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High-Resolution Mapping of Genotype-Phenotype Relationships in Cri du Chat Syndrome Using Array Comparative Genomic Hybridization

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We have used array comparative genomic hybridization to map DNA copy–number changes in 94 patients with cri du chat syndrome who had been carefully evaluated for the presence of the characteristic cry, speech delay, facial dysmorphology, and level of mental retardation (MR). Most subjects had simple deletions involving 5p (67 terminal and 12 interstitial). Genotype-phenotype correlations localized the region associated with the cry to 1.5 Mb in distal 5p15.31, between bacterial artificial chromosomes (BACs) containing markers *D5S2054* and *D5S676;* speech delay to 3.2 Mb in 5p15.32-15.33, between BACs containing *D5S417* and *D5S635;* and the region associated with facial dysmorphology to 2.4 Mb in 5p15.2-15.31, between BACs containing *D5S208* and *D5S2887*. These results overlap and refine those reported in previous publications. MR depended approximately on the 5p deletion size and location, but there were many cases in which the retardation was disproportionately severe, given the 5p deletion. All 15 of these cases, approximately two-thirds of the severely retarded patients, were found to have copy-number aberrations in addition to the 5p deletion. Restriction of consideration to patients with only 5p deletions clarified the effect of such deletions and suggested the presence of three regions, MRI–III, with differing effect on retardation. Deletions including MRI, a 1.2-Mb region overlapping the previously defined cri du chat critical region but not including MRII and MRIII, produced a moderate level of retardation. Deletions restricted to MRII, located just proximal to MRI, produced a milder level of retardation, whereas deletions restricted to the still-more proximal MRIII produced no discernible phenotype. However, MR increased as deletions that included MRI extended progressively into MRII and MRIII, and MR became profound when all three regions were deleted.

Introduction

Deletions on chromosome 5p lead to a variety of developmental defects, with most cases classified as cri du chat syndrome (MIM 123450) (Niebuhr 1978*a*). These deletions may be terminal or interstitial and occasionally occur in the context of a cytogenetically complex karyotype (Sreekantaiah et al. 1999). Cri du chat syndrome has several phenotypic components, including the characteristic cry that gives the syndrome its name, facial dysmorphology, speech delay, and mental retardation (MR). Several previous studies have associated the extent of the deleted segment on 5p with the phenotype (Overhauser et al. 1994; Church et al. 1997; Marinescu et al. 1999*a*; Mainardi et al. 2001). However, these studies have produced somewhat inconsistent results and substantial controversy concerning the relationship of MR to the deleted region. The differing results are likely due to a combination of factors, including inconsistent evaluation of the phenotype by multiple observers, lack of consideration for the age dependence of the prominence of some phenotypic characteristics, and limitations of the analytical techniques used to assess the genotype (Wilkins et al. 1983; Church et al. 1995; Marinescu et al. 1999*a*; Johnson et al. 2000). In the present study, we address all of these potential problems.

Previous genotypic analyses have employed conventional cytogenetics, FISH, polymorphic markers, and, more recently, chromosome-based comparative genomic hybridization (CGH) (Marinescu et al. 1999*b*; Levy et al. 2002) to detect and define the nature of the aberrations. Conventional cytogenetic analysis provides an overview of the entire genome but has limited resolution. Thus, aberration boundaries may be inaccurately established, small deletions overlooked, and complex aberrations improperly identified. FISH and polymorphic markers allow accurate assessment of aberrations and mapping of the aberrations relative to the genome sequence, but they require substantial effort because

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the probes have to be evaluated individually or in small groups. Thus, studies of large numbers of loci and samples are very labor intensive. Although chromosome CGH is capable of screening the entire genome for DNA copy–number alterations in a single hybridization, its resolution is limited to ~5–10 Mb, and the results cannot be mapped directly onto the genome sequence.

Recently, we and others have developed methods of performing microarray CGH (Solinas-Toldo et al. 1997; Pinkel et al. 1998; Pollack et al. 1999; Snijders et al. 2001; Fiegler et al. 2003). Our arrays use array elements made from large-insert genomic clones, such as BACS and PACS, and have sufficient measurement precision to permit reliable detection of single-copy aberrations affecting individual clones. Each clone's location on the genome sequence is determined using a sequence tag, such as an STS or end sequence. The resolution obtained with such an array is determined by the genomic spacing between the clones and by the clone length. Resolution of a fraction of the length of a BAC can be obtained by using overlapping clones (Albertson et al. 2000).

In the present study, we applied array CGH to the analysis of genomic DNA from 94 patients, most of whom had been determined, by use of conventional cytogenetics and FISH, to have deletions on 5p. These specimens were selected from a collection of cases with 5p deletions that had undergone detailed phenotypic evaluation with consistent criteria (Niebuhr 1978*b*; authors' unpublished results), prior to our analysis. Assessments were performed at several ages for most patients, so that phenotype could be established at the development stage at which it is best evaluated. Subsets of these cases have been included in previous studies of genotype-phenotype correlations with the effect of 5p deletions (Overhauser et al. 1990, 1994; Church et al. 1995, 1997). Our results demonstrate the value of array CGH for evaluation of these patients and allow us to clarify and refine the genotype-phenotype correlations for MR, speech delay, facial dysmorphology, and the cry, in cri du chat syndrome.

Methods

Patients with Cri du Chat Syndrome

Subjects were selected from a group of >150 patients with 5p deletions who have been extensively studied by one of us (E.N.) over the past several decades. Subjects were included if detailed clinical data and genomic DNA were available. To facilitate additional studies, we analyzed only those subjects for whom previous studies had provided knowledge of the parental origin of the aberrant chromosome and somatic cell hybrids with the aberrant chromosome 5 and for whom familial genomic DNA was available. For most subjects, results of conventional cytogenetic analysis were available. Table 1 lists the 94 qualifying subjects and includes the phenotypic assessment, conventional cytogenetic assessment, and the array results. (Table A1 in appendix A [online only] contains an electronic version of table 1; it can be opened in Microsoft Excel for data manipulation and plotting.) Patient numbers 1–69 refer to consecutive (time at diagnosis) Danish families. Subjects with numbers ⩾100 are from other countries—patients 100–117 from Norway, patients 201–256 from England, patient 300 from Czechoslovakia, and patient 402 from Australia—and were referred for a more-detailed clinical and cytogenetic evaluation. Thus, these cases represent a quasirandom sampling of individuals with 5p deletions. The array CGH measurements on these specimens were performed after appropriate approval from the institutional review board of the University of California San Francisco was obtained.

Phenotypic features were evaluated by a single observer (E.N.) so that classification criteria were uniformly applied. Since aspects of the phenotype may change during development, most patients were evaluated when aged <5 years and again when aged >5 years. The presence or absence of a phenotypic characteristic is labeled by a "Y" or "N," respectively, in table 1. Lack of data is indicated by "ND." Because phenotypic characteristics may be age dependent, the presence of a "Y" for either age group classified a subject as positive for that phenotypic characteristic. For example, the cry and facial dysmorphology are most distinct when the patients are young, so that the loss of those phenotypes in the older group was not deemed significant. Several patients did not show a particular phenotype at one age range (absence indicated by "N"), and data were not available at the other, which resulted in uncertainty in their phenotypic status. Such cases were excluded from the determinations of the relationship of that characteristic to genotype. Thus, patients 18, 45, 56, 112, and 117 were not used to define the cry region, and patients 56 and 112 were not used to determine the genomic region associated with facial dysmorphology. Patients with the most-severe mental defects were not used in establishing the speech-delay region, since their ability to speak may have been impaired for other reasons. Thus, patient 45 was not used to localize the speech component of the phenotype.

