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RESEARCH

Regional Assignment of 68 New Human Gene Transcripts on Chromosome 11

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We have tested 80 expressed sequence-tagged site (eSTS) markers assigned to human chromosome 11 by the Genexpress program on a panel of somatic cell hybrids containing parts of this chromosome, characterized by cytogenetic data, reference markers, and with respect to the Généthon microsatellite genetic map. Sixty-eight new gene transcripts have been assigned to 25 subregions, one of which was newly defined by five of the eSTS markers. The markers are distributed on the short and long arms in agreement with their physical length. The genic map thus obtained has been integrated with the cytogenetic, genetic, and disease maps. Two eSTS markers have been further mapped with respect to a yeast artificial chromosome (YAC) contig close to the brain-derived neurotrophic factor (BDNF) gene and thus provide potential candidate genes for the mental retardation phenotype of WAGR (Wilms' tumor, aniridia, genitourinary abnormalities and mental retardation) syndrome. Altogether, the 68 new gene transcripts localized here represent more than a threefold increase in the number of unknown regionalized genes that could reveal potential candidate genes for the numerous orphan pathologies associated with chromosome 11.

Identification of the genes responsible for human genetic disorders is a major goal of the human genome project. The availability of a high resolution genetic map based on microsatellite markers provides a basis for the positional cloning efforts and the integration of the physical map based on ordered contigs of cloned DNA fragments (Gyapay et al. 1994; Murray 1994). Once an interval of several hundred kilobases to several megabases is identified, it is necessary to catalog all of the genes in this interval and identify to one that is mutated in relation to the disease. This is usually performed by direct testing of candidate genes that have been previously characterized and mapped to the interval, or by direct selection, exon amplification, or exon trapping methods to characterize new genes (for review, see Gardiner and Mural 1995)

Sequencing of cDNA clones has allowed the characterization by structural data of a large fraction of all human gene transcripts. This provides a basis to develop expressed STS markers (eSTS; Auffray et al. 1995) that can be used to build a genic map of the human genome and integrate it with the genetic and physical maps. Thus, in addition to the 3700 genes registered previously in the Genome Data Base (GDB), the Genexpress, and other programs (Polymeropoulos et al. 1993; Auffray et al. 1995 and references therein) have recently developed >4000 new eSTS markers and assigned them to specific chromosomes using panels of human–rodent somatic cell hybrids containing one or a limited number of human chromosomes. Based on the collection of 2801 eSTS markers developed by the Genexpress program, we have undertaken the building of a genic map of the human genome in collaboration with laboratories participating in the IMAGE Consortium. Regional assignment can proceed using collections of somatic cell hybrids defining breakpoint or rearrangements along one chromosome, providing a resolution in the range of 1 to several Mb (Couillin et al. 1994; Withmore et al. 1994). Positioning in a genetic map interval is rarely obtained directly when the marker is not polymorphic, and in most cases is obtained indirectly us-

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ing resources such as radiation hybrid panels (Cox et al. 1990; James et al. 1994). The definition of clones containing the corresponding gene and integrated in the physical map provides the tool for further studies at the genomic level. Although the availability of eSTs markers assigned to a given interval represents an attractive resource for the establishment of the expression maps aimed at identifying genes responsible for genetic disorders (Chiannilkulchai et al. 1995).

Here we describe a contribution to the genic map of chromosome 11 based on a panel of somatic cell hybrids. This chromosome represents ~4.8% of the human genome and extends over ~144 Mb (Morton 1991). It should therefore encode ~2800–3500 of the estimated 60,000–80,000 genes believed to represent the coding capacity of the human genome (Antequera and Bird; Fields et al. 1994). To date, 202 identified genes have been localized on chromosome 11 as well as 29 transcribed sequences (van Heyningen and Little 1995; GDB). Sixty-nine diseases are linked to chromosome 11 and for 31 of them no responsible gene has been identified so far. These diseases will be considered here as "orphan pathologies" by analogy with orphan receptors, which lack identified ligands. They include Beckwith–Wiedemann syndrome, long QT syndrome, hypertrophic cardiomyopathy 4, Bardet–Biedl syndrome, and part of a complex pathology, the WAGR syndrome (Wilms tumor, aniridia, genitourinary abnormalities and mental retardation). We tested for the presence of 80 eSTS markers assigned to chromosome 11 by the Genexpress program on DNA from a panel of 25 somatic cell hybrids bearing different rearrangements of human chromosome 11 that delineate 24 subregions. These subregions have been characterized previously with respect to their microsatellite marker content (Couillin et al. 1994; S. Fauré and J. Weissenbach, pers. comm.).

