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Cloned DNA probes regionally mapped to human chromosome 21 and their use in determining the origin of nondisjunction

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ABSTRACT

A number of unique sequence recombinant DNA clones were isolated from a recombinant DNA library constructed from DNA enriched for chromosome 21 by flow sorting. Of these, five were mapped to chromosome 21 using a somatic cell hybrid. Regional mapping of these probes and of a probe previously assigned to chromosome 21, was carried out with the aid of chromosome 21 rearrangements using both chromosome sorting and a somatic cell hybrid. Three probes were shown to be located on either side of the breakpoint 21q21.2. Two of the probes were shown to identify restriction fragment length polymorphisms (RFLPs) with high rare-allele frequencies (0.46 and 0.43). A Bgl II RFLP revealed the parental origin of non-disjunction in three of ten families with Down's syndrome.

INTRODUCTION

The isolation of chromosome-specific DNA probes has been achieved in recent years by the use of chromosome sorting and DNA cloning[1,2,3]. Such probes can be used to study genetic defects associated with particular chromosomes. If these probes identify restriction fragment length polymorphisms (RFLPs) they can then be used as scorable markers defining a locus on that chromosome. When several probes have been accumulated for a particular chromosome, or region of a chromosome, the linkage relationship between their loci and disease and other loci can be determined. Botstein et al.[4] discuss the theoretical aspects of this type of procedure and Wilcox et al.[5] give a practical example.

Mapping of recombinant DNA probes can be carried out using somatic cell hybrids[6], or chromosome sorting [2,7,8,9]. Both these methods can be used for chromosomal and regional assignment using cell lines carrying appropriate translocations.

Trisomy for chromosome 21 is the most common autosomal trisomy in liveborn infants and although the clinical condition has been recognised for over a century, nothing is known about how the presence of the extra

chromosome leads to the dysmorphic phenotype and severe psychomotor retardation. The isolation of chromosome 21 specific sequences provides an opportunity to study the molecular pathology of Down's syndrome and the origin of the nondisjunctional event.

MATERIALS AND METHODS

Human genomic DNA was prepared from cord blood[10] and from the somatic cell hybrids THYB133R and A1WBf2 obtained from C. Bostock and P. Pearson respectively. THYB133R contains chromosome 21 as the only known human genetic material, and A1WBf2 has the region 21q21-qter but not the reciprocal product nor the normal chromosome 21. Translocated metaphase chromosomes prepared from a cell line by the method of Sillar and Young [11], containing a reciprocal translocation involving chromosomes 2 and 21 were sorted on a Becton Dickinson FACS IV cell sorter using the method of Davies et al.[1] and DNA extracted from them using a method similar to that of Kunkel et al.[2]. The flow karyotype of this cell line is shown in Figure 1. The chromosome constitution of the cell lines was verified by cytogenetic analysis.

Probes were isolated from the λgtWES.λB library of Krumlauf et al.[3] which was constructed from DNA enriched for chromosome 21 by flow sorting of metaphase chromosomes.

DNA was prepared from recombinant phage, grown in E.coli strains LE831 by a method similar to that of Cameron et al.[12] and purified insert electro-eluted from agarose by the method of McDonnell et al.[13].

Restriction enzymes were purchased from BRL (Xmn I from New England Biolabs) and digests carried out according to the directions of the suppliers. Gel electrophoresis was carried out in 0.8% agarose (Sigma) in Tris/acetate buffer and DNA transferred to nitrocellulose (Schleicher and Schuell)[14]. Nick-translations[15] were carried out using DNA polymerase supplied by BRL or Boehringer to label the DNA to a specific activity of 1×10^6-5×10^8 cpm/μg using radionucleotides supplied by Amersham (410 or 3000 ci/mmol). Hybridisations were carried out in 50% formamide (BDH, AnalaR) using the protocol of Wahl et al.[16]. Autoradiography was performed for 12-120 hours using Kodak XAR-5 or XRP film at -20°C or -70°C.

