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SHORT COMMUNICATION

A High-Resolution Interval Map of the q21 Region of the Human X Chromosome

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In a previous study, we have developed a panel of chromosomal rearrangements for the physical mapping of the q13–q21 region of the human X chromosome (Philippe *et al., Genomics* 17: 147–152, 1993). Here, we report the physical localization of 36 additional polymorphic markers by polymerase chain reaction analysis. The high density of chromosomal breakpoints in Xq21 allows us to map 58 DNA loci in 22 intervals. As a result, this segment of the X chromosome is saturated with approximately three sequence tagged sites per megabase of DNA, which will facilitate the construction of a YAC contig of this region. © 1995 Academic Press, Inc.

The Xq21 band is the most prominent, dark staining Giemsa band of the human X chromosome. This segment seems to be relatively devoid of functionally important genes, as demonstrated by the existence of male-viable deletions spanning almost the entire Xq21 region (3). These deletions very often are associated with contiguous gene syndromes consisting of choroideremia (CHM), X-linked mixed deafness (DFN3), and nonspecific mental retardation (MR). Together with X;autosome translocations (t(X;A)), the deletions were instrumental for positional cloning of the CHM gene (4, 15), and more recently, the DFN3 gene (6). Genetic linkage studies in families with syndromic forms of mental retardation, most notably the Allan–Herndon–Dudley syndrome locus (21), the Juberg–Marsidi syndrome (20), and X-linked α thalassemia/mental retardation (10), suggest a location of these genes in the Xq12–q21 region. Whether any of these syndromes and nonspecific MR are caused by mutations in the same gene remains to be investigated. In addition, the Xq21 region contains a gene underlying X-linked cleft palate and/or ankyloglossia (CPX) (22).

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Deletions and translocations have been very useful for the improvement of the physical map of the human genome, especially for the X chromosome. This is particularly true for the Xp22, Xp21, and Xq21 regions. We have previously developed a panel of 24 X-chromosomal rearrangements that, by means of Southern blot analysis, has allowed us to map physically more than 30 DNA markers within 18 intervals of Xq21 (16). Here we report the localization of an additional 26 DNA loci in Xq21 with special emphasis on polymorphic markers. These new markers have been mapped by polymerase chain reaction (PCR) analysis on genomic DNA from either lymphoblastoid cell lines of males with interstitial deletions or somatic cell hybrids derived from females with X;autosome translocations. The PCR data were combined with the hybridization data, which yielded a high-resolution interval map for Xq21.

A reaction volume of 50 μl was used with 200 ng of genomic DNA, 0.25 μg of each primer, 200 μM deoxynucleotide triphosphates, 1× PCR buffer specified by the *Taq* DNA polymerase suppliers, and 1 unit of *Taq* DNA polymerase. In most cases, the PCR conditions were those described in the original descriptions of the different markers. Occasionally, to improve PCR specificity, we modified either the annealing temperature or the $MgCl_2$ concentration. For each DNA marker mapped by PCR analysis, controls were carried out with a normal female cell line, a hamster cell line, and GMO6318B, a hybrid cell line containing the X chromosome as the sole human component (NIGMS Human Genetic Mutant Cell Repository). Fifteen microliters of the PCR reaction was analyzed on a 3% agarose gel (1.5% Seakem GTG agarose + 1.5% Nusieve GTG agarose).

A total of 36 sequence tagged sites, STSs, (Table 1) including 20 microsatellite markers from the Généthon second-generation genetic linkage map (9) have been physically mapped to the proximal region of Xq. Most of these markers represent short tandem repeats (STR) with high heterozygosity that are of interest for linkage

