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Physical Map of 1p36, Placement of Breakpoints in Monosomy 1p36, and Clinical Characterization of the Syndrome

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Monosomy 1p36 is the most common terminal deletion syndrome. This contiguous gene deletion syndrome is presumably caused by haploinsufficiency of a number of genes. We have constructed a contig of overlapping large-insert clones for the most distal 10.5 Mb of 1p36, evaluated the deletion sizes in 61 subjects with monosomy 1p36 from 60 families, and created a natural deletion panel. We found pure terminal deletions, interstitial deletions, derivative chromosomes, and more complex rearrangements. Breakpoints were "binned" into 0.5-Mb regions. Analyses revealed some clustering of breakpoints but no single common breakpoint. Determination of the parental origin showed that 60% of de novo 1p36 terminal deletions arose from the maternally inherited chromosome. Of the 61 subjects, 30 were examined systematically through a protocol at the Texas Children's Hospital General Clinical Research Center. Specifically, we report hearing evaluations, palatal and ophthalmological examinations, echocardiograms, neurological assessments, and thyroid function tests. To our knowledge, this systematic molecular and clinical characterization of monosomy 1p36 is the largest and most comprehensive study of this deletion syndrome to date. Many cytogenetically visible, apparent terminal deletions are more complex than anticipated by cytogenetics, as revealed at the molecular level by our study. Our clinical findings allow for the more accurate recognition of the syndrome and for proper medical evaluation.

Introduction

Chromosomal rearrangements occur in ~0.6% of live births (Shaffer and Lupski 2000). Unbalanced chromosomal abnormalities account for a substantive portion of mental retardation. A frequent class of cytogenetic abnormalities is deletion of the telomeric regions of chromosomes. These may cause substantial phenotypic abnormalities, because human telomeric regions are relatively gene rich as compared with other regions of the genome (Saccone et al. 1992). Each human chromosome is capped with 3–20 kb of repetitive $(TTAGGG)_n$ sequence (Moyzis et al. 1988). Just proximal to this sequence is ~100–300 kb of repetitive sequence called "telomere-associated repeats" (TAR) (Flint et al. 1997). The TAR sequence can be shared on multiple chromosome ends. The chromosome-specific unique sequence is proximal (centromeric) to the TAR sequence. Because most human chromosomes end in light-staining GTG bands, the telomeric regions are difficult to visualize cytogenetically. Thus, telomere region–specific probes for FISH have been developed to identify small terminal deletions that otherwise might not be seen with conventional cytogenetic techniques (Knight et al. 1997).

The constitutional deletion of 1p36 results in a syndrome with multiple congenital anomalies and mental retardation (Shapira et al. 1997). The frequency of monosomy 1p36 was estimated initially to be 1 in 10,000 births, making it one of the most common chromosomal deletions (Shapira et al. 1997). Recently, we refined this estimated prevalence to 1 in 5,000 (Shaffer and Lupski 2000), on the basis of the epidemiology of our large group of patients. As we first described in 13 subjects (Shapira et al. 1997), facial characteristics include deep-set eyes, flat nasal bridge, asymmetric ears, and pointed chin (fig. 1). Additional clinical characteristics include seizures, cardiomyopathy, developmental delay, and hearing impairment (reviewed by Slavotinek et al. 1999; Shaffer and Heilstedt 2001).

Chromosome 1p36 alterations, mostly deletions, have been reported to occur in various neoplasms, including neuroblastoma, prostate cancer, lung cancer, malignant melanoma, hepatoma, cervical carcinoma, breast cancer, colorectal adenocarcinoma, ovarian cancer, and non-

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Figure 1 Six subjects with 1p36 deletions. *A,* A boy of age 6 years 4 mo, subject 59; *B,* A girl of age 4 years 6 mo, subject 52; *C,* A boy of age 3 years 5 mo, subject 55a; *D,* a girl of age 10 years 11 mo, subject 55b; *E,* a girl of age 8 years 8 mo, subject 51; *F,* A girl of age 3 years 3 mo, subject 47. Note the flat nasal bridge and nose, asymmetric ears, and pointed chin.

Hodgkin lymphoma. The identification of deletions of 1p36 in a subset of neuroblastoma (White et al. 1995; reviewed by Blatt 2001), ovarian carcinoma (Thompson et al. 1997), and malignant melanoma (Nelson et al. 1999, 2000) led to the hypothesis that the 1p36 region contains a number of tumor-suppressor genes and that deletion of one or more of these genes is involved in the chain of events that results in malignancy (Blatt 2001).

Previously, we reported molecular findings in 33 subjects with 1p36 constitutional deletions (Wu et al. 1999; Ballif et al. 2000*a*) and found variability in the parental origin, deletion size, and complexity of the chromosomal rearrangements. We have expanded this study to 61 subjects with 1p36 deletions, with the ultimate goal of understanding the mechanism(s) that generate and stabilize terminal deletions. In addition, we have assembled a physical map of the most telomeric 10.5 Mb of 1p36 and have constructed a natural deletion panel from our study-subject cell lines. We have also collected clinical information on 30 of these subjects, through the Texas Children's Hospital General Clinical Research Center (TCH-GCRC). The clinical features in monosomy 1p36 have been defined more clearly, which may allow phenotype/genotype correlations to narrow the regions in which to search and identify causative genes that yield the various features of this syndrome.

Subjects and Methods

Study Population

Sixty-one subjects from 60 families were ascertained for this study, through a protocol and consent form approved by the Institutional Review Board for Human Subject Research at the Baylor College of Medicine. One family had two affected children, because of malsegregation of a parental translocation between the short arm of chromosome 1 and the long arm of chromosome 9.

Twenty-three subjects were male, and 38 were female. Although at a lower resolution than the current study, the molecular characterization for the first 30 subjects was reported elsewhere (Shapira et al. 1997; Wu et al. 1999). Ballif et al. (2000*a*) reported the results of subtelomeric region–specific FISH for the first 33 subjects. We obtained blood samples from all subjects and available parents and established lymphoblastoid cell lines. In subjects 29 and 31, amniocytes were used for the molecular investigation. Thirty subjects had clinical evaluations at the TCH-GCRC. The clinical investigation included evaluations by at least one geneticist (H.A.H. and S.K.S.), an ophthalmologist (R.A.L.), an audiologist, and a plastic surgeon (S.S.). Other tests included echocardiogram, electroencephalogram (EEG), auditory brainstem evoked response (ABER), and thyroid function tests.