In addition, we used the data of the so created genic map to study the genetic context of the human brain-derived neurotrophic factor (BDNF) gene localized at 11p13 (Maisonpierre et al. 1991). Deletion of the BDNF gene has been shown to correlate with the severity of the mental retardation phenotype of the WAGR syndrome that maps in the 11p13 region (Hanson et al. 1992). The presence of two eSTS markers in the corresponding subregions was tested on a 1.7-Mb yeast artificial chromosome (YAC) contig constructed around the BDNF gene (Rosier et al. 1994). One eSTS corresponding to an as yet unknown transcript was localized on the BDNF YAC contig and represents a potential candidate gene for the mental retardation phenotype.

The 70 gene transcripts newly localized in particular subregions define potential candidate genes for the 31 orphan pathologies associated with chromosome 11.

RESULTS

Description of the eSTS Markers

The ESTS markers used in this study are from the collection established at Généthon by the Genexpress program and assigned to chromosome 11 (Auffray et al 1995). These eSTS markers have been derived from selected partial cDNA sequences established upon the sequencing of two cDNA libraries constructed from skeletal muscle and infant brain mRNA. The GDB D numbers of the 80 eSTS markers studied here are listed in Table 1. In the case of multiassignments (8/80 eSTS markers, corresponding to pseudogenes or gene families), only the D number corresponding to chromosome 11 is indicated. The cDNA clone (name and sequence accession number in the EMBL data base) from which eSTS markers are derived are indicated, as well as any similarity to other known sequences. Detailed sequence analysis will be described elsewhere (R. Houlgatte, R. Marriage-Samson, S. Duprat, A. Tessier, S. Bentolila, B. Lamy, and C. Auffray, in prep.). Most eSTS markers (69/80) have been designed from cDNA sequences that did not match any known sequence (no identity). Among these, nine cDNA clones have been classified in four distinct families according to internal sequence similarities (R. Houlgatte et al., in prep.). They are indicated by numbers in Table 1. Seven eSTS markers correspond to known gene transcripts (id in Table 1) of which five (D11S2241E, D11S2242E, D11S2244E, D11S2245E, and D11F194S1E corresponding to ACAT1, CRYAB, SRPR, NDUFV1, and HBB, respectively) had already been assigned to a precise location on chromosome 11. They serve as controls for our regionalization procedure. The sixth and seventh eSTS markers corresponding to the transcripts for acidic ribosomal phosphoprotein P2 and small nuclear ribonucleotide polypeptide C, respectively, had not been mapped previously. Finally, four eSTS markers correspond to transcripts with similarities with known sequences. D11S2269E and D11S2274E correspond to sequences homologous to mouse flap endonuclease-1 and rat LL5 protein, respectively;

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Table 1. List of eSTS markers used in this study

eSTS D numbers[a]	Chromosome assignment	Corre-sponding cDNA clones[b]	EMBL sequence accession number	Status[c]	Gene product	Subregions
cdyObh07 (submitted)	11	c-0bh07	F05464	id	small nuclear ribonucleotide polypeptide C	21
D11S2227E	11	b-14g12	Z19327	no id		22
D11S2228E	11	b-30a01	Z28525	no id		22
D11S2229E	11	b-31a10	Z28534	no id		10
D11S2230E	11	b-63a06	Z28611	no id		22
D11S2231E	11	b-72c08	Z28653	no id		22
D11S2232E	11	b-73g08	F00203	no id		21
D11S2233E	11	b-80e02	F00215	no id		20
D11S2235E	11	b-84a05	Z28721	no id		20
D11S2236E	11	b-93d06	Z28772	no id		24
D11S2238E	11	b-99d07	F00394	no id		20
D11S2239E	11	b-b6c05	Z28895	no id		20
D11S2240E	11	b-c1a09	Z28919	no id		20
D11S2241E	11	b-06d09	F00504	id	mitochondrial acetoacetyl-CoA thiolase	22
D11S2242E	11	b-17g01	F00595	id	docking protein alpha	24
D11S2243E	11	b-30e05	F00674	id	acidic ribosomal phosphoprotein P2	1
D11S2244E	11	b-32d09	F00690	id	alpha B-crystallin	22
D11S2245E	11	b-34h10	F00706	id	human NADH dehydrogenase (ubiquinone flavoprotein 1 (51 kD)	19
D11S2246E	11	b-48d01	F00755	no id		22
D11S2247E	11	b-48e02	Z24835	no id		3
D11S2248E	11	b-57g10	Z24847	no id		18
D11S2249E	11	b-63c08	Z24869	no id		20
D11S2250E	11	b-71e04	Z28648	no id		24
D11S2251E	11	b-71g02	F00845	no id		22
D11S2252E	11	b-80f06	Z24987	no id		3 or 15
D11S2255E	11	c-02b04	Z38186	no id		20
D11S2256E[1]	11	c-02h07	F01414	no id		19
D11S2257E	11	c-0af02	Z38414	no id		1, 2, 3, 15, or 16
D11S2258E[2]	11	c-0cc01	F01735	no id		3
D11S2260E[2]	11	c-0cf09	F01755	no id		3
D11S2261E	11	c-0ch08	Z38477	no id		3
D11S2262E	11	c-0da02	Z38480	no id		24
D11S2263E	11	c-0ed05	Z38520	no id		15
D11S2264E	11	c-0gf10	F01880	no id		19
D11S2265E	11	c-0me10	Z38713	no id		3
D11S2266E	11	c-0pa04	Z38779	no id		2
D11S2267E	11	c-0pg04	F02133	no id		21
D11S2268E	11	c-0qd11	Z38820	no id		3
D11S2269E	11	c-0sh09	Z38906	homol	mouse flap endonuclease-1	18

