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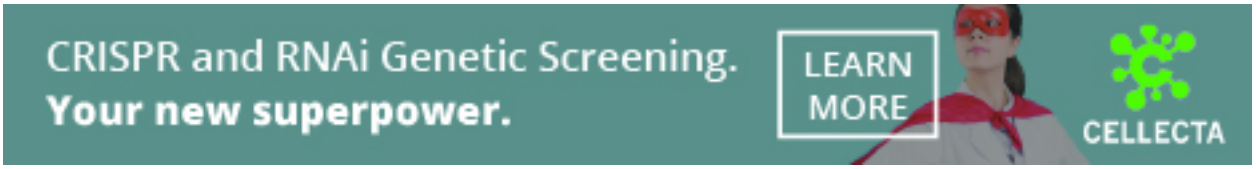

RESEARCH

Construction of a YAC Contig Encompassing the Usher Syndrome Type 1C and Familial Hyperinsulinism Loci on Chromosome 11p14–15.1

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The Usher syndrome type 1C (USH1C) and familial hyperinsulinism (HI) loci have been assigned to chromosome 11p14–15.1, within the interval D11S419–D11S1310. We have constructed a yeast artificial chromosome (YAC) contig, extending from D11S926 to D11S899, which encompasses the critical regions for both USH1C and HI and spans an estimated genetic distance of ≈4 cM. A minimal set of six YAC clones constitute the contig, with another 22 YACs confirming the order of sequence-tagged sites (STSs) and position of YACs on the contig. A total of 40 STSs, including 10 new STSs generated from YAC insert-end sequences and inter-*Alu* PCR products, were used to order the clones within the contig. This physical map provides a resource for identification of gene transcripts associated with USH1C, HI, and other genetic disorders that map to the D11S926–D11S899 interval.

Human chromosome 11p has been the focus of extensive studies as it contains a number of genes involved in disorders of clinical significance. These disorders include the WAGR (Wilm's tumor, aniridia, genitourinary abnormalities, mental retardation) syndrome (Rosier et al. 1994), Beckwith-Weidemann syndrome (Ping et al. 1989), Romano-Ward long-QT syndrome type 1 (Keating et al. 1991), atrophia areata syndrome (Fossdal et al. 1995), Usher syndrome type 1C (USH1C; Smith et al. 1992), and familial hyperinsulinism (HI; Glaser et al. 1994; Thomas et al. 1995a). In addition, evidence for loss of heterozygosity on chromosome 11p has implicated this region in various human neoplasias such as Wilm's tumor, rhabdomyosarcoma, hepatoblastoma, bladder carcinoma, and breast cancer (Seizinger et al. 1991). The interval between the microsatellite markers D11S419 and D11S1310 on 11p14–15.1 encompasses the critical regions for both USH1C and HI. USH1C is an autosomal recessive disorder characterized by profound congenital sensorineural deafness, vestibular dysfunction and progressive pigmentary retinopathy with noticeable visual loss occurring in childhood or adolescence. Initially the USH1C locus was mapped by genetic linkage analyses in the French-Acadian population to a ≈3 cM region flanked by D11S861 and D11S899 (Ayyagari et al. 1994; Keats et al. 1994). Recently, the location for USH1C was refined to the interval between D11S902 and D11S1888 (Ayyagari et al. 1995; Marietta et al. 1996). Familial HI is characterized by excessive insulin secretion in the presence of severe hypoglycemia and occurs predominantly in neonates. Genetic linkage analyses localized

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the HI gene to the interval between D11S419 and D11S1310 (Glaser et al. 1995), which also encompasses the USH1C critical region. Although mutations in the gene encoding the sulfonylurea receptor (SUR) were recently shown to be associated with HI (Thomas et al. 1995b), the precise physical location of this gene has not yet been described.

Several physical (Compton et al. 1988; Gessler and Bruns 1989; Rose et al. 1990; Cohen et al. 1993; Redeker et al. 1994; Sellar et al. 1994; Chumakov et al. 1995; Gawin et al. 1995; Quackenbush et al. 1995), genetic (Weissenbach et al. 1992; Litt et al. 1993; Gyapay et al. 1994; Litt et al. 1995), and radiation hybrid (RH) (James et al. 1994) maps of chromosome 11 have been constructed. Fantes et al. (1995) described an integrated physical, cytogenetic, and genetic map of the distal p13 to proximal p15.1 region of chromosome 11 in which several cosmids and yeast artificial chromosomes (YACs) were identified and localized to the D11S861–D11S899 interval. More recently, progress towards construction of physical maps spanning the entire chromosome 11 has been reported (Chumakov et al. 1995; Quackenbush et al. 1995). Quackenbush et al. (1995) used 455 sequence-tagged sites (STSs) to identify 909 chromosome 11 mega-YAC clones that were assembled into 109 contig islands. These contigs, of which 90 comprise two or more YAC clones, are estimated to span a total length of 203 Mb and thus, potentially represent ≈160% coverage of chromosome 11 (Quackenbush et al. 1995). Chumakov et al. (1995) assembled YAC contigs of chromosome 11, primarily by STS content mapping of YACs for genetic markers and confirmed overlaps between clones by *Alu*–PCR hybridization and fingerprint analyses.

In previous studies, we localized the HI and USH1C loci to the D11S419–D11S1310 and D11S902–D11S1888 intervals, respectively (Ayyagari et al. 1994; Glaser et al. 1995). To facilitate identification of potential candidate genes for these disorders, we initiated construction of a YAC contig as a first stage towards assembly of a high-resolution cosmid and/or P1 contig and transcript map of the D11S419–D11S1310 interval. Although the physical map of Chumakov et al. (1995) provided a framework for the construction of such contigs, the density of markers used to order YAC clones encompassing the D11S419–D11S1310 region was sparse, so that the extent of overlap between clones and the localization of other available STSs suitable for screening cosmid and P1 libraries was not defined. In this report we describe the construction of a YAC contig, extending from D11S926 to D11S899, which encompasses both the HI and USH1C critical regions. A total of 28 YACs were analyzed for the presence of 40 STSs, providing an average STS resolution of ≈1/0.1 cM. Twelve of these STSs are microsatellite markers, 12 are derived from previously identified genes, and 10 are new chromosome 11-specific STSs developed from YAC insert-terminal and inter-*Alu* PCR product sequences. The data described here verifies and expands upon other physical maps of this region (Sellar et al. 1994; Chumakov et al. 1995). The development of this physical map should provide a useful resource for construction of a high-resolution physical map and transcript map of the D11S926–D11S899 region and thus, facilitate the identification of additional candidate genes for USH1C and HI.

