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Molecular Definition of Deletions of Different Segments of Distal 5p That Result in Distinct Phenotypic Features

Deanna M. Church,[1] Ulla Bengtsson,[1] Kirsten Vang Nielsen,[4] John J. Wasmuth,[1,2,3] and Erik Niebuhr[4]

Departments of [1]Biological Chemistry and [2]Pediatrics and [3]Human Genome Center, University of California—Irvine, Irvine; and [4]Department of Medical Genetics, Human Genome Group, Panum, University of Copenhagen, Copenhagen

Summary

Cri du chat syndrome (CDC) is a segmental aneusomy associated with deletions of chromosome 5p15. In an effort to define regions that produce the phenotypes associated with CDC, we have analyzed deletions from 17 patients. The majority of these patients had atypical CDC features or were asymptomatic. Using these patients, we have mapped several phenotypes associated with deletions of 5p, including speech delay, catlike cry, newborn facial dysmorphism, and adult facial dysmorphism. This phenotypic map should provide a framework with which to begin identification of genes associated with various phenotypic features associated with deletions of distal 5p. We have also analyzed the parental origin of the de novo deletions, to determine if genomic imprinting could be occurring in this region. In addition, we have isolated cosmids that could be useful for both prenatal and postnatal assessments of del5(p) individuals.

Introduction

Cri du chat syndrome (CDC) is associated with deletions of 5p15 and is one of the most common contiguous-gene disorders, with an incidence of 1/50,000 live births (Niebuhr 1978). Hallmarks of this syndrome include mental and developmental retardation, microcephaly, hypertelorism, epicanthal folds, high palatal arch, microretrognathia, and a plaintive, high-pitched cry similar to the mewing of a cat (Lejeune et al. 1963; Niebuhr 1978). There have been no reports of CDC without a gross chromosome rearrangement, implicating at least a 1–2-Mb region that is important in rendering the phenotype. Previous studies indicate that there is a CDC critical region in 5p15.2-15.3 (Niebuhr 1978). In addition, patients have been described who present with only the catlike cry in the absence of the other hallmarks of the syndrome (Smith et al. 1990; Overhauser et al. 1994). Analysis of the abnormal chromosomes from these individuals suggests that the gene(s) involved in producing the abnormal cry maps to the proximal region of 5p15.3, whereas genes involved with other aspects of the phenotype are located in 5p15.2 (Overhauser et al. 1994).

Individuals with 5p deletions show a great deal of phenotypic heterogeneity (Breg et al. 1970; Niebuhr 1978; Wilkins et al. 1980, 1983). *Smith's Recognizable Patterns of Human Malformation* (Jones 1988) states that only four characteristics are present in all CDC individuals: the catlike cry, slow growth, microcephaly, and mental retardation. Other features, such as hypertelorism, may be present with varying frequency. Wilkins et al. (1983) studied a large group of home-reared CDC individuals and found a large degree of variation in all aspects of their phenotypes, with the largest variation found in the severity of mental retardation. This variation could not be correlated with the size of the 5p deletion. It is also important to note that the facial appearances of CDC change as the child matures. Newborn CDC individuals have round faces with apparent hypertelorism. As the child matures, the length of the face increases, giving the appearance of a long, slender face rather than a round face. The inner canthal distances normalize, sometimes becoming shorter than normal (Niebuhr 1978). In addition, cases have been described in which an infant demonstrates developmental delay and the catlike cry without any of the typical facial dysmorphism associated with CDC (Smith et al. 1990; Overhauser et al. 1994). Families have been described in which deletions of 5p are segregating without any manifestation of CDC (Kushnick et al. 1984; Overhauser et al. 1986; Baccichetti et al. 1988). The phenotype of speech delay has been noted in two cases with terminal deletions of 5p15.32 (Baccichetti et al. 1988). Thus we have attempted to localize subphenotypes of CDC, in order to better understand the heterogeneity seen in this syndrome.

In this report, we describe a molecular analysis of 17 cases of 5p deletions, most of whom have either an atypical CDC phenotype or no features of the syndrome. We have also determined the parental origin of these breakpoints, to assess a potential role in the phenotypic

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Address for correspondence and reprints: Dr. John W. Wasmuth, Department of Biological Chemistry, College of Medicine, Plumwood Room H131, University of California—Irvine, Irvine, CA 92717.

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heterogeneity seen in individuals with del(5p). The location of the deletion breakpoints is correlated with the presence or absence of various phenotypic features. Molecular and phenotypic analyses of these individuals have enabled us to assign specific features associated with del(5p) to subregions of 5p, including such features as the catlike cry, newborn and/or adult facial dysmorphism, and speech delay. Genes involved in mental retardation are likely to be in these regions as well. In particular, the feature of profound speech delay has not classically been noted as a feature of CDC, although many individuals were noted to have no speech. However, as shown in this report, speech delay is perhaps the most common feature seen in individuals with terminal deletions of 5p. Given that most cases of typical CDC have severe mental and/or physical retardation, it is not surprising that speech delay has not previously been described as a distinct clinical entity.

These studies have also enabled us to develop a set of cosmid probes for molecular cytogenetic (i.e., FISH) studies that define the different segments of distal 5p associated with the distinct phenotypes reported here. These probes will be of assistance in counseling cases of 5p deletions.

