Am. J. Hum. Genet. 77:89–96, 2005

Identification of a Major Recombination Hotspot in Patients with Short Stature and *SHOX* Deficiency

Katja U. Schneider,[1] Nitin Sabherwal,[1] Karin Jantz,[1] Ralph Röth,[1] Nadja Muncke,[1] Werner F. Blum,[2,3] Gordon B. Cutler, Jr.,[4] and Gudrun Rappold[1]

[1]Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany; [2]Lilly Research Laboratories, Eli Lilly and Company, Bad Homburg, Germany; [3]University Children's Hospital, Giessen, Germany; and [4]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis

Human growth is influenced not only by environmental and internal factors but also by a large number of different genes. One of these genes, *SHOX,* is believed to play a major role in growth, since defects in this homeobox-containing gene on the sex chromosomes lead to syndromal short stature (Léri-Weill dyschondrosteosis, Langer mesomelic dysplasia, and Turner syndrome) as well as to idiopathic short stature. We have analyzed 118 unrelated patients with Léri-Weill dyschondrosteosis and >1,500 patients with idiopathic short stature for deletions encompassing *SHOX.* Deletions were detected in 34% of the patients with Léri-Weill dyschondrosteosis and in 2% of the patients with idiopathic short stature. For 27 patients with Léri-Weill dyschondrosteosis and for 6 with idiopathic short stature, detailed deletion mapping was performed. Analysis was performed by polymerase chain reaction with the use of pseudoautosomal polymorphic markers and by fluorescence in situ hybridization with the use of cosmid clones. Here, we show that, although the identified deletions vary in size, the vast majority (73%) of patients tested share a distinct proximal deletion breakpoint. We propose that the sequence present within this proximal deletion breakpoint "hotspot" region predisposes to recurrent breaks.

Introduction

Short stature has been defined as height >2 SD below the mean for age and sex. This corresponds to the shortest 2.3% of children. Although it is clear that multiple factors contribute to final height, genetic factors play a crucial role. Deletions within the distal short arm of the human X chromosome, for example, lead to multiple phenotypes, including short stature, chondrodysplasia punctata, mental retardation, steroid sulfatase deficiency, and Kallmann syndrome, and have been found as isolated entities and as part of a contiguous gene syndrome (Ballabio et al. 1989). Deletions that are normally restricted to the pseudoautosomal region (PAR1) encompassing the short-stature homeobox gene (*SHOX* [MIM 312865]) have been found in patients with Léri-Weill dyschondrosteosis (LWD [MIM 127300]) and in those with idiopathic short stature (ISS [MIM 604271]) (Ellison et al. 1997; Rao et al. 1997*b*; Belin et al. 1998; Shears et al. 1998; Cormier-Daire et al. 1999, 2001; Spranger et al. 1999; Blaschke and Rappold 2000; Grigelioniene et al. 2000; Rappold et al. 2002; Binder et al. 2003; Morizio et al. 2003). LWD is a mesomelic short-stature syndrome characterized by a skeletal forearm abnormality termed "Madelung deformity." Approximately 50%–100% of cases of LWD are due to complete deletions or intragenic mutations of the *SHOX* gene (Grigelioniene et al. 2000; Schiller et al. 2000; Cormier-Daire et al. 2001; Ross et al. 2001). Evidence suggests that roughly two-thirds of *SHOX*-deficient patients with LWD have deletions, whereas one-third of *SHOX*-deficient patients with LWD have point mutations (Cormier-Daire et al. 2001; Falcinelli et al. 2001; Grigelioniene et al. 2001; Huber et al. 2001; Ross et al. 2001; Flanagan et al. 2002). In the cohort of patients with ISS, *SHOX* mutations were found in 2.4% of the patients, of which 80% (2.0% of the cohort) have been identified as carrying complete gene deletions (Rappold et al. 2002). Binder et al. (2003) confirmed these findings in a screen of individuals with ISS that revealed complete *SHOX* gene deletions in >2% of the affected individuals. On the basis of this prevalence of *SHOX* deletions in patients with ISS and under the assumption that the disorder found in most people with short stature is idiopathic, *SHOX* deletions are estimated to occur in ~1 in 2,000 individuals in the total population (Rappold et al. 2002; Binder et al. 2003). Moreover, others have found *SHOX* deletions in up to 7.1% of patients with short stature of unknown cause (Morizio et al. 2003).