RESULTS

Isolation of YAC Clones

The Centre d'Etude du Polymorphisme Humain (CEPH) libraries (Albertsen et al. 1990; P. Ougen, pers. comm.) were initially screened with six microsatellite markers, D11S861, D11S419, D11S902, D11S921, D11S1310 and D11S899, located within or flanking the proximal or distal boundaries of the USH1C and HI critical regions (Ayyagari et al. 1995; Glaser et al. 1995; Marietta et al. 1996). A total of 47 YAC clones were identified as positive for at least one of the six STSs used for screening. A subset of these YACs were selected for further construction of the contig on the basis of size and apparent lack of large deletions. Potential overlap between these YACs was examined by PCR analysis of all clones for the presence or absence of each of the six markers used for library screening. With the exceptions of YACs 966e8 and 776e7, each YAC was positive only for the STS used in its isolation (Fig. 1). YAC 966e8 was positive for both D11S902 and D11S921, and YAC 776e7 contained D11S902, D11S921, and D11S1310. None of those clones positive for D11S861 (770c6, 808b11, 24a6, 477e9) were positive for D11S419. Both D11S861 and D11S902 were absent from D11S419-positive clones (916b5, 742c8, 628e5, 652c9, 724e6). Similarly, D11S899 was not amplified from those clones positive for D11S1310 (776e7, 693b10)

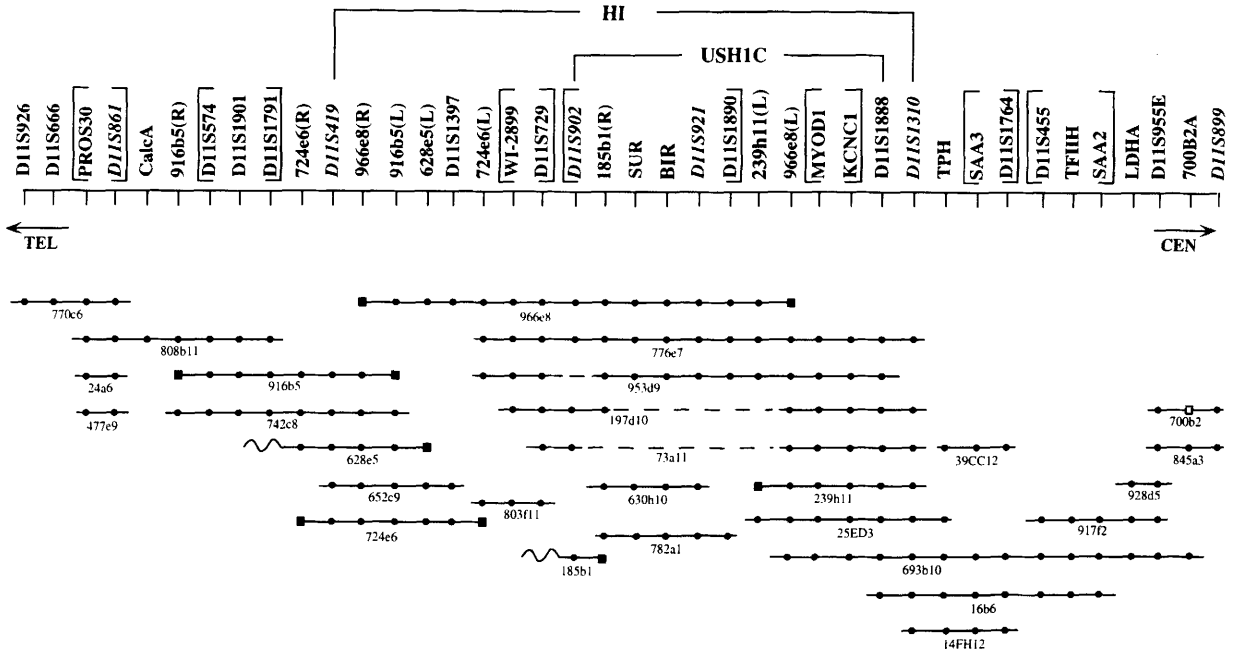

Figure 1 YAC contig encompassing the USH1C and HI loci. YAC clones are represented by horizontal lines. (●) The YAC was amplified by the STS listed directly above. STSs developed from sequences for YAC insert-termini or inter-*Alu* PCR products are represented by ■ and □, respectively. Dashed lines indicate regions of YACs that were negative by PCR analysis for the STS listed above, such YACs may contain internal deletions, rearrangements, or chimeric regions. Uneven lines denote chimeric YAC end-sequences. STSs are placed equidistant to each other as the physical distance between adjacent STSs has not been determined. Adjacent STSs placed within brackets are those for which the relative orders could not be established definitively from YAC STS-content analyses. The six microsatellite markers used in initial screens of the CEPH YAC libraries are shown in italics. Data are shown for a subset of the total number of YACs identified by YAC library screening.

and D11S1310 was absent from clones positive for D11S899 (845a3, 700b2).

STS Development

As the density of microsatellite markers in the D11S861–D11S899 interval was initially insufficient to establish map closure, new STSs were developed from YAC end-fragment sequences of six clones and from inter-*Alu* PCR product sequences to (1) determine overlap between clones, (2) screen YAC libraries for additional clones, and (3) investigate whether selected YACs were chimeric. To establish overlap between YACs positive for D11S419 and those positive for D11S902 or D11S861, STSs were developed from the left- and right-end insert terminal sequences of YACs 916b5, 628e5, 724e6, 966e8, and 185b1. An additional STS was developed from the left-end insert terminal sequence of YAC 239h11 and an STS was also derived from the inter-*Alu* PCR product of YAC 700b2 to detect overlap between D11S1310–positive and D11S899–positive clones. The chromosomal location of each STS was determined by PCR analysis of a rodent/human somatic cell hybrid (SCH) panel [National Institutes of General Medical Sciences (NIGMS)]. With the exceptions of the STSs 185b1(L) and 628e5(R), all STSs were specific for chromosome 11 (Table 1). The STS 185b1(L) was assigned to chromosomes 1 and 12, whereas 628e5(R) mapped to chromosome 10, indicating chimerism in YACs 185b1 and 628e5, respectively. All 10 chromosome 11-specific STSs as well as adjacent microsatellite markers were regionally assigned to the appropriate location on chromosome 11 (intervals f or g) by PCR analysis of the J1 SCH panel (Glaser et al. 1989) (Fig. 2). These results are consistent with the previously described genetic and cytogenetic locations (Weissenbach et al. 1992; Gyapay et al. 1994; Litt et al. 1995) of adjacent genetic markers. Comparison of these new STSs with sequences deposited in the EMBL/