Table 1

PCR Primers and Cosmids Used in Analysis of Patients

Bin	Marker(s)[a]	Cosmid(s)[b]
I	D5S392, 84C11, 124G12	124G12, C125
II		C1-7
III	D5S12	210B3
IV	D5S18, D5S74	24D4
V	D5S88, D5S23, D5S721, D5S821	C194
VI	D5S432, D5S667, D5S478, LA480, D5S24	62 E6
VII	D5S713, D5S817	
VIII	EX2, 100EA	143F8
IX	LA361, LA448, LA457, D5S17	133A3
X	D5S737	
XI	D5S257, D5S268	
XII	D5S416	12G7A

[a] Sequences of primers with the "D5S" prefix are available from the Genome Data Base, and primers with the "LA" prefix were provided by D. Grady and R. Moyzis (both of Los Alamos National Laboratories); the sequences of the two remaining pairs of primers are as follows: EX2—forward, ACATCTTTATAGATGCTTTGC; and reverse, AGTCTTCAATTCTTGGTGAAGG; and 100EA—forward, CCAGAAGCTAGTTATTTGG; and reverse, CTGAGTATCATGGACTTGC.

[b] All of the cosmids were obtained from a chromosome 5–specific library (Los Alamos National Laboratory) by using a subset of the PCR primers indicated.

Material and Methods

Cell Lines from Individuals with Deleted Chromosomes 5

Lymphoblastoid cultures were established from peripheral blood samples by using Epstein-Barr virus and standard procedures. In most cases, the deleted chromosome 5 from a given individual was segregated into somatic cell hybrids, as described elsewhere (Dana and Wasmuth 1982). In all cases, lymphoblasts and cell hybrids were analyzed using both G-banding and FISH, to confirm the karyotype analysis of patients and to ensure that the lymphoblast cultures and the derived cell hybrids contain the deleted chromosome 5.

FISH

Metaphase chromosome preparations from lymphoblasts and cell hybrids were prepared as described elsewhere (Winokur et al. 1994). Cosmid probes used for FISH were labeled and visualized on metaphase chromosome preparations as described elsewhere (Winokur et al. 1994). The various cosmids from 5p that were used in these studies are described in table 1.

PCR Analysis of Cultured Cell Lines

Approximately 100 ng of genomic DNA was amplified in a mixture containing 1.5 μM of each primer, 200 μM dNTPs, and 1.25 U of *Taq* polymerase (Perkin Elmer Cetus), in buffer containing 10 mM Tris-HCL (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% gelatin in a total reaction volume of 25 μl. Products were electrophoresed through either 6% nondenaturing polyacrylamide gels or 2% agarose gels. These gels were stained with ethidium bromide and were visualized with an Eagle Eye still video camera and UV transilluminator (Stratagene). The oligonucleotide primers used to amplify the various loci are listed in table 1.

Parental Origin of Deletions

Using DNA from either lymphoblastoid cultures or somatic cell hybrids from probands and parents, we typed polymorphic loci on the normal and deleted chromosomes 5. In all but one of the de novo cases reported here, it was possible to determine unequivocally the parental origin of the deletions by using one or more polymorphic loci. Determination of the parental origins for HHW 962, HHW 950, and HHW 909 have been described elsewhere (Overhauser et al. 1990).

Clinical Data

A brief clinical description and the reason for ascertainment are shown in table 2. IQ designations are as follows: profound, <25; severe, 25–50; moderate, 50–70; and mild, 70>90. All Danish patients were evaluated by one of the authors (E.N.), as were the pictures and clinical history of patient 7. All other cases were evaluated in various genetics clinics. IQ tests and developmental examinations were performed in accordance with requirements of local authorities and were appropriate for the age of the proband.

Table 2

Patients with Deletions of 5p, Grouped According to Haplotype

Group and Patient	Cell Line	Derivative 5	Parental Origin	Speech Delay	Catlike Cry
I:					
1	HHW1185	46XX del(5)(p15.3)	Not determined[d]	+[e]	–
2	HHW1273	46XX del(5)(p15.3)	Not determined[d]	+	–
3	*HHW1798*	46XY del(5)(p15.3)	Paternal	+	–
4	HHW1687	46XY del(5)(p15.3)	Not determined[d]	+	–
5	HHW1170	46XY del(5)(p15.3)	Not determined[d]	+	–
II:					
6	*HHW1799*	46XY del(5)(p15.2)	Maternal	+	+
7	HHW962	46XX del(5)(p15.2)	Paternal	+	+
III:					
8	*HHW909*	46XX del(5)(p15.2)	Paternal	+	+
9	*HHW1796*	46XX del(5)(p15.2)	Paternal	+	+
10	*HHW1296*	46XX del(5)(p15.2)	Paternal	+	+
IV:					
11	HHW1249	46XY der(5)T(5;7)(p15.2;p21)MAT	Maternal[f]	?	+
12	*HHW900*	46XY del(5)(p15.1)(T-D)?	Paternal	+	–
V:					
13	HHW750	46XY intdel(5)(p15.2P15.3)	Not determined[g]	–	–
14	*HHW950*	46XY intdel(5)(p14.1p15.3)	Paternal	–	+
15	*HHW1904*	46XY intdel(5)(p14.1p15.2)	Paternal	–	–
16	HHW1778	46XY intdel(5)(p14.2p15.2)	Paternal	Mild[h]	–
17	*HHW1295*	46XX intdel(5)(p14.3p15.2)	Paternal	–	–

[a] Cell lines HHW1798, HHW1799, HHW909, HHW1796, HHW1296, HHW900, HHW950, HHW1904, and HHW1295 are of Danish origin. Cell lines HHW1798, HHW1799, and HHW1796 are lymphoblastoid lines; all others are somatic-cell hybrids.