Molecular Analysis

Genomic DNA from blood, lymphoblastoid cell lines, or cultured amniocytes was prepared by standard protocols. DNA samples from 61 subjects and available parents were analyzed with 25 microsatellite markers (table 1). The initial marker order was obtained from the Human Chromosome 1 Home Page, and sequence analysis is from the Wellcome Trust Sanger Institute (Human Chromosome 1 Home). During the study, the marker order was adjusted on the basis of new information from sequencing, from our mapping efforts, and from the construction of our natural deletion panel. These 25 polymorphic markers were used in PCRs to determine the extent of deletion and the parent of origin. The PCR products were electrophoresed in 6% denaturing polyacrylamide gels and were visualized by autoradiography (Shaffer et al. 1993).

FISH

Metaphase chromosomes were prepared using standard protocols, and FISH was performed with various large-insert clones from our physical map. Large-insert clones from 1p36 were selected as FISH probes, on the basis of the microsatellite results from each study subject, to narrow the breakpoint regions to 0.5-Mb bins. These probes were labeled with digoxigenin and were detected as published previously (Shaffer et al. 1994). The TAR clone (GS-63M14) was obtained from Genome Systems (Knight et al. 2000). A chromosome 1q probe (GS-160H23) (Knight et al. 2000) served as a control and was labeled with biotin and detected as described previously (Shaffer et al. 1994). Telomeric FISH for 41 subtelomeric regions was performed on metaphase chromosomes from 60 subjects, through use of the Chromoprobe Multiprobe-T System (Cytocell), as published previously (Knight et al. 1997; Ballif et al. 2000*a*). DAPI counterstain was applied, and cells were viewed with a Zeiss Axiophot fluorescent microscope equipped with both single-band pass filters and a triple-band pass filter. Digital images were captured by a Power Macintosh G3 system and MacProbe version 4.0 or 4.3 (Perceptive Scientific Instruments). In each subject in whom a derivative chromosome 1 was found, the parent of origin was screened by FISH to determine whether the 1p36 deletion was caused by malsegregation of a parental translocation or a de novo event, or if it was perhaps associated with a familial polymorphism (Ballif et al. 2000*b*).

Table 1

Microsatellite Markers Used for Initial Screening of Deletion Size

Microsatellite Marker	Approximate Distance from 1p Telomere (Mb)	Marker-Containing Clone
D1S243	1.75	RPCI11-181G12
D1S468	3.25	RPCI5-1092A11
D1S2845	3.75	RPCI11-168B8
D1S2893	4.0	RPCI1-37J18
D1S2660	4.25	RPCI11-493P12
D1S1608	4.35	RPCI5-1096P7
D1S2795	5.0	RPCI3-491M17
D1S2145	5.25	RPCI5-1098C18
D1S2633	5.6	RPCI1-233K16
D1S2870	5.7	RPCI1-120G22
D1S253	5.75	RPCI1-120G22
D1S3041	6.0	RPCI11-58A11
D1S2731	6.1	RPCI1-126A5
D1S2642	6.2	RPCI11-312B8
D1S214	6.35	RPCI11-312B8
D1S2663	6.7	RPCI3-505B13
D1S2694	6.75	RPCI3-453P22
D1S548	6.8	RPCI3-453P22
D1S2666	6.85	RPCI3-453P22
D1S508	7.1	RPCI5-1045M14
D1S1615	7.75	RPCI5-1115A15
D1S160	8.3	RPCI5-963K15
D1S244	10.0	RPCI11-108G8
D1S2736	10.1	RPCI11-108G8
D1S1635	10.4	RPCI11-340B24

Results

Molecular Characterization of Deletions

Physical Map of 1p36 and Construction of a Natural Deletion Panel.—Marker order was initially assigned on the basis of information available through the Wellcome Trust Sanger Institute and the Human Chromosome 1 Home Page. Deletion sizes were determined in all 61 subjects. Deletion sizes were characterized initially from analyses of 25 microsatellite markers in each subject. The deletions ranged from 1.5 Mb to >10.5 Mb. We then assembled a minimal tiling path contig of 99 large-insert clones over the most distal 10.5 Mb of 1p36. This region was chosen because it contains most of the breakpoints from our subjects (fig. 2). This contig has five