Psychomotor-development assessments defining the severity of MR were based on personal observations (by E.N.), written information, psychological tests, school performance information, etc. MR is difficult to assess in young children, so only patients with data at age >5 years were used for correlations of MR with the deletion. Thus, patients 22, 225, and 231 were excluded from this analysis. The degree of MR was indicated by a numerical scale that ranged from 0 (unaffected) to 7 (profoundly

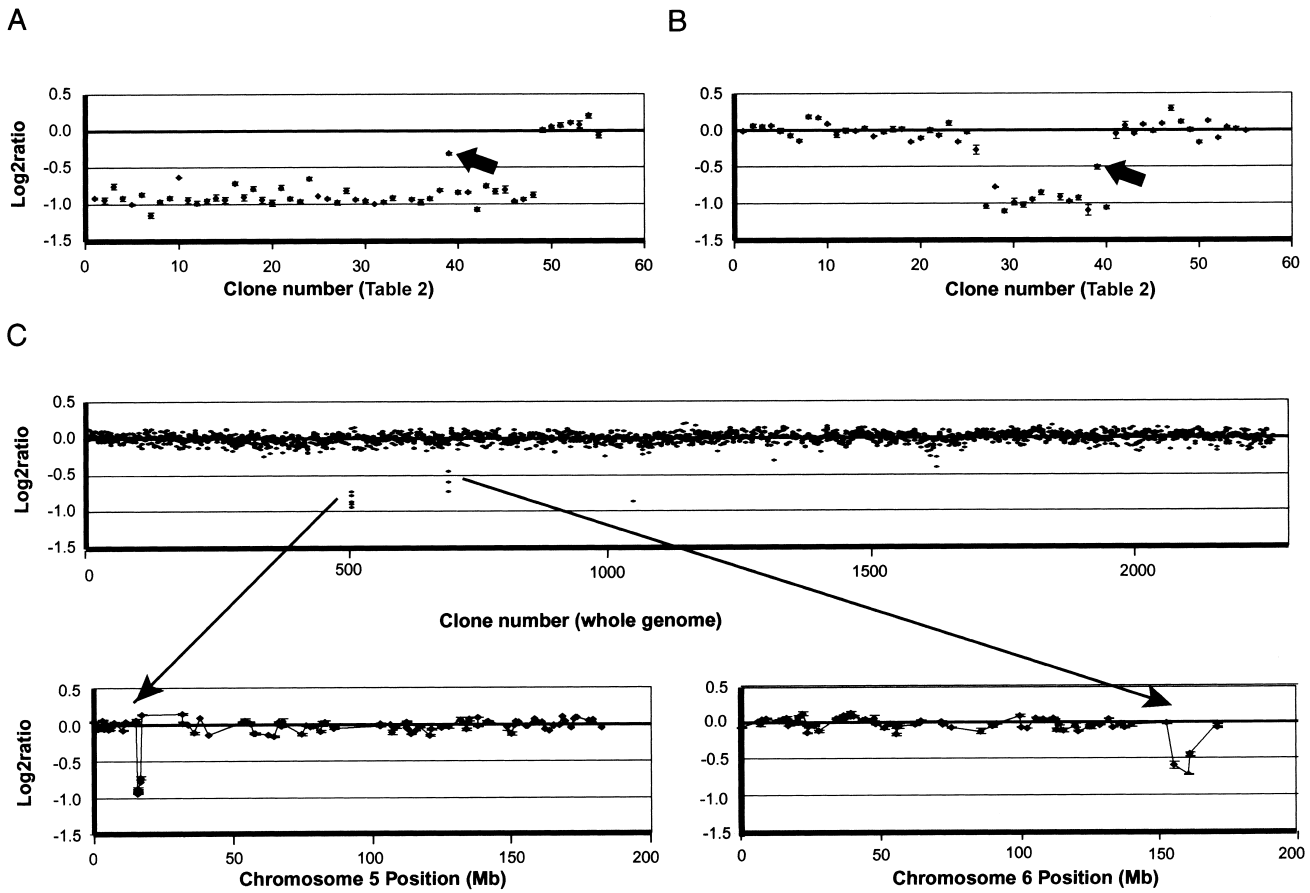

Figure 1 Array CGH analysis of patients with cri du chat syndrome. *A,* Terminal deletion, patient 27. The $\log_2$ of the measured ratio is plotted versus the order of clones on chromosome 5p, starting from the telomere at the left. Map positions of the clones are shown in table 3. Data are normalized so that $\log_2$ratio = 0 for portions of the genome that contain two copies of each sequence. Error bars, which, in most cases, are the same size as the dots in the figure, indicate the SD of the measurements on the replicate spots for each clone. The deletion boundary occurs between clones 48 and 49. The arrow indicates clone 39 that showed ratios intermediate between normal and deleted in subjects (*panel B*); deletion included clone 39 but did not include the region around clones 51 and 52. The $\log_2$ratio on clone 39, ~ -0.4, is consistent with a deletion from four to three copies of the sequence in that clone, suggesting it is duplicated in the human genome. FISH analysis confirms this duplication, as do some freezes of the human genome sequence. *B,* Interstitial deletion, patient 114. Clone 39 (*arrow*) shows an intermediate ratio in this case also. *C,* Whole-genome analysis of patient 45. The upper panel shows data for the whole genome, with the clones plotted in genomic order. Two small deleted regions, each involving several contiguous clones, are detected. The lower panels show the detailed data, plotted according to sequence position, for chromosomes 5 and 6, which contained the deletions. This array did not include the complete 5p clone set listed in table 3, so that the 5p deletion involves fewer clones than in the high-resolution analysis. The occasional single clones with ratios that differ from 0 either indicate copy-number polymorphisms between the test and reference genomes (Albertson and Pinkel 2003; Iafrate et al. 2004; Sebat et al. 2004) or small additional aberrations, noise.

affected). The general criteria for each classification are listed in table 2. The uncertainty of these assessments is estimated to range from ± 0.5 at the lower end of the scale to ± 1.0 at the upper end.

Arrays.—Arrays for high-resolution analysis of 5p contained elements produced from BAC, P1, and PAC clones that were selected using genetically mapped STS markers (Hudson et al. 1995; Peterson et al. 1999), as well as variable numbers of clones located on other chromosomes that were used for normalization and for detection of other aberrations. Locations of all clones are based on the July 2003 University of California–Santa Cruz (UCSC) freeze of the human genome. The density of coverage on 5p was highest in regions previously defined as important in cri du chat syndrome. The 55 clones that encompassed all of the 5p aberrations found in these patients are listed in table 3. (Table A2 in appendix A [online only] contains an electronic version of this table; it can be opened in Microsoft Excel for data manipulation and plotting.) With completion of the genome sequence, we were able to accurately map most clones. However, the sequence positions of several had to be interpolated on the basis of genetic-map information. All patients were analyzed on arrays that contained the 55 clones on 5p, plus an additional 100–300 clones at other locations. Thirty-seven of the patients were also analyzed on various versions of arrays that contained ~1,750–2,000 clones distributed over the entire genome (Snijders et al. 2001), to determine whether there were aberrations involving other chromosomes. Some of these larger arrays did not include the full set of 5p clones.

DNA for the array elements was isolated from the clones and was amplified by linker-adapter PCR. The PCR products were suspended in 20% dimethyl sulfoxide in water at a concentration of ~0.8 μg/μl (Snijders et al. 2001) and were spotted onto chromium-coated slides by use of a custom-built printer employing capillary-tube printing pins (authors' unpublished material). Neighboring triplicate spots were printed for each clone. For the smaller arrays, two sets of triplicates at widely separated locations on the array were printed.

Array CGH hybridization and analysis.—All genomic DNA was isolated from peripheral blood of patients and control subjects by use of standard techniques. DNA was labeled using either nick translation (1 μg) (Pinkel et al. 1998) or random primer extension (0.5 μg) (Snijders et al. 2001). Fluorochromes directly coupled to dCTP were used for labeling. Some measurements employed fluorescein and Texas-red (or Alexa 594), whereas others used Cy3 and Cy5 to label the test and reference genomes, respectively.

Labeled test and reference genomic DNAs along with 50 μg of Cot-1 DNA, included to suppress the hybridization of the repetitive sequences, were ethanol precipitated and resuspended in hybridization mix to a final composition of 50% formamide/10% dextran sulfate/2 × SSC/1%–4 % (v/v) SDS to a total volume of ~50 μl. The hybridization mix was heated to 70°C to denature the DNA, and the temperature was lowered to 37°C for ~1 h to allow reassociation of the repetitive sequences. For hybridization, a low barrier made from rubber cement was formed around the array. The hybridization mix was then applied to the array, and the array was placed in a sealed chamber that left the upper surface of the fluid free. Hybridization proceeded for 16–48 h on a slowly rocking table at 37°C (Pinkel et al. 1998). After hybridization, arrays were rapidly washed with a stream of PN buffer (0.1 M sodium phosphate; 0.1% nonidet P40; pH 8) to remove most of the hybridization solution; they were then immersed in 50% formamide/2 × SSC at 45°C for 15 min, followed by a final wash in room-temperature PN buffer for 15 min. After arrays were washed, a solution of 90% glycerol and 10% phosphate buffer, pH 9, containing 1 μM of the DNA stain DAPI (4′,6-diamidino-2-phenylindole) was applied to each array, and a cover slip was added. The DAPI stained the array spots, rendering them visible independent of the hybridization signals.