Although large deletions rarely account for the majority of mutations at a given locus (Cooper et al. 1998;

Received March 18, 2005; accepted for publication May 4, 2005; electronically published June 1, 2005.

Address for correspondence and reprints: Dr. Gudrun Rappold, Institute of Human Genetics, Im Neuenheimer Feld 366, 69120 Heidelberg, Germany. E-mail: gudrun_rappold@med.uni-heidelberg.de

0002-9297/2005/7701-0009$15.00

Antonarakis et al. 2000), a high frequency of deletions has been found in several inherited disorders. For instance, large gene deletions occur in Duchenne muscular dystrophy (DMD), in which two-thirds of all affected individuals display a deletion of one or more exons of the dystrophin gene (*DMD*) (Muntoni et al. 2003), and in X-linked ichthyosis, in which the majority of the patients carry a 1.9-Mb deletion encompassing the *STS* locus (Hernandez-Martin et al. 1999).

To gain a better understanding of the mechanism underlying *SHOX* deletions, we analyzed deletion breakpoints in 27 patients with LWD and in 6 patients with ISS. We identified a high incidence of breakpoints (73%) in a 5-kb region proximal to the *SHOX* gene, suggesting that this breakpoint may represent a hotspot for deletions leading to short stature.

Material and Methods

Subjects

Peripheral blood samples were taken from 118 individuals with LWD and from >1,500 individuals with ISS after informed consent was obtained. Thirty-three patients (27 patients with LWD and 6 patients with ISS) were studied in detail for deletion sizes. Six patients with ISS and *SHOX* deletions were from a previous screen (Rappold et al. 2002 and authors' unpublished data). The patients were of both sexes (22 females and 11 males) and were of European origin (17 from Germany, 5 from the Netherlands, 5 from France, 3 from Spain, 1 from the Czech Republic, 1 from Croatia, and 1 from Poland). All individuals were unrelated and had been examined by endocrinologists. Short stature was diagnosed when height, adjusted for sex and chronological age, was below the 3rd percentile or <2 SDs of national height standards. Exclusion criteria for this study were growth hormone deficiency, growth hormone receptor defect, malignant neoplastic disease, chronic infectious disease, active rheumatoid arthritis, diabetes mellitus, renal insufficiency, hepatic disease, congestive heart failure, gonadal dysgenesis, or abnormal diet. LWD diagnosis was based on the presence of Madelung deformity, bowing of the forearms, and mesomelic short stature. For some of the patients, X-ray analysis of the forearm was performed.

PCR-Based Microsatellite Analysis

Genomic DNA was extracted from blood samples and was used for amplification with the primers DXYS201 (SHOX 5CA forward: CAT GTC ATA TAT ATA TGT GAT CC; SHOX 5CA reverse: GAC ACA GAA ATC CTT CAT AAA), DXYS233 (233 forward: TGG GAA TTC GAG GCT G; 233 reverse: TGA TTT CCA TCC TGG GGT), and CAII (CAII forward: ATA TCA GAA AAG ACT GTG TTC TAC; reverse: TAG TAG GTT GCA AAG GCA TCT G). Twenty picomoles of each primer were used with 100 ng of DNA—together with 2 U of Goldstar DNA polymerase (Eurogentec), 10 pmol dNTP (Fermentas), and 75 mmol $MgCl_2$—in the buffer provided with the enzyme. PCR was performed in a Peltier Thermal Cycler (PTC-200), starting with denaturation (95°C for 5 min), followed by 35 cycles of amplification (95°C for 30 s, 55°C for 30 s, and 72°C for 45 s) and a final elongation step (72°C for 5 min). The PCR products were loaded on a polyacrylamide gel (10%) and were stained with $AgNO_3$ solution.

FISH Analysis

Biotinylated cosmid DNA was hybridized to interphase and metaphase chromosomes of lymphocytes of patients, as described elsewhere (Lichter and Cremer 1992). FISH was performed using cosmids 11D2 (LLN0YCO3′M′11D2), 56G10 (LLN0YCO3′M′56G10), HO32 (LLNLc110HO32), 25D5 (LLN0YCO3′M′25D5), 110A7 (LLN0YCO3′M′110A7), 110E3 (LLN0YCO3′-M′110E3), 43C11 (LLN0YCO3′M′43C11), 15D10 (LLN0YCO3′M′15D10), 34F5 (LLN0YCO3′M′34F5), G0411 (ICRFc104G0411), 9E3 (LLN0YCO3′M′9E3), 29B11 (LLN0YCO3′M′29B11), P0117 (ICRFc104P0117), ANT3 (ICRFc104D0137), and 12E11 (LLN0YCO3′-M′12E11). Hybridization signals were detected via avidin-conjugated fluorescein isothiocyanate (FITC). Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Images of FITC- and DAPI-stained chromosomes were taken separately, using a cooled charge-coupled–device camera system (Photometrics).