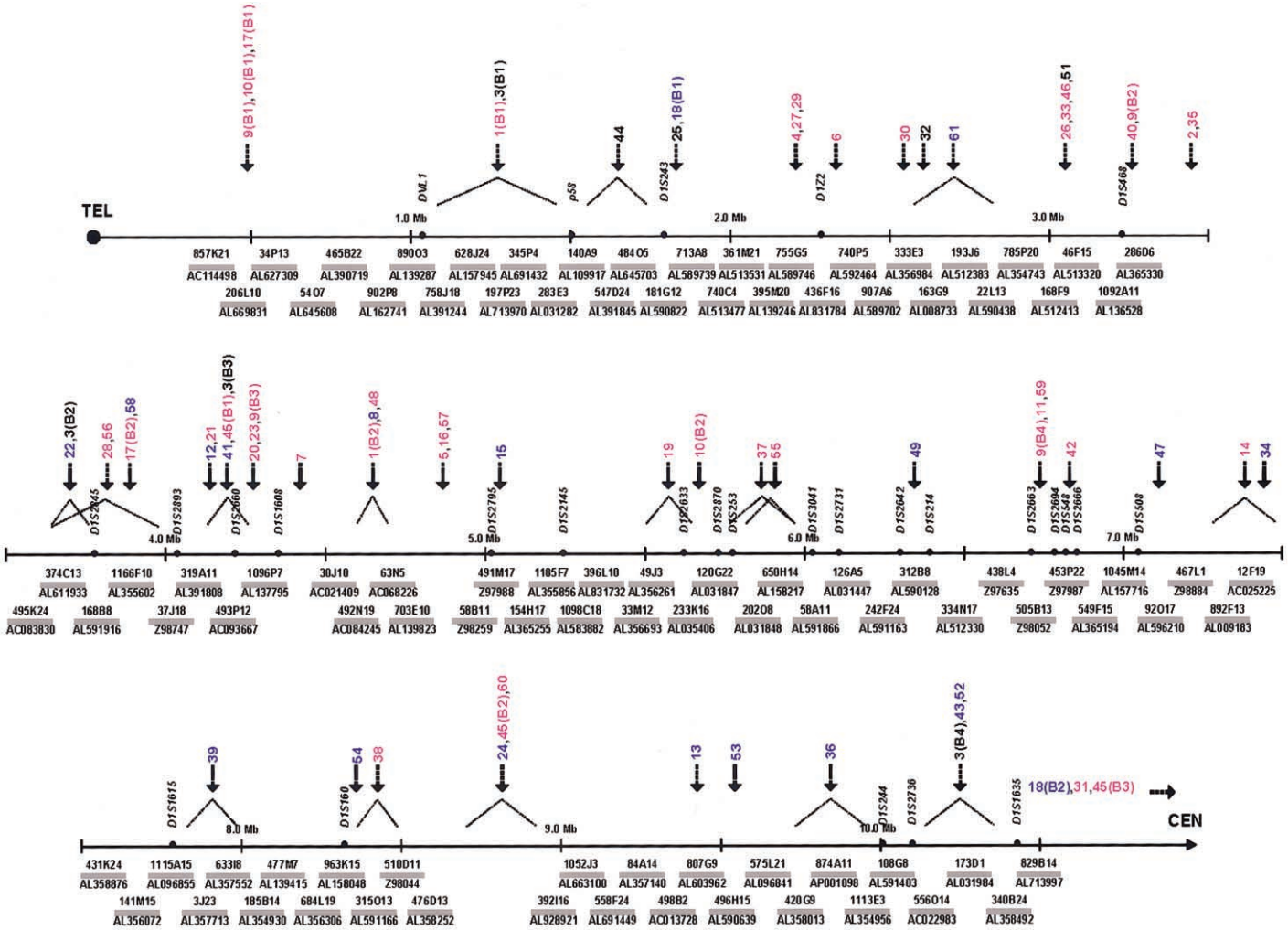

Figure 2 Breakpoints of 60 1p36 deletion cases. The genomic sequence of the most distal 10.5 Mb of 1p36 is shown as solid horizontal lines, beginning with the terminal telomeric repeat sequences. The minimum tiling path over the region is shown below the genomic sequence as a series of shaded boxes with BAC or PAC clone names and GenBank accession numbers above and below each box, respectively. Microsatellite and other marker locations are indicated as blackened dots along the genomic sequence line. The location of each deletion breakpoint is indicated with an arrow. Dotted lines indicate regions within which a subject's breakpoint must be located, as assessed from microsatellite and FISH analyses. Breakpoints ("B") for interstitial and complex deletion cases are numbered in parentheses—for example, "3(B2)" refers to subject 3, breakpoint 2—to distinguish each breakpoint location, because the rearrangements have more than one breakpoint. Pink patient numbers indicate maternally derived deletions, and blue patient numbers indicate paternally derived deletions. Black patient numbers indicate those in whom the parental origin could not be determined. Five sequence gaps are shown roughly to scale from data obtained from fiber FISH experiments performed by the Wellcome Trust Sanger Institute (S. Gregory, personal communication).

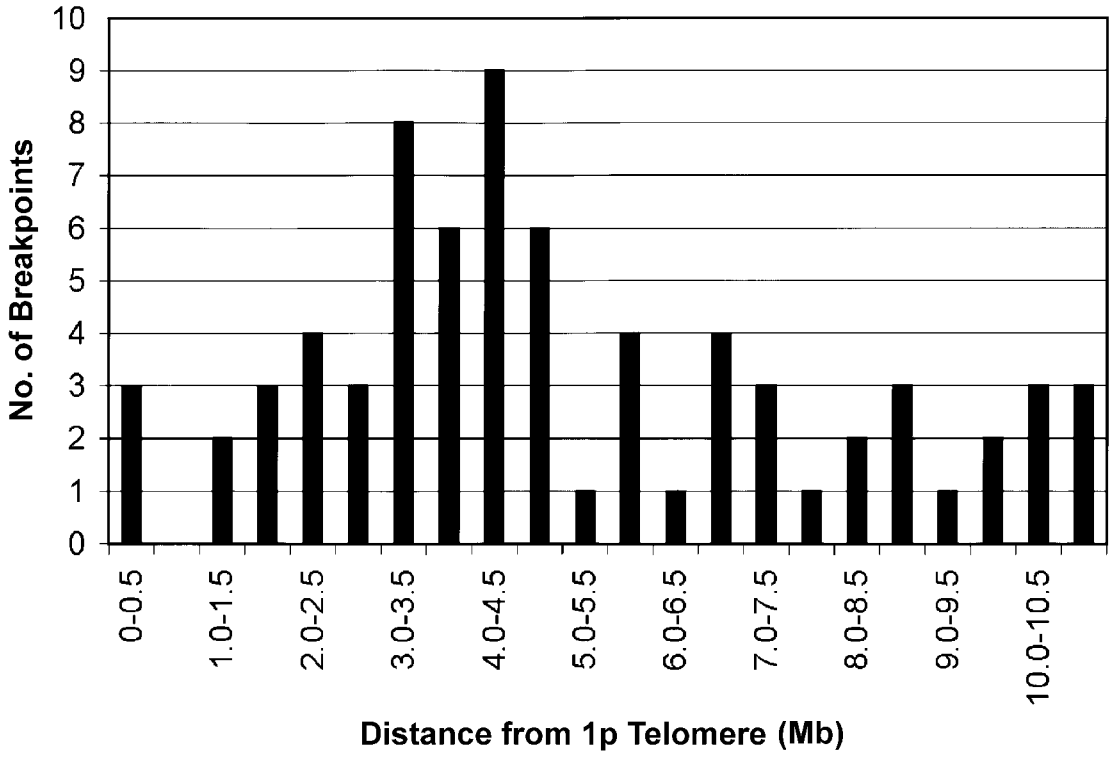

Figure 3 Breakpoint locations of 60 1p36 deletions. Breakpoint clustering was plotted as a histogram of the total number of breakpoints within each 0.5-Mb bin of the terminal 10.5 Mb of 1p36. All breakpoints are included for those subjects with multiple breakpoints due to interstitial deletions or complex rearrangements.