Table 1. Primer Sequences and Chromosomal Assignments for STSs Developed from YAC Insert-terminal Sequences and Inter-*Alu* PCR Products

YAC [a]	STS [b]	PRIMER SEQUENCE (5'-3')	Annealing Temp.(°C)	Size (bp)	Chromosomal Location [c]
185b1	185b1(L)	FOR: TGACTGGGTTTCTTCACTTG REV: GGATGGATAGTTATCCAGAAAG	60	177	1, 12
	185b1(R)	FOR: GTGCAGACATTGCTAATTGTTC REV: CCTGGCCTAGTTTTATCTCATG	60	125	11
966e8	966e8(L)	FOR: AGAAATGTCTGGAACATACCAG REV: CAGATCTCTCCTCAGCCTTG	58	150	11
	966e8(R)	FOR: CTTTACCATGAGAACAGTATGG REV: CCTGTGCACAAAAGAGAAAAC	60	174	11
916b5	916b5(L)	FOR: TAAAGTGTTGGACACATAGCTG REV: GTCATTGTAGCTACATGTACCC	60	125	11
	916b5(R)	FOR: ATTTGTATTTCCTGGCATGC REV: TGGGGTCCAAAGATAGTATAAC	55	307	11
724e6	724e6(L)	FOR: AGGGAGGCTATAGTAAGCAAAC REV: TCAGCTAGTAGGAGTGAAATTG	55	195	11
	724e6(R)	FOR: TATCAGCAGTATTTAGCAGTG REV: CTAAGGAGGTAATGCTTTGT	58	91	11
628e5	628e5(L)	FOR: GATTCCAGGATAGCATGACAG REV: GCCCTTTTATACCAGTGAAAAG	60	169	11
	628e5(R)	FOR: TTATTGTGCTACAGATGTTGGC REV: GCTTACCCAAATAGATTGCC	58	107	10
700b2	700b2A	FOR: TCCTCTACCCAATATGCTCC REV: CAAGGAGATAGTGCCAACAGC	52	80	11
239h11	239h11(L)	FOR: ATGGGGAAATGAGAGATGTCG REV: CGGAATTAATTTGCGAAATGA	50	90	11

[a]YAC names refer to the plate number and position of the clone within CEPH YAC libraries.
[b]STSs are designated as the YAC name followed by the suffix A for STSs derived from inter-*Alu* PCR products or by the suffix L or R (in parenthesis) for STSs developed from YAC-end fragments, denoting the left or right arms, respectively, of the YAC vector.
[c]Chromosomal assignments were determined by PCR analysis of a rodent/human somatic cell hybrid mapping panel (NIGM).

GenBank data base failed to reveal significant levels of homology to any previously described gene (data not shown). The STS 966e8(L) was used to rescreen both the CEPH mega-YAC (P. Ougen, pers. comm.) and Washington University YAC (Brownstein et al. 1989) libraries, resulting in the identification of four additional clones that were incorporated into the contig.

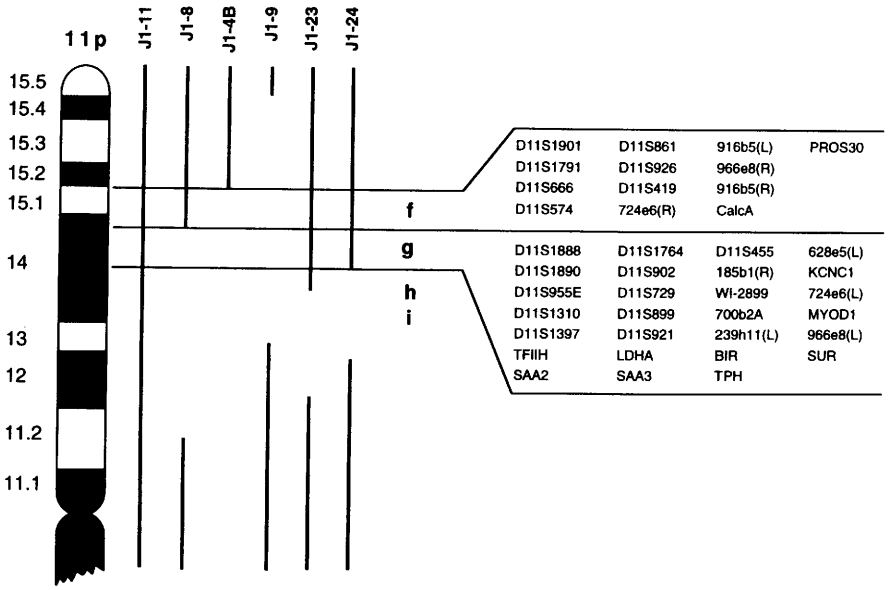

Figure 2 Regional localization of STSs used in YAC STS content analyses. STSs were assigned, by PCR analysis, to intervals on chromosome 11p based on their presence or absence in six hybrid cell lines of the J1 series (Glaser et al. 1989). Bold vertical lines represent portions of chromosome 11p retained in each hybrid. Absence of such lines indicates deleted regions within chromosome 11p as characterized by Glaser et al. (1989). Regions of chromosome 11q retained in these cell lines are not indicated.

YAC STS-content Analysis and Contig Closure

To establish closure of the contig, each YAC was screened for the presence of the 10 novel chromosome 11 STSs. YACs 808b11, 916b5, and 742c8 were amplified by the STS 916b5(R), establishing closure of the gap between D11S861 and D11S419. The STSs 916b5(L), 628e5(L), and 724e6(L) were present on YAC 966e8 as well as the respective parent YACs, allowing closure of the contig between D11S419 and D11S902. Overlap between YACs positive for D11S419 and those containing D11S902 was further verified by the presence of the STS 966e8(R) on all YACs positive for D11S419 (916b5, 742c8, 628e5, 652c9, 724e6). Continuity of the physical map between D11S1310 and D11S899 was demonstrated by the presence of the STS 700b2A on YACs 693b10, 845a3, and 700b2.