[b] A question mark (?) indicates that no records were available.

[c] The reason for chromosome study is considered the reason for ascertainment.

[d] Parental origin could not be determined, because of deletion segregating.

[e] Bilateral conductive hearing loss also was present.

[f] The mother is the carrier of a balanced translocation.

[g] No parental material was available.

[h] Speech delay was caused by paralyzed vocal cords.

Results

Table 2 contains a brief description of patients included in this report. Metaphase chromosomes from patient lymphoblasts were analyzed to confirm the 5p deletion. To determine the relative location of the various breakpoints, two techniques were used; FISH analysis was used on lymphoblast cell lines hybridized with the cosmids shown in table 1, and PCR analysis was performed on deleted chromosomes 5 that have been segregated into somatic cell hybrids (Dana and Wasmuth 1982) (table 1). Patients were grouped according to their clinical descriptions and were compared with respect to the location of their deletions. Groups I–IV are patients with terminal deletions. Group V patients all have interstitial deletions.

Group I (Patients 1–5)

All of these patients displayed speech delay with varying degrees of mental retardation, and two were ascertained for this reason. All of these patients except number 3 represent familial cases of del(5p), as shown in figure 1. Pedigrees of all multigenerational families are shown in figure 1. Members of these families who had a del(5p) karyotype showed varying degrees of developmental delay, ranging from none to moderate. Patient 1 was ascertained on the basis of global developmental delay. The proband has speech delay, but she also has profound hearing loss. Her mother and grandfather have the same deletion and are in the low-normal intelligence range. It is unknown whether the mother and grandfather had speech delay. Early clinical records on these individuals are unavailable. Both the grandfather and the mother of the proband are nondysmorphic. The mother has epilepsy. The proband is microcephalic and has preauricle skin tags.

Patient 2 did not have the catlike cry or facial dysmorphism. While she is not mentally retarded, other members of her family who have a del(5p) karyotype

Information on Patients[b]				
Infant/Early-Childhood Facial Dysmorphism	Older-Child/Adult Facial Dysmorphism	Retardation	Ascertainment[c]	Reference
–	–	Moderate	Developmental delay	Overhauser et al. 1994
–	–	None	Multiple miscarriages	Present study
–	–	Mild–moderate	Vaso-septal defect	Present study
–	–	None	Speech delay	Present study
–	–	None	Speech delay	Present study
?	–	Moderate–mild	Speech delay	Present study
?	–	Moderate–mild	Catlike cry	Overhauser et al. 1994
+	–	Moderate	Speech delay	Overhauser et al. 1987
+	–	Moderate	Speech delay	Present study
+	–	Moderate	Speech delay	Present study
+	+	Moderate–severe	Catlike cry	Berstein et al. 1993
+	+	Severe	Retardation	Overhauser et al. 1994
–	–	Moderate	Developmental delay and retardation	Present study
?	+	Severe	Retardation	Overhauser et al. 1994
–	–	Severe–moderate	Retardation	Present study
–	–	None–mild	Aspiration	Present study
?	+	Moderate	Retardation	Present study

are mildly retarded. Individuals in this family who have del(5p) have been noted to have a "breathy, raspy voice," which was considered diagnostic for the del(5p) karyotype. Patient 3 did not have the catlike cry or typical CDC facial dysmorphism, but he was mildly mentally retarded. He was noted to have speech delay. Patient 4 represents an extended multigenerational del(5p) family. There was no history of a catlike cry or dysmorphic features. There was a variable degree of developmental delay in individuals with del(5p), but there were no signs of mental retardation. Patient 5 had no sign of the catlike cry and did not have typical CDC features. He was slightly developmentally delayed. His mother also had a del(5p) karyotype. Both mother and son are of low-normal intelligence. His area of greatest delay was speech.

Figure 2 and tables 1 and 3 define bins based on deletion breakpoints and show the markers and cosmids in each bin. Patient 1 represents the most distal breakpoint in this set. Bin I is defined by this breakpoint and the telomere. Patient 2 has a more proximal breakpoint, as defined by the absence of cosmid c1-7 and the presence of cosmid 210b3 (bin II). Patients 3–5 have a more proximal breakpoint and define bin IV by the absence of markers D5S18 and D5S74 and the presence of markers D5S88, D5S23, D5S721, and D5S821.

Group II (Patients 6 and 7)

Patient 6 was suspected to have Williams syndrome, because of high levels of serum calcium at age 3 mo. He was reevaluated, at age 4 years, for speech delay, at which point a cytogenetic evaluation revealed a del(5p). His nonverbal IQ is estimated to be 65–70, but he has a high level of purposeless activity and persistently drools. He also has the catlike cry. All attempts at speech are unintelligible, and he has a very short attention span.

