Short Report

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An Xp22.1-p22.2 YAC Contig Encompassing the Disease Loci for RS, KFSD, CLS, HYP and RP15: Refined Localization of RS

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Abstract

To facilitate the positional cloning of the genes involved in retinoschisis (RS), keratosis follicularis spinulosa decalvans (KFSD), Coffin-Lowry syndrome (CLS), X-linked hypophosphatemic rickets (XLH, locus name HYP) and X-linked dominant cone-rod degeneration (locus name RP15), we have extended the molecular map of the Xp22 region. Screening of several YAC libraries allowed us to identify 156 YACs, 52 of which localize between markers DXS414 (P90) and DXS451 (kQST80H1). Analysis of their marker content facilitated the construction of a YAC contig from the region spanning (in this order): DXS414 – DXS987 – DXS207 – DXS1053 – DXS197 – DXS43 – DXS1195 – DXS418 – DXS999 – PDHA1 – DXS7161 – DXS443 – DXS7592 – DXS1229 – DXS365 – DXS7101 – DXS7593 – DXS1052 – DXS274 – DXS989 – DXS451. The region between DXS414 and DXS451 covers about 4.5–5 Mb. Two additional markers (DXS7593 and DXS7592) were placed in the region, thereby increasing the genetic resolution. Using the deduced marker order, the analysis of key recombinants in families segregating RS allowed us to refine the critical region for RS to 0.6 Mb, between DXS418 and DXS7161.

Key Words
Retinoschisis
Xp22
Contig

A number of disease loci have been mapped to the Xp22.1-p22.2 region [1], including spondylo-epiphyseal dysplasia (SEDL), retinoschisis (RS), keratosis follicularis spinulosa decalvans (KFSD), Coffin-Lowry syndrome (CLS), X-linked hypophosphatemic rickets (XLH, locus name HYP) and X-linked dominant cone-rod degeneration (locus name RP15) [2]. Contiguous gene syndromes and large deletions, which have greatly contributed to the unravelling of other regions of the X chromosome, have not been reported in the Xp22.1-p22.2 region. Linkage studies have placed the gene for RS, between DXS43 [3–5] and DXS999 [1, 6] and KFSD between DXS16 and DXS269 [7]. As a first step towards identification of RS and KFSD, we have used known STS markers, *Alu*-PCR products of yeast artificial chromosomes (YACs) and new Généthon markers to screen YAC libraries and isolate

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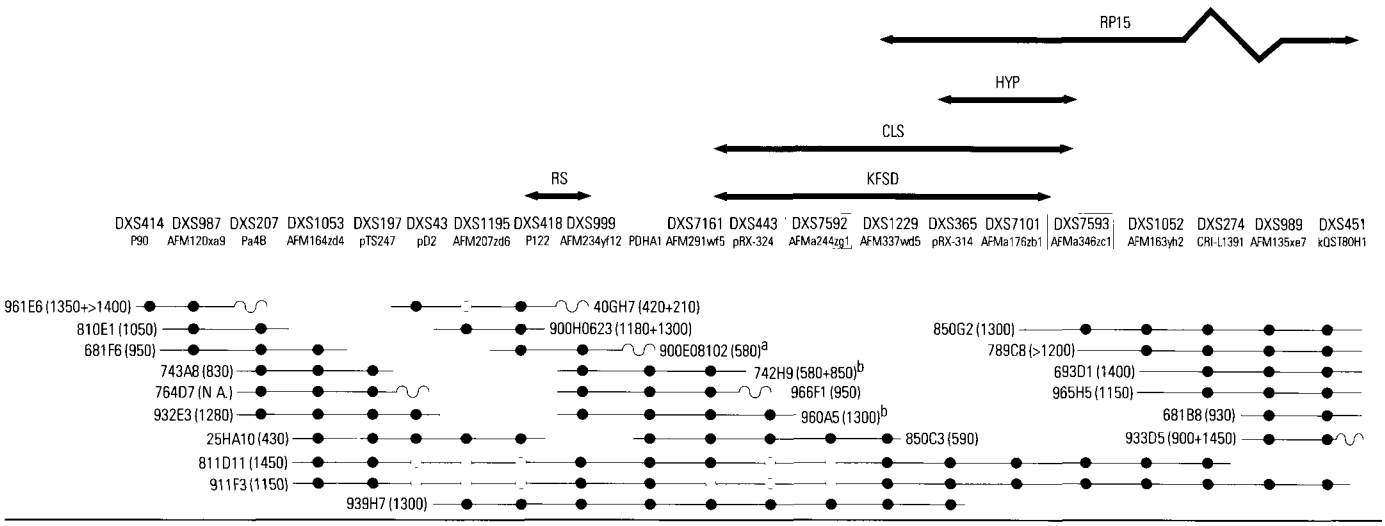