sequence gaps, with the first gap, between clone RPCI5-857K21 and the telomere, estimated to be <300 kb (Knight et al. 2000). The second gap is between RPCI1-286D6 and RPCI11-495K24 and is ~300 kb. The third gap, between RPCI5-1096P7 and RPCI11-30J10, and the fourth gap, between RPCI4-703E10 and RPCI1-58B11, are estimated to be <100 kb. The final gap, between RPCI11-476D13 and RPCI13-392I16, is ~400 kb. Gap size estimates (except for the most telomeric gap) are based on fiber FISH or fingerprinting experiments performed at the Wellcome Trust Sanger Institute (S. Gregory, personal communication). This contig provided a framework from which deletion sizes were further refined into 0.5-Mb "bins," by means of FISH, using these large-insert clones as probes. Many of the breakpoints were localized within a single clone or within two overlapping clones (fig 2). The analysis of the subject breakpoint locations and assessment of deletion sizes formed the natural deletion panel. This natural deletion panel aided in the correct ordering and orientation of BAC/PAC clones and subsequently confirmed or clarified our physical map.

During the construction of the natural deletion panel, each breakpoint was placed on the contig. Thus, for terminal deletions, one breakpoint was mapped. For four subjects with interstitial deletions (subjects 1, 10, 17, and 18), two breakpoints were placed on the map, and for three subjects with complex rearrangements (subjects 3, 9, and 45), each breakpoint—three or four, depending on the rearrangement—was placed on the map. Three breakpoints were not mapped within a 0.5-Mb bin, because they were >10.5 Mb from the telomere (fig. 2). Deletion sizes ranged widely, but most breakpoints (12.5%) clustered 4.0–4.5 Mb from the telomere. Forty percent of all breakpoints occurred 3.0–5.0 Mb from the telomere (fig. 3).

Classification of Rearrangements.—To distinguish between terminal deletions, interstitial deletions, and derivative chromosomes, telomere region–specific FISH probes further characterized 27 cases, since 33 subjects were characterized previously (Ballif et al. 2000*a*). In addition, a clone that maps to the 1p TAR region was used to further clarify the apparently pure terminal deletions. We found that 43 subjects (72%) with monosomy 1p36 appeared to have true terminal deletions in which the most distal known unique 1p subtelomeric sequence was absent and not replaced by another chromosome end (table 2; fig. 4*A*). In these cases, the

Table 2

Study Subjects Included in This Investigation

Study Subject	Age at Diagnosis	Sex	Clinical Protocol	Parental Origin[a]	Rearrangement
1	11 years	M	−	Maternal	Interstitial
2	10 years	F	+	Maternal	Terminal
3	Unknown	M	−	Unknown	Complex
4	5 years	F	+	Maternal	Terminal
5	2 years	M	−	Maternal	Derivative
6	2 years	F	+	Maternal	Terminal
7	2 years	M	+	Maternal	Terminal
8	Prenatal	F	+	Paternal	Terminal
9	1 mo	F	+	Maternal	Complex
10	Birth	M	+	Maternal	Interstitial
11	10 years	F	+	Maternal	Terminal
12	Birth	M	+	Paternal	Terminal
13	7 mo	M	+	Paternal*	Derivative
14	7 mo	F	+	Maternal	Terminal
15	8 years	F	+	Paternal	Derivative
16	1 year	F	+	Maternal	Terminal
17	1 year	F	−	Maternal	Interstitial
18	Unknown	F	−	Paternal	Interstitial
19	Prenatal	F	+	Maternal	Derivative
20	Unknown	F	−	Maternal	Terminal
21	2 years	F	+	Maternal	Terminal
22	Unknown	F	−	Paternal	Terminal
23	2 years	M	+	Maternal	Terminal
24	Unknown	F	−	Paternal	Terminal
25	14 years	F	−	Unknown	Terminal
26	Birth	F	−	Maternal	Derivative
27	Unknown	F	−	Maternal	Terminal
28	1.5 year	F	+	Maternal	Terminal
29	Prenatal	F	−	Maternal	Derivative
30	1.5 year	M	+	Maternal	Terminal
31	Prenatal	F	−	Maternal	Derivative
32	6 years	M	−	Unknown	Terminal
33	Unknown	M	−	Maternal	Terminal
34	Unknown	F	+	Paternal	Terminal
35	Unknown	M	+	Maternal	Terminal
36	9 mo	F	+	Paternal	Terminal
37	1 year	F	−	Maternal	Terminal
38	Birth	M	+	Maternal	Terminal
39	Birth	M	−	Paternal	Terminal
40	8 mo	F	+	Maternal	Terminal
41	14 mo	F	+	Paternal	Terminal
42	1 year	M	−	Maternal*	Derivative
43	6 mo	M	−	Paternal	Terminal
44	2 years	M	−	Unknown	Derivative
45	Birth	M	−	Maternal	Complex
46	Birth	F	−	Maternal	Terminal
47	7 mo	F	+	Paternal	Terminal
48	Unknown	M	−	Maternal	Terminal
49	Birth	F	−	Paternal	Terminal
50[b]	Prenatal	F	−	Unknown	Unknown
51	7 years	F	+	Unknown	Terminal
52	3 mo	F	+	Paternal	Terminal
53	Unknown	F	−	Paternal	Terminal
54	Unknown	F	−	Paternal	Terminal
55a	1 year	M	+	Maternal*	Derivative
55b	8 years	F	+	Maternal*	Derivative
56	10 mo	F	−	Maternal	Terminal
57	2 years	M	−	Maternal	Terminal
58	19 years	F	−	Paternal	Terminal
59	3 years	M	+	Maternal	Terminal
60	Unknown	M	−	Maternal	Terminal
61	1.5 years	F	−	Paternal	Terminal

[a] Parental origin of the chromosome with deletion of 1p36 is de novo in all cases, except those indicated with an asterisk (*).