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Table 1. (*Continued*)

eSTS D numbers[a]	Chromosome assignment	Corre-sponding cDNA clones[b]	EMBL sequence accession number	Status[c]	Gene product	Subregions
D11S2270E[3]	11	c-0tg06	Z38928	no id		18
D11S2271E	11	c-0ug06	Z38957	no id		22
D11S2273E	11	c-0wa07	Z38991	no id		3
D11S2274E	11	c-0xa02	F02357	homol	rat LL5 protein	24
D11S2275E	11	c-0xc11	F04857	no id		22
D11S2276E	11	c-0xe02	F02376	no id		22
D11S2277E[4]	11	c-0xg02	F02384	no id		23
D11S2278E	11	c-0zc01	Z39077	no id		5
D11S2279E	11	c-0zg08	Z39100	no id		5
D11S2280E	11	c-10b08	Z38142	no id		12
D11S2283E	11	c-12h10	Z39175	no id		16
D11S2284E	11	c-13e12	Z39199	no id		23
D11S2286E	11	c-14a01	Z39212	no id		21
D11S2287E	11	c-15b10	Z39242	no id		22
D11S2288E	11	c-17c12	F02702	no id		19
D11S2289E[1]	11	c-17e02	Z39328	no id		19
D11S2290E	11	c-19b09	Z39395	no id		18
D11S2291E	11	c-19f07	Z39415	no id		18
D11S2292E[3]	11	c-19g08	F02763	no id		18
D11S2293E	11	c-1ag04	Z39462	no id		23
D11S2294E[4]	11	c-1ah07	F02791	no id		23
D11S2313E	11	c-zph05	Z41301	no id		25
D11S2314E	11	c-0de01	Z42258	no id		22
D11S2316E	11	c-01e10	Z42491	no id		16
D11S2317E	11	c-0na10	F05806	no id		24
D11S2318E	11	c-0pa09	Z42603	no id		24
D11S2319E	11	c-0xf10	Z42911	no id		18
D11S2320E	11	c-0ya09	Z42924	no id		22
D11S2321E[2]	11	c-0ya12	Z42925	no id		3
D11S2322E	11	c-10c09	Z42994	no id		15 or 16
D11S2323E	11	c-13e02	Z43105	no id		12
D11S2325E	11	c-1aa05	Z46134	no id		22
D11S2329E	11	c-zpe07	Z45606	no id		22
D11F194S1E	11/9	b-b0d01	F01190	id	hemoglobin beta	1
D11F217S1E	11/4	c-13c10	Z39189	no id		17–19
D11F223S1E	11/12	c-17d03	Z39321	no id		15 or 16
D11F231S1E	11/7	c-zrf04	Z41357	rel	human DNA J protein homolog HSJ1	1, 21, or 22
D11F241S1E	11/19	c-0kf07	F05736	rel	mouse substrate of protein tyrosine kinase receptors and p60v-src	17
D11F250S1E	11/13	c-0yc08	Z42934	no id		1, 2, or 3
D11F271S1E	11/12	b-15h06	Z28463	no id		20
D11F278S1E	11/12	b-37h03	Z19512	no id		3

[a]F instead of S means multiassignment; members of the four cDNA families (see text) are indicaed by 1–4.
[b]Prefixes b and c are for cDNA derived from skeletal muscle and infant brain libraries, respectively.
[c](id) Identical; (homol) homologous; (rel) related; (no id) unknown.

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D11F241S1E and D11F231S1E to sequences related to the mouse substrate of protein tyrosine kinase receptors or p60v-src and human DNA J protein homolog HSJ1, respectively. Taking into account the 7 known genes and the fact that 9 markers are derived from four transcripts, the 80 eSTS markers tested define 68 new human gene transcripts.