Patient 7(HHW962) was originally reported to have

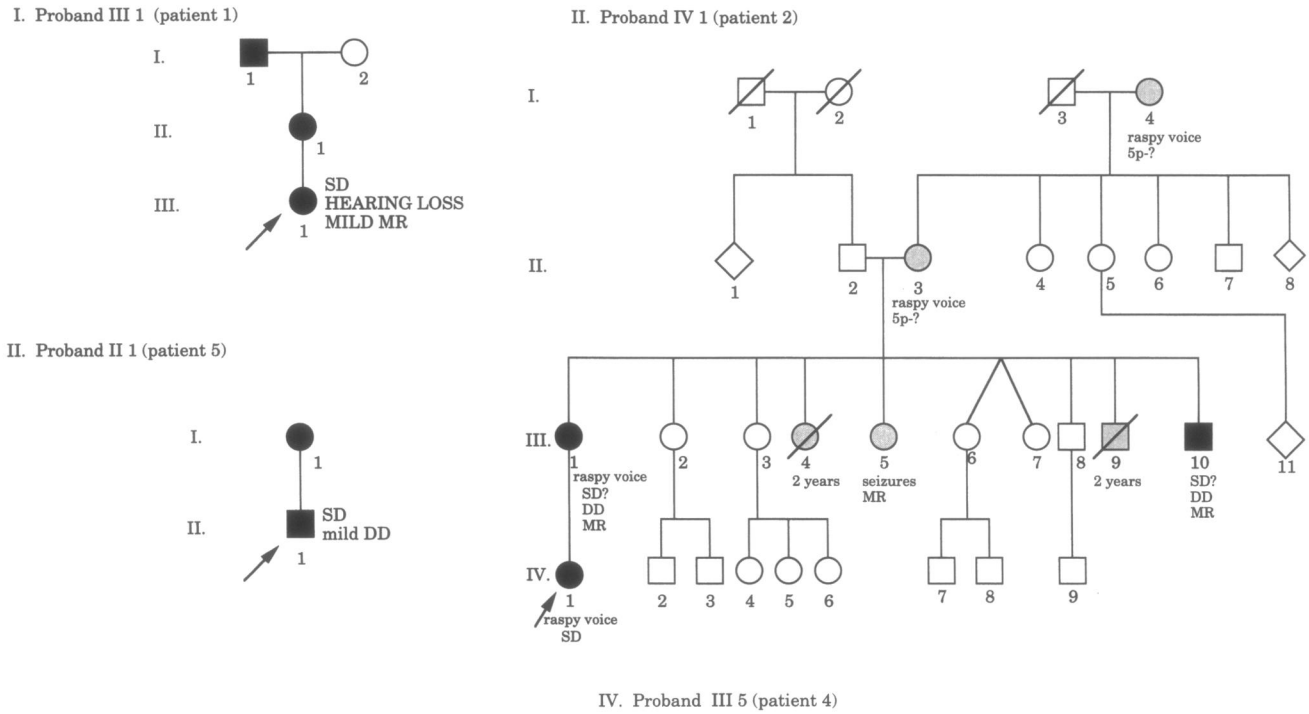

Figure 1 Pedigrees of four del5(p) multigenerational families. Blackened boxes represent members in which a 5p− phenotype has been confirmed; and boxes with slashes represent individuals suspected of 5p− but for whom there is no karyotype information. Members of patient 2's family have a wide range of other clinical symptoms, including seizures and a characteristic raspy voice heard in all members with del(5p). SD = speech delay; DD = developmental delay; and MR = mental retardation.

typical CDC (Overhauser et al. 1994). Reevaluation of this patient (by E.N.) indicated that, although she does have the catlike cry, she does not have typical CDC facial features. She is moderately retarded, with an IQ of 60, and she has attention-deficit disorder (ADD). There is some indication that ADD and her oppositional behavior deflated her actual IQ score. Speech and language development have been her areas of greatest delay. Her physical development has been relatively normal, although she does demonstrate some muscle weakness. She also has mild pes planus and mild pronation. Patient 6's breakpoint is one of two that define bin VIII, as evidenced by the presence of markers ex2 and 100ea and the absence of markers D5S817 and D5S713. Patient 7 has markers D5S88, D5S23, D5S721, and D5S821 missing and has markers D5S432, D5S667, D5S478, LA480, and D5S24 present, defining the proximal border of bin V.

Group III (Patients 8–10)

All of these individuals showed profound speech delay and moderate mental retardation. This group is interesting with regard to dysmorphology. All were noted as having typical CDC facial features as newborns. When assessed later, they had not developed the typical dysmorphisms usually seen in older CDC patients. All of these patients were referred for chromosome study, because of speech delay. Although all of these patients are moderately retarded, their speech is delayed relative to their psychomotor development.