Fig. 1. Marker-based YAC contig. Closed circles indicate presence of the marker (after PCR or hybridization analysis), open circles indicate absence of the marker. Wavy lines indicate chimeric YACs. [a]900E08102 does not hybridize to chromosome 3 in all metaphases. [b]YACs 742H9 and 960A5 were found to be nonchimeric by FISH, but were found to be chimeric by Alitalo et al. [9] by analysis of YAC endclones. Boxed markers are reagents additional to those previously located in contigs covering the region. The 23 YACs range in size from 210 to over 1,400 kb with an average size of 1,050 kb. Sizes for specific YACs were usually consistent with available data (i.e. Généthon database [17] and [9, 10]). The disease gene candidate regions indicated for HYP, CLS and RP15 are as published [1, 2]. During preparation of this paper, the gene mutated in HYP patients has been identified [18].

YACs from the Xp22 region. An *Alu*-PCR-based fingerprinting method [8; Coffey et al., unpubl. data] was used to assemble crude contigs and to determine overlaps between the YACs. Subsequently, PCR and hybridization analysis were used to refine the contig, order the markers and construct a physical map. The deduced map spans about 4.5–5 Mb and includes the loci for RS, KFSD, CLS, and HYP. Several groups published overlapping Xp22 contigs [9–11], which are in agreement with the map presented here. We place two additional markers (DXS7592, DXS7593) in the region. The mapping data, combined with linkage analysis, enabled refinement of the localization of RS, between DXS418 and DXS7161 and KFSD, between DXS7161 and DXS1226 [12].

The CEPH [13], ICI [14] and ICRF [15] YAC libraries were screened with 21 probes which resulted in the isolation of 156 positive clones. To rapidly obtain a rough physical map, all 156 YACs were *Alu*-PCR fingerprinted, revealing the presence of 10 *Alu*-PCR-based contigs, containing a total of 52 YACs. Four of these contigs were located in the Xp22.1-p22.2 region. Subsequently, the YACs from these contigs were tested for the presence of Xp22 markers including new Généthon markers which could not be ordered by genetic mapping. Out of the 156 YACs, 23 were positive for two or more markers. FISH analysis of the 23 YACs showed that 17 hybridized to Xp22 only, while 6 gave more than one hybridization signal. The marker order that was obtained (fig. 1), is in agreement with the consensus map of the 5th X-chromosome Workshop [1] and as published by Alitalo et al. [9]. The available genetic data indicate that the region analyzed here has a highly increased recombination frequency. The estimated physical distances, in combination with the published genetic distances [16, 17], indicate an increased recombination frequency of, on average, 0.2 Mb/cM for the region between DXS987 and DXS989.

The candidate regions for the HYP, CLS, RP15, RS and KFSD disease genes are indicated in the physical map (fig. 1). The new data on the marker order generated were used to refine the localization of the candidate regions for RS and KFSD. For RS we performed an extended linkage analysis in 21 RS families [3]. Two key recombination events were identified (fig. 2). In family P 22.337, the recombination between RS and DXS418 in patient III-3 places the disease gene proximal to DXS418. In family P 24.130, the recombination between RS and DXS7161 in