Arrays were imaged in a custom-built charge-coupled device (CCD) imaging system (Pinkel et al. 1998; authors' unpublished data). We acquired DAPI plus Cy3 and Cy5 or fluorescein and Texas-red images, depending on which dye pair was used to label the test and reference genomes. The entire array was contained within a single CCD image. The image-analysis software UCSF SPOT (Jain et al. 2002) was used for most analyses. This program determined the location of the array spots by use of the DAPI image and calculated the test and reference intensities of each pixel in each spot, after subtraction of local background. We use the ratio of the total (background subtracted) test intensity to total (background subtracted) reference intensity as the measure of relative copy number for each spot, and we averaged the ratios of the replicates. No computational adjustments of any type (e.g., shading corrections, lowess normalization, spatially dependent normalizations, and clone-specific normalizations) were made to either the images or the ratio data. Quality criteria, including the correlation of test and reference signals within a spot, were applied to recognize problematic signals. Generally, spots with a correlation $r < 0.8$ were rejected. Clones with an SD >0.2 for the replicates were removed from the analysis. In most cases, the SD of replicate spots was <0.02. Measurements of clones with only one of the replicates surviving quality checks were also rejected. An overall normalization factor was applied, so that the median of the $\log_2$ratios, or median linear ratio, of array elements at two copies per cell was set equal to 0 or 1, respectively.

Table 1

Summary of Primary Data for 94 Subjects with Cri du Chat Syndrome

Patient	Phenotype			MR Level	Conventional Cytogenetics[a]	Boundary (Mb/clone no.[b])	
	Cry[c]	Facial Dysmorphology[c]	Speech Delay			Proximal[d]	Distal
1	Y/Y	Y/Y	Y	5	46,XY,del(5)(p14.3)*	20.837/39	
3	Y/Y	Y/Y	Y	6.5	46,XY,del(5)(p13.3)*	30.726/51	
4	Y/Y	Y/Y	Y	6	46,XY,der(5)t(5;22)(p14.2;p13)pat	30.022/44	
5	Y/Y	Y/Y	Y	5.5	46,XY,del(5)(p14.3)*	17.500/37	
6	Y/Y	Y/Y	Y	6	46,XX,del(5)(p14.1)*	23.276/41	
8	Y/Y	Y/Y	Y	6	46,XY,del(5)(p14.1)*	25.841/42	
9	Y/Y	Y/Y	Y	7	45,XX,dic(5;13)(p13.3;p12)*	31.942/45	
10	Y/Y	Y/Y	Y	5.5	46,XX,del(5)(p14.2)*	21.797/40	
11	ND/Y	Y/Y	Y	5.5	46,XX,del(5)(p14.1)*	30.022/44	
12	ND/Y	Y/Y	Y	5.5	46,XX,del(5)(p14.3)*	18.336/38	
13	Y/Y	Y/Y	Y	6	46,XX,del(5)(p13.3)*	33.428/49	
14	Y/Y	Y/Y	Y	6	46,XY,del(5)(p13.3)*	30.022/44	
15	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.3)*	20.837/39	
16	Y/Y	Y/N	Y	6.5	46,XX,der(5)t(5;8)(p14.1;p22)*	31.751/46	
17	Y/Y	Y/Y	Y	6	46,XX,del(5)(p14.1)*	25.841/42	
18	ND/N	Y/N	N	6	46,XY,del(5)(p14.1p15.31)*IN	30.022/44	8.021/15
20	Y/Y	Y/Y	Y	6	46,XX,del(5)(p14.1)*	31.751/46	
21	Y/Y	Y/Y	Y	5.5	46,XX,del(5)(p14.3)*	18.336/38	
22	Y/ND	Y/ND	Y	NA[e]	46,XX,add(5)(p14.3)*	21.797/40	
23	Y/Y	Y/Y	Y	6	46,XY,del(5)(p14.1)*	30.022/44	
24	Y/Y	Y/N	Y	7	46,XX,add(5)(p14.1)mat	21.797/40	
25	Y/Y	Y/N	Y	7	46,XX,add(5)(p14.2)*	18.336/38	
26	Y/Y	Y/Y	Y	6	46,XY,del(5)(p14.1)*	31.751/46	
27	Y/Y	Y/Y	Y	6	46,XY,del(5)(p13.3)*	33.428/49	
28	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.3)*	20.837/39	
30	Y/Y	Y/Y	Y	5.5	46,XX,del(5)(p14.3)*	20.837/39	
31	Y/Y	Y/Y	Y	6	46,XX,del(5)(p13.3)*	31.942/45	
35	Y/ND	Y/ND	Y	6.5	45,XY,der(5)t(5;14)(p14.2;q12),-14,mat	23.276/41	
36	Y/Y	Y/Y	Y	6	46,XX,del(5)(p14.1)*	30.022/44	
37	Y/Y	Y/Y	Y	6	46,XX,del(5)(p14.1)*	30.022/44	
38	Y/Y	Y/Y	Y	5	46,XX,del(5)(p15.1)*	18.336/38	
39	Y/Y	Y/Y	Y	6	46,XX,del(5)(p14.1)*	30.022/44	
40	Y/Y	Y/Y	Y	5	46,XY,del(5)(p15.1)*	16.684/33	
42	Y/Y	Y/ND	Y	3	46,XX,del(5)(p15.2)*	11.035/28	
44	Y/Y	Y/ND	Y	3	46,XX,del(5)(p15.2)*	11.035/28	1.207/1
45	ND/N	N/N	Y	7	46,XY,der(14)t(5;14)(q11;p13) del(5)(p14.3p15.31)*	17.500/37	14.929/30
48	Y/N	Y/N	Y	6.5	46,XY,add(5)(p14.2)*	16.684/33	
49	Y/Y	Y/Y	N	5	46,XX,der(5) ins(15;5)(q22;p14.3p15.31)mat	26.794/43	5.945/12
50	Y/Y	Y/Y	Y	6	46,XX,del(5)(p14.1)*	31.942/45	
51	N/N	N/N	N	0	46,XX,del(5)(p13.3p14.1)mat	31.942/45	18.336/38
52	Y/Y	Y/Y	Y	6.5	46,XY,del(5)(p13.3)*	31.751/46	
54	Y/Y	Y/Y	Y	5	46,XX,del(5)(p15.1)*	16.684/33	
55	Y/Y	Y/Y	Y	5.5	46,XX,der(5)t(5;13)(p14.3;p12)mat	20.837/39	
56	ND/N	ND/N	ND	6	46,XY,der(5)t(5;7)(p14.1p21)? del(5)(p14.1p15.1)?del(7)*	26.794/43	8.021/15
58	Y/Y	Y/Y	Y	6	46,XX,del(5)(p14.1)*	30.022/44	
59	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.3)*	23.276/41	
60	Y/Y	Y/Y	Y	5	46,XY,del(5)(p14.2)*	20.837/39	
63	Y/Y	Y/Y	Y	5	46,XX,rec(5)del(5)(p14.2) inv(5)(p14.1p15.1)mat	21.797/40	
66	Y/Y	Y/Y	Y	6	46,XX,del(5)(p14.2)*	23.276/41	
68	Y/Y	Y/Y	Y	6	46,XY,del(5)(p14.1)*	25.841/42	
101	Y/Y	Y/ND	Y	2.5	46,XX,del(5)(p15.2)*	11.035/28	
102	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.2p15.33)*	23.276/41	3.174/4
104	Y/Y	Y/Y	Y	6.5	46,XY,del(5)(p13.3)*	32.162/48	
105	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.3)*	17.224/36	
107	Y/Y	Y/Y	Y	6	46,XY,del(5)(p13.3)*	33.428/49	
109	Y/Y	Y/Y	Y	5.5	mos 46,XX,del(5)(p14.1)/del(5)(p15.2)*	25.841/42	
110	Y/Y	Y/Y	Y	6.5	46,XX,del(5)(p13.3)*	30.726/51	
111	Y/Y	Y/Y	Y	6	46,XY,del(5)(p14.1)*	25.841/42	
112	ND/N	ND/N	N	1	46,XX,del(5)(p14.2p15.2)*	23.276/41	11.435/27
113	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.1)*	23.276/41	
114	N/N	N/N	N	2	46,XY,del(5)(p14.2p15.2)*	23.276/41	11.360/26
115	Y/Y	Y/Y	Y	5	46,XX,del(5)(p15.2)*	11.035/28	
117	ND/N	N/N	Y	3.5	46,XY,del(5)(p15.31)*	9.288/18	
201	Y/Y	Y/N	Y	7	46,XY,add(5)(p14.3)*	17.224/36	
202	Y/Y	Y/ND	Y	2.5	46,XY,del(5)(p15.2)*	11.435/27	

(continued)