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TABLE 1

STR and RFLP Loci Mapped by PCR in This Study

Locus	Name	STR	Heterozygosity	Reference
DXS262	KZ040	—	0.27 (RFLP)	1
DXS345[a]	RX72R1	—	0.38 (RFLP)	This study
DXS346	RX86	$(CA)_{17}$	0.20	1
DXS355	RX167	$(TAAAA)_4$	0.38 (RFLP)	1
DXS364	RX272	$(AC)_{14}$	0.46	1
DXS441/DXS566[b]	1 and 2/HX60	$(AC)_{19}/(AC)_{19}$	0.76/0.68	17, 18
DXS453/DXS983[c]	Mfd66/AFM078za1	$(AC)_{22}/(AC)_n$	0.63/0.64	9, 25
DXS454	Mfd72	$(AC)_{17}G(GA)_8$	0.59	25
DXS456	XG30B	$(AC)_n$	0.77	14
DXS458	Mfd79	$(AC)_{15}A$	0.62	25
DXS571/DXS1105[d]	HX79(B1B2)/AFM263wc1	$(CA)_{16}/(AC)_n$	0.46/0.38	5, 9
DXS738	MIT-E114	$(CA)_{17}$	0.71	12
DXS986	AFM116xg1	$(AC)_n$	0.75	9
DXS988	AFM123xd4	$(AC)_n$	0.60	9
DXS990	AFM136yc7	$(AC)_n$	0.75	9
DXS991	AFM151xf6	$(AC)_n$	0.81	9
DXS995	AFM207zg5	$(AC)_n$	0.60	9
DXS1000	AFM248te9	$(AC)_n$	0.34	9
DXS1002	AFM249vh5	$(AC)_n$	0.70	9
DXS1066	AFM234tf8	$(AC)_n$	0.37	9
DXS1120	L21174	$(AC)_{23}$	0.74	7
DXS1124	L21177	$(TG)_6TT(TG)_{17}$	0.54	7
DXS1167	D3	$(AC)_{5,5,7}(CA)_7$	0.03	1
DXS1168	XKL10	$(CA)_{16}G(CA)_{14}$	0.53	1
DXS1169	A1	$(CA)_{19}$	0.53	1
DXS1170	B8	$(CA)_{24}$	0.67	1
DXS1196	AFM056yb8	$(AC)_n$	0.79	9
DXS1197	AFM072za5	$(AC)_n$	0.64	9
DXS1203	AFM262vg1	$(AC)_n$	0.65	9
DXS1209	AFM273zd5	$(AC)_n$	0.45	9
DXS1217	AFM288ye9	$(AC)_n$	0.62	9
DXS1221	AFM303wd1	$(AC)_n$	0.59	9
DXS1222	AFM308xb5	$(AC)_n$	0.65	9
DXS1225	AFM311vg5	$(AC)_n$	0.74	9
DXS1230	AFM337zb1	$(AC)_n$	0.62	9
DXS1231	AFM340ye1	$(AC)_n$	0.49	9

Note. The characteristics of the DNA loci are those reported in the original description of the PCR markers. While our work was in progress, several dinucleotide repeats with different DXS number were shown to be identical (see footnotes *b–d*).

[a] The novel STS primer pair for probe RX-72R1 (DXS345) has been developed by D. F. Barker, Utah, (pers. comm.) and is as follows (5′ → 3′): RX72R1(a) F, AGCAACAGCTAATTTCTATGG; RX72R1(a) R, ACAAACTTTTACCCCTGCT. The PCR was performed as described in the text with a final $MgCl_2$ concentration of 1.5 m*M*. After a first denaturation step (5 min at 94°C), 40 cycles (94°C for 40 s, 53°C for 40 s) were performed. A final elongation step (5 min at 72°C) ended the process.

[b] Lindsay *et al.* (13) have demonstrated that the microsatellite at DXS566 corresponds to the dinucleotide repeat at DXS441.

[c] Rider and Monaco (19) have proved that the dinucleotide repeat at the locus DXS453 is identical to that designated at locus DXS983.

[d] Gyapay *et al.* (9) noted that microsatellites at DXS571 and DXS1105 are identical.

analysis in families with X-linked diseases located in the proximal region of the long arm of the human X chromosome. Of the 36 new STSs integrated in the physical map, 10 are located either proximal to the C56N breakpoint or distal to the SN breakpoint. Therefore, with the exception of DXS441/566, which is deleted in patient RvD, they are not included in Fig. 1. Loci DXS453/983, DXS988, DXS991, and DXS1000 map proximal to the PMI breakpoint (GDB breakpoint 84 not depicted in Fig. 1). Loci DXS1124 and DXS1221 are located within Xq13 between the PMI and the C56N breakpoints. Among the 36 DNA markers tested, DXS456, DXS571, and DXS1120 are distal to the SN breakpoint (the SN breakpoint is located between DXS87 and DXS571 in Xq22.3).

