LETTER

Integrated YAC Contig Map of the Prader–Willi/Angelman Region on Chromosome 15q11–q13 with Average STS Spacing of 35 kb

Susan L. Christian,[1] Nehal K. Bhatt,[1] Scott A. Martin,[1] James S. Sutcliffe,[2] Takeo Kubota,[3], Bing Huang,[4] Apiwat Mutirangura,[5] A. Craig Chinault,[6] Arthur L. Beaudet,[6,7] and David H. Ledbetter[1,8]

[1]Department of Human Genetics, The University of Chicago, Chicago, Illinois 60637 USA; [2]Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232 USA; [3]Department of Hygiene and Medical Genetics, Shinshu University, Nagano, Japan; [4]Genzyme Genetics, Long Beach, California 90806 USA; [5]Genetics Unit, Department of Anatomy, Chulalongkorn University, Bangkok, Thailand; [6]Department of Molecular and Human Genetics and [7]Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030 USA

Prader–Willi syndrome and Angelman syndrome are associated with parent-of-origin-specific abnormalities of chromosome 15q11–q13, most frequently a deletion of an ~4-Mb region. Because of genomic imprinting, paternal deficiency of this region leads to PWS and maternal deficiency to AS. Additionally, this region is frequently involved in other chromosomal rearrangements including duplications, triplications, or supernumerary marker formation. A detailed physical map of this region is important for elucidating the genes and mechanisms involved in genomic imprinting, as well as for understanding the mechanism of recurrent chromosomal rearrangments. An initial YAC contig extended from D15S18 to D15S12 and was comprised of 23 YACs and 21 STSs providing an average resolution of about one STS per 200 kb. To close two gaps in this contig, YAC screening was performed using two STSs that flank the gap between D15S18 and 254B5R and three STSs located distal to the *GABRA5*–149A9L gap. Additionally, we developed 11 new STSs, including seven polymorphic markers. Although several groups have developed whole-genome genetic and radiation hybrid maps, the depth of coverage for 15q11–q13 has been somewhat limited and discrepancies in marker order exist between the maps. To resolve the inconsistencies and to provide a more detailed map order of STSs in this region, we have constructed an integrated YAC STS-based physical map of chromosome 15q11–q13 containing 118 YACs and 118 STSs, including 38 STRs and 49 genes/ESTs. Using an estimate of 4 Mb for the size of this region, the map provides an average STS spacing of 35 kb. This map provides a valuable resource for identification of disease genes localized to this region as well as a framework for complete DNA sequencing.

Chromosome 15q11–q13 serves as an important paradigm for genomic imprinting in human disease as two distinct mental retardation disorders, Prader–Willi syndrome (PWS) and Angelman syndrome (AS), are associated with parent-of-origin-specific deficiencies of this region. PWS is caused by a paternal deficiency due to paternal deletions (~70%), maternal uniparental disomy (~25%), or imprinting center defects (<5%) (Reis et al. 1994; Buiting et al. 1995). AS is caused by a maternal deficiency due to maternal deletions (~70%), paternal uniparental disomy (~5%), imprinting defects (~1%–2%), maternal loss-of-function mutations in the imprinted gene *UBE3A* (~2%–4%) (Albrecht et al. 1997; Kishino et al. 1997; Matsuura et al. 1997; Rougeulle et al. 1997; Vu et al. 1997), or unknown defects (10%–20%). The identification of multiple genes or transcripts with paternal-specific expression patterns within this region (*SNRPN*, *IPW*, PAR-1, PAR-5, PAR-SN, and *ZNF127*) (Driscoll et al. 1992; Glenn et al. 1993; Sutcliffe et al. 1994; Wevrick et al. 1994; Ning et al. 1996) provides a number of candidate

[8]Corresponding author.
E-MAIL dhl@genetics.uchicago.edu; FAX (773) 834-0505.

 8:146–157 ©1998 by Cold Spring Harbor Laboratory Press ISSN 1054-9803/98 $5.00; www.genome.org

genes for PWS, although it is difficult at present to assess the role of individual genes in its pathophysiology. Additional studies are needed to identify all imprinted genes present on chromosome 15 and to explore their potential functional relationship to PWS. Only five additional genes (*P, GABRB3, GABRA5, GABRG3,* and *NDN*) have currently been identified for this ~4-Mb region (Gardner et al. 1992; Rinchik et al. 1993; Greger et al. 1995; Glatt et al. 1997; MacDonald and Wevrick 1997; Sutcliffe et al. 1997), whereas a typical region of this size might be expected to have 100 or more genes.

Chromosome 15q11–q13 is also associated with an unusually high frequency and diversity of recurring rearrangements. The ~4-Mb common deletion observed in PWS and AS occurs at a frequency of 1/10,000–1/20,000 live births. Additionally, >50% of supernumerary marker formation (~1/2500 live births) involves rearrangement of the regions at the proximal and distal ends of 15q11–q13 (Huang et al. 1997). Other rearrangements observed less often include duplications, triplications, balanced reciprocal translocations, and jumping translocations. Evidence suggests the presence of at least four hot spots of chromosomal breakage in this region that require detailed physical maps to eluciate the mechanism(s) of rearrangement involved (Christian et al. 1995; Robinson et al. 1997).

One of the goals of the Human Genome Project is the construction of a physical map with sufficient ordered landmarks to provide an average resolution of 100 kb. Most landmarks are sequence-tagged-sites (STSs) that can be assayed using PCR (Olson et al. 1989; Green and Olson 1990). An initial STS-based YAC contig was developed in 1993 with a density of about one STS per 200 kb, but this physical map contained two gaps (Mutirangura et al. 1993b). Several genome-wide mapping efforts have provided additional mapping information for 15q11–q13 (Adamson et al. 1995; Chumakov et al. 1995; Hudson et al. 1995; Sheffield et al. 1995; Dib et al. 1996; Schuler et al. 1996; Stewart et al. 1997). However, the simultaneous development of these maps with few framework markers in common has made integration of the data difficult. Additionally, analysis of the data from these multiple sources for the chromosome 15q11–q13 region has yielded inconsistensies in the ordering of markers, making detailed analysis of this region extremely difficult.

To resolve these inconsistencies and increase the STS density for the chromosome 15q11–q13 region, we have analyzed the available YACs and STSs to create a fully integrated YAC-based STS content map. We now report an integrated map containing 118 YACs and 118 STSs, including 38 short tandam repeats (STRs) and 49 genes/ESTs, which resolves the discrepancies present within the other maps. This map has an average STS spacing of 35 kb, thus surpassing the goal of one STS per 100 kb. Additionally, this map will be a valuable resource toward elucidating the mechanism of rearrangement, aiding positional cloning of disease genes, as well as serving as an important framework for the systematic sequencing of the region.

RESULTS

One of the greatest advantages of the whole genome mapping effort is the availability of a large number of new reagents (i.e., STSs and YACs) for fine mapping of specific regions of the human genome. To create the integrated YAC contig of 15q11–q13, we pooled STSs developed by our laboratory and others (see Table 1 for references) and YACs developed through whole-genome mapping efforts (Chumakov et al. 1995; Hudson et al. 1995).