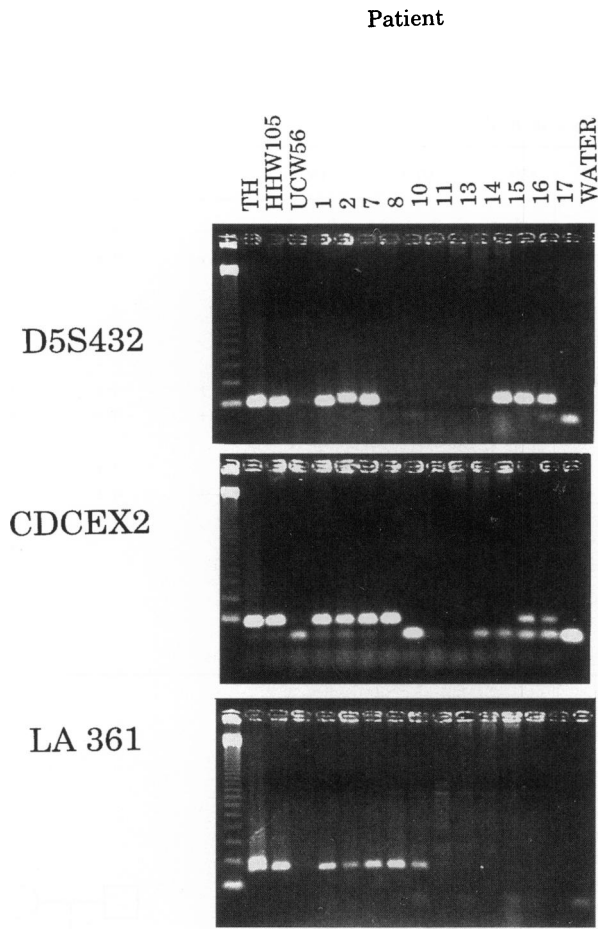

Figure 2 Examples of PCR data when primers are used in three different bins. Table 1 shows the cosmids or primers contained within each bin.

These patients have similar breakpoints. Patient 8 defines bin VII, with the absence of markers D5S713 and D5S817 and the presence of markers ex2 and 100ea. Patients 9 and 10 have indistinguishable breakpoints and define bin VIII, since markers ex2 and 100ea are absent and since markers LA361, LA448, LA457, and D5S17 are present.

Group IV (Patients 11 and 12)

These patients are the only two with terminal deletions who retained typical CDC facial features as they matured. Patient 11 is moderately retarded; his language development is unknown. Patient 12 is severely retarded, with no language skills.

These patients have the most proximal breakpoints in this set. Patient 11's derivative chromosome is the result of inheriting an unbalanced translocation from his mother, resulting in a 46XY der(5)T(5;7)(p15.2″1) karyotype. This breakpoint establishes the proximal breakpoint of bin IX, with the absence of markers LA361, LA448, LA457, and D5S17 and with the presence of marker D5S737. Patient 12 appears to have a terminal deletion in distal p15.1, with a possible translocation/duplication of unknown material. This breakpoint marks the proximal boundary of bin X, with the absence of D5S737 and with the presence of D5S257 and D5S268.

Group V (Patients 13–17)

These patients, all of whom have interstitial deletions, are distinguished by the fact that they have few, if any, characteristics of CDC. Patient 13 does not have speech delay or the catlike cry. He is considered to be slightly dysmorphic but has never had features diagnostic for CDC. He is only mildly mentally retarded and has ADD. Patient 14 does not have speech delay, but he did have the catlike cry. There are no clinical data on his appearance as an infant, but as an adult he has features considered diagnostic of CDC; although his head is larger than those of most CDC individuals. He is also >6 feet tall, which is very atypical of CDC individuals. He is severely mentally retarded. Patient 15 does not have speech delay or the catlike cry. Although he is dysmorphic, he has never had typical CDC features, either as an infant or as he matured. He has moderate to severe mental retardation. Patient 16 had mild speech delay, but this was thought to be due to a paralyzed left vocal cord and lack of oral/motor stimulation. He has neither the catlike cry nor any facial dysmorphism associated with CDC. He has mild developmental delay, with the greatest delay in expressive language. He has swallowing problems and an Arnold-Chiari type I brain anomaly. Patient 17 never had speech delay or the catlike cry. She was identified as CDC, in the presence of a group of CDC individuals and their normal sibs. However, like patient 14, her facial size was larger than those of most CDC individuals. She is also only moderately mentally retarded.

These patients are distinguished by the fact that they all have interstitial deletions. Patient 13 has an interstitial deletion of 5p15.3-5p15.2. He has marker D5S12 present and has bin IV markers missing distally. Proximally he is missing marker D5S737 but has marker D5S416. Patient 14 has a large interstitial deletion, from 5p15.3 to 5p14.1. His proximal breakpoint is not molecularly established, but his distal breakpoint is defined by the presence of bin IV markers and by the absence of bin V markers. Patients 15–17 all have undefined proximal breakpoints in 5p14, and distally their breakpoints are very similar. Patient 15 is missing bin VII markers but has bin VI markers present. Patients 16 and 17 both have bin VIII markers present and have bin IX markers missing.