Table 1 (continued)

Patient	Phenotype			MR Level	Conventional Cytogenetics[a]	Boundary (Mb/clone no.[b])	
	Cry[c]	Facial Dysmorphology[c]	Speech Delay			Proximal[d]	Distal
203	Y/Y	Y/Y	Y	6	46,XY,del(5)(p13.3)*	30.022/44	
205	Y/Y	Y/Y	Y	5	46,XY,del(5)(p14.2)*	21.797/40	
206	Y/Y	Y/ND	Y	7	46,XX,add(5)(p14.3)pat	18.336/38	
209	Y/Y	Y/Y	Y	6	46,XX,del(5)(p13.3)*	31.751/46	
210	Y/Y	Y/Y	Y	7	46,XX,del(5)(p14.1)pat	25.841/42	
212	Y/Y	Y/Y	Y	6.5	46,XY,del(5)(p14.1)*	30.022/44	
213	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.2)*	20.837/39	
214	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.2)*	17.224/36	
215	Y/Y	Y/N	Y	7	46,XX,der(5)t(4;5)(q32;p14.3)*	14.929/30	
216	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.2)*	20.837/39	
218	Y/Y	Y/Y	Y	6.5	46,XX,add(5)(p14.3)pat	17.500/37[f]	
219	Y/Y	Y/Y	Y	6.5	46,XY,del(5)(p14.1)*	25.841/42	1.207/1
221	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.2)*	18.336/38	
222	Y/Y	Y/Y	Y	6	46,XY,del(5)(p14.1)*	26.794/43	
223	Y/Y	Y/Y	Y	6	46,XX,del(5)(p13.3)*	33.428/49	
225	Y/ND	Y/ND	Y	4[g]	46,XY,del(5)(p14.3p15.31)*	21.797/40	3.174/4
228	Y/Y	Y/Y	Y	6	46,XY,add(5)(p15.2)pat	11.035/28	
229	Y/Y	Y/Y	Y	7	45,XX,dic(5;22)(p13.2 p12)*	33.428/49	
231	Y/ND	Y/ND	Y	3.5[g]	46,XX,del(5)(p15.1)*	15.679/31	
232	Y/Y	Y/Y	Y	6.5	46,XX,del(5)(p13.3)*	31.942/45	
250	Y/Y	Y/Y	Y	5	46,XX,del(5)(p14.1)*	26.794/43	
251	Y/Y	Y/Y	Y	6.5	46,XX,del(5)(p13.3)*	30.022/44	
252	N/N	N/N	N	5	46,XY,del(5)(p15.1p15.31)*	15.872/32	6.365/13
253	Y/Y	Y/Y	Y	5	46,XY,r(5)(14.3q35.3)*	20.837/39	
254	Y/Y	Y/Y	Y	5	46,XY,del(5)(p14.3)*	20.837/39	
255	Y/Y	Y/Y	Y	6.5	46,XY,add(5)(p14.2)*	20.837/39	
256	Y/Y	Y/Y	Y	5.5	46,XX,del(5)(p13.3)*	30.022/44	
300	Y/Y	Y/Y	Y	5	46,XY,del(5)(p14.1)*	25.841/42	
402	Y/Y	Y/ND	Y	3	46,XY,del(5)(p15.2)*	11.035/28	

Note.—Y = phenotype present. N = phenotype absent. ND = not determined.

[a] An asterisk (*) indicates de novo cases (83 total; 78% paternal, 22% maternal). The other 11 cases are familial.

[b] Clone number is from table 3.

[c] Presence or absence when patient is aged <5 years/presence or absence when patient is aged >5 years.

[d] Undeleted clone at 5p deletion boundary. If interstitial deletion is present, proximal and distal deletion boundaries are indicated. Boundary clones may be at elevated copy number, since there are some duplications at deletion boundaries (table 4).

[e] Patient is deceased; MR level not available (NA).

[f] Noisy sample. Multiple hybridizations indicate boundary could be clone 36 or 37.

[g] Assessment for patients 225 and 231 was at age 3.5 years and 3 years, respectively.

Results

Array CGH Measurements

Representative copy-number profiles for chromosome 5 and a whole-genome scan are shown in figure 1. Single-copy deletions and gains in a homogeneous sample would ideally have $\log_2$ratios of -1.0 and $+0.58$, respectively. In practice, the ratios for the deletions had a range of -1 to -0.7, with deviations from ideal presumably due to incomplete suppression of signals from highly repeated sequences, cross hybridization, imperfect definition of background levels, etc. With this nearly quantitative relationship of ratio to copy number and with the noise levels (indicated in fig. 1), deletions could be detected with very high reliability by use of a simple threshold set at a $\log_2$ratio equal to -0.4. In most cases, sufficient precision to establish one or both deletion boundaries of the interval between two array clones was achieved in a single hybridization. If the initial measurement contained too much noise to identify clones flanking a deletion boundary, the measurement was repeated. Measurements with dye reversal were not performed, since they provided no more information than a rehybridization. Occasionally, it was found that repurification of the specimen DNA improved data quality, indicating that unknown contaminants in some specimens affected the ability to consistently label or hybridize the genomic DNA.

Table 1 summarizes the deletion data, conventional cytogenetic analysis, and phenotypic characteristics for the 94 patients. The patients are listed in numerical order, corresponding to previous numbering (Niebuhr 1978*a*; authors' unpublished material). The deletions in 82 subjects were terminal, whereas, in 12, the deletions were interstitial, for a total of 106 deletion boundaries

Table 2

Classification of MR

MR Level	Description (IQ)	Phenotype	
		Adult	Child
0	Normal	No MR	No MR
1	Borderline (<70)	Attends standard school for many years; requires minor/major support	Normal developmental milestones; minor retardation obvious during the first school years
2	Very mild (<65)	Attends standard school for a few years; requires major support; has simple reading, writing, and math ability	Normal developmental milestones during the first years of life; minor retardation obvious from age 2–3 years
3	Mild (<50)	Understands everything, including long sentences; has very simple reading, writing, and math ability	Developmental milestones delayed a few mo; retardation obvious from age 1–2 years
4	Moderate (<35)	Understands almost everything; makes use of small sentences and lots of signs	Developmental milestones delayed several mo; retardation obvious from age 1 year
5	Severe (<20)	Understands simple, daily sentences and single words; uses sentences of 2–3 words, and many signs; walks	Developmental milestones delayed several mo to 1 year; MR obvious before age 1 year
6	Very severe (<10)	Understands a few words; usually walks, unsteadily, if supported; has no language or only a few words	Developmental milestones delayed several years; MR obvious before age 6 mo
7	Profound	Shows minor or no response; may sit and stand unaided; walking is rare	Cannot sit unaided at age 5 years

on 5p. As discussed more fully below, 15 of the subjects were found to have copy-number aberrations in addition to 5p deletions. Three of those aberrations were duplications of 5p sequences adjacent to the deletion boundary, whereas the other aberrations involved genomic regions other than 5p, as shown in table 4.

Two indications of interesting DNA-sequence features were found on the basis of our measurements. First, a significant breakpoint cluster was detected. All of the 106 deletion boundaries found in these patients occurred within the distal ~33 Mb of the chromosome, resulting in an average density of about three boundaries per megabase. Of the 94 cases, 9 had distal or proximal boundaries in the ~0.5 Mb region of clones 26, 27, and 28 in 5p15.2, yielding a density of ~18 boundaries per megabase. Moreover, if the clones in this region are ordered as indicated in the July 2003 sequence freeze, then the deletion boundaries appear complex, having oscillations in the ratio of neighboring clones at the boundaries. Reordering the clones simplifies the apparent structure of the deletion boundary in all cases. In table 3, we list the clones in our revised order, but we indicate sequence positions based on the genome-sequence assembly. These results suggest that there may be some sequence motifs in this region that facilitate aberration formation and that complicate proper assembly of the genome sequence. Alternatively, if the sequence assembly is correct, then the cluster of deletion boundaries in this region has a recurrent complex copy-number structure that reflects local sequence features. Higher density clone coverage would potentially narrow the size of this cluster region and would potentially reveal other breakpoint clusters in 5p.