During construction of the contig, an additional 23 STSs, including six microsatellite markers (D11S1901, D11S1791, D11S1397, D11S1890, D11S1888, D11S1764), were assigned by RH mapping to the D11S926–D11S899 interval (James et al. 1994). YACs 25ED3, 14FH12, and 39CC12, reported to contain some of these additional STSs (Sellar et al. 1994), were obtained from the ICI Diagnostics YAC library (Anand et al. 1990) and were integrated into the existing contig (Fig. 1). To further establish the degree of overlap between individual YACs and also to define their STS content, all YACs were screened by PCR analysis for the presence or absence of a total of 40 STSs, including the six STSs used for initial screening of YAC libraries. Results of the STS-content screening are summarized in Figure 1 and Table 2. The insert sizes of selected YACs were determined by pulsed-field gel electrophoresis (PFGE) (Table 2).

Figure 1 shows a contiguous set of 28 YAC clones that extends from D11S926 to D11S899, encompassing both the USH1C and HI loci. The minimal contig is defined by the six overlapping YACs 770c6, 808b11, 916b5, 966e8, 693b10, and 700b2.

DISCUSSION

We have assembled a YAC contig that extends from D11S926 through D11S899, encompassing both the HI and USH1C loci. This physical map comprises 28 YACs and incorporates 40 STSs, spanning an estimated genetic distance of ≈4 cM (Gyapay et al. 1994). Continuity of the contig was established by STS-content analysis of YACs, including STSs developed from YAC end-fragments and inter-*Alu* PCR products. Physical overlap was also detected by comparison of inter-*Alu* or L1 PCR fingerprints (data not shown). The contig is ordered on the genetic map by seven previously mapped microsatellite markers [11pter–D11S926–D11S861–D11S419–(D11S902–D11S921)–D11S1310–D11S899–11pcen] (Weissenbach et al. 1992; Gyapay et al. 1994; James et al. 1994). With the exceptions of the (PROS30, D11S861)–916b5(R), (D11S1764, SAA3)–(D11S455, TFIIH, SAA2) and 700b2A–D11S899 intervals, the density of YACs comprising the contig is at least threefold. All YACs com-

Table 2. STS Content of YACs

CLONE	Insert size (kb)	D11S926	D11S666	PROS30	D11S861	CalcA	916b5(R)	D11S574	D11S1901	D11S1791	724e6(R)	D11S419	966e8(R)	916b5(L)	628e5(L)	D11S1397	724e6(L)	WI-2899	D11S729	D11S902	1855b1(R)	SUR	BIR	D11S921	D11S1890	239h11(L)	966e8(L)	MYOD1	KCNC1	D11S1888	D11S1310	TPH	D11S1764	SAA3	D11S455	TFIIH	SAA2	LDHA	D11S955E	700B2A	D11S899
770c6	270	+	+	+	+																																				
24A6			+	+																																					
477e9	360		+	+																																					
808b11	1350			+	+	+	+	+	+	+																														
742c8	1400						+	+	+	+	+	+	+	+																										
916b5	1400						+	+	+	+	+	+	+	+																										
628e5	750, 285														+	+																								
724e6	785										+	+	+	+	+	+	+																							
652c9	1100											+	+	+	+	+																							
966e8	2400												+	+	+	+			+	+							+	+											
776e7	680																		+	+							+	+				+							
803f11	1000																		+	+	+																		
953d9	1100																		+	+	+	+					+	+				+							
73a11	460																			+	+	+					+	+				+							
197d10	300																		+	+	+	+					+	+				+							
185b1	440																			+		+																	
782a1	1600																					+			+														
630h10	560, 450																					+			+														
239h11	530																									+	+	+	+	+	+								
25ED3	610																									+	+	+	+	+	+	+							
693b10	>2000																										+	+	+	+	+	+	+							
16b6	670																													+	+		+	+	+	+			
14FH12	385																																	+						
39CC12																																								
917f2																																			+						
928d5																																				+	+				
700b2	750																																					+	+	+	+
845a3	500																																					+	+	+	+

Data are shown for a subset of the total number of YACs analyzed by STS content screening.
A plus sign (+) indicates that a YAC was positive by PCR analysis for the STS listed directly above. Absence of an STS is indicated by a blank square or a minus sign (−).

prising the minimal path (770c6, 808b11, 916b5, 966e8, 693b10, 700b2) were localized to chromosome 11p14–15.1 by fluorescent in situ hybridization (FISH) (data not shown), consistent with the regional assignment of STSs developed from the insert-termini of YACs 916b5 and 966e8 to intervals f or g on 11p (Fig. 2).

A total of 40 STSs were positioned on the physical map, providing an estimated average resolution of ~1 STS /100 kb. The relative order of STSs depicted in Figure 1 is tentative and is inferred solely from the STS content of each YAC. In some cases, the order of adjacent STSs could not be established definitively, specifically for those STSs within the groups (PROS30, D11S861), (D11S574, D11S1901, D11S1791), (WI-2899, D11S729), (D11S902, 185b1R, SUR, BIR, D11S921, D11S1890), (MYOD1, KCNC1), (SAA3, D11S1764), and (D11S455, TFIIH, SAA2). In addition, the relative placement of STSs in some regions is complicated by the apparent loss of STSs in several YACs. For example, an area of uncertainty is the placement of the STSs WI-2899 and D11S729 distal to D11S902 because the YAC clones 953d9, 73a11, and 197d10 appear to possess various internal deletions based upon the apparent absence of one or more STSs located within the regions these YACs encompass. Subsequent rescreening of these YACs consistently gave negative results, suggesting that the absence of these STSs represents internal rearrangements or deletions within these YACs. As not all YACs comprising the contig were examined for chimeric end-sequences, and the presence of small rearrangements, internal chimerism, and/or small internal deletions within YACs would not have been detected in these studies, a more precise determination of the relative STS order and average resolution of STSs awaits construction of a cosmid contig and long-range restriction map of this region.