YAC Screening to Fill Gaps

The initial YAC contig of the PWS/AS region provided a one- to fourfold coverage of the ~4-Mb 15q11–q13 region but contained two gaps (Mutirangura et al. 1993b). Prior to the development of the whole genome level YAC contigs, the CEPH Mark I and II YAC libraries were screened using markers D15S541 and D15S543, known to flank this region, to fill the gap between D15S18 and 254B5R (Fig. 1) (Christian et al. 1995). Screening the CEPH libraries with D15S541 identified YAC y705C2. Marker D15S543 amplifies two fragments of different sizes, one from chromosome 15 and the other from chromosome 16 (Christian et al. 1995). Screening the CEPH Mark I library with D15S543 identified YACs y55F10, y166G7, y254B5, y338B6, y368H3, and y409C4 from chromosome 15 and YACs y42D4, y64C11, y64E4, y391E3, and y474H1 from chromosome 16.

To fill the gap between *GABRA5* and 149A9L, an STS developed from the left end of YAC 149A9 was used to screen the CEPH Mark I YAC library. The YACs identified were y97F4, y128G7, y149A9, y162C1, y369B10, y406B2, y406C2, y469E3, and y483H8. Two additional STRs, D15S156 and D15S219, were identified that mapped between *GABRB3* and D15S165 (Beckmann et al. 1993). Screening with D15S156 identified YACs y97F4, y128G7, y180F2, y369B10, y407B1, y483H8, and y493C4, and screening with D15S219 identified YACs y97F4, y128G7, y162C1, y483H8, and

Table 1. Chromosome 15q11–q13 STSs

Locus	Alias	GDB ID#	GenBank #	Reference
	NIB1540	GDB:1222663	T16604	Hudson et al. 1995
D15S1239	SHGC-13938	GDB:736182	G14918	Stewart et al. 1997
BCL8				Dyomin et al. 1997
A002B45	THC55220	GDB:563498	T36044	Schuler et al. 1996
D15S912	WI-9582	GDB:588623	Z39166	Hudson et al. 1995
SGC32610	EST392818	GDB:4582898	H90407	Hudson et al. 1995
D15S18	pIR39	GDB:190060	AF017580	Kuwano et al. 1992
D15S1035	AFM344te5	GDB:613155	Z51470	Dib et al. 1996
D15S541	SC2	GDB:328759	AF017561	Christian et al. 1995
D15S542	SC3	GDB:328772	AF017562	Christian et al. 1995
D15S16	pTD135	GDB:9786985	AF017568	Tantravahi et al. 1989
	SHGC 17218	GDB:1231343	G19381	Stewart et al. 1997
D15S1259	SHGC 15126	GDB:736248	G15008	Stewart et al. 1997
A008B26	THC104585	GDB:4588898	T89271	Schuler et al. 1996
D15F37S1	MN7		X69635	Buiting et al. 1992
A006B10	THC117982	GDB:4571055	T90793	Schuler et al. 1996
D15F375S1	368h3L	GDB:9786987	AF017565	Huang et al. 1997
D15S15	pTD3-16	GDB:9786989	AF017567	Tantravahi et al. 1989
D15F376S1	116G7R	GDB:9786991	AF017564	Huang et al. 1997
D15S17	pIR29-1	GDB:9786993	AF017569	Tantravahi et al. 1989
D15S543	SC1	GDB:340835	AF017563	Christian et al. 1995
ZNF127	D15S9	GDB:192783	U19107	Kuwano et al. 1992
SGC 35648	ZNF127	GDB:4581508	U19107	Hudson et al. 1995
WI-15987	EST277595	GDB:4577955	H17657	Hudson et al. 1995
SGC30582	NDN	GDB:4581616	F11033	Hudson et al. 1995
D15S1523	71B11L	GDB:9786995	AF017570	Mutirangura et al. 1993b
	A156E1R	GDB:190068	AF017573	Kuwano et al. 1992
D15S11(CA)	4-3R (CA)	GDB:185468	X63671	Mutirangura et al. 1992b
D15S646	ATA10C01	GDB:683607	G07887	Sheffield et al. 1995
D15S11(STS)	IR4-3R	GDB:190026	AF017583	Kuwano et al. 1992
D15S817	GATA81F03	GDB:685707	G07908	Sheffield et al. 1995
	307A12L	GDB:195171	AF017576	Mutirangura et al. 1993b
D15S1524	189-1-2	GDB:9786997	AF017579	new
D15S1525	189-1-3	GDB:9786999	AF017579	new
D15S675	326F6R	GDB:376580	AF029058	Wevrick et al. 1994
D15S13(STS)	TD189-1	GDB:190017	AF017582	Kuwano et al. 1992
D15S1526	189-1-1	GDB:9787001	AF017584	new
D15S673	11H11R	GDB:376575		Wevrick et al. 1994
D15S1021	AFMb344wc5	GDB:611550	Z53764	Dib et al. 1996
D15S63	PW71	GDB:192784		Kuwano et al. 1992
D15S677	457B4R	GDB:376584	AF029059	Wevrick et al. 1994
	B58C7R	GDB:189988	AF017574	Kuwano et al. 1992
D15S128	AFM273yf9	GDB:188608	Z17197	Dib et al. 1996
D15S674	32F6LF	GDB:376578	AF029057	Wevrick et al. 1994
SNRPN			U41384	Nakao et al. 1994
SGC31492	snRP SM-D	GDB:4581528	J04615	Hudson et al. 1995
D15S1527	PAR-SN	GDB:9787003	U55937	Ning et al. 1996
D15S226E	PAR-5	GDB:9787005	AF019618	Sutcliffe et al. 1994
D15S631E	Bda77b11	GDB:364505	Z28671	Richard et al. 1994
WI-15028	EST280908	GDB:4573853	H20970	Hudson et al. 1995
WI-15655	EST207144	GDB:4585489	R37082	Hudson et al. 1995
D15S612E	Cdy0he12	GDB:364448	Z45998	Richard et al. 1994
WI-13791	EST226878	GDB:4578563	R42946	Hudson et al. 1995
D15S626E	Bda12c11	GDB:364490	Z19301	Richard et al. 1994
D15S229E	PAR-7	GDB:9787007		Sutcliffe et al. 1994
D15S672	11H11LF	GDB:376572		Wevrick et al. 1994
IPW		GDB:424492	U12897	Wevrick et al. 1994
D15S903	WI-6780	GDB:588262	Z38790	Hudson et al. 1995
WI-14946	EST277487	GDB:4577556	H17549	Hudson et al. 1995
D15S227E	PAR-1	GDB:9787009	AF019616	Sutcliffe et al. 1994
stSG3525	EST122569	GDB:4564318	F06848	Schuler et al. 1996

Table 1. (*Continued*)