[b] Study subject 50 is included in the table for completeness but was not evaluated for deletion size, parental origin, or type of rearrangement.

$(TTAGGG)_n$ telomeric repeat probe was present in each (data not shown). Of 60 deletions, 4 (7%) were interstitial, retaining either the 1p subtelomeric region or the 1p TAR (table 2; fig. 4*B*). Ten subjects (17%) had a derivative chromosome 1 in which the 1p telomeric region was replaced by another chromosome end (table 2; fig. 4*C*); three of these resulted from malsegregation of balanced parental translocations, and seven were de novo. The derivative chromosomes included addition of the telomeric regions from 1q in three cases, 22q in two cases, and one case each of 2p, 8p, 9q, 15p, and Xp. In addition, three subjects (subjects 3, 9, and 45) had more complex rearrangements (fig. 4*D*). Subjects 3 and 9 had interstitial deletions, which also retained a small piece of distal chromosome 1p36. Subject 45 had a terminal deletion of chromosome 1p36, retention of some proximal 1p36 material, and another, more proximal deleted region. Both microsatellite analysis and FISH confirmed each of these complex rearrangements.

Parental Origins of Deletions.—The parental origin of the chromosome with the deletion was determined in 40 of the 43 subjects with de novo terminal deletions (table 2). In two cases, we were unable to identify informative markers in the deleted region, and, in one case, neither parental sample was available. Twenty-four (60%) were derived from the maternally inherited chromosome, and 16 (40%) arose from the paternally inherited chromosome. In general, deletions of paternal origin were larger than deletions derived from the maternally inherited chromosome. Of those with de novo terminal deletions, 75% of maternal deletions were <5.0 Mb, whereas 62.5% of paternal breakpoints were >5.0Mb. We applied a Fisher's exact probability test (Fisher's Exact Statistical Analysis Web site) to compare the number of de novo maternal and paternal breakpoints that were <5.0 Mb to those that were >5.0 Mb. We found a significant difference ($P = .02$), with more maternally derived smaller deletions.

If all rearrangements are considered, 65% of deletions were from the maternally derived chromosome, and 35% were from the paternally derived chromosome. When we compared all breakpoints for size and parental origin by Fischer's exact probability test, including multiple breakpoints from those with complex deletions and interstitial deletions, a significant difference was found. Most maternally derived deletions were <5.0 Mb, and most paternally derived deletions were >5.0 Mb ($P = .013$).

Clinical Characterization of 30 Subjects with Monosomy 1P36

Craniofacial and Ophthalmological Features.—The facial characteristics of these subjects are remarkably similar. Common features include microcephaly, brachy-