SNP Analysis

SNPs that mapped to the critical breakpoint region in PAR1 were identified by searching dbSNP. Additional SNPs in this area were identified during the sequencing process and were further used for confirming heterozygosity. SNPs within the markers *DXYS86, DXYS59* (dbSNP accession number rs6644571), C7/SNPs (dbSNP accession numbers rs1016964 and rs3995647), C8/SNPs (dbSNP accession numbers rs2399945 and rs2399946), C9/SNPs, and C10/SNPs in the respective patients and their families were analyzed for heterozygosity by sequence analysis with the use of the following primers: P131 (forward: TCT GCC ATC CTA ATG ATG GT; reverse: AGA AGT TTC TTC TCC ACC TG), DXYS59 (forward: CAT TAA GCT GAT TAG CC AAT; reverse: AGA GCT CCA ATA TAT GCC AT), C7SNPs (forward: TAC GTG GTA GGG AGG AAG ATT GGA; reverse: ATC CAA GCA ATT GTC AGG GAT GGC), C8SNPs (forward: TCC TCC CAG TTG TCC CAG TTG TAT; reverse: GCT GCC CAT GAC AGG GGA CTA ACG), C9SNPs (forward: GCT GCT ACT AAG

TTA ACT GG; reverse: CAG ATA GAC AAT ATT TTC AG), and C10SNPs (forward: CGT CTC TTC CTT ATC ACT TG; reverse: TAA CCG CAG TGA AAC GTC CC). Sequencing was performed on a MegaBACE sequencer (Amersham Bioscience) by use of the DYEnamic ET Terminator Cycle Sequencing kit, in accordance with the manufacturer's instructions.

Secondary-Structure Prediction of the Hotspot Region by Computational Analysis

For secondary-structure prediction, a 6-kb sequence encompassing the hotspot region as well as 6-kb sequences of the direct proximal and distal adjacent regions were submitted to the Mfold server (Zuker 2003). Structures with the lowest free-energy values were selected for analysis.

Results

Deletion analysis was performed for 118 unrelated patients with LWD, by use of three polymorphic markers—*CAII, DXYS201* (CA repeat), and *DXYS233*—that reside between 520 kb and 870 kb from the telomere in PAR1 (see fig. 1). In cases of homozygosity of at least two of the three markers, deletion mapping was performed by FISH with the use of cosmids from a cosmid contig that was previously established by our group (Rao et al. 1997*a*).

In total, we found deletions encompassing *SHOX* in 40 individuals (34% of the 118 patients with LWD). For 27 patients with LWD, we were able to perform a detailed deletion mapping with the use of 15 PAR1 cosmids in total (fig. 1). The deletion sizes vary extensively, with the smallest comprising ~90 kb (patient LWD21) and the largest comprising >2.5 Mb (patients LWD25–LWD27) (table 1). However, most interestingly, 20 (74%) of 27 patients share a distinct breakpoint that is defined by the presence of a hybridization signal of cosmid P0117 and the absence of a signal of cosmid 29B11, which maps the breakpoint within the genomic sequence represented within cosmid P0117 (fig. 2).

This high incidence prompted us to investigate whether patients with ISS present similar deletion breakpoints, since 2%–3% of cases of ISS result from *SHOX* mutations (Rappold et al. 2002; Binder et al. 2003). We determined that the proximal deletion breakpoint similarly localizes within cosmid P0117 in four (67%) of the six patients with *SHOX* deletions, which indicates the clustering of the deletion breakpoint within a relatively small interval. Cosmid 29B11 harbors the probe AK1 (*DXYS163*), which detects a variable number of tandem repeats (VNTR) locus ~65 kb proximal to the *SHOX* gene (Klink et al. 1993). We used AK1 for Southern hybridization on *Taq*I-digested genomic DNAs and found that AK1 is absent in all patients carrying the deletion (data not shown). Cosmid P0117, however, was found by FISH analysis to be present in all the patients with deletions, on both alleles of the sex chromosomes.