The second sequence feature we found was a region of sequence duplication on 5p in the normal human genome. Data for 58 cases (e.g., fig. 1*A* and 1*B*,) indicated that clone 39 had a $\log_2$ratio of ~ -0.4, intermediate between that expected for a normal region and that for a deletion, when the surrounding clones were clearly deleted. However, if the deletion extended proximally to include the region of clones 51 and 52, then the ratio on clone 39 decreased to the expected value. This suggests that a substantial fraction of the sequence of clone 39 was duplicated on 5p between clones 51 and 52, so that deletions including only clone 39 would represent a change from four copies to three ($\log_2[3/4] \sim -0.4$). FISH analysis with this BAC as a probe confirmed this supposition, with the discovery of two closely spaced signals on 5p. Some freezes (June 2002, November 2002, and April 2003) of the human genome sequence have indicated the presence of this duplication, locating the marker in this BAC at both 20.791 Mb and 34.070 Mb (April 2003). The July 2003 freeze we have used does not indicate the duplication. Large duplications of this type occur frequently in the genome (Lupski et al. 1996; Eichler 2001). No polymorphism in DNA copy number (Albertson and Pinkel 2003; Iafrate et al. 2004; Sebat et al. 2004) that involved clone 39 was found in this set of samples. Further indication of the likely sequence complexity in this region is the fact that the sequence assembly disagrees with the clone order indicated by the simplest interpretation of our deletion boundaries near clone 51, as noted in table 3.

Dependence of MR on Deletions

Figure 2*A* shows the relationship of deletions on 5p to the MR status of the patients, with the patients ordered by increasing level of retardation. The relationship appears complex and somewhat perplexing. There is a clear increase in the severity of retardation with an in-

Table 3

Array Clones for 5p Analysis

Clone Number	Marker		Position			Clone Name
	Symbol	Name	Cytogenetic[a]	Sequence (Mb)[a]	Genetic (cM)[b]	
1	*D5S1981*	*AFMa217zh1*	5p15.33	1.207	1	CTC-326E20
2	*D5S2005*	*AFMB002xc1*	5p15.33	1.395	0	RP11-94J21
3	*D5S1970*	*AFMa183wh5*	5p15.33	2.497	5	CTB-116F8
4	*D5S417*	*AFM205wh8*	5p15.33	3.174	6	RP11-20B3
5	*D5S1980*	*AFMA217yh1*	5p15.33	3.449	7	RP11-82M24
6	*D5S675*	*AFM336tc1*	5p15.33	3.998	9	RP11-103L11
7	*D5S1906*	*WI-2725*	5p15.33	4.258		CTB-27O23
8	*D5S405*	*AFM154xa3*	5p15.33	3.995	9	RP11-227M19
9	*D5S406*	*AFM154xg3*	5p15.32	5.047	12	RP11-58A5
10	*D5S1921*	*WI-2897*	5p15.32	5.200		CTC-263B18
11	*D5S464*	*AFM112xe3*	5p15.32	5.890	15	CTC-248O4
12	*D5S2054*	*AFMB355wb1*	5p15.32	5.945		RP11-53K22
13	*D5S635*	*AFM276yb9*	5p15.32/15.31	6.365	16	RP11-36H5
14	*D5S676*	*AFM347yg9*	5p15.31	7.492	18	RP11-46O23
15	*D5S18*[c]		5p15.31	8.021		CTB-16G3
16	*D5S1957*	*AFMa124wg5*	5p15.31	8.550	20	CTB-27I9
17	*D5S208*		5p15.31	9.039	21	DPA-896A3
18	*D5S74*[d]		5p15.31	9.288		DPA-671G7
19	*D5S630*	*AFM268zd9*	5p15.31	9.614	21	DPA-255H3
20	*D5S759*	*AFM204ze1*	5p15.31/15.2	10.100		DPA-1398C5
21	*D5S1850*	*WI6722*	5p15.2	10.328		DPA-1104F2
22	*D5S23*[e]		5p15.2	10.364		DPA-1349G2
23		*AFM042xa11*	5p15.2	10.403	23	RP11-145B1
24	*D5S2480*	*WI7320*	5p15.2	10.732		DPA-941C2
25	*D5S432*	*AFM255xb9*	5p15.2	10.746		RP11-72C10
26	*D5S117*		5p15.2	11.360		DPB-70G7
27	*D5S2887*	*AFMa240xf9*	5p15.2	11.435	25	RP11-29N3
28	D5S478[f]	*AFM179xd10*	5p15.2	11.035	24	CTC-305H11
29	*D5S2081*	*AFM347ta5*	5p15.2	13.530	26	CTC-305H17
30	*D5S1991*	*AFMa282wa5*	5p15.2/15.1	14.929	29	RP11-5N8
31	*D5S1989*	*AFMa247wc1*	5p15.1	15.679	30	RP11-135M13
32	*D5S1954*	*AFMA114xf9*	5p15.1	15.872	30	RP11-261B20
33	*D5S1963*	*AFMA140vd1*	5p15.1	16.684	31	RP11-269O14
34	*D5S416*	*AFM205wh10*	5p15.1	16.723	31	RP11-260E18
35	*D5S2114*	*AFMa090yh5*	5p15.1	16.949	32	CTB-28D11
36	*D5S486*	*AFM206zc1*	5p15.1	17.224	34	CTB-33B3
37	*D5S2096*	*AFM105xg1*	5p15.1	17.500	35	RP11-88L18
38		*WI-4804*	5p15.1/14.3	18.336		CTB-34B4
39	*D5S2419*[g]	*WI-10830*	5p14.3	20.837		CTC-253L1
40	*D5S411*	*AFM193xe11*	5p14.3	21.797	39	CTB-55P22
41	*D5S1868*	*WI-9400*	5p14.3/14.2	23.276		CTB-115E13
42	*D5S648*	*AFM292yg5*	5p14.1	25.841	42	CTB-100A5
43	*D5S627*	*AFM217ye1*	5p14.1	26.794	43	CTC-296N5
44	*D5S661/D5s2061*	*AFMC011xb1*	5p14/13	30.022	44	CTB-161H9
45	*D5S477*[f]	*AFM177xb4*	5p13.3	31.942	49	
46	*D5S1993*	*AFMa286ya9*	5p13.3	31.751	48	CTB-13O21
47	*D5S1986*	*AFMA238za9*	5p13.3	31.776	49	RP11-5N11
48	*D5S1996*	*AFM297wa5*	5p13.3	32.162	51	RP11-67P13
49	*D5S651*	*AFM302wd5*	5p13.3	33.428	51	CTC-221E3
50	*D5S2062*	*AFM277yh9*	5p13.3	33.806	55	RP11-94E6
51	D5S395[f]	*AFM284vc1*	5p13.3	30.726		CTB-62F24
52	*D5S1506*	*GATA63c02*	5p13.3	33.873		CTB-38G24
53	*D5S395*	*AFM063yb6*	5p13.2	35.843	57	RP11-85N3
54	*D5S2025*	*AFMb297za5*	5p13.2	36.018	57	CTB-107L17
55	*D5S1994*	*AFMa286ze9*	5p13.2	36.465	56	CTB-18O17

NOTE.—An electronic version of this table (table A2) is available in appendix A (online only).

[a] Position is based on the July 2003 UCSC sequence.

[b] Position is based on the STS-based map of the human genome (Hudson et al. 1995).

[c] Interpolation between *D5S676* and *D5S1957*.

[d] Interpolation between *D5S208* and *D5S630*.

[e] Interpolation between *D5S1850* and *afm042xa11*.

[f] Position is based on ordering by deletion data. The order disagrees with the genome sequence.

[g] Our data indicate that this sequence is duplicated in the genome. July 2003 freeze does not show duplication; April 2003 indicates second copy located at ~34.070 Mb.