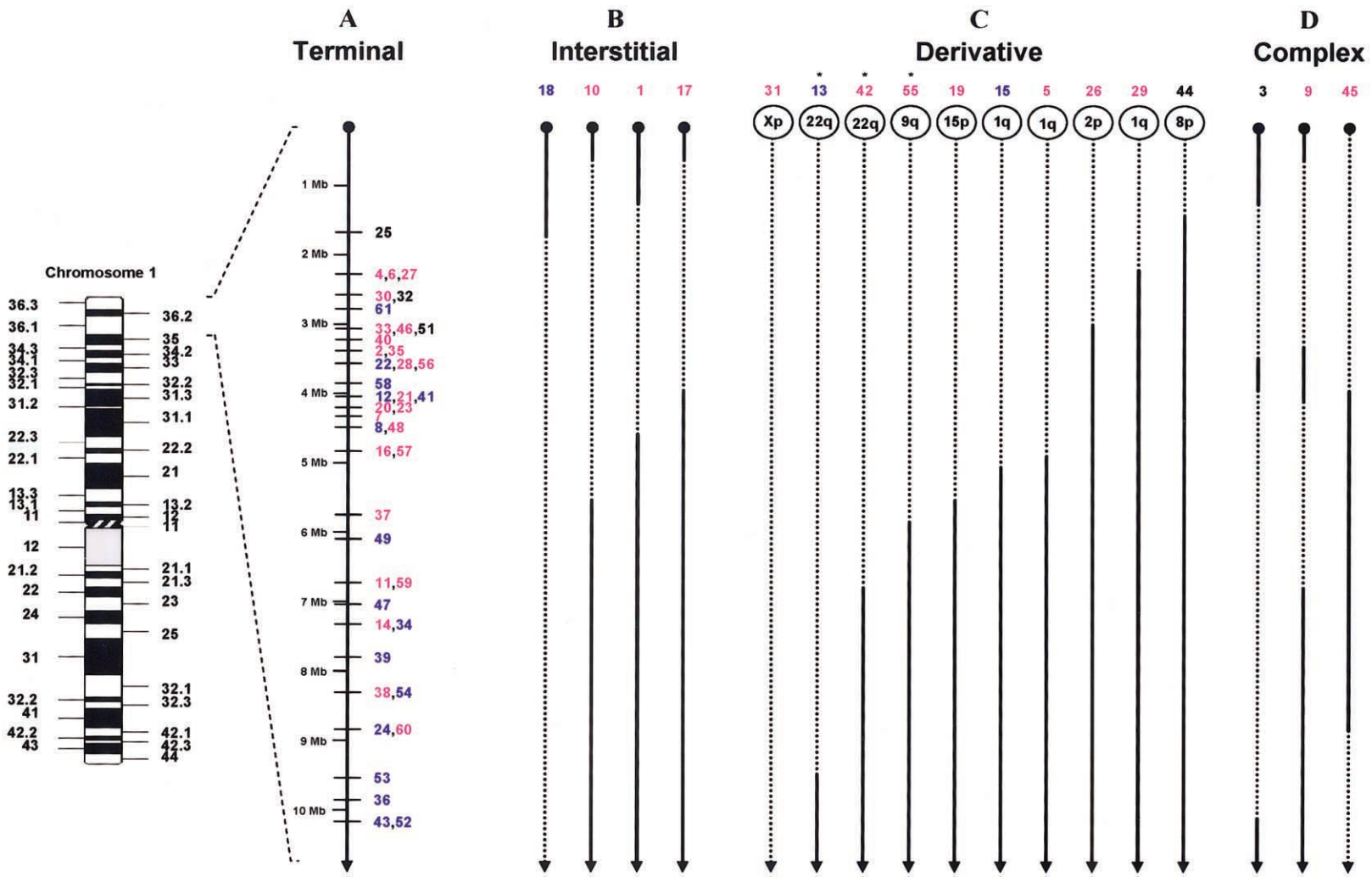

Figure 4 Classes of 1p36 deletions. The genomic sequence of the most distal 10.5 Mb of 1p36 is depicted as an extended vertical arrow. *A*, The breakpoint locations of the 43 subjects with apparently pure terminal deletions are indicated on a single solid arrow. The breakpoints of the 4 interstitial deletions (*B*), the 10 derivative chromosomes (*C*), and the 3 complex rearrangements (*D*) are represented as individual arrows. Solid lines indicate regions of 1p36 that are not deleted. Dotted lines indicate regions of 1p36 that are deleted. Pink patient numbers indicate maternally derived deletions; blue patient numbers indicate paternally derived deletions. Black patient numbers identify those whose parental origins could not be determined. The origin of the chromosomal material (as identified by FISH) that is translocated to the end of 1p36 in the derivative chromosome cases is listed within the circle at the telomeric end of each arrow. Asterisks indicate derivative chromosomes that are not de novo in origin.

cephaly, deep-set eyes, flat nose and nasal bridge, and pointed chin (fig. 1; table 3). By history or direct physical examination, 85% had a large or late-closing anterior fontanel. Other features include overt clefting abnormalities, in 17% of subjects. This included one patient with cleft lip and palate, one with cleft lip only, and three with minor palatal clefting abnormalities such as submucous occult cleft and bifid uvula. Five subjects had a high-arched palate but no overt clefting on examination, and one patient had a history of a cricoid cleft. Visual inattentiveness, defined as absence of attentive visual behavior with fixation and following movements, was seen in 30% of subjects. Other ophthalmological findings are shown in table 3.

Endocrinological Findings.—Two subjects had previously diagnosed hypothyroidism and were on thyroid supplementation at the time of this survey. Baseline thyroid studies on the remaining subjects showed that four others had elevated thyroid stimulating hormone (TSH) levels with corresponding low T4 levels; thus, six subjects (20%) had chemical hypothyroidism.

Neurological Manifestations.—All subjects had mental retardation of varying degrees, as assessed with a number of neuropsychiatric tests (Shapira et al. 1997), depending on the age of the subject. Fifty-eight percent of subjects had a history of seizures, including 48% with chronic seizures (epilepsy) who required continuing anticonvulsant medications. The types of seizures included generalized tonic-clonic, myoclonic, partial complex, and infantile spasms. The seizure histories and EEGs in the first 24 subjects have been reported elsewhere (Heilstedt et al. 2001). By report, 87% of subjects were described as hypotonic in infancy, and 63% had histories of feeding problems, including poor suck and swallow, reflux, and vomiting in infancy. Three subjects required gastric tubes for feeding difficulties, and 72% had oropharyngeal dysphasia on swallow studies, which ranged from mild to severe.

Cardiovascular Findings.—Seven subjects (23%) had a history of dilated cardiomyopathy in infancy. None worsened over time, although three subjects continued on medication at the time of the evaluations. Thirteen subjects (43%) had a structural heart defect, most frequently patent ductus arteriosus (table 3).

Hearing Evaluation.—Complete auditory evaluation, which included testing at high frequencies (6–8 kHz), was obtained on 28 subjects. Testing was performed by ABER or sound field evaluation. Eighty-two percent of subjects had some hearing impairment. Eleven subjects had mild high-frequency sensorineural hearing impairment (>30–50 db loss by tone burst), and 12 subjects had more severe hearing impairment at all frequencies. Only two had conductive hearing loss.

Table 3

Frequency of Clinical Features of Monosomy 1p36

Clinical Feature	No. of Study Subjects/Total Evaluated (%)
Physical characteristics:	
Large anterior fontanel	22/26 (85)
Microcephaly	18/30 (60)
Brachycephaly	18/30 (60)
Deep-set eyes	24/30 (80)
Flat nasal bridge	23/30 (77)
Flat nose	20/30 (67)
Thickened ear helices	16/30 (53)
Asymmetric ears	16/30 (53)
Posteriorly rotated ears	7/30 (23)
Low-set ears	7/30 (23)
Pointed chin	20/30 (67)
Short fifth finger	26/30 (87)
Fifth-finger clinodactyly	12/30 (40)
Clefting abnormalities:	
Orofacial clefting anomalies	5/30 (17)
Ophthalmological findings:	
Hypermetropia	20/30 (67)
Visual inattentiveness	9/30 (30)
Strabismus	9/30 (30)
Myopia	5/30 (17)
Nystagmus	4/30 (13)
Endocrinological findings:	
Hypothyroidism	6/30 (20)
Neurological findings:	
Developmental delay/mental retardation	30/30 (100)
Hypotonia	26/30 (87)
Oropharyngeal dysphasia	21/29 (72)
Feeding difficulties in infancy	19/30 (63)
Epilepsy[a]	15/31 (48)
Cardiovascular findings:	
Dilated cardiomyopathy	7/30 (23)
Patent ductus arteriosus	5/30 (17)
Ventricular septal defect	4/30 (13)
Dilated aortic root	3/30 (10)
Atrial septal defect	2/30 (7)
Left-ventricular dilation without cardiomyopathy	2/30 (7)
Bicommisural aortic valve	2/30 (7)
Ebstein anomaly	1/30 (3)
Auditory findings:	
Hearing loss	23/28 (82)

[a] One patient did not participate in the full evaluation but did have a history of seizures and therefore was included.