On the basis of these findings, further mapping of the breakpoint region was achieved for seven patients (LWD1, LWD6, LWD7, LWD10, LWD15, LWD16, and ISS30) by use of SNP analysis. Cosmid P0117 contains the microsatellite marker *P117* (*DXYS417*) (Wapenaar et al. 1992), and further SNP markers were selected in the vicinity of *P117* (three distal and three proximal markers [see fig. 3]). SNP analysis mapped the breakpoints to an interval of 5 kb that is directly distal to P117 in all affected individuals. Sequence analysis of this critical region by use of the Tandem Repeat Finder of the University of California–Santa Cruz (UCSC) Genome Browser revealed a high occurrence of STRs (~85%) within the hotspot region.

Since tandem repeats can form unusual secondary structures, secondary-structure–prediction analysis was performed using the Mfold web server. Unlike the neighboring regions, the hotspot region showed (in Mfold analysis) an extensive secondary structure that is characterized by strong DNA bending and a free energy of −617.9 kcal/mol, together with hairpins and loops (fig. 4). Sequence and structure analysis of the tandem repeats revealed that they can vary in length but that they consist mostly of homologous units of 27 bp in a head-to-tail arrangement. In conclusion, the high incidence of breakpoints on cosmid P0117 is very likely due to the accumulation of STR sequences that may cause genomic instability by abnormal secondary-structure formation, including DNA bending, which, in turn, might explain the hotspot of deletions observed in the analyzed patients with short stature.

Discussion

The distal short arm of the human X chromosome is frequently subjected to deletion events that lead to genetic disorders such as short stature, chondrodysplasia punctata, mental retardation, ichthyosis, and Kallmann syndrome (Ballabio et al. 1989). We were interested in defining the deletion breakpoints in the vicinity of the *SHOX* gene, and we found that 24 (73%) of the 33 analyzed individuals share a common deletion breakpoint within a 5-kb interval. *SHOX* deletions account for 2.0%–7.1% of short stature of unknown cause (Rappold et al. 2002; Binder et al. 2003; Morizio et al. 2003), indicating that the major breakpoint that we identified contributed to the deletions in possibly >1% of patients with idiopathic short stature. Of all the tested patients with LWD and Xp deletions, the majority (73%) harbor this breakpoint on cosmid P0117. This leaves the