RESULTS

Isolation of Probes

384 recombinant phage were isolated at random from the chromosome 21

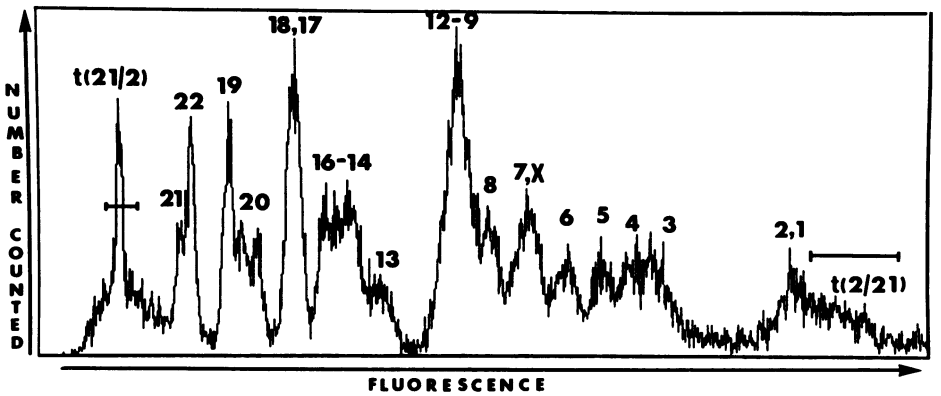

Figure 1. Flow karyotype of the cell line carrying the 2/21 reciprocal translocation chromosomes. The bar marked above each translocation indicates the window settings used for sorting in each case.

library. These were transferred to nitrocellulose[17] and probed with nick-translated total genomic human DNA. 54 did not show strong hybridisation. These were picked and used for further analysis as this group would be expected to contain a sub-group of recombinants representing unique sequence human DNA. 14 of these contained apparent unique sequence DNA when hybridised to EcoRI digested human genomic DNA and of these 10 were used in subsequent mapping experiments. A further 4 clones contained DNA which gave rise to several bands, suggesting that the insert contained a mildly repetitive sequence.

Mapping of Probes

Five of the single copy probes were mapped to and five excluded from chromosome 21 on the basis of their hybridisation to DNA of the cell line THYB133R. The assignment of probe D21K9[18] was confirmed. No cross-hybridisation of these probes with the DNA of the mouse parental line BW5147 was observed at high stringency washing of filters. Probes which had been mapped to chromosome 21 were assigned names in accordance with Shows and McAlpine [19].

Once probes had been assigned to chromosome 21 they were regionally mapped on the chromosome using both chromosome sorting and a somatic cell hybrid. A filter containing DNA from sorted fractions of a chromosome suspension containing either a 2/21 or a 21/2 reciprocal translocation (breakpoint 21q21.2), digested with EcoRI and Southern blotted was successively hybridised with each probe assigned to chromosome 21. The results

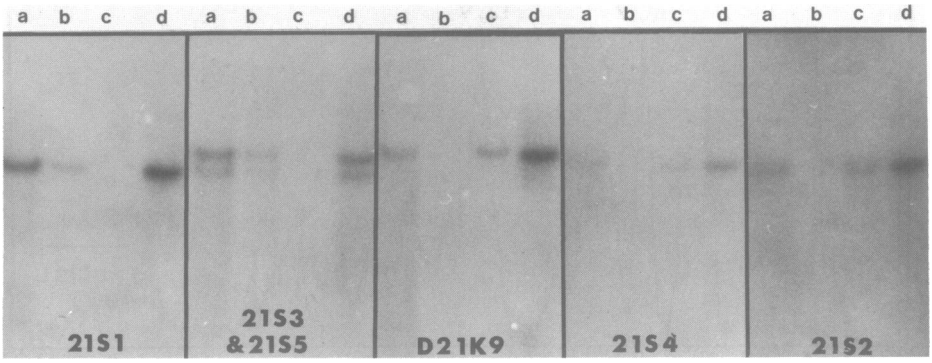

Figure 2. Regional mapping of chromosome 21-specific probes using sorted DNA. (a) - total DNA from cell line carrying the 2/21 translocation, (b) - sorted DNA containing the region 21q21-qter, (c) - sorted DNA containing the region 21q21-pter, (d) - genomic DNA. Each DNA sample has been digested with Eco RI,transferred to nitrocellulose and probed as indicated.

of this are shown in Figure 2. Three probes mapped to the region 21q21.2-qter and 3 to 21q21.2-pter. These results are confirmed by the hybridisation of probes to the DNA of cell line A1WBf2, shown in Figure 3 which, cytogenetically, has the same breakpoint.