Locus	Alias	GDB ID #	GenBank #	Reference
D15S174	pB11	GDB:9787015		Greger et al. 1993
D15S228E	PAR-4	GDB:9787017	AF019617	Sutcliffe et al. 1994
D15S676	457B4LF	GDB:376582	AF029060	Wevrick et al. 1994
D15S613E	Cda0jb12	GDB:364451	Z38637	Richard et al. 1994
D15S1506	TK CA-17	GDB:5218343	AF018071	Sutcliffe et al. 1997
D15S871E	DOS197E	GDB:581448	M85504	Richard et al. 1994
stSG3346	EST186117	GDB:4564278	R16323	Schuler et al. 1996
D15S839	WI-6654	GDB:458841	Z38637	Hudson et al. 1995
D15S1089	NIB1419	GDB:626296	T16516	Hudson et al. 1995
A005C48	THC117852	GDB:4587534	R41400	Schuler et al. 1996
	B230E3L	GDB:194758		Mutirangura et al. 1993b
D15S824	WI-4277	GDB:458233	G03501	Hudson et al. 1995
UBE3A (3′)	E6-AP		L07557	Nakao et al. 1994
D15S837	WI-6519	GDB:458830	Z38495	Hudson et al. 1995
WI-11918	EST197498	GDB:1222743	R27453	Hudson et al. 1995
D15S225E	PAR-2	GDB:9787019	L25446	Nakao et al. 1994
D15S122	AFM200wb4	GDB:188289	Z16882	Dib et al. 1996
D15S10 (CA)	TD3-21	GDB:181189	L23501	Lindeman et al. 1991
D15S664	WI-1147	GDB:355254	G03480	Hudson et al. 1995
D15S10 (STS)	TD3-21	GDB:190002	AF017581	Kuwano et al. 1992
	B230E3R	GDB:194573	AF017575	Mutirangura et al. 1993b
D15S210	AFM320vd9	GDB:200358	Z24360	Dib et al. 1996
WI-13724	EST222705	GDB:4580326	R52640	Hudson et al. 1995
D15S835	WI-5696	GDB:458630	G03512	Hudson et al. 1995
WI-16777	EST157778	GDB:4583546	T87657	Hudson et al. 1995
D15S986	AFMa284za9	GDB:608280	Z52771	Dib et al. 1996
D15S1528	A229A2R	GDB:9787021	AF017585	Mutirangura et al. 1993b
D15S1364	TK4	GDB:1128023	AF017586	new
D15S113	LS6-1#1	GDB:196476	X68440	Mutirangura et al. 1993a
D15S891	WI-6134	GDB:588000	G05027	Hudson et al. 1995
D15S1365	JS208	GDB:1128024	AF019771	new
D15S540	UT7715	GDB:315737	L30335	Adamson et al.1995
D15S1529	A229A2L	GDB:9787024	AF017577	Mutirangura et al. 1993b
WI-9991	MR12918	GDB:1222711	G11833	Hudson et al. 1995
	AFMa309yg1	GDB:1222704	Z67472	Hudson et al. 1995
WI-15959	EST236624	GDB:4581234	R59869	Hudson et al. 1995
	B25E9R	GDB:194754		Mutirangura et al. 1993b
D15S740	WI-2610	GDB:450930	G03492	Hudson et al. 1995
GABRB3		GDB:188666	X63670	Mutirangura et al. 1992a
D15S97	MS14#2	GDB:182257	M87695	Beckmann et al. 1993
D15S511	85CA	GDB:304610	L25823	Glatt et al. 1994
GABRA5	A55CA-1	GDB:304613	L25838	Glatt et al. 1994
GABRA5		GDB:196394	M93435	Greger et al. 1995
GABRG3		GDB:593252	S82769	Greger et al. 1995
D15S822	GATA88HO2	GDB:685854	G07911	Sheffield et al. 1995
D15S975	AFMa216zc9	GDB:607038	Z52524	Dib et al. 1996
WI-10300	MR13302	GDB:1222666	G11805	Hudson et al. 1995
D15S156	AFM214xg11	GDB:189402	Z17258	Dib et al. 1996
D15S219	mfd209	GDB:214861	L20034	Beckmann et al. 1993
D15S1530	149A9L	GDB:9787026	AF017578	Mutirangura et al. 1993b
WI-14097	EST232495	GDB:4574880	R55789	Hudson et al. 1995
WI-15852	EST224544	GDB:4575218	R54479	Hudson et al. 1995
D15S217	gata8b06	GDB:686877	G07914	Sheffield et al. 1995
D15S661	GATA8D05	GDB:365525	G09148	Sheffield et al. 1995
D15S1531	93C9L	GDB:9787028	AF017566	new
D15S737	WI-5568	GDB:450865	G03489	Hudson et al. 1995
SGC32570	EST384265	GDB:4580300	H81966	Hudson et al. 1995
OCA2	D15S12	GDB:679062	G06453	Kuwano et al. 1992
D15S931	WI-4477	GDB:589111	G04645	Hudson et al. 1995

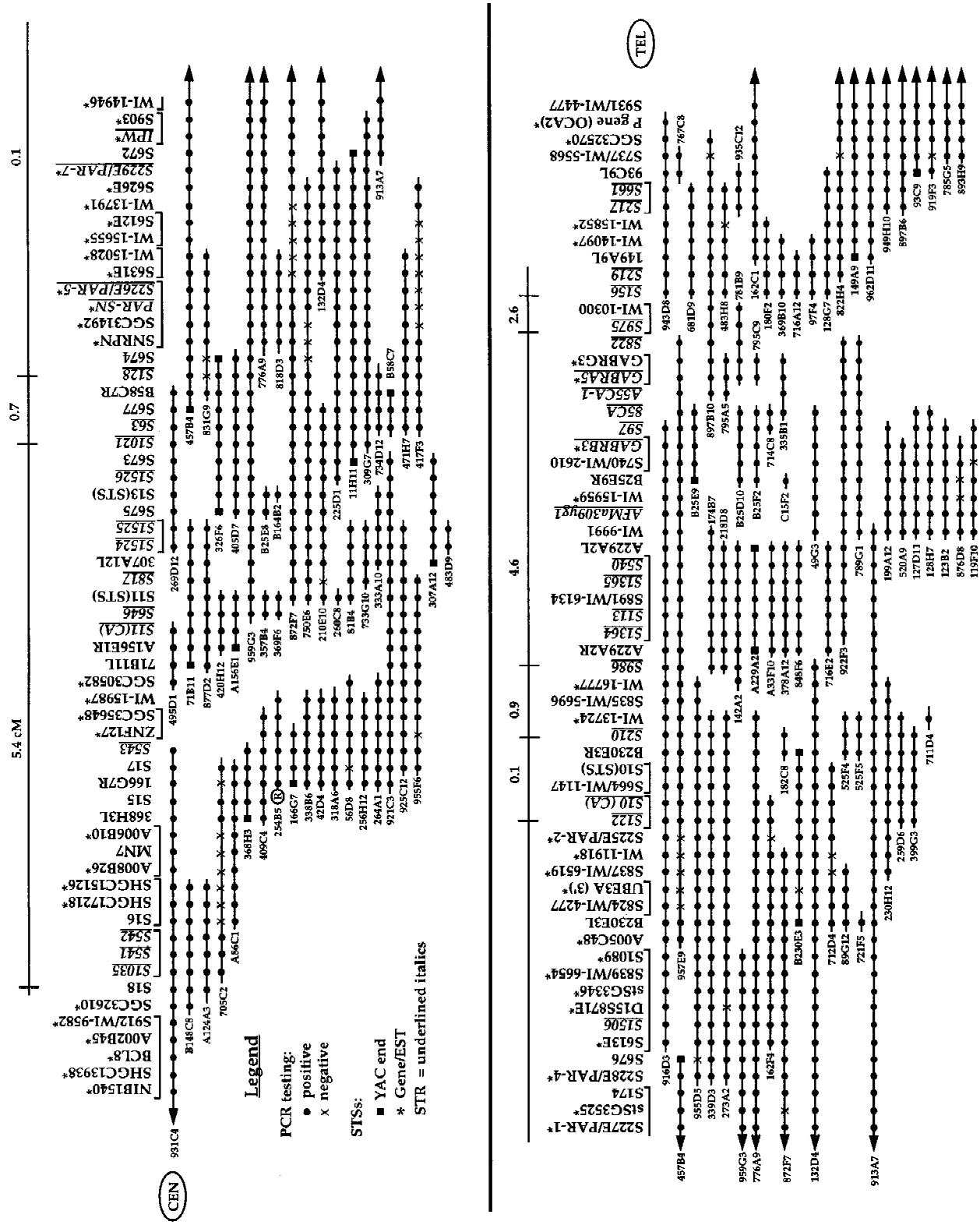

Figure 1 (*See facing page for legend.*)