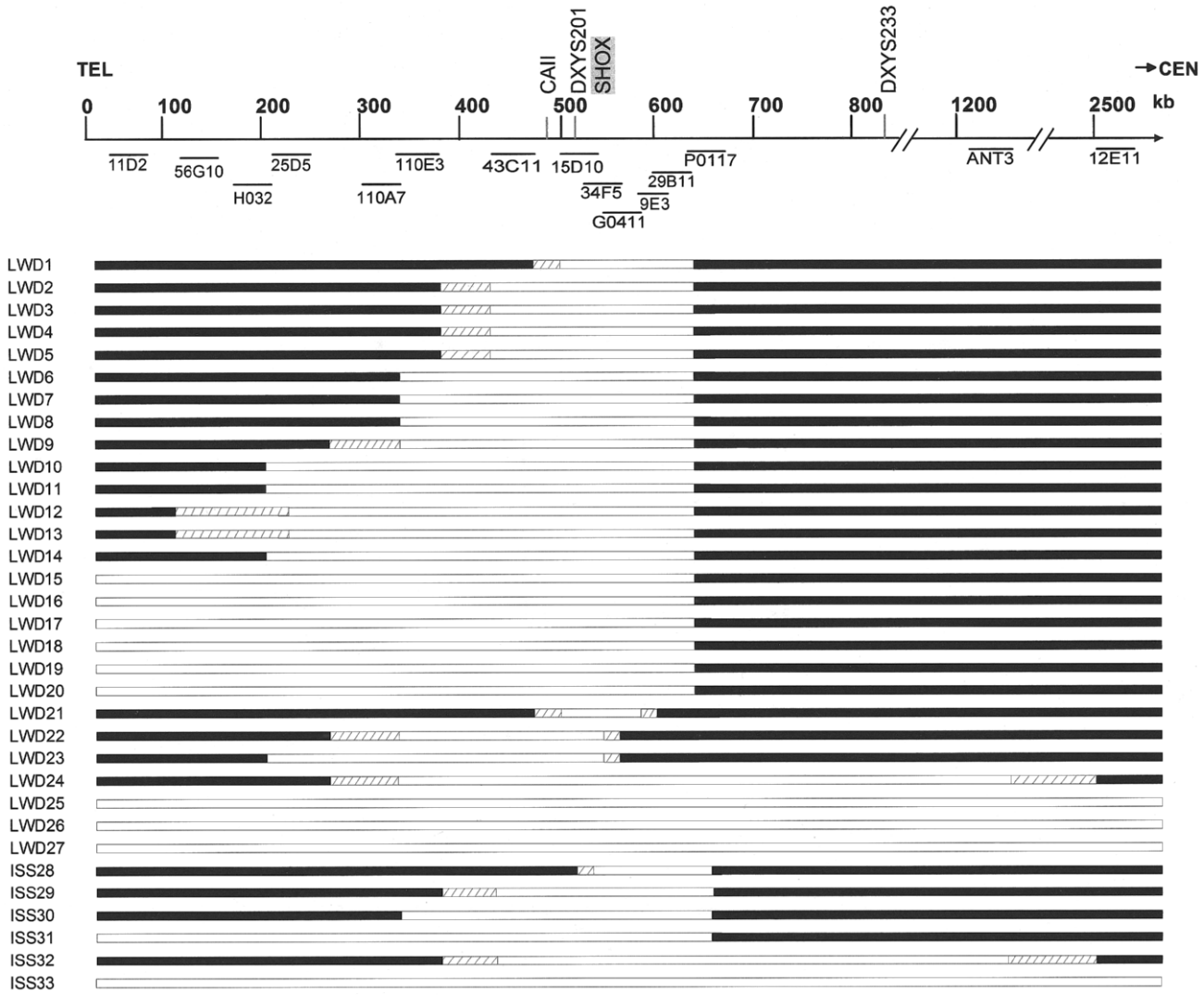

Figure 1 Deletion mapping of 27 patients with LWD (patients LWD1–LWD27) and 6 patients with ISS (patients ISS28–ISS33). Patients LWD2, LWD3, LWD4, LWD5, LWD8, LWD9, LWD19, LWD20, LWD22, and LWD28 have been described elsewhere as presenting a *SHOX* deletion (Schiller et al. 2000); however, the exact extent of the deletion sizes as well as the breakpoints were not defined. Blackened areas indicate the presence of the respective cosmid clone on both of the patient's sex chromosomes; unblackened areas indicate the absence of a cosmid clone on one sex chromosome; shaded areas indicate the breakpoint region. The map positions of the cosmid clones are indicated by horizontal lines and the clone abbreviation (see the "Material and Methods" section). The approximate distance from the telomere is given in kb. Microsatellite markers are indicated with vertical lines at their respective map position. The genomic locus of the *SHOX* gene is indicated in gray and resides ~555.1–582.3 kb from the telomere. The deletion hotspot is defined by the presence of cosmid P0117 and the absence of cosmid 29B11.

DXYS233 locus intact (fig. 1), which, in some screens, was used as an indicator of a *SHOX* deletion (e.g., Binder et al. 2003), suggesting that the true incidence of *SHOX* deletions in those studies may be considerably higher than previously estimated. A diagnostic test for *SHOX* deletions should therefore encompass markers next to the gene (e.g., CA markers *CAII* or *DXYS201*) and should not consider *DXYS233*.

The DNA instability that we observed in the overlap of cosmid clones P0117 and 29B11 is most likely due to the abundance of STRs that cause an extensive secondary-structure formation, as shown by the Mfold analysis (fig. 4). In contrast to the frequent proximal deletion breakpoint, the distal breakpoints vary considerably, with at least seven different breakpoints, as defined by FISH analysis. This variation might be due to weaker genomic instabilities at the respective positions in PAR1. PAR1 itself is a region that is highly active in recombination (Rappold et al. 1994). Crossovers are not distributed randomly but cluster in specific recom-