Our study resolves the Xq21 band of the X chromosome into 22 ordered intervals from the RvD proximal deletion boundary (BXP 93) to the distal deletion breakpoints in patients RvD and MBU (BXP 94 and BXP 110, respectively) (Fig. 1). Interval 23 corresponds to almost the entire Xq22 band, as DXS17 has been mapped to Xq22.3 (26). The gradual integration of 24 STRs and 2 conventional RFLP markers allowed us to create 4 additional intervals since the previously published map (16). According to the most updated consensus map of the X chromosome (26), BXP 93 is located between PGK1 and DXS566 at the boundary between Xq13.3 and Xq21.1, while both BXP 94 and BXP 110 (distal deletion breakpoints in RvD and MBU, respectively) are flanked by DXS118 and DXS454 in the proximal part of Xq22.1.

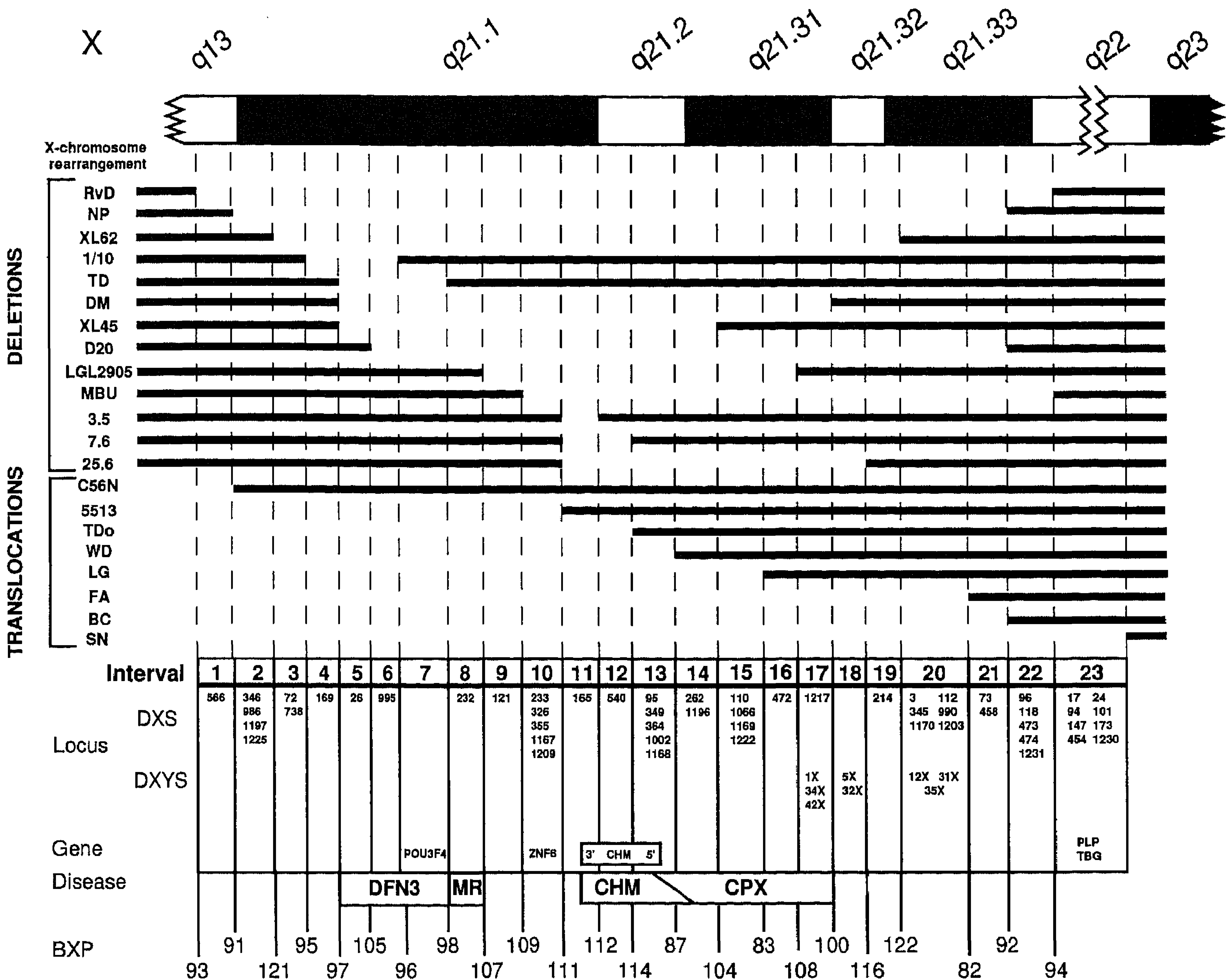

FIG. 1. Detailed physical mapping of marker loci in Xq21. Since the previously published map (16), six X-rearrangements have been removed from this deletion translocation panel: duplications CN and KM, deletions SD and 33.1, and translocations CIII-1 and HR10. We have added one rearrangement, 5513, found in a female carrying a balanced X;autosome translocation, 46,X,t(X;22)(q21.2;p12), which is not associated with any abnormal phenotype. EBV-transformed cells from this female were fused with Wg3h hamster cells by using polyethylene glycol. Hybrid clones containing the der22 as the sole human X component were obtained under HAT (hypoxanthine, aminopterine, thymidine) selection essentially as described by Benham *et al.* (2). At the top an idiogram of the Xq21 region in G-banding is presented. For each X-rearrangement the segment of the X chromosome present is indicated by a bar. Within each interval the probes are arranged arbitrarily in increasing numerical order. The different disease loci in Xq21 as well as the GDB (Genome Data Base) breakpoint numbers (BXP) are depicted at the bottom of the figure. For the BXP numbers, when more than one deletion/translocation breakpoint defines the boundary between two intervals, we have chosen a BXP number corresponding to the first X-rearrangement from the top to the bottom of the figure (for example, BXP 91 corresponds to the proximal breakpoint of the deletion NP).