y493C4. The overlapping YACs identified for these three STSs placed D15S156 and D15S219 within the *GABRA5*–149A9L gap but did not close it. The gap was closed when YACs y897B10 and y781B9—localized to this region by radiation hybrid mapping (Hudson et al. 1995)—were analyzed using STSs *GABRA5* and WI-10300.

Development of STSs

Although most of the STSs utilized in construction of this map have been published previously (Table 1), 11 new STSs, including 7 polymorphic STRs, have been developed that have not been described previously. Three STRs (S1524, S1525, and S1526) were identified from YAC 307A12 in a region previously limited in STR coverage. D15S1526, located distal to D15S13, contains a tetranucleotide repeat of $[CTAT]_{12}$. D15S1524 and S1525, located proximal to D15S13, are overlapping repeats where S1524 contains a $[TG]_{16}$ repeat, whereas S1525 contains a $[TC]_{17}[CA]_{15}$–{111 bp}–$[TG]_{16}$ repeat. Two additional STRs have been developed from cosmids isolated from YAC A229A2. D15S1364 contains a $[CA]_{16}$ repeat developed from a cosmid that also contains D15S113, whereas D15S1365, located distal to D15S113, contains a tetranucleotide repeat of $[GAAA]_{15}GAGAAAA[GAAA]_{17}$. Two additional polymorphic regions were found within the sequence of the PAR-5 and PAR-7 transcripts (Sutcliffe et al. 1994).

Four nonpolymorphic STSs have also been developed. An STS for the left end of YAC 93C9 (D15S1531) was developed following publication of the earlier contig. Three additional STSs (D15S15, D15S16, and D15S17) were developed by end-sequencing clones originally isolated from an inv dup(15)(q13)-enriched phage library (Donlon et al. 1986; Tantravahi et al. 1989).

YAC–STS Map Construction

To construct this map, the initial YAC contig was used as the starting framework. The order of YACs had been established previously using interphase fluorescence in situ hybridization (FISH) (Kuwano et al. 1992) and confirmed in other fine-mapping experiments (Nakao et al. 1994; Sutcliffe et al. 1994, 1997; Christian et al. 1995; Huang et al. 1997). STSs present within the mega-YAC contigs (Chumakov et al. 1995; Hudson et al. 1995) were mapped by PCR analysis against all YACs in the framework map to integrate the three maps. Once a preliminary integrated map was established, 12 subregions were created to establish the precise order of markers. Each STS, including those present on the original contig, was analyzed against all YACs that mapped to that subregion. Table 1 provides a list of all STSs utilized in this map. Based on the positive and negative PCR results for each STS, the final map was constructed (Fig. 1). Several YACs identified in the CEPH library screenings, described above, either gave negative PCR results with all STSs analyzed (y55F10 and y493C4), or the clones did not grow (y406B2, y406C2, and y469E3) and do not appear on the contig.

Although the current map represents the data as accurately as possible, certain caveats in interpretation of map order need to be considered. The primary concern is the presence of false-positive and false-negative PCR results as a possible source of errors in building YAC contigs (Bouffard et al. 1997). To minimize this problem, any questionable PCR results, either positive or negative, were repeated to provide the most accurate data possible. However, interstitial deletions within individual YACs exist that could influence the ordering of STSs. Therefore, four YACs, (y755A2, y785E7, y843F4, and y965G9), each containing two to five interstitial deletions that could not be mapped clearly, were excluded from the contig. The level of density of other YACs within this region, which showed consistent results, increased our level of confidence that these four YACs were of poor quality. Additionally, the map construction program SEGMAP also segregates YACs with inconsistent data (Bouffard et al. 1997).

Features of the Map

STRs

The current map contains 38 polymorphic markers extending across ~4 Mb. Of these, eight represent

Figure 1 Refined YAC STS content map of chromosome 15q11–q13. STSs are listed vertically with STRs italicized and underlined. The region is oriented toward the centromere on the *upper left* and toward the telomere on the *lower right.* An asterisk (*) is used to indicate STSs, which also represent either known genes or ESTs. Below the STSs are horizontal lines representing YACs with the YAC name indicated. (●) A positive PCR reaction between the STS and YAC indicated. An *X* is used to represent a negative PCR reaction. (■) STSs developed from YAC ends. Above the STSs is a horizontal line indicating the genetic distances between STRs present on the Genethon genetic map. The right end of y254B5, used as a hybridization probe in Mutirangura et al. (1993b), is indicated with a circled R. The brackets indicate STSs that cannot be uniquely ordered.

markers present on the Genethon genetic map (Gyapay et al. 1994; Dib et al. 1996). The genetic distance between these markers is indicated above the YAC contig to integrate the genetic and physical maps (Fig. 1). The localization of 30 additional STRs in this region between the Genethon markers provides the resources for improved linkage analysis and disease gene identification.

ESTs

Of the 49 genes/ESTs present on the current map, 14 represent known genes, 4 represent ESTs for known genes, 1 represents an EST for a new gene, and the other 30 are ESTs that have not yet been characterized. The four ESTs identifying previously characterized genes are SGC35648 (*ZNF127*), SGC31492 (*SNRPN*), D15S903 (*IPW*), and WI-15852 (*P*). One EST, SGC30582, was identified in a BLAST search as the human homolog of the mouse necdin-encoding gene (*Ndn*), which has been identified recently as an imprinted gene in both human and mouse (MacDonald and Wevrick 1997; Sutcliffe et al. 1997). The 30 uncharacterized ESTs will provide candidate genes for studies showing linkage to a particular subregion within this area.

Nonrandom Distribution of ESTs

The region from SGC30582 to *SNRPN*, representing ~1 Mb in physical distance, contains no genes or ESTs. In contrast, the region between *SNRPN* and UBE3A, representing ~500 kb in physical distance, contains 22 genes/ESTs, of which 8 represent known genes. The other 14 ESTs represent two of these known genes and 12 new uncharacterized ESTs. The domain from SNRPN to UBE3A is known to be imprinted (Nakao et al. 1994; Sutcliffe et al. 1994; Wevrick et al. 1994; Ning et al. 1996; Albrecht et al. 1997), so it will be of interest to examine the imprinting status of these ESTs and determine the relationship, if any, of these genes to the phenotype of PWS.