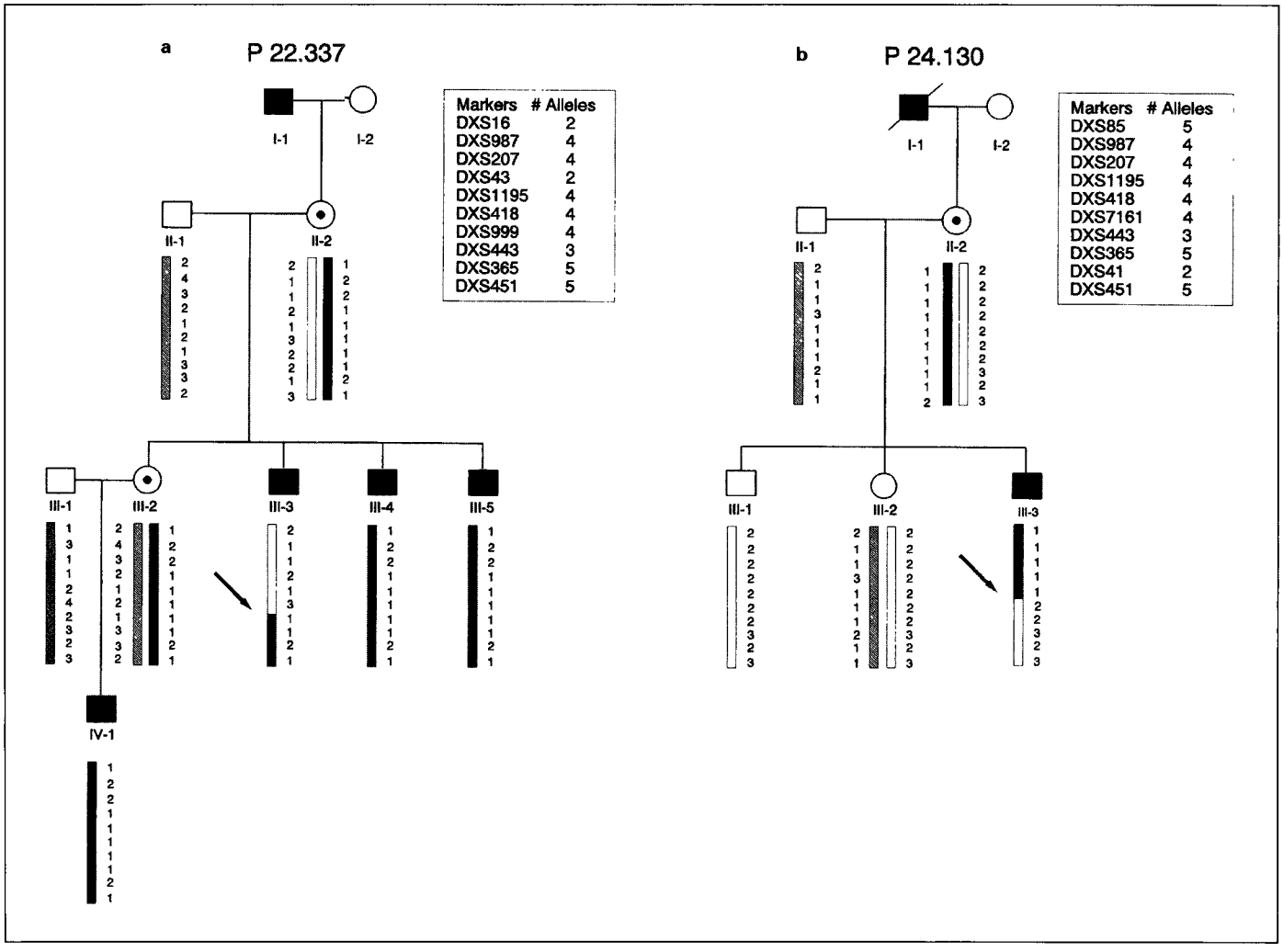

Fig. 2. Refined critical region for RS. **a** In family P 22.337, the RS disease locus cosegregates, with haplotype 1-2-2-1-1-1-1-1-2-1. The recombination observed in patient III-3 places RS proximal to DXS16, DXS987, DXS207, DXS43, DXS1195 and DXS418. **b** In family P 24.130, the phase of the markers could be obtained through the analysis of the haplotypes of male III-1 and female III-2. Healthy male III-1 inherited the X chromosome 2-2-2-2-2-2-2-3-3-2 from his mother (II-2). The phase of the maternal X chromosomal alleles of female III-2 is also 2-2-2-2-2-2-2-2-3-2, since she inherited the X chromosome characterized by 2-1-1-3-1-1-1-2-1-1 from her father. Thus, patient III-3 is recombinant for 2 (DXS7161) - 2 (DXS443) - 3 (DXS365) - 2 (DXS41) - 3 (DXS451). The analysis of the recombination breakpoints in this family suggests that RS localizes distal of DXS7161. DXS999 was not informative.

patient III-3 places the gene distal to DXS7161. DXS999, the most distal marker according to George et al. [6] was not informative in this family. Together, these data refine the localization of RS to between DXS418 and DXS999. In a parallel study, using the marker order established here, we were able to refine the localization of KFSD to between DXS7161 and DXS1226 [12]. The candidate regions for both RS and KFSD have now become small enough to establish a transcriptional map directed at the isolation of the gene(s) involved in these diseases.

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