hybridises with the acrocentric p arm telomeres showed that the mother had a balanced reciprocal translocation involving the 9q and 13p subtelomeric regions.⁶² The proband had inherited an unbalanced rearrangement being monosomic

Table 3 Polymorphisms and cross hybridisations of first generation telomere clones

	Telomere	Clone
Polymorphisms	2q	210E14 (P1), 2112b2 (cosmid)
	XpYp	CY29 (cosmid)
Cross hybridisations	5p with acrocentric p arms	114j18 (BAC)
	5q with acrocentric centromeres	B22a4 (cosmid)
	8p with 1p	2205a2 (cosmid)
	11p with 17p	2209a2 (cosmid)
	12p with 6p and 20q	496a11 (PAC)

for 9q and trisomic for the 13p telomere. Thus, in this situation the availability of the acrocentric probe was critical for the full clinical evaluation of this family.

Rearrangements involving satellited DNA are not the only examples of rearrangements which may be missed when performing subtelomeric FISH analyses. Two other forms of rearrangement, uniparental isodisomy and small subtelomeric tandem duplications, are also undetectable. Of these, uniparental isodisomy is likely to be very rare and, as yet, there is no alternative technique available which would reliably detect all small subtelomeric duplications. Interestingly, there has been one report of a subtelomeric duplication which was detected using high resolution chromosome analysis and yet remained undetected using the subtelomeric FISH approach.⁶³ By contrast, there are reports in which subtelomeric anomalies were detected using the FISH method and yet 850 band level cytogenetic examinations failed to detect any abnormalities even with the knowledge of where to look. Knight *et al*³³ noted that 18 out of 22 subtelomeric rearrangements remained undetected on 850 band analysis, even with the benefit of hindsight.

As alluded to previously, one problem which may arise through using the subtelomeric probes is that we do not know the diagnostic implications of previously undocumented small subtelomeric rearrangements. In the studies of Knight *et al*,³³ the sizes of previously undescribed de novo rearrangements were so large that they were unlikely to be without phenotypic effect. However, the ascertainment of smaller rearrangements will be much more problematical since it is not yet possible to predict whether these have phenotypic consequences. Killos *et al*⁶⁴ noted an apparently normal person with a satellited chromosome 17p who was also deleted for the 17p subtelomeric probe and Joyce *et al*⁶⁵ have identified two anomalies in 40 phenotypically normal controls. As it may not be feasible for diagnostic laboratories to perform studies to size de novo rearrangements occurring in regions previously unknown to cause mental retardation, the diagnostic implications of these cases are likely to remain unknown until the region of interest can be further characterised or until further cases are identified.

Second generation set of telomere specific clones

The first generation set of telomere clones reported in 1996 included six clones (1p, 6p, 9p, 12p, 15q, and 20q) which were only designated as telomeric by virtue of the fact that they contained the most distal marker known at that time.²⁷ One clone, 5p, also had not been mapped. Therefore, the exact distance of these markers from the telomere was uncertain. This is important because if these clones are located a long distance from the telomere then it is likely that more distal rearrangements involving these telomeres will be missed. Indeed, interphase mapping has since confirmed that the 9p clone is less than 1.2-1.5 Mb from the

telomere and the 12p clone is more than 800 kb from the telomere, whereas the 15q clone may be less than 100 kb from the telomere.⁶⁵ The distances for the 5p, 6p, and 20q clones are still unknown. The problem of large gap sizes between such clones and the corresponding telomere, together with the problems of polymorphism and cross hybridisation prompted a second generation set of telomere specific clones to be developed. Lese *et al*⁶⁵ have now isolated clones for 9p and 12p which map less than 200 kb and 600-800 kb distal to revised distal markers. In addition, Knight *et al* (manuscript in preparation) have isolated a complete second generation set of telomere specific clones. Unlike the first generation set of clones which are mainly cosmids with a few PACs and P1s, the second generation sets of clones are entirely made up of PACs, P1s, and BACs. The larger insert size of these improves the hybridisation signal without compromising sensitivity and also solves some of the problems of polymorphism.

Conclusion

Until recently, no useful diagnostic test existed for the routine detection of submicroscopic rearrangements in patients who are suspected of having a chromosomal anomaly, but for whom there are no clues regarding the chromosomal origin. FISH technology has made new approaches available for cytogenetic screening and the development of the first and second generation sets of telomere specific FISH clones has made the prospect of subtelomeric screening a realistic aid to clinical diagnosis. Although the era of subtelomeric FISH probes is still in its infancy, there are already several examples of where they have had a profound impact, particularly in providing diagnoses and allowing accurate genetic counselling in families with members affected by mental retardation. As subtelomeric screening becomes more widespread, the characteristics of the probes used will become better defined and the full scope of their potential for clinical diagnosis realised. In addition, the availability of the probes is expected to increase our understanding of telomeres at the molecular level, giving insight into their evolution, structure, and function and enabling us to identify dosage sensitive genes involved in human genetic disease.

Telomere screening is, however, still a first step towards the goal of high resolution analysis of the entire genome for chromosomal rearrangements. The possibility of high resolution screening has come much closer to reality with the advent of DNA arrays. It has recently been shown that copy number changes in the genome can be detected by comparative genomic hybridisation to cDNA arrays. In this technique, patient and normal DNA is differentially labelled and cohybridised to arrays of DNA clones which span the entire genome. A comparison of the hybridisation signals is expected to yield a ratio of 1:1, indicating equal DNA copy numbers in both samples. Ratios greater or less than this indicate possible DNA copy number changes in the patient sample.

The technique is relatively straightforward and quick to perform, but limited to those laboratories with access to the gridding technology and scanners that can interpret the results. Once it becomes more widely available, we expect it will replace the FISH based approaches described in this review.

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