Depth of Coverage

The initial YAC contig represented a minimal tiling path through the 15q11–q13 region with a one- to fourfold coverage (Mutirangura et al. 1993b). This updated YAC contig has increased the depth of coverage to 3- to 15-fold for most of the q11–q13 region. However, three regions with only one- to twofold coverage are still present on the current map. One lies at the most proximal end of the map, one lies between D15S1259 and 368H3L, and the third lies between D15S822 and D15S156. The most proximal region, including markers D15S1239, A002B45, and D15S912, contains the single YAC clone y931C4. Screening of the CEPH library with these most proximal markers failed to identify any additional YACs. It is possible that this region, which resides near the centromere of chromosome 15, may be under-represented in the YAC libraries. Screening of PAC, BAC, and cosmid libraries may be necessary to complete the physical map of this region. Weak coverage of the centromeric regions has also been observed for chromosomes 7 (Bouffard et al. 1997) and X (Nagaraja et al. 1997). Only the centromeric region of chromosome 10 appears to have been mapped successfully (Jackson et al. 1996).

Two additional regions with poor coverage lie in the same locations as the gaps present in the initial map (Mutirangura et al. 1993b). The region encompassing STSs MN7, A006B10, and A008B26 is associated with one of the four hot spots for chromosome breakage, involved in approximately half of PWS/AS deletion patients and half of the small marker 15 chromosomes (Christian et al. 1995; Huang et al. 1997). This region also demonstrates high genetic recombination, as ovarian teratoma mapping showed a 2.7-cM distance between S541/S542 and S543 (Christian et al. 1995). The low depth of coverage between S822 and S156 is also a region with high recombination frequencies (Robinson and Lalande 1995). The problem of underrepresentation of genomic regions in the YAC libraries has also been observed for other chromosomes (Chumakov et al. 1992; Foote et al. 1992; Qin et al.1996; Bouffard et al. 1997). The potential relationship between high recombination rates and underrepresentation in YAC libraries is unclear at present.

STS Density Determination

The region of common deletion observed in PWS and AS has been estimated to be ~4 Mb by cytogenetic and FISH analysis (Kuwano et al. 1992). On the YAC contig this region extends from NIB1540 to *P* for class I patients or S543 to *P* for class II patients (Kuwano et al. 1992; Christian et al. 1995; S. Christian, unpubl.). Therefore, we estimate the physical distance of the region covered by this contig as ~4 Mb. Using this distance, the current contig with 118 STSs would therefore provide an average spacing of 35 kb. However, some of these STSs cannot be uniquely ordered, as indicated by the brackets in

Figure 1. Using the 83 uniquely ordered groups of STSs provides a larger average spacing of 48 kb between STSs; however, this value is still well within the goal of one STS per 100 kb.

DISCUSSION

The primary impetus for construction of this YAC contig of chromosome 15q11–q13 was the difficulty in integrating the data from multiple sources into a single, cohesive physical map. By analyzing all YACs and STSs from these multiple sources, the current map integrates the genetic and physical maps, resolves the inconsistencies in the order of markers, and places new STRs and ESTs for this region onto a single map.

Integration of Maps and Resolution of Discrepancies

Cytogenetic/FISH Map

The common deletion region observed in PWS extends from 15q11 to q13 and contains the G-positive q12 band and the G-negative q13 band (Ledbetter et al. 1981). This region corresponds to the D15S543–*P* region on the physical map, which has been confirmed by both interphase FISH (Kuwano et al. 1992) and microsatellite analysis (Mutirangura et al. 1993a; Christian et al. 1995). The association of GC-rich regions containing CpG islands with G-negative chromosome bands has been well established (Saccone et al. 1993, 1996). Although it is difficult to definitively integrate the cytogenetic and physical maps, it would be interesting to speculate that the G-positive q12 region correlates with the gene-poor region extending from SGC30582 to *SNRPN*.

Genetic Map

The comprehensive genetic map of the human genome contains >5000 microsatellites, of which 8 are present in chromosome 15q11–q13 (Dib et al. 1996). For almost all markers, there was complete concordance in the order of Genethon markers with the order on the physical map. However, one discrepancy has been resolved by the physical mapping data. This contig places S122 proximal to S210, whereas the genetic map has the order reversed (Dib et al. 1996). Additional fine mapping using PACs and cosmids confirms that S122 is proximal to S210. D15S122 was found to lie within the *UBE3A* genomic region, whereas S210 is located within PAC 14I12 but distal to cosmid 24 (Sutcliffe et al. 1997; T. Kubota, unpubl.).

Additionally, Pàldi et al. (1995) analyzed six Genethon markers relative to the CEPH YACs in the initial contig (Mutirangura et al. 1993b). Although we confirmed the locations published for S128, S122, S210, and S986, the locations for S1035 and S975 differed. Analysis of S1035 placed it in the proximal YACs (yA124A3, yB148C8, and y931C4) rather than y254B5 and y264A1. A comparison of the sequences of S1035 and S542 indicates that these STRs overlap each other, confirming the more proximal location for S1035. D15S975 was found to map only to YACs y897B10 and y781B9, placing it within the *GABRA5*–A149A9L gap of the earlier map.

Genetic vs. Physical Distances

The overall genetic distance of 14.4 cM for the region from S1035 to S156 is significantly larger than expected for a physical distance of ~4 Mb (Dib et al. 1996). Two subregions, which account for a significantly higher genetic distance compared to the physical distance, include S542–S543 (2.7 cM) and S975–S156 (2.3 cM).

Radiation Hybrid Maps

The Whitehead/MIT radiation hybrid map has proven to be a valuable resource that has allowed integration of STSs from multiple genome-wide mapping efforts (Hudson et al. 1995). The general localization of STSs within the 15q11–q13 region on the radiation hybrid map has been accurate; however, additional fine mapping is required to order these markers precisely. Comparison of the Genethon markers between the genetic (Dib et al. 1996) and radiation hybrid maps (Hudson et al. 1995) indicates a discrepancy in the ordering of the markers S128-S122-S156. The radiation hybrid map places the markers in the order S156-S128-S122, whereas the genetic map places the markers in the order S128-S122-S156. Data presented here indicate the marker order as S128-S122-S156, consistent with the genetic map.

The first 26 STSs on the most proximal contig for chromosome 15, WC 15.0 (Hudson et al. 1995), have been placed on this YAC contig. The Whitehead contig does not contain the most proximal region from D15S1239 to D15S128. Additionally, the WC 15.0 contig begins with marker *GABRA5*, located in the distal region of this map, and proceeds with markers S986-S122-S128. FISH experiments have confirmed the correct order of markers within this region as D15S18-S9-S11-S63-S13-S10-

S113-*GABRB3*-S12 (Kuwano et al. 1992). Therefore, this map indicates an inversion of the ordering of the markers present in the most proximal portion of contig WC 15.0.