Genotype-Phenotype Correlation

The relative order and approximate location of 90 known genes or full-length mRNAs were identified by a comparative analysis of the annotated genomic sequence of 1p36 from three genome browsers (Ensembl; NCBI Home Page; UCSC Genome Bioinformatics Home). A clone-by-clone sequence analysis of the 99 clones in our 10.5-Mb contig of 1p36 was also performed with the U.K. Human Genome Mapping Resource Centre's bioinformatics application, NIX (NIX Web site). NIX uses BLAST analyses and multiple gene-prediction programs to iden-

tify various important elements within an unknown nucleic acid sequence. Fifty-one of the genes were identified by each of the four approaches, 29 were identified by three of the four approaches, and only two of the four approaches identified 10 of the genes. This list of the gene content of 1p36 is not exhaustive, because only known genes and full-length mRNAs were included. Many other novel genes may reside within 1p36, but, because of the extensive variations in the annotations of the human genome by the browsers used, putative novel genes were not included in our analyses. For those subjects in whom adequate clinical information is available, the critical regions for certain features are depicted in figure 5. These critical regions are based on the subject with the smallest deletion with a given phenotypic feature.

Discussion

To define more precisely the types and sizes of deletions of 1p36, we constructed a physical map of the most distal 10.5 Mb of 1p36. From this physical map, we created a natural deletion panel of subjects with 1p36 deletions and have localized the breakpoints to within 0.5-Mb bins. This deletion panel assigned the appropriate location and orientation of large-insert clones and markers found on the various public databases. In several subjects, the breakpoints were contained within large-insert clones. The deletion sizes in subjects with monosomy 1p36 were quite variable, although there was some breakpoint clustering 4.0–4.5 Mb from the telomere. This variability confounds the prediction of a common mechanism for these deletions, as has been postulated for other deletion syndromes, such as DiGeorge syndrome (Edelman et al. 1999) and Smith-Magenis syndrome (Chen et al. 1997), in which low-copy repeats mediate homologous recombination between nonallelic regions. The reason that deletions of 1p36 are observed with a relatively high population frequency of 1 in 5,000 (Shaffer and Lupski 2000) remains unknown.

We identified true terminal deletions, interstitial deletions, derivative chromosomes, and complex rearrangements of 1p36. Conventional cytogenetics may not distinguish these different rearrangements, especially those that are derivative chromosomes. In our study, three subjects had a derivative chromosome 1 inherited from a parent with a balanced translocation. Only two of these were seen cytogenetically. The third derivative chromosome could be detected only with subtelomere region–specific FISH probes. A balanced parental translocation substantially increases the risk of recurrence of an unbalanced event in a family. Therefore, FISH with subtelomeric region–specific probes should be considered in all cases of monosomy 1p36, to detect double segmental imbalances and to identify parental rearrangements. However, ~10% of our subjects had interstitial deletions, and most would be interpreted as normal if just the subtelomeric FISH probe were used. Thus, additional probes may be necessary to uncover deletions. Our results show that some cytogenetically defined terminal deletions of 1p36 may be more complex than simple telomeric truncations, and, on the basis of this observation, we suggest that these terminal deletions may be formed by a variety of mechanisms.

The de novo deletions occurred mostly on the maternal chromosomes, although the larger deletions tended to occur on the paternal chromosomes. The sex-specific breakpoint clustering is currently under further investigation. No obvious differences in phenotype could be interpreted solely from the parent-of-origin studies.

The clinical phenotypes in 30 subjects with monosomy 1p36 have been defined carefully in this study. No subject in our study had a history of cancer (10 mo to 13 years of age at evaluation). This observation may be due to (i) the age of our patient population, (ii) the small number of subjects in this study compared with the incidence of various cancers such as neuroblastoma, or (iii) possible parent-of-origin effects among varying-sized deletions in the development of cancer, most notably neuroblastoma (Wu et al. 1999).

Double segmental imbalance was present in the 10 subjects who had derivative chromosomes. Clinical information is available on only four of these subjects, those who were included in the GCRC protocol. Subject 13 has duplication of distal 22q in addition to deletion of 1p36, and subject 15 has duplication of distal 1q in addition to deletion of 1p36. These subjects are somewhat more delayed than comparable subjects with pure 1p36 deletion, although this is a nonspecific finding, in that the degree of delay is quite variable in this syndrome in patients with similar-sized deletions. Subject 19 has duplication of 15p in addition to deletion of 1p36. Because the short arm of chromosome 15 contains the ribosomal RNA genes and repetitive DNA, addition of this genetic material is unlikely to contribute to any phenotypic findings. Subjects 55a and 55b have duplication of distal 9q in addition to deletion of 1p36. These unbalanced translocations are due to malsegregation of a maternal translocation. Any contribution of 9q trisomy to the phenotype cannot be identified. These two subjects are somewhat different in their clinical presentations, with subject 55a having the characteristic large anterior fontanelle, which was absent in subject 55b, who is more delayed than subject 55a. However, these findings are within the usual variation seen in this syndrome. The variability in their clinical presentations is interesting, given that these two subjects have different fathers. This family demonstrates the difficulty in identifying small regions in which to search for genes