Regional Localization of the eSTS Markers on Chromosome 11

The 80 eSTS markers were tested on the DNA from a panel of 25 somatic cell hybrid lines, each containing different deletions of chromosome 11 (Couillin et al. 1994; van Heyningen and Little 1995). PCR assays enabled us to distribute the 80 eSTS markers among the different subregions all along chromosome 11 (Fig. 1; Table 1). The Mar57 and Lev55.3 lines allowed assignment of 25 and 54 eSTS markers on the short and long arms of chromosome 11, respectively, a distribution that reflects their physical length (58 and 86 Mb.) Ambiguous localization (see below) of eSTS marker D11F231S1E hindered its assignment to a specific area of chromosome 11. The panel of somatic cell hybrids originally delineated 24 subregions. An additional subregion (Fig. 1, subregion 19) was defined here by five eSTS markers (D11S2245E, D11S2256E, D11S2264E, D11S2288E, and D11S2289E) that are present in the Lev55.3 line but absent in both G35F1a and CF5246. Thus, these eSTS markers distinguished breakpoints in the G35F1 and CF5246 somatic hybrid cell lines that could not be differentiated with markers reported previously (Couillin et al. 1994). According to these data our panel now difines 25 subregions, 16 on the short arm and 9 on the long arm of chromosome 11. This biased distribution is related to the fact that the panel was constructed primarily when studying the genetic disorders implicating the 11p13–11p15 region (Wilms' tumor, WAGR syndrome). Correspondence between our subregions and the intervals defined previously (van Heyningen and Little 1995) can be deduced easily from common breakpoints and microsatellite markers.

Validation of our procedure is provided by the concordant localization of five eSTS markers (D11S2241E, D11S2242E, D11S2244E, D11S2245E, and D11F194S1E) corresponding to known transcripts already mapped within chromosome 11. The sixth and seventh known gene transcripts encoding acidic ribosomal phosphoprotein P2 and small nuclear ribonucleotide polypeptide C are now assigned to subregion 1 and 21, respectively, of chromosome 11 (Fig. 1)

Ambiguous Localizations

Among the 80 assigned eSTS markers, 7 could not be assigned unambiguously to a unique subregion (Table 1). We assigned D11S2322E to the short arm of chromosome 11 but could not distinguish between subregions 15 and 16 because the hamster control was positive, thus preventing us from interpreting the results obtained with the hamster-derived lines and particularly with the G35Fla line. Other ambiguities came from multiassignment of the eSTS markers or/and from the ambiguous results obtained with some hybrid cell lines. D11F217S1E, D11F2231E, and D11F231S1E have been assigned to other chromosomes in addition to chromosome 11. (see Table 1). Owing to the presence of these chromosomes in some of our nonmonochromosomal somatic cell hybrids, none of these three eSTS markers could be assigned to a unique subregion. Some eSTS markers gave an ambiguous response with more than one hybrid cell line, impairing interpretation of the results. For example, D11S2252E does not amplify DNA from the LHV1PS or LHV1PH lines; D11S2257E amplifies DNA from neither of these two lines nor from the STO28, Pel40, or Pel16 lines. Localization of D11F250S1E presents the two difficulties mentioned above, multiassignment to chromosomes 11 and 13 and ambiguous responses with the STO28, Pel40, and Pel16 lines.

A Resource of Potential Candidate Genes for Orphan Pathologies

Correlation between the so-created genic map and the orphan disease map of chromosome 11 is schematized in Figure 1. Assignment of genetic disorders to given cytogenetic intervals (van Heyningen and Little 1995; GDB) makes it possible to delineate the subregions of our somatic cell hybrid panel involved in these pathologies. The number of potential candidate genes is given in Table 2 for the 31 orphan pathologies associated with chromosome 11. The genes coding for acidic phosphoriboprotein P2 and small nuclear ribonucleotide polypeptide C have been in-

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cluded in the calculation because they were localized in this study and may not have been considered earlier as candidate genes for diseases associated with subregions 1 and 21. (Fig 1; Table 2).