Parental Origin

Given both the phenotypic heterogeneity associated with deletions of 5p15 and the recent importance of genomic imprinting in deletion syndromes (Ohlsson et

Table 3

FISH and PCR Results

	Results for Bin[b]											
Patient[a]	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1		+	++	++	++	++	++	++	++	++	++	++
2			++	++	++	++	++	++	++	++	++	++
3					+	+	+	+	+			+
4					+	+	+	+	+			+
5					+	+	+	+	+			+
6								+	+			+
7						++	++	++	++	++	++	++
8								++	++	++	++	++
9									+			+
10									++	++	++	++
11										++	++	++
12											++	++
13	++		++									++
14	++		++	++								
15	++		++	++	++	++						
16	++		++	++	++	++	++	++				
17	++		++	++	++	++	++	++				

[a] For patients 3–6, lymphoblastoid cell lines were used; for all other patients, somatic-cell hybrids were used.
[b] A plus sign (+) denotes a FISH result; a double plus sign (++) denotes a PCR result.

al. 1993; Reed and Leff 1994), we analyzed the parental origin of the derivative chromosomes. In four cases (patients 1, 2, 4, and 5) it was not possible to determine the parental origin of the de novo deletion, since the deleted chromosome had been segregating in the family. Parental material could not be obtained from patient 13. Figure 3 shows a typical PCR analysis used to determine parental origin. In the remaining patients, 10 of 11 de novo deletions were from the paternal line. This proportion is slightly higher than those in previous studies, which indicate that ~80% of de novo deletions of 5p are of paternal origin (Overhauser et al. 1990; E. Niebuhr, unpublished data).

Discussion

Figure 4 shows an ideogram of chromosome 5, with approximate locations of chromosome breakpoints and associated phenotypes. Overhauser et al. (1994) recently placed the critical region for the CDC facial dysmorphism in the region between the breakpoints bordered by patients 14 and 7. However, more clinical data on patient 7 have indicated the absence of the typical facial dysmorphism associated with CDC. She did have moderate mental retardation and behavioral problems and was noted to have a high-pitched cry as a child. Analyses of patient 7 and other patients seem to place the facial dysmorphism region in a location more proximal than previously described. In addition, we have been able to sublocalize the phenotypes of speech delay, the catlike cry, an area that involves the typical facial dysmorphism

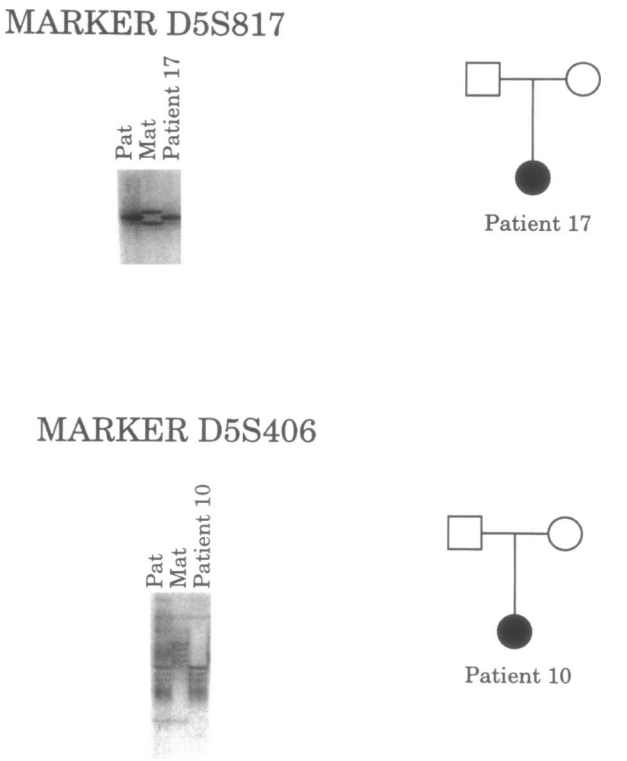

Figure 3 Parental origin analysis performed on patients with de novo deletions. Lymphoblast DNA was used for parental analysis, and somatic cell hybrids were used in analysis of the proband. The family information is shown on the right, and marker analysis is shown to the left. D5S817 is a tetranucleotide-repeat marker, and D5S406 is a dinucleotide repeat marker.

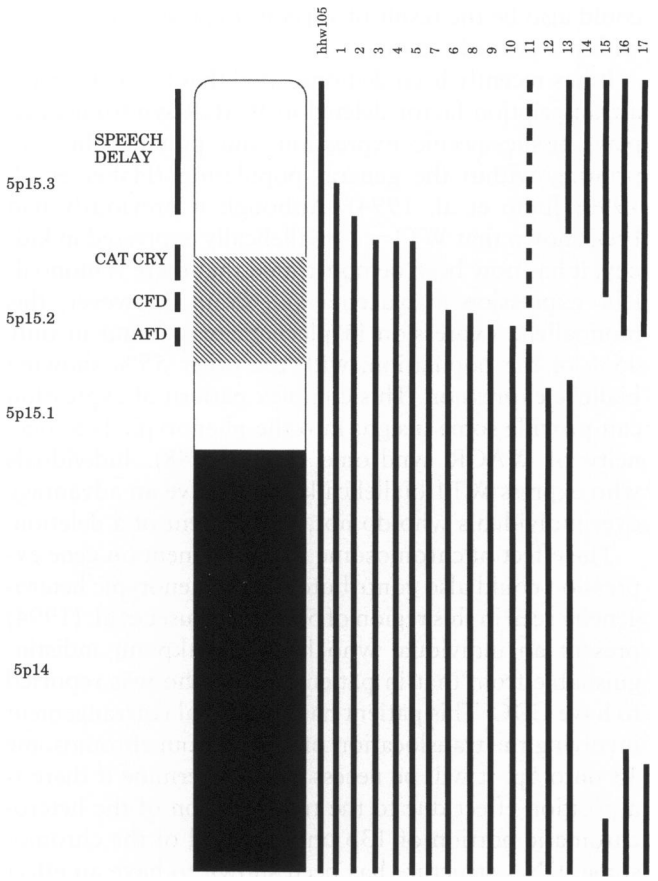

Figure 4 Phenotypic map for 5p15. HHW105 is a somatic cell hybrid with a normal chromosome 5 as its only human component. The dotted line for patient 11 indicates a translocation. AFD = Older-child/adult facial dysmorphism; and CFD = infant/early childhood facial dysmorphism.