Disease Gene Identification

An important goal of the Human Genome Project is to map and characterize all human genes and determine how defects in these genes are associated with human disease. Three human diseases, PWS, AS, and oculocutaneous albinism type II (*OCA2*), have known associations with genes within chromosome 15q11–q13. PWS involves the deficiency of one or more paternally expressed genes in this region, AS is associated with deficiencies of *UBE3A* (Kishino et al. 1997; Matsuura et al. 1997), whereas OCA2 is associated with mutations of *P* (Rinchik et al. 1993). In addition, there are several human diseases that have shown linkage to this region, although the specific gene has not yet been identified. These include spastic paraplegia 6 (Fink et al. 1995), obsessive–compulsive disorder (Dykens et al. 1996), bipolar illness (Edenberg et al. 1997), and autism (Cook et al. 1997). The development of a single physical map for chromosome 15q11–q13 with ordered ESTs provides candidate genes for analysis and will allow earlier identification of disease genes localized to this region of the human genome.

METHODS

Chromosome 15 STSs

Five STRs (D15S1364, S1365, S1524, S1525, and S1526) were developed using methods described previously (Mutirangura et al. 1993a). An STS for the left end of YAC 93C9 was obtained using vectorette PCR (Riley et al. 1990; Green 1993). The clones D15S15, S16, and S17 (Tantravahi et al. 1989) were acquired from the ATCC and used to create STSs. All other STSs were identified from previously published papers. Table 1 shows the complete list of all STSs used to develop the map, the GDB amplimer file number to access the PCR primer sequences, GenBank accession numbers for sequence information, if available, and the reference for the STS.

YAC Clones

YAC screening of the CEPH library was performed at the Baylor College of Medicine Genome Center. Other sources of YACs mapping to chromosome 15q11–q13 were identified through YAC contigs developed by CEPH (Cohen et al. 1993; Chumakov et al. 1995) and the Whitehead Institute (Hudson et al. 1995). The YAC clones from the CEPH libraries were acquired from the Baylor College of Medicine Genome Center, the National Human Genome Research Institute (Bethesda, MD), and Research Genetics, Inc. (Huntsville, AL). YACs from the St. Louis libraries were acquired from the Baylor College of Medicine Genome Center.

PCR Analysis

PCR was performed in 10-µl reactions containing 1 µl of Perkin Elmer buffer I, 200 µM dNTP mix, 0.5 µM primers, 0.5 unit of Amplitaq Gold (Perkin Elmer, Foster City, CT) and 5–25 ng of YAC DNA or 20–40 ng of genomic DNA contol. The reactions were carried out in a Perkin Elmer 9600 thermocycler using a program of initial denaturation at 95°C for 4 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 50°C–55°C for 30 sec, extension at 72°C for 30 sec, followed by a final extention at 72°C for 5 min. The authenticity of the PCR analysis was monitored using genomic DNA as a positive control. The reaction products were separated on a 2.0% agarose gel, stained with ethidium bromide, and visualized using a UVP GDS8000 gel documentation system.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Eric Green for critical reading of the manuscript.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked ''advertisement'' in accordance with 18 USC section 1734 solely to indicate this fact.

REFERENCES

Adamson, D., H. Albertsen, L. Ballard, P. Bradley, M. Carlson, P. Cartwright, C. Council, T. Elsner, D. Fuhrman, S. Gerken et al. 1995. A collection of ordered tetranucleotide-repeat markers from the human genome. *Am. J. Hum. Genet.* 57: 619–628.

Albrecht, U., J.S. Sutcliffe, B.M. Cattanach, C.V. Beechey, D. Armstrong, G. Eichele, and A.L. Beaudet. 1997. Imprinted expression of the murine Angelman syndrome gene *Ube3a* in hippocampal and Purkinje neurons. *Nature Genet.* 17: 75–78.

Beckmann, J.S., J. Tomfohrde, R.I. Barnes, M. Williams, O. Broux, I. Richard, J. Weissenbach, and A.M. Bowcock. 1993. A linkage map of human chromosome 15 with an average resolution of 2 cM and containing 55 polymorphic microsatellites. *Hum. Mol. Genet.* 2: 2019–2030.

Bouffard, G.G., J.R. Idol, V.V. Braden, L.M. Iyer, A.F. Cunningham, L.A. Weintraub, J.W. Touchman, R.M. Mohr-Tidwell, D.C. Peluso, R.S. Fulton et al. 1997. A physical map of human chromosome 7: An integrated YAC contig map with average STS spacing of 79 kb. *Genome Res.* 7: 673–692.

Buiting, K., V. Greger, B.H. Brownstein, R.M. Mohr, I. Voiculescu, A. Winterpacht, B. Zabel, and B. Horsthemke. 1992. A putative gene family in 15q11-13 and 16p11.2: Possible implications for Prader-Willi and Angelman syndromes. *Proc. Natl. Acad. Sci.* 89: 5457–5461.

Buiting, K., S. Saitoh, S. Gross, B. Dittrich, S. Schwartz, R.D. Nicholls, and B. Horsthemke. 1995. Inherited microdeletions in the Angelman and Prader-Willi syndromes define an imprinting centre on human chromosome 15. *Nature Genet.* 9: 395–400.

Christian, S.L., W.P. Robinson, B. Huang, A. Mutirangura, M.R. Line, M. Nakao, U. Surti, A. Chakravarti, and D.H. Ledbetter. 1995. Molecular characterization of two proximal deletion breakpoints in both Prader-Willi and Angelman syndrome patients. *Am. J. Hum. Genet.* 57: 40–48.

Chumakov, I., P. Rigault, S. Guillou, P. Ougen, A. Billaut, G. Guasconi, P. Gervy, I. LeGall, P. Soularue, L. Grinas et al. 1992. Continuum of overlapping clones spanning the entire human chromosome 21q. *Nature* 359: 380–387.

Chumakov, I.M., P. Rigault, I. Le Gall, C. Bellanne-Chantelot, A. Billault, S. Guillou, P. Soularue, G. Guasconi, E. Poullier, I. Gros et al. 1995. A YAC contig of the human genome. *Nature* (Suppl.) 377: 175–297.

Cohen, D., I. Chumakov, and J. Weissenbach. 1993. A first-generation physical map of the human genome. *Nature* 366: 689–701.

Cook, E.H., Jr., V. Lindgren, B.L. Leventhal, R. Courchesne, A. Lincoln, C. Shulman, C. Lord, and E. Courchesne. 1997. Autism or atypical autism in maternally but not paternally derived proximal 15q duplication. *Am. J. Hum. Genet.* 60: 928–934.

Dib, C., S. Faure, C. Fizames, D. Samson, N. Drouot, A. Vignal, P. Millasseau, S. Marc, J. Hazan, E. Seboun et al. 1996. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380: 152–154.

Donlon, T.A., M. Lalande, A. Wyman, G. Bruns, and S.A. Latt. 1986. Isolation of molecular probes associated with the chromosome 15 instability in the Prader-Willi syndrome. *Proc. Natl. Acad. Sci.* 83: 4408–4412.

Driscoll, D.J., M.F. Waters, C.A. Williams, R.T. Zori, C.C. Glenn, K.M. Avidano, and R.D. Nicholls. 1992. A DNA methylation imprint, determined by the sex of the parent, distinguishes the Angelman and Prader-Willi syndromes. *Genomics* 13: 917–924.

Dykens, E.M., J.F. Leckman, and S.B. Cassidy. 1996. Obsession and compulsions in Prader-Willi syndrome. *J. Child Psychol. Psychiatry Allied Discip.* 37: 995–1002.