The human choroideremia gene (CHM) spans at least 150 kb in Xq21.2 between DXS165 and DXS1002 (23, 24). Based on the distal deletion breakpoint (BXP 188) in patient MS (24), DXS1002 is located proximal to DXS95 in interval 13. From fine mapping of Xq21 deletions associated with complex syndromes (11), interval 8 contains a gene involved in MR. The X-linked cleft palate and/or ankyloglossia (CPX) locus has been mapped by linkage analysis between DXS95 and DYXS1X (22). The physical map reported in this study should facilitate the positional cloning of disease genes from the Xq21 region. The positioning of the microsatellite marker AFM207zg5 (DXS995) in interval 6 is a good example, as it has been very useful for cloning and characterization of the critical region for DFN3 (11). Very recently, by combining positional cloning and candidate gene approaches, the POU domain gene BRN4 (8) was shown to be implicated in X-linked mixed deafness (6).

The high density of naturally occurring chromosomal rearrangements in this segment of the X chromosome allowed us to build a high-density map. The physical size of the X-chromosomal segment between DXS566 and DXS118 is estimated to be roughly 15–20 Mb, which brings the average size for each interval to less

than 1 Mb. Moreover, as each interval contains on average 2.6 DNA markers, this Xq21 map should contain 1 marker per every 350 kb.

In establishing this translocation/deletion map, we have assumed that each rearrangement is not more complex than a simple translocation or interstitial deletion. Furthermore, the use of hybrid cell lines for mapping needs vigilance as tiny rearrangements are quite frequent. For every DNA marker localized, our findings are in agreement with all genetic and physical data published so far in the proximal region of Xq, and no single deletion or translocation gave inconsistent results when compared to other X-rearrangements, indicating that these rearrangements are not complex. Some YAC contigs have been built in Xq21, but several gaps remain (26). The detailed physical map presented here will be of great value in generating a complete YAC contig of the Xq21 region and provides a very efficient way to map precisely any new microsatellite or expressed sequence tag positioned in the proximal part of the long arm of the human X chromosome. Any new STS localized in the Xq proximal region can be sent to the authors for refined mapping, or, alternatively, DNA samples from the deletion/translocation panel are available upon request. This work demonstrates the importance of chromosome rearrangements in human gene mapping.

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