In general, the physical map described here is in agreement with genetic (Weissenbach et al. 1992; Litt et al. 1993; Gyapay et al. 1994; Litt et al. 1995), RH (James et al. 1994) and other physical maps (Sellar et al. 1994; Chumakov et al. 1995; Whitehead Institute/MIT Center for Genome Research, 1995) of the region. The proposed order of genetic markers, tel–D11S926–D11S861–D11S419–(D11S902–D11S921)–D11S1310–D11S899–cen (Fig. 1) is consistent with that established by linkage analysis (Weissenbach et al. 1992; Litt et al. 1993; Gyapay et al. 1994; Litt et al. 1995) and RH mapping (James et al. 1994). For the STSs D11S574, D11S1901, and D11S1791, which could not be ordered relative to D11S419 by RH mapping (James et al. 1994), the data described here support a location distal to D11S419. This order is in agreement with the integrated map of Fantes et al. (1995), in which D11S574 is also placed distal to D11S419. Recently, the Whitehead Institute/MIT Center for Genome Research (1995) constructed an integrated map of chromosome 11 comprising YAC/STS contigs anchored to genetic linkage (Gyapay et al. 1994) and RH maps. Comparison of this integrated map with the physical map described here revealed that nine STSs (D11S926, D11S1791, D11S419, D11S1397, D11S902, D11S1890, D11S1888, D11S899, WI-2899) and 15 CEPH mega-YACs were common to both maps. With the exception of YAC 803f11, the YAC STS content data described here for these common STSs and YACs is in agreement with the Whitehead Institute/MIT integrated map (1995). YAC 803f11 was negative for the STSs D11S1397 and D11S1890 but positive for these markers in the Whitehead/MIT map (1995). This discrepancy may represent the accumulation of deletions and/or rearrangements in the clone used in the studies described here. More recently, Chumakov et al. (1995) published a second-generation physical map of chromosome 11 in which mega-YACs were initially identified by screening for genetic markers and overlap among YACs was established by hybridization with inter-*Alu* PCR probes derived from a subset of YACs and/or fingerprint analyses. Comparison of the map constructed here with that of Chumakov et al. (1995) indicates that several identical YACs from the CEPH YAC libraries had been incorporated into both contigs. For these YACs, the STS content data described here for the five genetic markers common to both studies (D11S419, D11S902, D11S921, D11S1310, and D11S899) is consistent with that of Chumakov et al. (1995). In addition to these YACs, the contig described here incorporates additional clones, identified by YAC library screening, positive for these 5 genetic markers. The contig described here also differs from that of Chumakov et al. (1995) with respect to YAC STS-content analyses. All YACs in this physical map were analyzed for the presence of 40 STSs and overlap between clones was verified by screening of YACs with novel STSs developed from several YAC insert-terminal sequences (Table 2) as well as by inter-*Alu* PCR and L1 fingerprint analyses (data not shown).

A number of discrepancies exist between the STS order described here and the RH map of James et al. (1994). Although CalcA is placed distal to D11S861 on the RH map, the data shown here suggest a more proximal location for this STS. Both CalcA and D11S861 are present on YAC 808b11 whereas YAC 770c6, which extends distal to YAC 808b11, is positive for D11S861 but negative for CalcA. However, the possibility that CalcA is distal to D11S861 cannot be excluded because (1) only one YAC clone (808b11) in the contig spans the CalcA region, and (2) YAC 770c6 may contain chimeric proximal end-sequences or possess an internal deletion for CalcA. Although the relative order of STSs at the proximal end of the contig, 11pter–(MYOD1, KCNC1)–D11S1888–D11S1310–TPH–(SAA3, D11S1764)–(D11S455, TFIIH, SAA2)–LDHA–D11S955E–11pcen is consistent with the integrated map of Fantes et al. (1995) and the physical map of Sellar et al. (1994), the order described here differs in the placement of these STSs relative to the microsatellite markers D11S902 and D11S921. Based on the FLPter measurements of individual YACs and cosmids containing some of these loci, Fantes et al. (1995) assigned this group of STSs to the interval between D11S902 and D11S921. However, members of this group of STSs were not detected in YACs (966e8, 185b1, 630h10, 782a1) that are partly encompassed within or span the D11S902–D11S921 interval. Furthermore, YACs 25ED3, 239h11, and 693b10 are negative for D11S902 and D11S921 but contain the proximal end of 966e8 (966e8L) as well as the STSs MYOD1, KCNC1, D11S1888, D11S1310, and TPH. Centromeric placement of the above group of STSs is also supported by results obtained with YACs 16b6, 14FH12, and 39CC12 which are positive for all or some of the STSs D11S1888, D11S1310, TPH, SAA3, D11S1764, D11S455, TFIIH, SAA2, and LDH but are negative for D11S902 and D11S921. Together, these data support a location for D11S902 and D11S921 distal to this group of STSs. Another discrepancy between the RH map and the data described here is the placement of MYOD1 proximal to D11S1310 by RH mapping. MYOD1 is placed distal to D11S1310 on the physical map because (1) YAC 953d9, which contains D11S902, D11S921, and MYOD1, is negative for D11S1310, and (2) YACs 14FH12 and 16b6 contain D11S1310 but are negative for MYOD1.

USH1C is characterized by sensorineural hearing loss and progressive pigmentary retinopathy. As both the retina and inner ear (cochlea), which are affected by the Usher gene defect, are neural in origin (Rubel 1978), the expressed-sequence tag (EST) D11S955E derived from a brain cDNA library (Polymeropoulos et al. 1993) and localized to the g segment of chromosome 11p (Fig. 2) was considered a candidate gene for USH1C. However, PCR analysis of YACs comprising the contig places D11S955E proximal to the marker D11S1310, excluding this EST as a candidate gene for USH1C. Recently, mutations in the gene encoding myosin VIIA were shown to be associated with Usher syndrome type 1B (USH1B) (Weil et al. 1995). Because USH1C and USH1B possess similar clinical features, all YACs spanning the D11S902–D11S1310 interval were analyzed for the presence of myosin VIIA-related genes by Southern blot hybridization under conditions of reduced stringency. However, no YAC sequences homologous to the myosin VIIA gene were detected (data not shown). Additional candidate genes in the USH1C critical region are those encoding the potassium channel protein KCNC1 and myogenic factor 3 (MYOD1), although Marietta et al. (1996) failed to detect mutations by single-strand conformation polymorphism (SSCP) analysis of the KCNC1 gene in USH1C-affected individuals.