Localization of Two eSTS Markers within a YAC Contig at llp13

A 1.7-Mb YAC contig carrying the human BDNF gene has been constructed and integrated into

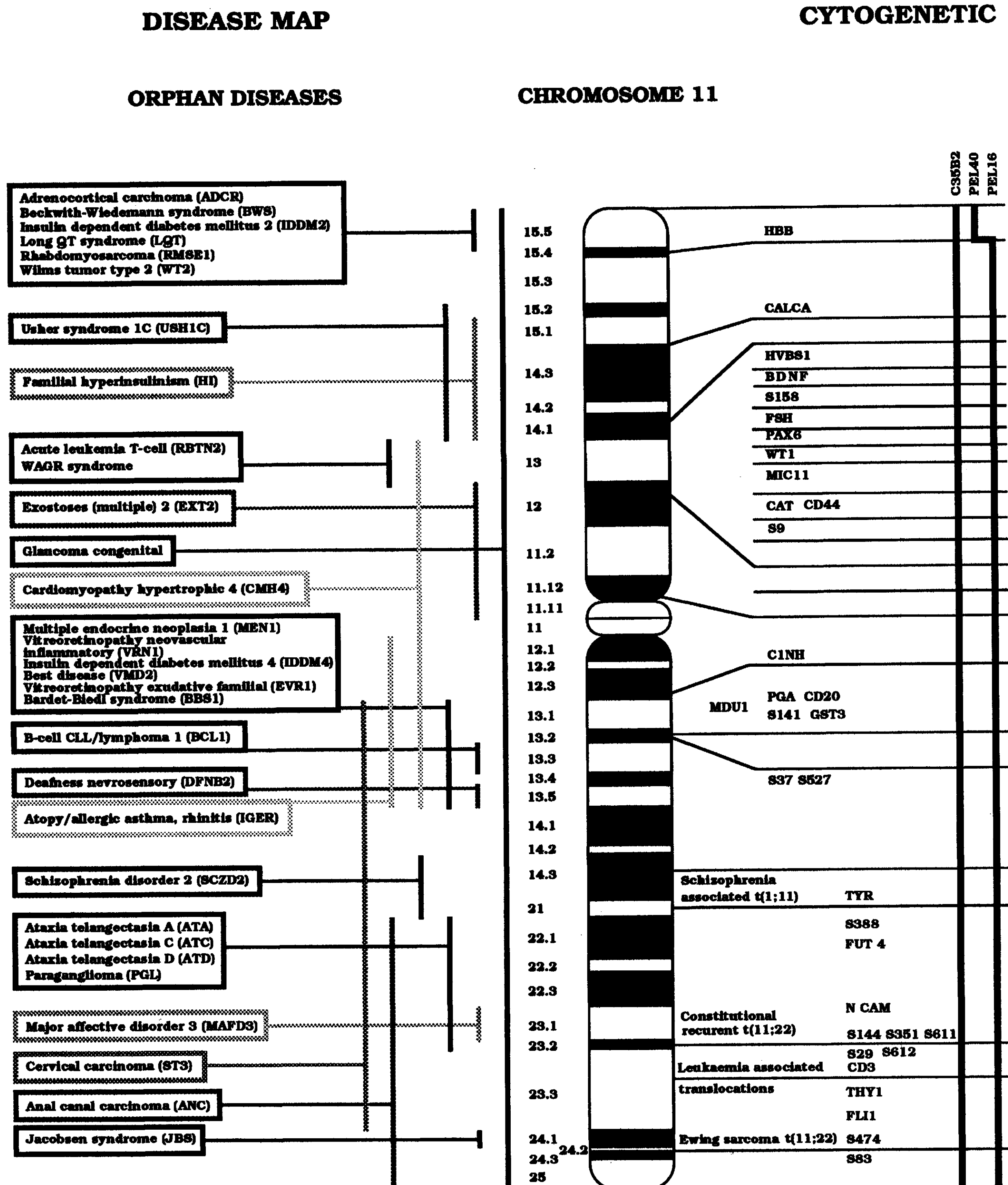

Figure 1 *(Fig. 1 and legend continued on following page.)*

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MAP | GENIC MAP | GENETIC MAP

HYBRID LINES | eSTSs | MICROSATELLITES

STO 28, BID7, RAN68, SOA54, LEV22-1, LEV14-13, ANX6-14, LHV1F8, LHV1FH, DAR15-14, RGE8 20, G35F1a, MAR57, LEV55-3, J1-44, CF5246, MAR1, MAR12, ALE4, M11-X, A3RS12B, A3EW2 3B