Table 4

Additional Aberrations

Patient	MR Level	Cytogenetic Position	Copy Number	Upper Boundary		Lower Boundary		Size (Mb)	Revised Karyotype[a]
				Retained Clone[b]	Position (Mb)[c]	Retained Clone[b]	Position (Mb)[c]		
16	6.5	8pter-21.3	3	pter	0	RP11-89M8	22.745	22.745	46,XX,der(5)t(5;8)(p14.1;p21)
24	7	13q32.2-ter	3	RP11-40H10	93.26	qter	113.042	19.782	46,XX,der(5)t(5;13)(p14.1;q32.2)
25	7	5q35.3-ter	3	RP11-125L2	173.496	qter	181.034	7.538	46,XX,der(5)t(5;5)(p14.2;q35.3)mat
35	6.5	14q11.2	1	RP11-152G22	19.227	RP11-68M15	21.467	2.24	45,XY,der(5)t(5;14)(p14.2;q11.2), -14,mat
45	7	6q25.2-6qter	1	GS-59B4	152.455	RP1-57H24	170.695	18.24	46,XY,der(14)t(5;14)(q11;p13) del(5)(p15.1p15.2)del(6)(q25.2)
48	6.5	5p15.11-5p14	3	RP11-261B20/32[d]	15.872	CTC-296N5/43	26.794	10.922	46,XY,del(5)(p14.3)dup(5)(p14.2p15.1)
104	6.5	5p13.3	4	CTB-13O21/46	31.715	RP11-67P13/48	32.162	.447	46,XY,del(5)(p13.3)dup(5)(~p13.3)[e] × 2
201	7	5q35.1-ter[f]	3	RP11-15F10	169.122	qter	181.034	11.912	46,XY,der(5)t(5;5)(p14.3q35.1)
206	7	20pter-12.2	3	pter	0	DuPontA-967A8	10.282	10.282	46,XX,der(5)t(5;20)(p14.3;p12.2)pat
210	7	1qter	3	RP11-194F13	238.741	qter	246.127	7.386	46,XX,der(5)t(1;5)(q43;p14.1)pat
215	7	4q31.1-ter	3	RP11-40D5	135.249	qter	191.731	56.482	46,XX,der(5)t(4;5)(q31.1;p14.3)
218	6.5	8pter-8p23.2	3	pter	0	RP11-112O08	11	11	46,XX,der(5)t(5;8)(p14.3;p23.2)pat
228	6	8q23.1-ter	3	CTD-2013D21	110.377	qter	146.308	35.931	46,XY,der(5)t(5;8)(p15.2;q23.1)pat
251	6.5	5p13.3	3	CTB-115E13/41	23.276	RP11-94E6/50	33.806	10.53	46,XY,del(5)(p13.3)dup(5)(p13.3~14.1)[e]
255	6.5	1qter	3	RP11-210E16[g]	229.578				46,XY,der(5)t(1;5)(q42~43;15.2)

Note.—Aberrations that apparently extend to the telomere are listed as pter or qter.

[a] Karyotype is revised on the basis of array data. Initial assessment is shown in table 1.

[b] Clone at upper (closest to pter) or lower (closest to qter) boundary of aberration but not included in the aberration.

[c] Position is based on the July 2003 UCSC sequence; positions without decimals are estimated.

[d] Clone number from table 3.

[e] Defined by array data.

[f] Noisy data due to poor DNA quality. Other aberrations may be present.

[g] Gain includes this clone. There were insufficient clones on array to estimate the size of the gained region.

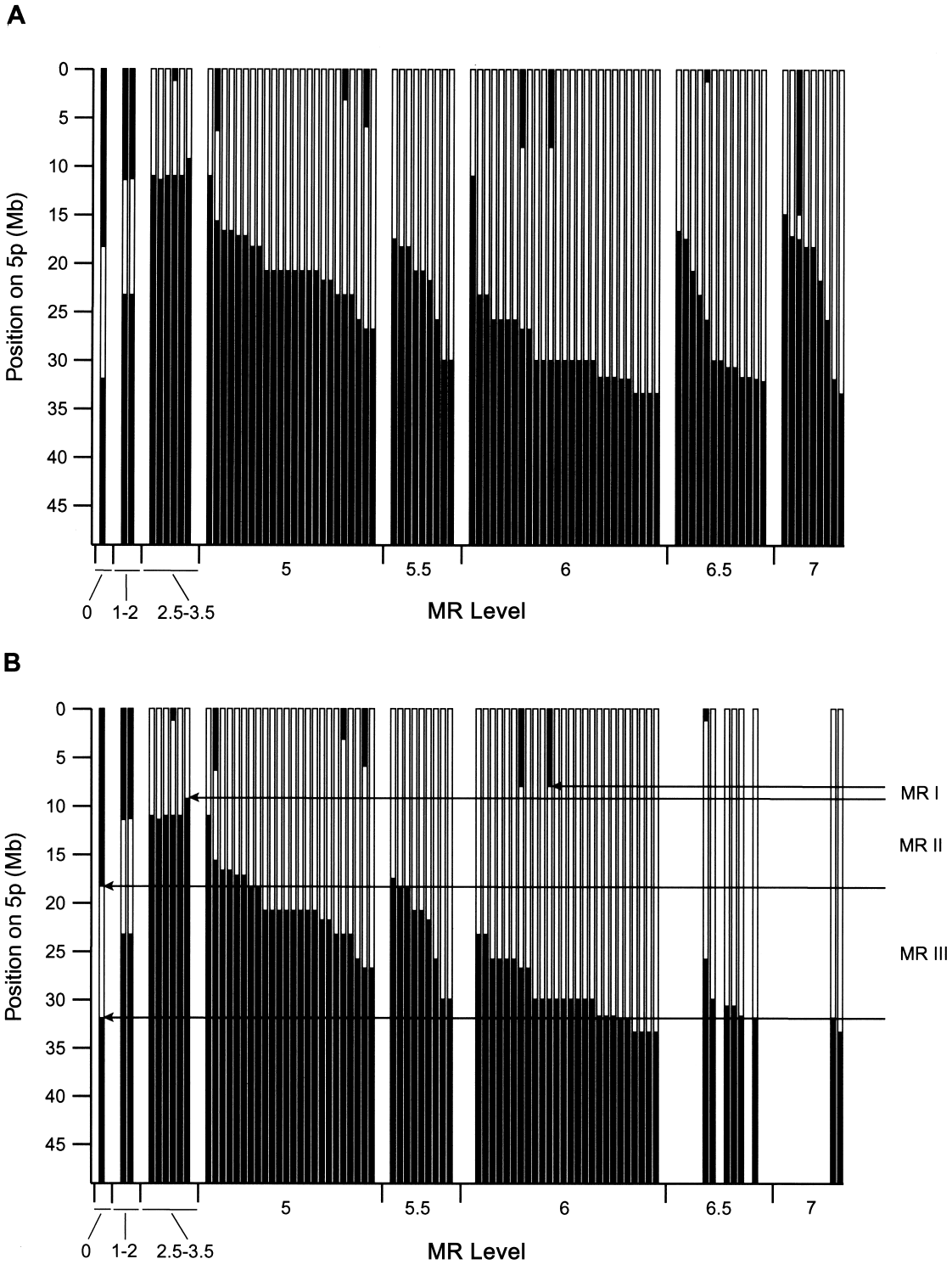

Figure 2 Dependence of MR level on 5p deletion. *A,* Data from all 91 patients for whom retardation assessment was available. Blackened portions of the bars indicate the chromosomal region(s) that is retained in each case, plotted versus physical position from 5pter. The centromere is just below the bottom of the graph. The dependence of retardation level on deletion is evident, but there are many cases with deletions that appear too small for the general trend. For MR level ≥ 5, data are plotted in the order of the proximal deletion boundary within each level. There is an estimated uncertainty of ± 0.5–1 in MR-level assignment of retardation phenotype from these severely and profoundly affected patients. *B,* The same plot for patients in whom we detected only 5p deletions. Note that most of the severely retarded patients are no longer present because they have additional aberrations (fig. 2*A*). The dependence of retardation on 5p deletion is much more consistent. Three regions of 5p—MRI, MRII, and MRIII—with differential effects on retardation are indicated. These regions are discussed in the text.