Although mutations postulated to cause aberrant RNA splicing in SUR were recently shown to be associated with HI in nine different families (Thomas et al. 1995b), it is unknown whether HI is a genetically heterogeneous disorder. The YAC contig shown here indicates that SUR and a member of the small inward rectifier K^+ channel family (BIR) are clustered within the HI critical region. Recent co-expression and electrophysiological studies suggest that BIR and SUR are subunits of the pancreatic β-cell K_{ATP} channel (Inagaki et al. 1995). Because HI is characterized by persistent hyperinsulinism and pancreatic β-cell K_{ATP} channels play a crucial role in regulation of glucose-induced insulin secretion (Ashcroft et al. 1988), BIR may be considered an additional candidate gene for HI. The YAC contig described here will further assist the identification and characterization of other potential candidate genes for both USH1C and HI.

METHODS

YAC Library Screening

YAC clones were obtained by screening total human ge-

nomic YAC libraries constructed at the Center for Genetics in Medicine (CGM; Washington University School of Medicine, St. Louis) (Brownstein et al. 1989) and the CEPH (Paris, France) (Albertsen et al. 1990; P. Ougen, pers. comm.). YAC libraries were screened with the microsatellite markers D11S861, D11S419, D11S902, D11S921, D11S1310, D11S899, and the STS 966e8(L) using a PCR-based library pooling strategy as described by Green and Olsen (1990). Primer sequences for the six microsatellite markers were obtained from Genome Data Base and Smith et al. (1993). Oligonucleotide sequences for the STS 966e8(L) are shown in Table 1. Additional YACs positive for the six microsatellite markers were identified from the CEPH YAC database (Cohen et al. 1993). YACs 14FH2, 25ED3, and 39CC12, previously shown to contain the STSs MYOD1, TPH, SAA2, SAA3, LDH, or TFIIH (Sellar et al. 1994), were obtained from the ICI YAC library (Anand et al. 1990).

Isolation of YAC DNA

YAC colonies were inoculated into 50–100 ml AHC medium and incubated for 2–3 days at 30°C. Total yeast genomic/YAC DNA was isolated as previously described (Chandrasekharappa et al. 1992). Briefly, yeast cells were pelleted by centrifugation and washed in SCE buffer (0.9 M sorbitol, 0.1 M sodium citrate at pH 7.0, 0.06 M EDTA, 90 mM β-mercaptoethanol). Cells were treated with yeast lytic enzyme, pelleted, and then resuspended in lysis solution containing 5 mM β-mercaptoethanol and 2% SDS. DNA from lysed cells was isolated by phenol-chloroform extraction and ethanol precipitation.

Sequence Analyses of YAC-insert Termini and STS Development

STSs were developed from DNA sequences obtained from YAC-insert termini and inter-*Alu* PCR products. YAC-end fragments were generated by a ligation-mediated PCR method as described by Kere et al. (1992). Briefly, 1 μg yeast genomic/YAC DNA was digested with *Rsa*I, *Alu*I, *Pvu*II, *Eco*RV, or *Sca*I in a 15-μl reaction. Ligation buffer, linker, and T4 DNA ligase were added and the reactions were incubated at 16°C for 16 hr. To amplify YAC insert termini, ligation reaction products (1 μl) were amplified by PCR using a linker primer and a YAC vector arm-specific primer. An aliquot of the resultant PCR product was diluted and reamplified using the linker primer and an internal YAC vector arm-specific primer. PCR products were isolated from 1% low melting point (LMP) agarose gels, purified, and sequenced directly by double-stranded DNA cycle sequencing (Perkin-Elmer) according to the manufacturer's protocol. Both strands of the PCR product were sequenced using either linker or YAC vector arm primers end-labeled with ^{32}P.

For generation of STSs from inter-*Alu* PCR products, YAC DNA/yeast genomic DNA was amplified by PCR with 5′- and 3′-*Alu*-PCR primers as described by Tagle and Collins (1992). Three independent PCR reactions were carried out for each clone using the 5′- and 3′- primers alone or in combination. Unique inter-*Alu* PCR products were purified using Magic PCR prep columns (Promega) and subcloned into a TA cloning vector (Invitrogen). Insert sizes were determined by PCR amplification of recombinant plasmid DNA using primers complementary to SP6 and T7 promoter sequences flanking the insert. Inserts >300 bp were sequenced by double-stranded DNA cycle sequencing (Promega) with SP6 or T7 primers.

Sequences obtained from YAC insert termini and inter-*Alu* PCR products were analyzed for homology to known DNA sequences using BLASTN (Altschul et al. 1990). Sequences identified as human repetitive DNA elements (SINE, LINE) were excluded from further STS development. Primer sequences for STSs were selected with the computer program PRIMER (provided by E. Lander, Whitehead Institute, Cambridge, MA) and are listed in Table 1. STSs are designated as the YAC number followed by the origin of the insert DNA relative to the left (L) or right (R) YAC vector arms.

The chromosomal origins of YAC end-fragments and inter-*Alu* sequences were determined by PCR amplification of the STSs against genomic DNAs from a rodent/human SCH mapping panel (NIGMS Human Genetic Mutant Cell Repository, Camden, NJ) and a panel of somatic cell hybrids containing various human chromosome 11 deletions (Glaser et al. 1989).

STS-content Screening of YACs

With the exception of those STSs listed in Table 1, primer sequences and PCR amplification conditions for STSs were obtained from Genome Data Base or by anonymous FTP from ftp.well.ox.ac.uk. (James et al. 1994). An STS, designated SUR, for the sulfonylurea receptor gene was developed from the cDNA sequence (provided by Dr. J. Bryan, Baylor College of Medicine, Houston, TX) using the following primers: 5′-CACATCATCATTGATGGCATT-3′ and 5′-CTCTCAGGGTCCAGGTTAAA-3′. Primer sequences for the STS BIR, 5′-CAATGACATGGTAGATGATCAG-3′ and 5′-CAAGAGCATGATCATCAGC-3′, were derived from the cDNA sequence (provided by Dr. S. Seino, Chiba University School of Medicine, Japan). PCR reactions were done in a 10 μl reaction volume containing 20 ng of total yeast DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.0), 1.5 mM $MgCl_2$, 200 μM each dNTP, 10 pmole of each primer, and 1.0 unit of *Taq* polymerase (GIBCO). Cycling conditions were an initial denaturation at 94°C (4 min), followed by 35 cycles of 94°C (1 min), optimal annealing temperature (1 min) and 72°C (1 min). Annealing temperatures for novel STSs developed from YAC end-fragment sequences and inter-*Alu* PCR products are shown in Table 1.