Interval	eSTSs
1	D11F19481E D11S2243E
2	D11S2266E
3	D11F27881E D11S2247E D11S2258E[2] D11S2260E[2] D11S2261E D11S2265E D11S2268E D11S2273E D11S2321E[2]
4	
5	D11S2278E D11S2279E
6	
7	
8	
9	
10	D11S2229E
11	
12	D11S2280E D11S2323E
13	
14	
15	D11S2263E
16	D11S2283E D11S2316E
17	D11F24181E
18	D11S2248E D11S2269E D11S2270E[3] D11S2319E D11S2290E D11S2291E D11S2292E[3]
19	D11S2245E D11S2256E[1] D11S2264E D11S2288E D11S2289E[1]
20	D11F27181E D11S2249E D11S2233E D11S2235E D11S2238E D11S2239E D11S2240E D11S2255E
21	D11S2232E Cdy0bh07 D11S2267E D11S2286E
22	D11S2227E D11S2228E D11S2230E D11S2231E D11S2241E D11S2244E D11S2246E D11S2251E D11S2271E D11S2275E D11S2276E D11S2287E D11S2314E D11S2320E D11S2325E D11S2329E
23	D11S2277E[4] D11S2284E D11S2293E D11S2294E[4]
24	D11S2236E D11S2242E D11S2250E D11S2262E D11S2274E D11S2317E D11S2318E
25	D11S2313E

Figure 1 Integrated view of the disease, cytogenetic, genic, and genetic maps of chromosome 11. (Disease map) Orphan pathologies are listed at *left*. Diseases associated with the same cytogenetic interval are boxed. The cytogenetic localization of each group is represented by vertical lines linked to the corresponding boxes. (Cytogenetic map) Bold, vertical lines indicate the portion of human chromosome 11 that each hybrid cell line contains. The hybrid cell panel divides chromosome 11 into 25 intervals illustrated by horizontal lines. (Genic map) In each subregion, the tested eSTS markers are displayed in alphabetical and numerical order, with no implication about physical order. The eSTS markers that are derived from transcripts of the same gene (cDNA families) are indicated by numbers. (Genetic map) Shown are the best-supported order for 107 microsatellite markers (4: S. Fauré and J. Weissenbach, pers. comm.). The genetic position of each microsatellite on chromosome 11 is schematized at *right* by lines.

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Table 2. List of orphan pathologies localized on chromosome 11 and number of potential candidate genes

Orphan pathologies	Cytogenetic localization	Subregion concerned	Number of new potential candidate genes
Adrenocortical carcinoma	11p15.5	1	4
Beckwith–Wiedemann syndrome	11p15.5	1	4
Insulin-dependent diabetes mellitus	11p15.5	1	4
Long QT syndrome	11p15.5	1	4
Rhabdomyosarcoma	11p15.5	1	4
Wilms tumor type 2	11p15.5	1	4
Usher syndrome 1C	11p15.2-p14	2–14	16
Familial hyperinsulinism	11p15.1-p14	2–14	16
Acute leukemia T-cell	11p13	5–14	5
WAGR syndrome	11p13	5–14	5
Cardiomyopathy hypertrophic 4	11p13-q13	5–20	31
Exostoses (multiple) 2	11p12-cen	5–17	14
Atopy/allergic asthma, rhinitis	11q12-q13	17–20	19
B-cell CLL/lymphoma 1	11q13.3	20	8
Deafness neurosensory	11q13.5	20	8
Bardet-Biedl syndrome	11q13	18–20	18
Best disease	11q13	18–20	18
Insulin dependent diabetes mellitus 4	11q13	18–20	18
Multiple endocrine neoplasia 1	11q13	18–20	18
Vitreoretinopathy exudative familial	11q13	18–20	18
Vitreoretinopathy neovascular inflmmatory	11q13	18–20	18
Cervical carcinoma	11q13-q23	18–24	46
Schizophrenia disorder 2	11q14-q21	20–22	27
Ataxia telangiectasia A, C, D	11q22-q23	22–24	24
Paraganglioma	11q22-q23	22–24	24
Anal canal carcinoma	11q22-qter	22–25	25
Major affective disorder 3	11q23.1	22	15
Jacobsen syndrome	11q24.1	24	6
Glaucoma congenital	11	1–25	70

Source: van Heyningen and Little (1995; GDB).

the physical, cytogenetic, and genetic maps of chromosome 11 (Rosier et al. 1994). It constitutes a basis for the isolation and characterization of genes located within this chromosomal region that may be involved in the WAGR syndrome and, more precisely, in the mental retardation phenotype associated with this syndrome. As the BDNF gene maps to subregion 6, there were only two eSTS markers (D11S2278E and D11S2279E) present in the neighboring subregion 5 that could be tested on the four YACs (245F2, 886H12, 892C12, 878D5) of the contig. D11S2279E was found to map on the contig, being positive with YAC 892C12 but not with YACs 245F2, 886H12, and 878D5, as expected, as these YACs do not extend over subregion 5. We thus colocalized D11S2279E with the microsatellite marker D11S904 between the YAC end STSs D11S1568 and D11S1566, respectively. In addition, D11S2278E, which did not map to any of the four YACs but is assigned to subregion 5, could thus be localized between the telomeric boundary of this subregion and the YAC end STS D11S1568 together with the microsatellite markers D11S929 and D11S930 (Fig. 2).