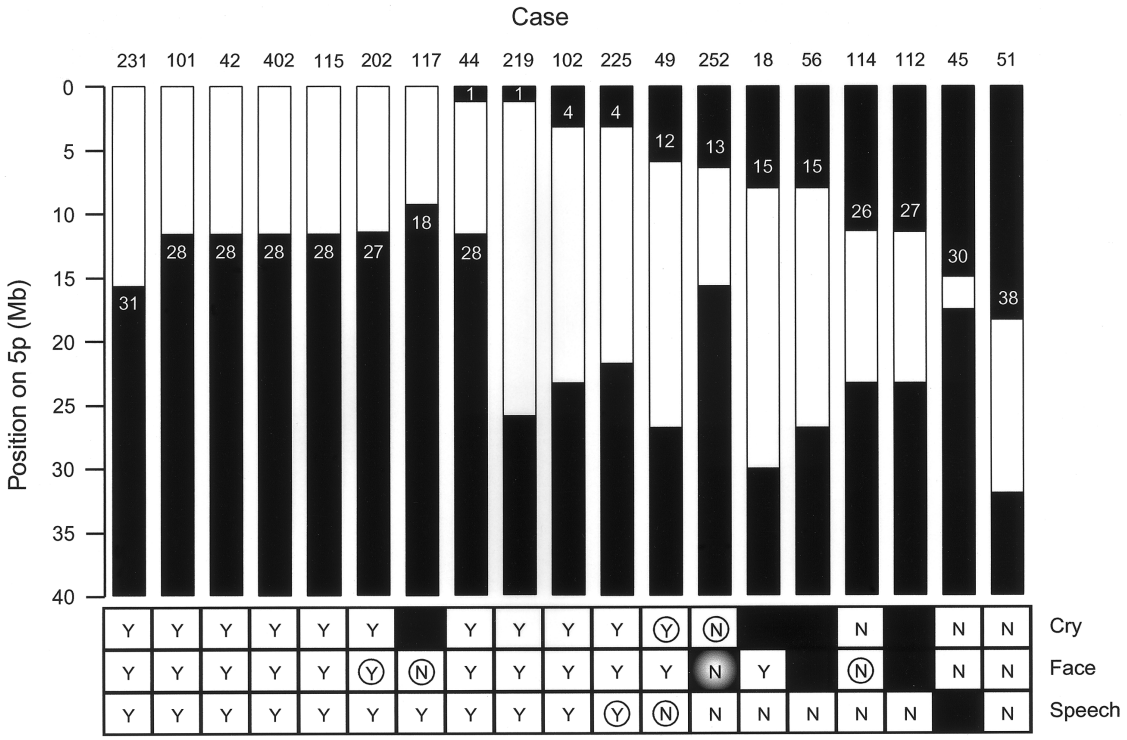

Figure 3 Summary of patient data that localize regions of 5p responsible for the cry, facial-dysmorphology, and speech-delay characteristics of the phenotype. Blackened portions of the bars indicate retained regions of the chromosome. The retained clones at the boundaries are indicated (see table 3). A "Y" indicates that the phenotypic characteristic was present in the patient, and an "N" indicates that it was absent. Blackened squares indicate that relevant information was not available for that patient. The circled letters indicate those patients who provide the most informative information on the location of the chromosome portion responsible for that characteristic. The shaded "N" for patient 252 indicates that this patient's deletion should have produced the phenotype by did not.

creasing extent of deletion, but there are some significant exceptions. For example, there are some patients with interstitial deletions on 5p who are either unaffected or minimally retarded, whereas others with smaller deletions in the same region are profoundly retarded.

Conventional cytogenetic and FISH analyses had previously been performed on these subjects and had shown that the chromosomal aberrations in some of the patients were complex, involving rearrangements of chromosomes in addition to chromosome 5. Therefore, we analyzed 37 of the patients, using arrays that provided genome-wide data. These cases included all those whose 5p deletions seemed too small to explain their retardation level, when compared with the general trend (shown in fig. 2*A*), plus an approximately equal number that fit the trend. All subjects with severe retardation and small 5p deletions were found to have additional gains or losses (e.g., patient 45 [shown in fig. 1C]). No differential phenotypic effects could be specifically attributed to any of these additional aberrations. Details of the subjects with the additional aberrations are listed in table 4. Because arrays with evolving clone compositions were used for these measurements, the precision with which the boundaries of the additional aberrations are defined is variable. However, it is clear that these aberrations typically involve at least several contiguous array elements, making them considerably larger in extent than the copy-number polymorphisms in unaffected individuals (Albertson and Pinkel 2003; Iafrate et al. 2004; Sebat et al. 2004). Some of these complex cases involve additional aberrations affecting chromosome 5, including several with duplications of material proximal to the deletion boundary. Figure 2*B* shows the relationship between deletion and MR level for all patients with 5p deletions and no other detected aberrations. The relationship between severity of MR and deletion size and location is now much more consistent.

Chromosomal Regions Affecting Cry, Speech Delay, and Facial Dysmorphology

The chromosomal regions affecting cry, speech, and facial features have previously been mapped to the distal portion of 5p (Overhauser et al. 1994; Gersh et al. 1995, 1997). Figure 3 shows our data for the subjects with deletion boundaries that provide information on these ge-

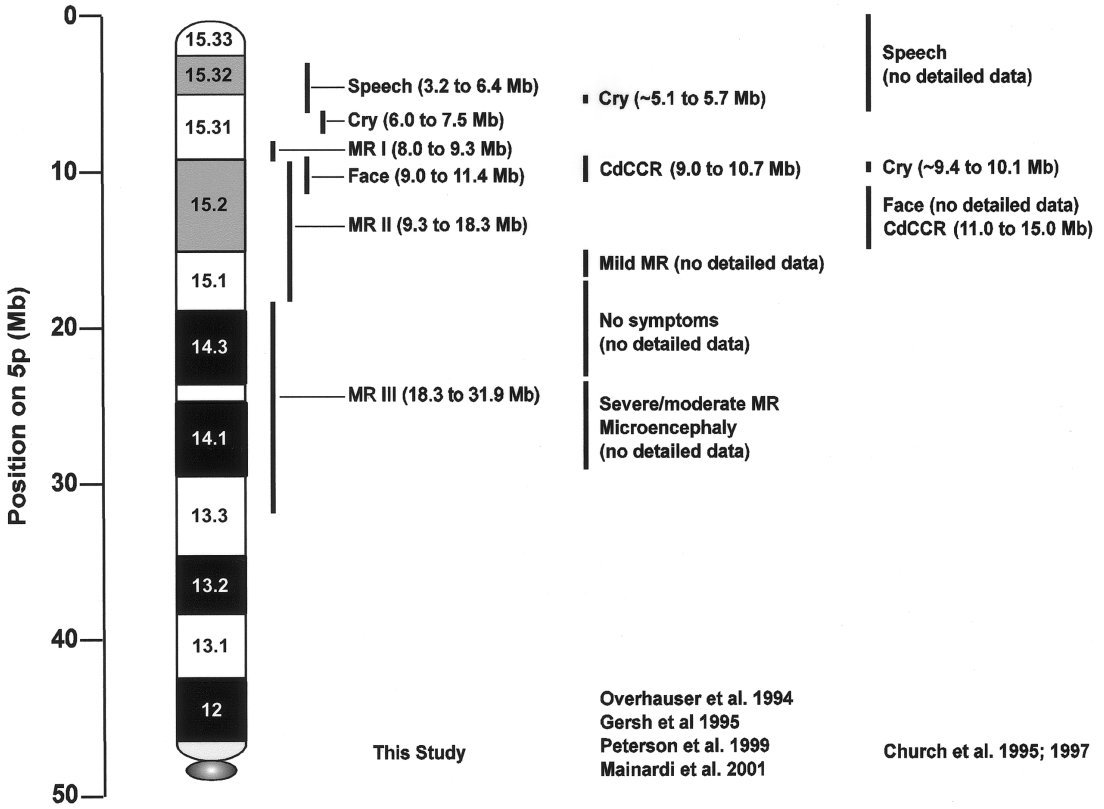

Figure 4 Summary of genotype-phenotype relationships from our data and data in previous publications. Chromosome bands are indicated relative to the physical map from the July 2003 freeze of the human genome sequence.

notype-phenotype relationships. The clones at the boundaries are indicated for each case. The region that affects the characteristic cry is most narrowly defined by the difference in patients 49 and 252, both of whom have interstitial deletions. For patient 49, who has the cry, the deletion begins proximal to clone 12, whereas for patient 252, who does not have the cry, the deletion may retain sequences up to clone 14. Thus, the chromosomal region responsible for this phenotype is located in the 1.5-Mb region between these clones.

The region affecting the characteristic facial features is most narrowly defined by patients 117, 202, and 114. Patient 117 has a terminal deletion beginning between clones 17 and 18 and has no facial phenotype. Thus, the important region must lie proximal to clone 17, since material may be retained until this position. Patient 202 has a larger terminal deletion, with a boundary between clones 26 and 27, and has the phenotype. This defines a 2.4-Mb region between clones 17 and 27 as the region responsible for the facial features. Patient 114 supports the proximal limit of this region. Patient 114 has an interstitial deletion, with the distal boundary between clones 26 and 27, and has no phenotype, which places the critical location for this phenotype distal to clone 27. We note that patient 252, who has a deletion that includes this proposed critical region, is discordant, because the patient does not have the phenotype.

The chromosomal region responsible for speech delay is best defined by the data from patients 102, 225, and 49, who have interstitial deletions. In patients 102 and 225, who have the phenotype, the deletion is proximal to clone 4. In patient 49, who does not have the phenotype, the distal portion of the chromosome may be retained up to clone 13. Thus, speech delay is due to deletion within a 3.2-Mb region between clones 4 and 13.

Discussion

The establishment of the relationship between patient genotype and phenotype in developmental syndromes is the first step toward discovery of the genetic mechanisms responsible for the symptoms and provides a basis for clinical management of the disease in patients. Numerous previous studies of chromosomal aberrations that lead to cri du chat syndrome have established the outlines of this relationship, as summarized in figure 4. Several regions on chromosome 5p that separately affect different components of the classical phenotype have been

defined, including one in 5p15.2 that has a strong effect on MR, termed "the cri du chat critical region" (CdCCR), and others associated with the characteristic cry, speech delay, and facial features. However, there have been significant discrepancies between the various studies, including controversy about the relationship between MR and deletion. Our study, which employed array CGH to give efficient high-resolution analysis of DNA copy-number alterations in a group of patients whose phenotypes were carefully assessed, has provided more-accurate information on specific aspects of the phenotype and has clarified some of the controversial issues.