Identification of RFLPs

The restriction endonucleases Bgl II, EcoRI, Hind III, MspI, PstI, PvuII, SstI, TaqI and XmnI were used to digest at least 8 human genomic DNA samples and after Southern blotting each set of samples was probed

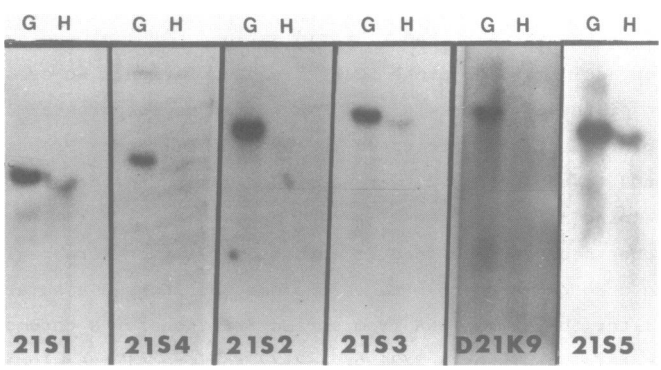

Figure 3. Regional mapping of chromosome 21-specific probes using DNA from a somatic cell hybrid, A1WBf2 containing the region 21q21-qter (H) and human genomic DNA (G). Samples digested with Eco RI.

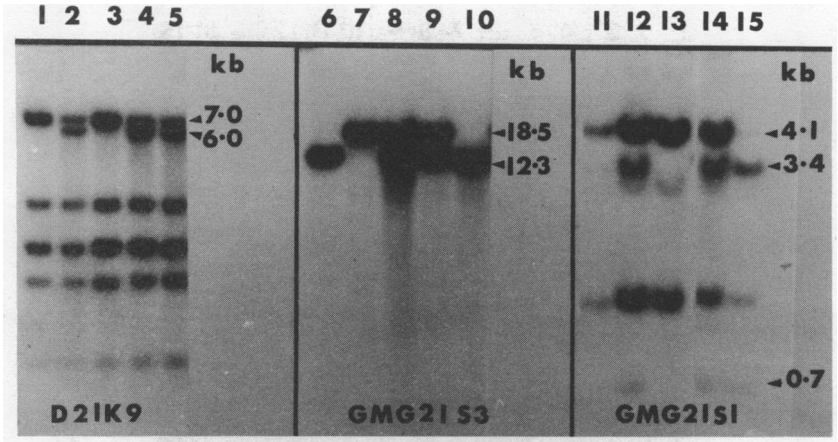

Figure 4. Polymorphic probes on chromosome 21. Various genomic DNA samples digested with: Taq I, lanes 1-5; Bgl II, lanes 6-10 and Msp I, lanes 11-15, transferred to nitrocellulose and hybridised with the probe indicated.

with the chromosome 21 probes in an attempt to identify RFLPs. Two such polymorphisms were found: one for MspI using probe GMG21S1, allele sizes 4.1 kb and 3.4/0.7 kb and one for Bgl II with probe GMG21S3, allele sizes 18.5 kb and 12.3 kb. The TaqI polymorphism for D21K9 of Davies et al.[18] was confirmed. These polymorphisms are shown in Figure 4. Extra samples were used to enable the allele frequencies to be calculated. In addition, family studies demonstrated the Mendelian inheritance of the alleles (see Table 1).

The Parental Origin of Nondisjunction

Fig.5 shows DNA samples from 10 Down's syndrome nuclear families

Table 1. Allele frequencies of RFLPs.

Probe	Number of Individuals A_1A_1	A_1A_2	A_2A_2	Totals	Frequencies A_1	A_2	SE	χ^2	PIC
GMG21S1	19	37	14	70	0.54	0.46	±0.04	0.43	0.37
GMG21S3	18	21	11	50	0.57	0.43	±0.05	0.64	0.37
D21K9	25	31	1	57	0.71	0.29	±0.04	4.72	0.33

fA_1 - common allele frequency, fA_2 - rare allele frequency

SE - standard error = $((fA_1 \times fA_2)/2N)^{1/2}$

χ^2 - Chi square to test that alleles are in Hardy-Weinberg Equilibrium

PIC - polymorphism information content = $1-fA_1^2-fA_2^2-2(fA_1^2 \times A_2^2)$ (from Botstein et al. 1980).