seen in neonates and infants, and the dysmorphism seen in older CDC individuals (fig. 4).

Localization of the Region Associated with Speech Delay

There is a subset of individuals, exemplified by group 1, who have profound speech delay but none of the other phenotypes of CDC. It appears that this phenotype is associated with deletions of the distal portion of p15.3. Baccichetti et al. (1988) reported similar findings for two families in which several individuals had a terminal deletion 5p15.32-pter. All of these individuals demonstrated speech delay. It has generally been understood that individuals with typical CDC have little or no speech, but many of these individuals were also severely mentally retarded. These patients provide clear evidence that speech delay can be distinct from mental retardation, since these individuals are only mildly retarded, if at all. In the analysis of group 5, it was found that none of the individuals with interstitial deletions had a speech problem. In fact, patient 17, who is the most severely retarded patient in this set, is able to produce many words and to make two-word combinations. The region for speech delay is assigned to distal 5p15.3. Patient 1 is not useful in narrowing this region, since she also has profound hearing loss. This would assign the speech-delay phenotype to bin I or bin II. The data from Baccichetti et al. (1988) point to a very distal localization of speech delay, in 5p15.33. Two known genes lie in this region. NHE-3 is an apical epithelial sodium-hydrogen exchanger (Brant et al. 1993). NHE-3 is not likely involved in speech delay, because of both its lack of expression in brain and its proposed role of transepithelial Na^+ absorption (Brant et al. 1993). DAT1 is a type I dopamine transporter exhibiting high levels of expression in various regions of the midbrain (Giros et al. 1992; Shimada et al. 1992; Vandenbergh et al. 1992). DAT1 is a possible candidate gene because of both its high level of expression in the brain and its role in neurotransmitter uptake (Shimada et al. 1992).

Localization of the Region Associated with the Catlike Cry

The catlike cry from which the syndrome gets its name is defined by patients in group 2, none of whom have the cry, and group 3, all of whom have the cry. This assigns the catlike cry to our bin V. Two patients do not fit with this localization, patients 12 and 13. It is possible that these individuals have undetected cryptic translocations involving this region that have not been detected by FISH. Alternatively, there could be other genetic aspects in these individuals that mask the phenotype. This localization of a region involved in the catlike cry appears to be consistent with previously published reports (Overhauser et al. 1994) that have localized the catlike cry to the proximal part of 5p15.3. Our region includes the very proximal portion of 5p15.3 and the distal region of 5p15.2. Patient 14, who has an interstitial deletion and the catlike cry, lends further support for localization of the catlike cry to this region. The etiology of the catlike cry is not well understood. It may be caused by a small or weak larynx. It has been noted that several patients with typical CDC including the catlike cry had relatively normal larynx development with only minor morphological changes (Niebuhr 1978). It is possible that in this region there is a gene(s) involved not only in larynx development but also in larynx physiology.

Localization of the Region Associated with Childhood Facial Dysmorphism

One of the more interesting cases to come out of this study has been patient 8. This case was diagnosed, at an early age, as having the typical facial features of CDC, but, when seen as an older child, she did not have the facial features associated with typical CDC. This indicates that there could be a region, in proximal p15.2, that is important in fetal development of the typical facial dysmorphism but that another area, on 5p, is im-

portant in the childhood developmental period and gives rise to the typical facies seen in older CDC individuals. The region important for the development of facial dysmorphism seen in infants is defined by patient 8, who has childhood facial features, and patient 7, who did not have the facial features as an infant (fig. 4 and table 1). It should also be noted that patients with deletions in this area are only moderately mentally retarded. This tentatively assigns this phenotype to bin VII. Some caution should be taken in this assignment, since the medical records on patient 7 are somewhat unclear as to her appearance as an infant. Infant pictures of patient 7 were not available, but the clinical description does not mention any severe dysmorphism.

Localization of the Region Involved in Facial Features of Older Individuals

Of the patients presented in this paper, only four—patients 11, 12, 14, and 17—seem to have shown the typical CDC facial features as they have matured. All of these, except for 17, are more severely retarded than are other patients in this set. No clinical records are available on the childhood appearances of either patient 14 or patient 17. Patient 11 is the carrier of a balanced translocation with the breakpoint in proximal 15.2. Patient 12 has a terminal deletion in either p15.1 or p15.2 and possibly a small amount of unknown material translocated onto the end of the deleted chromosome. Patient 17 has a large interstitial deletion with the distal breakpoint in p15.2 and with the proximal breakpoint in p14.2. On the basis of terminal deletions, this phenotype can tentatively be assigned to bin IX. However, patients 13, 15, and 16 do not fit with this, since they have the same region of p15.1 deleted and do not have typical CDC facial features. The lack of the typical facial features in these two individuals could be due to their genetic background. Alternatively, the placement of this phenotype could be more distal, and patients 14 and 17 could present with CDC-like features because of a position effect. Only further characterization of this region will unequivocally determine this localization.