The relationship of deletion size and location to MR has been difficult to ascertain; some studies have found a progressive increase in the severity of effect as the deletion size increases (Mainardi et al. 2001), whereas others have found no such relationship (Marinescu et al. 1999*a*). In our study, we analyzed a set of patients who had been assessed for MR status on a numerical scale—in a consistent manner by a single observer (E.N.)—many years prior to our measurements (Niebuhr 1978*a,* 1978*b*; Overhauser et al. 1990; Kjaer and Niebuhr 1999). Because assessment of retardation may be dependent on patient age, we have included only patients for whom evaluation was performed when they were aged >5 years. All analyses employed DNA isolated from peripheral blood rather than Epstein-Barr virus–transformed cell lines, so that transformation-induced aberrations did not confound our results.

Figure 2*A* shows the relationship of deletions on 5p to MR level. It is clear that patients with MR levels of ~3 have smaller deletions than those with MR levels >5, but the dependence of retardation on deletion size appears weak. For example, for MR levels >5, there is almost complete overlap of the deletion ranges among the different retardation levels, so that many patients with level-5 MR have 5p deletions similar to those patients with level-7 MR. Moreover, there are some striking apparent discordances. Patients 112 and 114 have ~10-Mb interstitial deletions, with MR levels of 1–2, whereas profoundly affected patient 45 has a much smaller interstitial deletion in the same region.

Cytogenetic analyses had previously shown that some patients with cri du chat syndrome have complex chromosomal rearrangements, with copy-number aberrations involving other regions of the genome. Therefore, we performed whole-genome–array CGH for 37 patients, including all those whose 5p deletion seemed too small to account for their retardation level. Aberrations in addition to the 5p deletions were found in 15 of these patients, as seen in table 4. Our results indicate that the majority (14/22) of the profoundly retarded subjects have aberrations in addition to 5p deletions. If one restricts attention to those subjects for whom the only aberrations we detected were deletions on 5p, the dependence of retardation on deletion size and location becomes much clearer. Figure 2*B* shows that, for retardation levels >3.5, retardation increases monotonically as the deletion size increases. The small departures from this general behavior may be due to modifying genetic factors in patients, uncertainty in the determination of MR level, or undetected small aberrations elsewhere in the genome. We note that the clarity of the relationship of MR to deletion as shown in figure 2*B* supports the accuracy of the phenotypic assessment of the patients, because it is highly unlikely that it could have occurred by chance. The lack of patients with deletion boundaries more proximal than 33 Mb from 5pter may indicate that such deletions are lethal during development.

Array CGH provides a more complete assessment of the genome than does standard cytogenetics. Overall, we find that ~16% of our subjects had complex aberrations, somewhat greater than the 12%–13% in previous studies (Niebuhr 1978*a*; Mainardi et al. 2001). Many—but not all—of the subjects with additional dosage aberrations found by array CGH (e.g., patient 206) had cytogenetically detected structural aberrations involving chromosome 5 and other chromosomes (table 1). Conversely, some subjects with interchromosomal cytogenetic aberrations did not have copy-number changes outside of chromosome 5p (e.g., patients 4 and 49). Thus, the higher resolution and efficiency of array CGH has significant advantages for assessment of dosage abnormalities in cri du chat syndrome and should have a significant diagnostic benefit, especially for severely retarded patients, of whom approximately two-thirds have modest 5p deletions and additional aberrations.

The dependence of MR on 5p deletions, as shown in figure 2*B,* suggests the presence of three chromosomal regions that affect this phenotype in different ways. The first, MRI, is included in the deletions of all subjects with retardation levels >2.5. Findings for patients 117 and 56 indicate that MRI may be confined to the 1.2-Mb region between clones 15 and 18 in 5p15.31, which is at the distal end of the CdCCR in 5p15.2, as defined elsewhere (Overhauser et al. 1994; Mainardi et al. 2001). It is not clear how many critical genes may be contained in MRI, but its limited size suggests that it may be a very small number.

The other two regions, MRII and MRIII, are located immediately proximal to MRI, as indicated in figure 2*B*. Deletions restricted to MRII have mild affects, whereas those affecting only MRIII (e.g., in patient 51) result in no discernible phenotype. However, patients with deletions that include MRI have increasingly severe retardation as the deletion extends into MRII and MRIII. Thus, it appears that a series of genes within MRII and MRIII contribute to the phenotype, possibly through modification of the affects of the gene(s) in MRI.

The defining of these boundaries is, of course, subject

to some uncertainty and depends on the underlying model one adopts of how the deletions affect MR. Our model basically assigns a dominant role to MRI, with additional aberrations in MRII and MRIII serving to dramatically increase the severity of the phenotype. The distal boundary of MRI is set by the fact that the deletion or retention of genes distal to clone 15 does not have a significant effect on MR level in our patients. However, there are no patients in our set with deletions restricted to the region distal to MRI, so this cannot be absolutely confirmed. The absence of the deletions may indicate the lack of ascertainment due to absence of the MR phenotype, but one would expect such individuals to have other characteristics of cri du chat syndrome.

Setting the proximal boundary of MRI is more problematic. Above, we have indicated a choice of the smallest possible region consistent with the data by using the boundary of patient 117. However, other subjects, who have both less-severe and more-severe retardation levels, have slightly larger deletions, with boundaries in the breakpoint cluster region near clones 26–28. Thus, patient 117 might be somewhat anomalous, so that MRI may extend distally an additional 2 Mb to clone 28. Analysis of a large number of additional cases with well-characterized phenotypes will be required to better define this region.

Deletions restricted to MRII and MRIII lead to mildly affected or phenotypically normal individuals. However, their relatively normal phenotypic states appear "fragile," because additional aberrations result in severe phenotypes. These additional aberrations may involve deletion of MRI, as discussed above, and/or gains or losses elsewhere on chromosome 5 (for patients 25, 48, 104, 201, and 251) or on other chromosomes (table 4). For example, profoundly retarded patient 45 has only a small deletion within MRII on 5p, which would be expected to produce a very mild retardation. Thus, the additional deletion on 6q (fig. 1*C*) presumably results in the very severe MR phenotype. We note that gains in MRII and MRIII may influence phenotype. Patient 48, with MR level 6.5, has a deletion of MRI and part of MRII that is consistent with retardation levels 4–5. Thus, its duplication (at the deletion boundary) of portions of MRII and MRIII has strong effects.

The fragility of the normal phenotype in individuals with deletions in MRIII is also indicated by family data. For example, the deletion in patient 51 is present in the mother and a grandparent, and all three are unaffected. However, there are reports that unaffected parents with deletions in MRIII may have an affected child who has inherited the deletion (Hand et al. 2000; Johnson et al. 2000). This change in phenotype may indicate that environmental or inheritable modifying factors that do not adversely affect people with normal genomes have significant consequences for those with MRIII deletions. Alternatively, the affected children may have additional aberrations that were not detected with the techniques used in those studies. High-resolution whole-genome array CGH might detect such aberrations and clarify the genotypic status of such children.

Our measurements have also provided improved localization of the regions on chromosome 5p that affect the characteristic cry, facial features, and speech delay. For the cry region, deletion of only one clone distinguished patients with and without the phenotype. That places the possible important gene(s) between the two flanking clones (containing markers *D5S2054* and *D5S676,* respectively) on the array. Thus, higher-resolution analysis of these two cases would allow placement of more stringent limits on the critical region. Higher-resolution analysis of the current cases will not substantially refine the speech-delay or facial regions, since multiple-array clones are contained within them. A comparison of our results on the genotype-phenotype relationships with those of previous publications is shown in figure 4.

In summary, we have mapped the aberrations in 94 patients with deletion of 5p, using array CGH to improve the understanding of the relationship between genotype and phenotype. We have shown that there are three regions of the chromosome that have differential effects on the MR level of the patients. Deletions involving all or parts of these three regions, coupled with other aberrations in the genome, interact to produce the full MR phenotype. Finally, our high-resolution data have permitted refinement of the locations of genes involved in the typical cry, facial features, and speech delay in cri du chat syndrome.

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Electronic-Database Information

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for cri du chat syndrome)
UCSC Genome Bioinformatics, http://genome.cse.ucsc.edu/

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