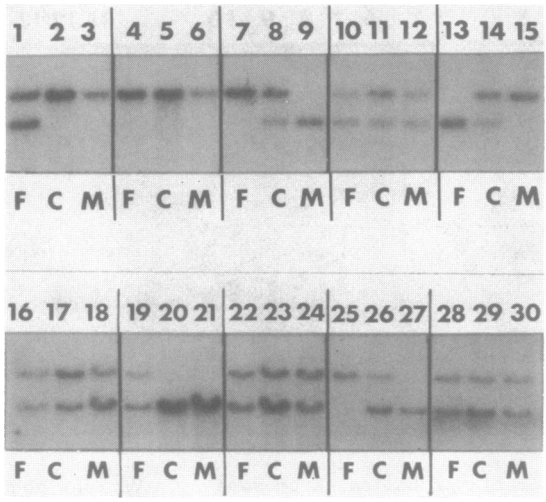

Figure 5. DNA samples from 10 Down's syndrome families (F = father, C = affected child, M = mother) restricted with Bgl II and probed with GMG21S3. In most tracks a dosage effect is apparent for one restriction fragment in the affected child. 7-9 illustrates nondisjunction of the paternal chromosome 21, and maternal nondisjunction is shown in 13-15 and 25-27.

(mother, father and trisomic child) restricted with Bgl II and probed with GMG21S3. Care was taken to load each track with 5µg DNA so that in almost every case a dosage effect is apparent involving one of the two chromosome 21 restriction fragments in the affected child. In two families (13-15 and 25-27) maternal nondisjunction is indicated by a double dose of the maternal allele in the affected child, and in one family (7-9) there is a double dose of the father's allele. It is not possible to distinguish either first and second meiotic nondisjunction or post-fertilisation nondisjunction in these examples.

DISCUSSION

Mapping of Probes

Six probes have been regionally mapped on chromosome 21. D21K9, GMG21S2 and GMG21S4 map to the region 21q21-pter and GMG21S1, GMG21S3 and GMG21S5 to the region 21q21-qter. The accuracy of the assignments is defined by the cytogenetic determination of the breakpoint at 21q21.2. The use of further cell lines would permit more precise localisation.

RFLPs

The RFLPs reported here both have high rare allele frequencies. 0.46 and 0.43 and as such are useful polymorphisms for linkage studies as there is a high chance that any given mating will be informative.

Use of Chromosome 21-Specific DNA Probes

Other workers have isolated probes for chromosome 21. Watkins et al.[20] have reported 12 chromosome 21-specific probes at least half of which identify RFLPs and Lieman-Hurvitz et al.[21] have a probe for the SOD-1 gene which is assigned to chromosome 21. There are therefore a minimum of 19 probes for chromosome 21, at least 9 of which identify RFLPs. It should therefore be possible to create a complete linkage map of the chromosome by use of chromosome sorting, somatic cell hybrids and by classical genetic techniques involving the measurement of recombination fractions between loci with polymorphic alleles. These probes could then be used to define breakpoints more accurately than by cytogenetic analysis. Further chromosome 21 probes could be fitted into the map as they are isolated.

It is possible to determine the parental origin of nondisjunction in Down's syndrome children using RFLPs to identify the parental chromosomes[18]. This should be possible in 37% of cases using the 3 RFLPs described here. The use of the probes of Watkins et al.(1984) as additional markers brings this figure up to 73%. This provides the basis for a non-subjective test for determining the parental origin of the extra chromosome in almost 3/4 of all cases. This proportion will increase as additional probes are isolated. Figure 5 illustrates the use of GMG21S3 to identify the parental origin of the extra chromosome 21 in 3 out of 10 families tested.

As there is a probability that at least some of the chromosome 21 probes in existence are located within the critical region which is duplicated in translocation Down's syndrome, it should be possible to use these as probes to examine gene expression in this condition. Such probes can also be used as tools for accurately mapping probes for expressed sequences in this region. Three of the probes reported here are not excluded from this region and of these, GMG21S1 and GMG21S3 exhibit polymorphisms.

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