Phenotypic Heterogeneity

Variable expressivity has been noted in several syndromes associated with aneuploidy. This is true for such syndromes as Down syndrome (trisomy 21) and WAGR (Wilms tumor, aniridia, urogenital abnormalities, and mental retardation) syndrome (Jones 1988). Several possibilities could account for the phenotypic heterogeneity seen in del(5p) individuals. Allelic variation of the normal chromosome affects the phenotype. This is especially apparent in the cases of familial del(5p), in whom there is often a variable phenotype among individuals with the same deletions. Recessive mutations on the normal chromosome will be expressed phenotypically in hemizygous regions. The phenotypic heterogeneity could also be the result of variable expressivity of genes within a critical region.

It has recently been demonstrated that the WT1 gene, a transcription factor deleted in WAGR syndrome, displays tissue-specific expression and polymorphic imprinting within the general population (Haber et al. 1990; Jinno et al. 1994). Although it previously had been shown that WT1 was biallelically expressed in kidney, it has now been demonstrated that there is monoallelic expression in placenta and brain. However, this monoallelic expression in placenta was found in only 45% of the population, with the other 55% showing biallelic expression. This complex pattern of expression can provide some insight into the phenotypic heterogeneity of WAGR syndrome (Jones 1988). Individuals who express WT1 biallelically could have an advantage over individuals who do not, in the event of a deletion.

The effect of chromosome rearrangement on gene expression could also contribute to the phenotypic heterogeneity seen in this region of 5p. Overhauser et al. (1994) present an individual who has a breakpoint indistinguishable from that in patient 7 and who was reported to have CDC. This patient has a terminal rearrangement involving the translocation of rDNA from chromosome 13 onto 5p. It will be necessary to determine if there is a position effect due to the translocation of the heterochromatic portion of 13p onto the end of the chromosome. DNA structure has been shown to have an effect on transcription of nearby genes, and it is unclear how this terminal rearrangement has affected the expression of nearby genes (Rivier and Pillus 1994). In addition, position effect could explain the differences seen between patients 16 and 17. They have indistinguishable distal breakpoints, but their proximal breakpoints are different. Both have breakpoints in 5p14, which is a heterochromatic region with many chromosome-specific repeats (U. Bengtsson and J. J. Wasmuth, unpublished data). It is unclear what effect these repeats could have on the transcription of nearby genes.

The next step to further define the causes of phenotypic heterogeneity will be to clone genes within this region of 5p15. However, implicating genes that are involved in the etiology of the disease will prove to be much more difficult. The assessment of the genes in this region, with regard to spatial and temporal expression, will be one way to possibly implicate candidate genes for CDC. Any patterns of allele-specific expression should also be noted. The consequences of any possible position effects in the deleted and derivative chromosomes should also be assessed.

Another consequence of these data is in the area of genetic counseling and prenatal diagnosis. We have presented individuals who have very similar breakpoints but who have different phenotypes. Wilkins et al. (1983) conducted a large study of individuals with CDC and found that there was no correlation between the size

of the deletion and the severity of mental retardation. Indeed, we and others have demonstrated that large amounts of the short arm of chromosome 5 can be missing, with no apparent phenotypic consequences. Overhauser et al. (1986) presented a family in which the entire cytogenetic band p14, which comprises ~10% of the short arm, was missing, with no apparent phenotype. In this paper, patient 2 does not have any of the facial features of CDC and is not retarded (fig. 4). Her level of developmental delay may have been perceived as worse than it really was, because of her speech delay. However, her mother has the same deletion and is mildly mentally retarded.

It is also apparent from this and other studies that a del(5p) karyotype does not necessarily indicate a diagnosis of CDC (Kushnick et al. 1984; Baccichetti et al. 1988; Smith et al. 1990; Overhauser et al. 1994). In fact, many of the patients in this study were referred for cytogenetic studies for reasons other than CDC. This emphasizes the necessity of a clinical evaluation without the knowledge of chromosomal analysis. Classical CDC generally has a bleak prognosis, since these individuals are severely mentally retarded and developmentally delayed. However, many del(5p) individuals are only minimally delayed and have a much better prognosis than do individuals with CDC. This is especially true of individuals with deletions of 5p15.3. A set of cosmid probes has been developed that could aid in diagnosis. However, we and others (Smith et al. 1990; Overhauser et al. 1994) have shown that even individuals with similar chromosomal deletions in 5p15.2 can have different phenotypes, making it difficult to give a prognosis for any individual with a deletion in this region. This is especially true of individuals with interstitial deletions, since they have a more complex DNA rearrangement. Further molecular study of this region will be necessary in order to obtain a better understanding of the etiology of this syndrome. In addition, the ascertainment of patients with partial CDC phenotypes, such as speech delay, without visible deletions would be useful in identifying genes that are involved with this syndrome.

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