PFGE of YAC DNA

High-molecular-weight yeast genomic/ YAC DNA was prepared from yeast cells embedded in LMP agarose plugs as described (Chandrasekharappa et al. 1992). YACs were separated from yeast chromosomes by PFGE through a 1% agarose gel in 0.5× Tris-borate-EDTA buffer (TBE), using a contour-clamped homogeneous electric field (CHEF) DRII apparatus (Bio-Rad, Hercules, CA), under the following conditions: 200 V for 24 hr with a linear 60 sec to 120 sec ramp and an included angle of 120°. Gels were blotted onto nylon membranes and YAC sizes were determined by

Southern blot hybridization using ^{32}P-labeled pBR322 as the probe (Chandrasekharappa et al. 1992).

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REFERENCES

Albertsen, H.M., H. Abderrahim, H.M. Cann, J. Dausset, D. Le Paslier, and D. Cohen. 1990. Construction and characterization of a yeast artificial chromosome library containing seven haploid human genome equivalents. *Proc. Natl. Acad. Sci.* **87:** 4256–4260.

Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215:** 403–410.

Anand, R., J.H. Riley, R. Butler, J.C. Smith, and A.F. Markham. 1990. A 3.5 genome equivalent multi access YAC library: Construction, characterization, screening and storage. *Nucleic Acids Res.* **18:** 1951–1956.

Ashcroft, F.M. 1988. Adenosine triphosphate-sensitive K^+-channels. *Annu. Rev. Neurosci.* **11:** 97–118.

Ayyagari, R., R.J.H. Smith, M. Polymeropoulos, A.C. Chinault, S.P. Daiger, M.Z. Pelias, L. Wozencraft, M. Kaiser-Kupfer, and J.F. Hejtmancik. 1994. *Invest. Ophthalmol.Vis. Sci.* (Abstr.) **35:** 2143.

Ayyagari, R., Y. Li, R.J.H. Smith, M.Z. Pelias, and J.F. Hejtmancik. 1995. Fine mapping of Usher syndrome type 1C to chromosome 11p14 and identification of flanking markers by haplotype analysis. *Mol. Vision* **1:** 95002.

Brownstein, B.H., G.A. Silverman, R.D. Little, D.T. Burke, S.J. Korsmeyer, D. Schlessinger, and M.V. Olson. 1989. Isolation of single-copy human genes from a library of yeast artificial chromosome clones. *Science* **244:** 1348–1351.

Chandrasekharappa, S.C., D.A. Marchuk, and F.S. Collins. 1992. In *Methods in Molecular Biology* (ed. M. Burmeister and I. Ulanovsky), pp. 235–257. The Humana Press Inc., Totowa, N.J.

Chumakov, I.M., P. Rigault, I. Le Gall, C. Bellane-Chantelot, A. Billault, S. Guillou, P. Soularue, G. Guasconi, E. Pouillier, I. Gros, et al. 1995. A YAC contig map of the human genome. *Nature* (Suppl.) **377:** 175–299.

Cohen, D., I. Chumakov, and J. Weissenbach. 1993. A first-generation physical map of the human genome. *Nature* **366:** 698–701.

Compton, D.A., M.M. Weil, C. Jones, V.M. Riccardi, L.C. Strong, and G.F. Saunders. 1988. Long range physical map of the Wilm's tumor-aniridia region on human chromosome 11. *Cell* **55:** 827–836.

Fantes, J.A., K. Oghene, S. Boyle, S. Danes, J.M. Fletcher, E.A. Bruford, K. Williamson, A. Seawright, A. Schedl, I. Hanson, et al. 1995. A high-resolution integrated physical, cytogenetic, and genetic map of human chromosome 11: Distal p13 to proximal p15.1. *Genomics* **25:** 447–461.

Fossdal, R., L. Magnússon, J.L. Weber, and Ó. Jensson. 1995. Mapping of the locus of atrophia areata, a helicoid peripapillary chorioretinal degeneration with autosomal dominant inheritance, to chromosome 11p15. *Hum. Mol. Genet.* **4:** 479–483.

Gawin, B., B. Klamt, A. König, C. Thäte, D. Le Paslier, I. Chumakov, R. Bhogal, G. Zehetner, G. Bruns, and M. Gessler. 1995. An integrated YAC clone contig for the WAGR region on human chromosome 11p13–p14.1. *Genomics* **30:** 37–45.

Gessler, M. and G.A. Bruns. 1989. A physical map around the WAGR complex on the short arm of chromosome 11. *Genomics* **5:** 43–55.

Glaser, T., D. Housman, W.H. Lewis, D. Gerhard, and C. Jones. 1989. A fine-structure deletion map of human chromosome 11p: Analysis of J1 series hybrids. *Somat. Cell Mol. Genet.* **15:** 477–501.

Glaser, B., K.C. Chiu, R. Anker, A. Nestorowicz, H. Landau, H. Ben-Bassat, Z. Shlomai, N. Kaiser, P.S. Thornton, C.A. Stanley, et al. 1994. Familial hyperinsulinism maps to chromosome 11p14–15.1, 30 cM centromeric to the insulin gene. *Nature Genet.* **7:** 185–188.

Glaser, B., K.C. Chiu, L. Liu, R. Anker, A. Nestorowicz, N.J. Cox, H. Landau, N. Kaiser, P.S. Thornton, C.A. Stanley, et al. 1995. Recombinant mapping of the familial hyperinsulinism gene to an 0.8 cM region on chromosome 11p15.1 and demonstration of a founder effect in Ashkenazi Jews. *Hum. Mol. Genet.* **4:** 879–886.

Green, E.D. and M.V. Olson. 1990. Systematic screening of yeast artificial chromosome libraries by use of the polymerase chain reaction. *Proc. Natl. Acad. Sci.* **87:** 1213–1217.

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 Généthon human genetic linkage map. *Nature Genet.* **7:** 246–339.

Inagaki, N., T. Gonoi, J.P. Clement, IV., N. Namba, J. Inazawa, G. Gonzalez, L. Aguilar-Bryan, S. Seino, and J. Bryan 1995. Reconstitution of I KATP: An inward rectifier subunit plus the sulfonylurea receptor. *Science* **270:** 1166–1170.

James, M.R., C.W. Richard III, J.J. Schott, C. Yousry, K. Clark, J. Bell, J.D. Terwilliger, J. Hazan, C. Dubay, and A. Vignal. 1994. A radiation hybrid map of 506 STS markers spanning human chromosome 11. *Nature Genet.* **8:** 70–76.