DISCUSSION

The 80 assigned eSTS markers are distributed throughout chromosome 11–25 (31%) on the short arm, 54 (67%) on the long arm, with the remaining marker presenting an ambiguous lo-

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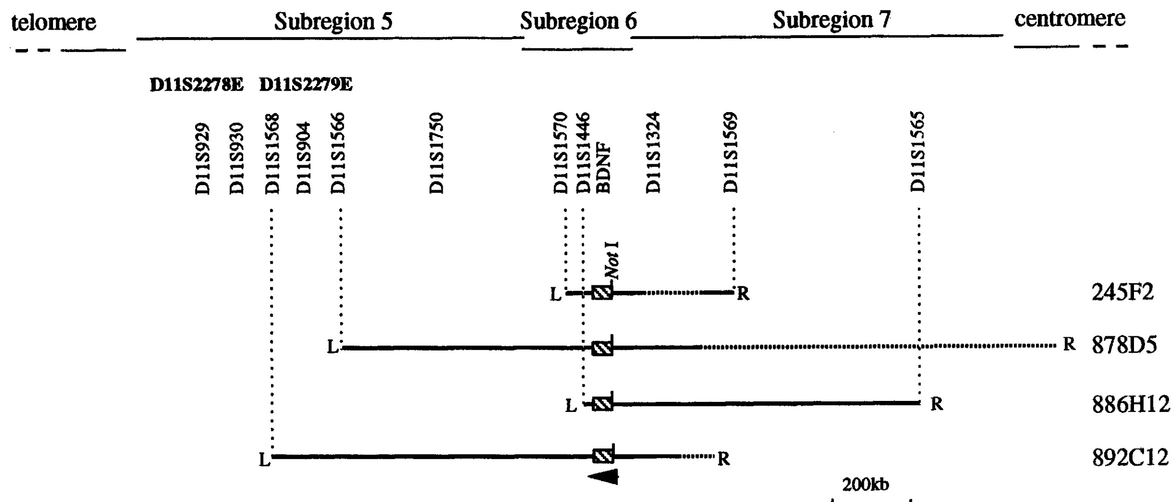

Figure 2 Refinement of the localization of two eSTS markers assigned to the vicinity of the BDNF gene with respect to the YAC contig at 11p13. Horizontal lines at the *top* cover the subregions defined by the hybrid cell lines beneath them. The names of the two eSTS markers are in boldface type at their approximate new localizations. The contig consists of four YACs named at *right* and depicted by horizontal lines. The approximate length of the chimeric parts is indicated by a dotted line. (L, R) Left and right arms, respectively, of the pYAC4 vector. Vertical dotted lines point out to the D numbers of STS markers obtained from each YAC extremity. The BDNF gene is represented by a box, and the *Not*I site by a short vertical line. The orientation of the BDNF gene (arrow) is indicated, as well as the D number of the microsatellite markers present in the different YACs and subregions.

calization (see Table 1). This distribution is similar to that observed either with known genes or with Généthon microsatellite markers on chromosome 11 (Gyapay et al. 1994; GDB) pointing out that p:q distribution of all markers assigned until now to this chromosome is in the proportion 1:2.

The localization of five eSTS markers corresponding to five of the seven known gene transcripts is in agreement with data available in the GDB. This represents a good validation of our mapping strategy. Moreover, the sixth and seventh genes, coding for acidic ribosomal phosphoprotein P2 and small nuclear ribonucleotide polypeptide C, respectively, have been newly localized in the 11p15.4–p15.5 and 11q14.3–q21 intervals, respectively. Among the 73 remaining eSTS markers, 4 correspond to cDNA sequences presenting similarities to known sequences and 69 to cDNA sequences with no putative identification, 9 of which can be clustered into families of overlapping sequences corresponding to four distinct human gene transcripts (R. Houlgatte, R. Marriage-Samson, S. Duprat, A. Tessier, S. Bentolila, B. Lamy, and C. Auffray, in prep.). In summary, the homologous, related, and unknown cDNAs appear to be derived from 68 new human gene transcripts that are now physically linked to known polymorphic markers. This represents more than a threefold increase in the number of unknown gene transcripts regionalized on chromosome 11 (van Heyningen and Little 1995). We assume that the corresponding chromosomal sites contain functional genes, but as the possibility exists that some of them might be pseudogenes, this has to be confirmed by genomic sequencing. Further cDNA sequence clustering analysis should also help in assessing the actual number of genes mapped in this study.