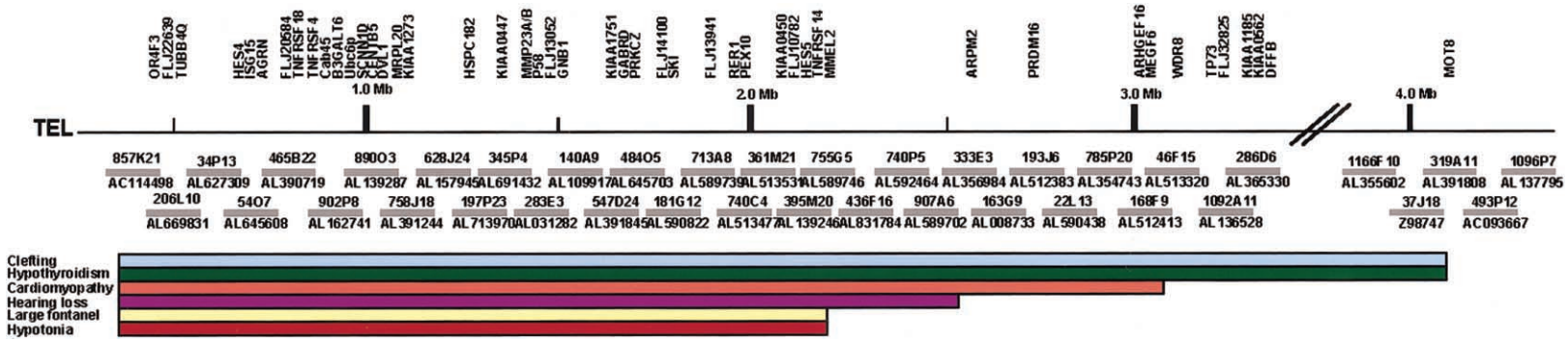

Figure 5 Gene content of critical regions within 1p36. The most distal 4.0 Mb of 1p36 is shown because the majority of critical regions are found in this segment. The known genes and full-length mRNAs are listed across the top. The telomere is at the far left of the figure. The slashes indicate a break in continuity of the distance. The names of the large-insert clones are listed as in figure 2. The approximate critical regions for the phenotypes are indicated by the horizontal colored bars.

to particular clinical features, because the clinical presentations can be variable even within families.

The constellation of facial features, including deep-set eyes, flat nasal bridge, flat nose, and pointed chin, leads to a clinically recognizable facial phenotype (fig. 1). The medical evaluations from the present study suggest that selected testing should be performed on all patients with monosomy 1p36. For example, hearing impairment appears to be quite common, occurring in 82% of study subjects. Many subjects had mild sensorineural hearing loss at the high frequencies (6–8 kHz). In more basic audiological evaluations, high-frequency hearing impairment would be missed, because these frequencies are not always tested, since they do not affect speech reception. However, the identification of mild hearing loss is important for appropriate follow-up testing. In addition, accurate diagnoses are critical to narrow the regions in which to search for causative genes. Thus, full audiometric evaluations, including testing at high frequencies, should be performed, and, because the hearing impairment has been progressive in some patients, yearly evaluations are suggested.

Seizures occurred in approximately half of subjects, so all patients should be monitored for seizure activity. Twenty percent of subjects in this study had chemical hypothyroidism requiring thyroxin supplementation. Hypothyroidism was reported recently in two unrelated patients with 1p36 deletions (Zenker et al. 2002). Therefore, we recommend that all patients with monosomy 1p36 be evaluated for thyroid dysfunction by T4, free T4, and TSH levels at birth, at 6 mo, and then annually. Palatal abnormalities were found in 17% of subjects and, thus, the palate should be evaluated, because these abnormalities can affect feeding and can compound both hearing loss and speech development. Oropharyngeal dysphagia was found in most study subjects (72%). Therefore, swallow-function studies should be included in the initial evaluation of patients with monosomy 1p36, to assess the need for oromotor intervention. Ophthalmological evaluation is encouraged, because 83% of study subjects had notable visual disorders, including substantial refractive errors. Dilated cardiomyopathy has been reported previously to occur in monosomy 1p36 (Keppler-Noreuil et al. 1995; Slavotinek et al. 1999); we found that nearly a quarter of our study subjects had this cardiac finding. However, a larger number of subjects (43%) had various structural cardiac abnormalities, suggesting that an echocardiogram should be included in the evaluation of patients with this deletion.

Because the deletions in monosomy 1p36 are variable, genotype-phenotype correlations may be useful to locate the genes responsible for several clinical features of the syndrome. The phenotypic features tend to vary with the size of the deletion, and study subjects with larger deletions tend to have more phenotypic features (Wu et al. 1999). Diligent clinical evaluation assists in the characterization of this genotype-phenotype correlation. We have used this approach to identify candidate genes associated with an epilepsy phenotype (Heilstedt et al. 2001) and clefting abnormalities (Colmenares et al. 2002). We anticipate that other genes, when haploinsufficient, result in other phenotypic features of the syndrome. Identification of genes involved in specific phenotypic features of this syndrome could potentially lead to more-precise treatments for individuals with 1p36 deletions and might identify mutations for single-gene disorders. We have elucidated potential critical regions for certain clinical findings in monosomy 1p36 (fig. 5). Although the terminal region of chromosome 1p36 is gene rich, only some of these genes will lead to a specific phenotype when deleted. Nonpenetrance, epigenetic, and stochastic factors may influence certain clinical features and would not allow the critical regions to be precisely defined. Characterization of more study subjects with small or complex deletions and accurate clinical assessment may refine some of these critical regions.

Because of diversity in both the types and sizes of cytogenetic anomalies in monosomy 1p36, these rearrangements should be investigated with a variety of FISH probes and/or polymorphic markers. Appropriate genetic counseling and accurate assessment of recurrence risks are contingent on a thorough analysis. Although monosomy 1p36 is the most commonly observed terminal deletion in the human population, the basis for its relatively high frequency remains unclear. Cloning and characterization of breakpoints should suggest mechanisms involved in generating deletions of 1p36.

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Electronic-Database Information

The URLs for data presented herein are as follows:

Ensembl, http://www.ensemble.org/
Fisher's Exact Statistical Analysis, http://faculty.vassar.edu/lowry/fisher.html
GenBank, http://www.ncbi.nlm.nih.gov/Genbank (for BAC and PAC clones listed in fig. 2)
Human Chromosome 1 Home Page, http://compgen.rutgers.edu/chr1/resources/index.shtml
NCBI Home Page, http://www.ncbi.nlm.nih.gov/
UCSC Genome Bioinformatics Home, http://genome.ucsc.edu
U.K. Human Genome Mapping Resource Center Bioinformatics Applications: NIX, http://www.hgmp.mrc.ac.uk/Registered/Webapp/nix/
Wellcome Trust Sanger Institute, Human Chromosome 1 Home, http://www.sanger.ac.uk/HGP/Chr1/

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