Keating, M., D. Atkinson, C. Dunn, K. Timothy, G.M. Vincent, and M. Leppert. 1991. Linkage of cardiac arrhythmia, the long QT syndrome, and the Harvey ras-1 gene. *Science* **252:** 704–706.

Keats, B.J., N. Nouri, M.Z. Pelias, P.L. Deininger, and M. Litt. 1994. Tightly linked flanking markers for the Usher syndrome type 1 locus on the short arm of chromosome 11. *Am. J. Hum. Genet.* **54:** 681–686.

Kere, J., R. Nagaraja, S. Mumm, A. Ciccodicola, M. D'Urso, and D. Schlessinger. 1992. Mapping human chromosomes by walking with sequence-tagged sites from end fragments of yeast artificial chromosome inserts. *Genomics* **14:** 241–248.

Litt, M., P. Kramer, X.Y. Hauge, J.L. Weber, Z. Wang, P.J. Wilkie, M.S. Holt, S. Mishra, H. Donis-Keller, and L. Warnich. 1993. A microsatellite-based index map of human chromosome 11. *Hum. Mol. Genet.* **2:** 909–913.

Litt, M., P. Kramer, E. Kort, S. Cox, D. Root, R. White, J. Weissenbach, H. Donis-Keller, R. Gatti, J. Weber, et al. 1995. The CEPH consortium linkage map of human chromosome 11. *Genomics* **27:** 101–112.

Marietta, J., K.S. Walters, R. Burgess, L. Ni, K. Fukushima, K.C. Moore, and J.F. Hejtmancik. 1996. *Ann. Otol. Rhinol. Laryngol.* (in press).

Ping, A.J., A.E. Reeve, D.J. Law, M.R. Young, M. Boehnke, and A.P. Feinberg. 1989. Genetic linkage of Beckwith-Weidemann syndrome to 11p15. *Am. J. Hum. Genet.* **44:** 720–723.

Polymeropoulos, M.H., H. Xiao, J.M. Sikela, M. Adams, J.C. Venter, and C.R. Merrill. 1993. Chromosomal distribution of 320 genes from a brain cDNA library. *Nature Genet.* **4:** 381–386.

Quackenbush, J., C. Davies, J.M. Bailis, J.V. Khristich, K. Diggle, Y. Marchuck, J. Tobin, S.P. Clark, A. Rodkins, S. Marcano, et al. 1995. An STS content map of human chromosome 11: Localization of 910 YAC clones and 109 islands. *Genomics* **29:** 512–525.

Redeker, E., J.M. Hoovers, M. Alders, C.J. van Moorsel, A.C. Ivens, S. Gregory, L. Kalikin, J. Bliek, L. de Galan, and R. van den Bogaard. 1994. An integrated physical map of 210 markers assigned to the short arm of human chromosome 11. *Genomics* **1:** 538–550.

Rose, E.A., T. Glaser, C. Jones, C.L. Smith, W.H. Lewis, K.M. Call, M. Minden, E. Champagne, L. Bonetta, and H. Yeger. 1990. Complete physical map of the WAGR region of 11p13 localizes a candidate Wilm's tumor gene. *Cell* **60:** 495–508.

Rosier, M.F., A.F. Goguel, A. Martin, D. Le Paslier, P. Couillin, R. Houlgatte, A. Bernheim, C. Auffray, and M.D. Devignes. 1994. A 1.7-Mb YAC contig around the human BDNF gene (11p13): Integration of the physical, genetic, and cytogenetic maps in relation to WAGR syndrome. *Genomics* **24:** 69–77.

Rubel, E.W. 1978. In *Development of sensory systems* (ed. M. Jacobson), pp. 135–238. Springer-Verlag, New York, NY.

Seizinger, B.R., H.P. Klinger, C. Junien, Y. Nakamura, M. Le Beau, W. Cavenee, B. Emmanuel, B. Ponder, S. Naylor, F.F. Mitelman, et al. 1991. Report of the committee on chromosome and gene loss in human neoplasia. *Cytogenet. Cell Genet.* **58:** 1080–1096.

Sellar, G.C., K. Oghene, S. Boyle, W.A. Bickmore, and A.S. Whitehead. 1994. Organization of the region encompassing the human serum amyloid A (SAA) gene family on chromosome 11p15.1. *Genomics* **23:** 492–495.

Smith, R.J.H., E.C. Lee, W.J. Kimberling, S.P. Daiger, M.Z. Pelias, B.J.B. Keats, M. Jay, A. Bird, W. Reardon, M. Guest, et al. 1992. Localization of two genes for Usher syndrome type 1 to chromosome 11. *Genomics* **14:** 995–1002.

Smith, M.W., S.P. Clark, J.S. Hutchinson, Y.H. Wei, A.C. Churkian, L.B. Daniels, K.L. Diggle, M.W. Gen, A.J. Romo, Y. Lin, et al. 1993. A sequence-tagged site map of human chromosome 11. *Genomics* **17:** 699–725.

Tagle, D.A. and F.S. Collins. 1992. An optimized *Alu*-PCR primer pair for human-specific amplification of YACs and somatic cell hybrids. *Hum. Mol. Genet.* **1:** 121–122.

Thomas, P.M., G.J. Cote, D.M. Hallman, and P.M. Mathew. 1995a. Homozygosity mapping, to chromosome 11p, of the gene for familial persistent hyperinsulinemic hypoglycemia of infancy. *Am. J. Hum. Genet.* **56:** 416–421.

Thomas, P.M., G.J. Cote, N. Wohlik, B. Haddad, P.M. Mathew, W. Rabl, L. Aguilar-Bryan, R.F. Gagel, and J. Bryan. 1995b. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* **268:** 426–429.

Weil, D., S. Blanchard, J. Kaplan, P. Guilford, F. Gibson, J. Walsh, P. Mburu, A. Varela, J. Levilliers, and M.D. Weston. 1995. Defective myosin VIIA gene responsible for Usher syndrome type 1B. *Nature* **374:** 60–61.

Weissenbach, J., G. Gyapay, C. Dib, A. Vignal, J. Morissette, P. Millaseau, G. Vaysseix, and M. Lathrop. 1992. A second-generation linkage map of the human genome. *Nature* **359:** 794–801.

Whitehead Institute/MIT Center for Genome Research, Human Genetic Mapping Project. 1995. Data release 8.

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