All orphan pathologies linked to chromosome 11 are listed and localized in Figure 1 (van Heyningen and Little 1995; disease map; GDB) and are defined by cytogenetic intervals. One exception is glaucoma, which is associated with the entire chromosome 11. Most assigned eSTS markers presented here are now mapped to an integrated cytogenetic and genetic map including 107 microsatellite markers developed by Généthon (Fig. 1; Couillin et al. 1994; S. Fauré and J Weissenbach, pers. comm.). D11S2279E has been mapped further onto one YAC, 892C12 (Fig. 2). The number of potential candidate genes for a given gene disorder depends on its precise localization (Table 2). For example, candidate genes

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for glaucoma should be searched among all 70 gene transcripts (68 unknown and 2 known but previously not assigned) newly regionalized to chromosome 11. In contrast, for the Jacobsen Syndrome localized to 11q24.1, only six gene transcripts should be considered as possible candidates.

D11S2278E and DS11S2279E have been mapped close to the BDNF gene. This region between FSHB and HBVS1 (Fig. 1) has been proposed previously to contain a locus specific for the WAGR mental retardation phenotype on the basis that individuals with deletions extending into this interval are mentally retarded, whereas those with deletions breaking proximal to it are not (Hanson et al. 1992). Thus, the two gene transcripts mapping to this region are potential candidates for the mental retardation phenotype associated with this complex pathology. Their involvement in the disease can now be assessed by comparison of expression and DNA sequence between normal and affected individuals. The availability of eSTS markers will facilitate RT–PCR assays and further screening of cDNA libraries to isolate full-length clones.

This work represents a contribution toward a complete and high resolution genic map of chromosome 11. It will be complemented by assignment of the eSTS markers using radiation hybrid panels and/or cloned DNA fragments to obtain further integration with the genetic and physical maps of chromosome 11. Additional eSTS markers are steadily being produced and integrated into the genic map and will continuously increase its coverage and the likelihood that the genes involved in specific physiological or pathological traits will have been cataloged, thereby validating the usefulness of this resource for their identification.

METHODS

Somatic cell hybrids and YAC contig

A panel of 25 somatic hybrid cell lines from different origins was used for this study. The main characteristics and references (human parental subjects, first characterization, and mapping data) are described by Couillin et al. (1994), with the exception of the ST028 line, which derives from patient STO 46,XY,t(1;11)(q11;p15.5) (S. Gilgenkrantz, unpubl.) and retains der(1). All hybrids were obtained by fusion between human and mouse cells except for the C35B2, G35F1a, and J1-44 lines, which are derived from hamster cells. The fragments of chromosome 11 present in each of these 25 hyprid lines and the subregions delineated with the whole panel are schematized in Figure 1. The number of subregions depends directly on the number and type of rearrangements, for example, an interstitial deletion defines two breakpoints. The subregions were characterized by cytogenetic data and reference markers. All hybrid lines were typed with 107 microsatellite markers developed by Généthon, allowing the integration of the cytogenetic and genetic maps.

YAC clones 245F2, 886H12, 892C12, and 878D5 belonging to a 1.7-Mb contig established at 11p13 around the BDNF locus are presented in Figure 2 (Rosier et al. 1994). Three negative control DNAs were used: DNA from mouse and hamster extracted from SP2 and CHO cell lines, respectively (Couillin et al. 1994), and DNA from *Saccharomyces cerevisiae* (Promega).

eSTS Markers

eSTS markers were designed from a collection of partial cDNA sequences derived from cDNA libraries from human skeletal muscle (Stratagene no. 936216) or infant brain (Soares et al. 1994). The D numbers of all eSTS markers used here are listed in Table 1. The eSTS markers have been assigned previously to chromosome 11 using a panel of nonochromosomic somatic cell hybrids and multi-assignments verified with a panel of multichromosomic somatic cell hybrids (Auffray et al. 1995).

PCR on Somatic Cell Hybrid and YAC DNA

Primers synthesized by Genset, Genosys, or DNAgency were from the same batches as those used for initial chromosomal assignment. Amplification conditions for all primer pairs used were as follows. Reaction mixtures (50 µl) containing 400 ng of DNA except for human genomic DNA (Clontech, 50 ng), 25 pmoles of each oligonucleotide primer, 0.2 mM dNTPs, and 2 mM $MgCl_2$ were incubated with 1.5 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus) under standard ionic conditions. Cycling was as follows: 5 min at 94°C; 30 cycles composed of 30 sec at 94°C, 30 sec at 59°C, and 30 sec at 72°C using the GeneAmp PCR System 9600 (Perkin-Elmer Cetus). Amplification products were analyzed by electrophoresis on a 3% agarose gel (NuSieve, FMC) in 1× TBE (90 mM Tris, 90 mM boric acid, 2 mM EDTA at pH 8) and visualized with ethidium bromide under UV light.

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