Dyomin, V.G., P.H. Rao, R. Dalla-Favera, and R.S.K. Chaganti. 1997. BCL8, a novel gene involved in translocations affecting band 15q11-q13 in diffuse large-cell lymphoma. *Proc. Natl. Acad. Sci.* 94: 5728–5732.

Edenberg, H.J., T. Foroud, P.M. Conneally, J.J. Sorbel, K. Carr, C. Crose, C. Willig, J. Zhao, M. Miller, E. Bowman et al. 1997. Initial genomic scan of the NIMH genetics initiative bipolar pedigrees: Chromosomes 3, 5, 15, 16, 17, and 22. *Am. J. Med. Genet.* 74: 238–246.

Fink, J.K., C.-t.B. Wu, S.M. Jones, G.B. Sharp, B.M. Lange, A. Lesicki, T. Reinglass, T. Varvil, B. Otterud, and M. Leppert. 1995. Autosomal dominant familial spastic paraplegia: Tight linkage to chromosome 15q. *Am. J. Hum. Genet.* 56: 188–192.

Foote, S.D. Vollrath, A. Hilton, and D.C. Page. 1992. The human Y chromosome: Overlapping DNA clones spanning the euchromatic region. *Science* 258: 60–66.

Gardner, J.M., Y. Nakatsu, Y. Gondo, S. Lee, M.F. Lyon, R.A. King, M.H. Brilliant. 1992. The mouse pink-eyed dilution gene: Association with human Prader-Willi and Angelman syndromes. *Science* 257: 1121–1124.

Glatt, K., D. Sinnett, and M. Lalande. 1994. The human γ-aminobutyric acid receptor subunit β3 and α5 gene cluster in chromosome 15q11–q13 is rich in highly polymorphic $(CA)_n$ repeats. *Genomics* 19: 157–160.

Glatt, K., H. Glatt, and M. Lalande. 1997. Structure and organization of GABRB3 and GABRA5. *Genomics* 41: 63–69.

Glenn, C.C., K.A. Porter, M.T.C. Jong, R.D. Nicholls, and D.J. Driscoll. 1993. Functional imprinting and epigenetic modification of the human SNRPN gene. *Hum. Mol. Genet.* 2: 1377–1382.

Green, E.D. 1993. Physical mapping of human chromosomes: generation of chromosome-specific sequence-tagged sites. In *Methods in molecular-genetics, vol. 1: Gene and chromosome analysis (part A)* (ed. K.W. Adolph), pp. 192–210. Academic Press, San Diego, CA.

Green, E.D. and M.V. Olson. 1990. Chromosomal region of the cystic fibrosis gene in yeast artificial chromosomes: A model for human genome mapping. *Science* 250: 94–98.

Greger, A., E. Woolf, and M. Lalande. 1993. Cloning of the breakpoints of a submicroscopic deletion in an Angelmen syndrome patient. *Hum. Mol. Genet.* 2: 921–924.

Greger, V., J.H.M. Knoll, E. Woolf, K. Glatt, R.F. Tyndale, T.M. DeLorey, R.W. Olsen, A.J. Tobin, J.M. Sikela, Y. Nakatsu et al. 1995. The γ-aminobutyric acid receptor γ3 subunit gene (GABRG3) is tightly linked to the α5 subunit gene (GABRA5) on human chromosome 15q11-q13 and is transcribed in the same orientation. *Genomics* 26: 258–264.

Gyapay, G., J. Morissette, A. Vignal, C. Dib, C. Fizames, P. Millasseau, S. Marc, G. Bernardi, M. Lathrop, and J. Weissenbach. 1994. The 1993–1994 Genethon human genetic linkage map. *Nature Genet.* 7: 246–249.

Huang, B., J.A. Crolla, S.L. Christian, M.E. Wolf-Ledbetter, M.E. Macha, P.N. Papenhausen, and D.H. Ledbetter. 1997. Refined molecular characterization of the breakpoints in small inv dup(15) chromosomes. *Hum. Genet.* 99: 11–17.

Hudson, T.J., L.D. Stein, S.S. Gerety, J. Ma, A.B. Castle, J. Silva, D.K. Slonim, R. Baptista, L. Kruglyak, S.-H. Xu et al. 1995. An STS-based map of the human genome. *Science* 270: 1945–1954.

Jackson, M.S., C.G. See, L.M. Mulligan, and B.F. Lauffart. 1996. A 9.75-Mb map across the centromere of human chromosome 10. *Genomics* 33: 258–270.

Kishino, T. M. Lalande, and J. Wagstaff. 1997. UBE3A/E6-AP mutations cause Angelman syndrome. *Nature Genet.* 15: 70–73.

Kuwano, A., A. Mutirangura, B. Dittrich, K. Buiting, B. Horsthemke, S. Saitoh, N. Niikawa, S.A. Ledbetter, F. Greenberg, A.C. Chinault, and D.H. Ledbetter. 1992. Molecular dissection of the Prader-Willi/Angelman syndrome region (15q11–13) by YAC cloning and FISH analysis. *Hum. Mol. Genet.* 1: 417–425.

Ledbetter, D.H., V.M. Riccardi, S.D. Airhart, R.J. Strobel, B.S. Keenan, and J.D. Crawford. 1981. Deletions of chromosome 15 as a cause of the Prader-Willi syndrome. *New Engl. J. Med.* 304: 325–339.

Lindeman, R., S. Kouts, T. Woodage, A. Smith, and R.J. Trent. 1991. Dinucleotide repeat polymorphism near D15S10 in the Prader-Willi chromosome region (PWCR). *Nucleic Acids Res.* 19: 5449.

MacDonald, H.R. and R. Wevrick. 1997. The necdin gene is deleted in Prader-Willi syndrome and is imprinted in human and mouse. *Hum. Mol. Genet.* 6: 1873–1878.

Matsuura, T., J.S. Sutcliffe, P. Fang, R.-J. Galjaard, Y.-H. Jiang, C.S. Benton, J.M. Rommens, and A.L. Beaudet. 1997. De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. *Nature Genet.* 15: 74–77.

Mutirangura, A., S.A. Ledbetter, A. Kuwano, A.C. Chinault, and D.H. Ledbetter. 1992a. Dinucleotide repeat polymorphism at the $GABA_A$ receptor β3 (GABRB3) locus in the Angelman/Prader-Willi region (AS/PWS) of chromosome 15. *Hum. Mol. Genet.* 1: 67.

Mutirangura, A., A. Kuwano, S.A. Ledbetter, A.C. Chinault, and D.H. Ledbetter. 1992b. Dinucleotide repeat polymorphism at the D15S11 locus in the Angelman/Prader-Willi region (AS/PWS) of chromosome 15. *Hum. Mol. Genet.* 1: 139.

Mutirangura, A., F. Greenberg, M.G. Butler, S. Malcolm, R.D. Nicholls, A. Chakravarti, and D.H. Ledbetter. 1993a. Multiplex PCR of three dinucleotide repeats in the Prader-Willi/Angelman critical region (15q11–13): Molecular diagnosis and mechanism of uniparental disomy. *Hum. Mol. Genet.* 2: 143–151.

Mutirangura, A., A. Jayakumar, J.S. Sutcliffe, M. Nakao, M.J. McKinney, K. Buiting, B. Horsthemke, A.L. Beaudet, A.C. Chinault, and D.H. Ledbetter. 1993b. A complete YAC contig of the Prader-Willi/Angelman chromosome region (15q11–q13) and refined localization of the SNRPN gene. *Genomics* 18: 546–552.

Nagaraja, R.S., MacMillan, J. Kere, C. Jones, S. Griffin, M. Schmatz, J. Terrell, M. Shomaker, C. Jermak, C. Hott et al. 1997. X chromosome map at 75-kb STS resolution, revealing extremes of recombination and GC content. *Genome Res.* 7: 210–222.

Nakao, M., J.S. Sutcliffe, B. Durtschi, A. Mutirangura, D.H. Ledbetter, and A.L. Beaudet. 1994. Imprinting analysis of three genes in the Prader-Willi/Angelman region: SNRPN, E6-associated protein, and PAR-2 (D15S225E). *Hum. Mol. Genet.* 3: 309–315.

Ning, Y., A. Roschke, S.L. Christian, J. Lesser, J. Sutcliffe, and D.H. Ledbetter. 1996. Identification of a novel paternally expressed transcript adjacent to SNRPN in the Prader-Willi syndrome critical reigon. *Genome Res.* 6: 742–746.

Olson, M., L. Hood, C. Cantor, and D. Botstein. 1989. A common language for physical mapping of the human genome. *Science* 245: 1434–1435.

Pàldi, A., G. Gyapay, and J. Jami. 1995. Imprinted chromosomal regions of the human genome display sex-specific meiotic recombination frequencies. *Curr. Biol.* 5: 1030–1035.

Qin, S., N.J. Nowak, J. Zhang, S.N.J. Sait, P.G. Mayers, M.J. Higgins, Y.-J. Cheng, L. Li, D.J. Munroe, D.S. Gerhard et al. 1996. A high-resolution physical map of human chromosome 11. *Proc. Natl. Aca. Sci.* 93: 3149–3154.

Reis, A., B. Dittrich, B. Greger, K. Buiting, M. Lalande, G. Gillessen-Kaesbach, M. Anvret, and B. Horsthemke. 1994. Imprinting mutations suggested by abnormal DNA methylation patterns in familial Angelman and Prader-Willi syndromes. *Am. J. Hum. Genet.* 54: 741–747.

Richard, I., O. Broux, N. Chiannilkulchai, F. Gougerousse, V. Allamand, N. Bourg, L. Brenguier, C. Devaud, P. Pasturaud, C. Roudaut et al. 1994. Regional localization of human chromosome 15 loci. *Genomics* 23: 619–627.

Riley, J., R. Butler, D. Ogilvie, R. Finniear, D. Jenner, S. Powell, R. Anand et al. 1990. A novel, rapid method for the isolation of terminal sequences from yeast artificial chromosome (YAC) clones. *Nucleic Acids Res.* 18: 2887–2890.

Rinchik, E.M., S.J. Bultman, B. Horsthemke, S.-T. Lee, K.M. Strunk, R.A. Spritz, K.M. Avidano, M.T.C. Jong, and R.D. Nicholls. 1993. A gene for the mouse pink-eyed dilution locus and for human type II oculocutaneous albinism. *Nature* 361: 72–76.

Robinson, W.P., and M. Lalande. 1995. Sex-specific meiotic recombination in the Prader-Willi/Angelman syndrome imprinted region. *Hum. Mol. Genet.* 4: 801–806.

Robinson, W.P., B. Horstkemke, S. Leonard, S. Malcolm, C. Morton, R.D. Nicholls, R.J. Ritchie, P. Rogan, R. Schultz, S. Schwartz et al. 1997. Report of the third international workshop on human chromosome 15 mapping 1996. *Cytogenet. Cell. Genet.* 76: 1–13.

Rougeulle, C., H. Glatt, and M. Lalande. 1997. The Angelman syndrome candidate gene, *UBE3A/E6-AP*, is imprinted in brain. *Nature Genet.* 75: 14–15.

Saccone, S., A. De Sario, J. Wiegant, A.K. Raap, G. Della Valle, and G. Bernardi. 1993. Correlations between isochores and chromosomal bands in the human genome. *Proc. Natl. Acad. Sci.* 90: 11929–11933.

Saccone, S. S. Caccio, J. Kusada, L. Andreozzzi, G. Bernardi.

1996. Identification of the gene-richest bands in human chromosomes. *Gene* 174: 85–94.

Schuler, G.D., M.S. Boguski, L.D. Stewart, G. Stein, K. Gyapay, K. Rice, R.E. White, P. Rodriguez-Tome, A. Aggarwal, E. Bajorek et al. 1996. A gene map of the human genome. *Science* 274: 540–546.

Sheffield, V.C., J.L. Weber, K.H. Buetow, J.C. Murray, D.A. Even, K. Wiles, J.M. Gastier, J.C. Pulido, C. Yandava, S.L. Sunden et al. 1995. A collection of tri- and tetranucleotide repeat markers used to generate high quality, high resolution human genome-wide linkage maps. *Hum. Mol. Genet.* 4: 1837–1844.

Stewart, E.A., K.B. McKusick, A. Aggarwal, W. Bajorek, S. Brady, A. Chu, N. Fang, D. Hadley, M. Harris, S. Hussain et al. 1997. An STS-based radiation hybrid map of the human genome. *Genome Res.* 7: 422–433.

Sutcliffe, J.S., M. Nakao, S. Christian, K.H. Orstavik, N. Tommerup, D.H. Ledbetter, and A.L. Beaudet. 1994. Deletions of a differentially methylated CpG island at the SNRPN gene define a putative imprinting control region. *Nature Genet.* 8: 52–58.

Sutcliffe, J.S., Y.-H. Jiang, R.-J. Galjaard, T. Matsuura, P. Fang, T. Kubota, S.L. Christian, J. Bressler, B. Cattanach, D.H. Ledbetter, and A.L. Beaudet. 1997. The E6-AP ubiquitin-protein ligase (UBE3A) gene is localized within a narrowed Angelman syndrome critical reigon. *Genome Res.* 7: 368–377.

Sutcliffe, J.S., M. Han, S.L. Christian, and D.H. Ledbetter. 1997. Neuronally-expressed necdin gene: An imprinted candidate gene in Prader-Willi syndrome. *Lancet* 350: 1520–1521.

Tantravahi, W., R.D. Nicholls, H. Stroh, S. Ringer, R.L. Neve, L. Kaplan, R. Wharton, D. Wurster-Hill, J.M. Graham Jr., E.S. Cantu et al. 1989. Quantitative calibration and use of DNA probes for investigating chromosome abnormalities in the Prader-Willi syndrome. *Am. J. Med. Genet.* 33: 78–87.

Vu, T.H. and A.R. Hoffman. 1997. Imprinting of the Angelman syndrome gene, *UBE3A,* is restricted to brain. *Nature Genet.* 75: 12–13.

Wevrick, R., J.A. Kerns, and U. Francke. 1994. Identification of a novel paternally expressed gene in the Prader-Willi syndrome region. *Hum. Mol. Genet.* 3: 1877–1882.

Received October 16, 1997; accepted in revised form January 5, 1998.