Table 1

Deletion Size in Patients with LWD or ISS

Patient	Presence or Absence of Hybridization Signal in Cosmid Clone															Estimated Deletion Size (kb)
	11D2 (~30 kb)	56G1 0 (~120 kb)	H032 (~180 kb)	25D5 (~240 kb)	110A7 (~300 kb)	110E3 (~320 kb)	43C11 (~440 kb)	15D10 (~490 kb)	34F5 (~530 kb)	G0411 (~560 kb)	9E3 (~580 kb)	29B11 (~600 kb)	P0117 (~650 kb)	ANT3 (~1,200 kb)	12E11 (~2,500 kb)	
LWD1							+	−	−			−	+			210
LWD2				+		+	−		−			−	+			330
LWD3				+		+	−		−			−	+			330
LWD4	+					+			−			−	+			330
LWD5				+		+	−		−			−	+			330
LWD6				+	+	−			−			−	+			350
LWD7				+	+	−	−		−			−	+			350
LWD8				+	+	−	−		−			−	+			350
LWD9				+	−	−			−			−	+			410
LWD10	+	+	+	−	−				−			−	+			470
LWD11	+	+	+	−				−	−	−		−	+			470
LWD12	+	−	−	−				−	−	−		−	+			620
LWD13	+			−			−		−	−		−	+			620
LWD14	+	+	+	−					−			−	+			470
LWD15	−			−					−			−	+			650
LWD16	−			−					−			−	+			650
LWD17	−			−				−	−	−		−	+			650
LWD18	−			−				−	−	−		−	+			650
LWD19	−								−			−	+			650
LWD20	−								−			−	+			650
LWD21						+		+/−	−	−	+	+	+			90
LWD22						+	−		−	+			+			230
LWD23	+	+	+	−		−	−	−	−	+	+	+	+			380
LWD24				+		−	−		−				−	−	+	>1,200
LWD25	−			−					−				−	−	−	>2,500
LWD26	−			−					−				−	−	−	>2,500
LWD27	−			−					−				−	−	−	>2,500
ISS28				+		+	+	−	−			−	+			210
ISS29				+		+	−		−			−	+			330
ISS30				+	+	−	−		−			−	+			350
ISS31	−			−					−			−	+			650
ISS32				+		+	−		−			−	−	−	+	>1,200
ISS33	−			−					−			−	−	−	−	>2,500

Note.—A plus sign (+) indicates the presence of a hybridization signal; a minus sign (−) indicates the absence of a signal. Cosmid clones 11D2, 25D5, 110E3, 43C11, 15D10, 34F5, G0411, 9E3, 29B11, P0117, ANT3, and 12E11 represent cosmids of PAR1, with their approximate position from the telomere (in kb). The estimated deletion size found in each patient is indicated on the right and ranges from 90 kb to >2,500 kb.

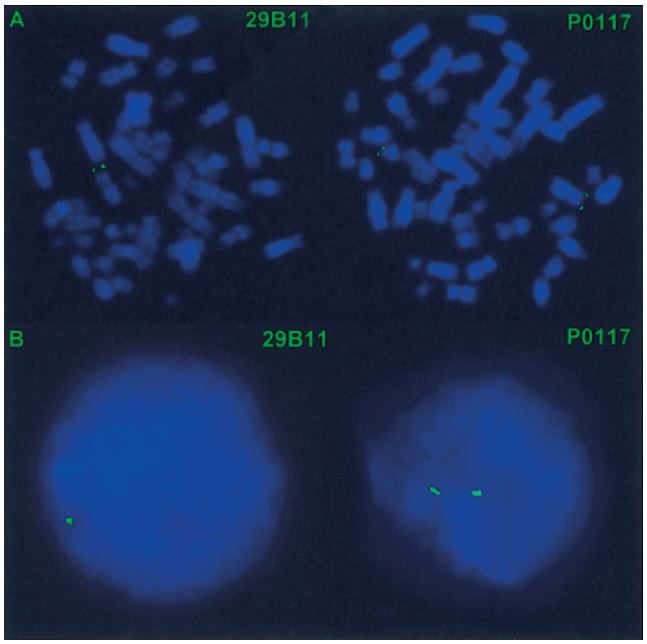

Figure 2 FISH of cosmids 29B11 and P0117 to metaphase (*A*) and interphase chromosomes (*B*) of patient LWD10. The absence of the signal of cosmid 29B11 and the presence of the signal of cosmid P0117 locates the breakpoint on cosmid P0117.

bination hotspots (May et al. 2002). Sequence comparisons of the breakpoint regions did not reveal sequence compositions that were similar to that the P0117 cosmid, suggesting that homologous recombination may not be the underlying mechanism for the deletions observed. Rather, we speculate that the unusual secondary structure of the proximal region, together, perhaps, with double-strand DNA breaks, may lead to recombination in this genomic region (Klein et al. 1996). The published sequence of the human X chromosome is 99.3% complete, and intrachromosomal duplications account for 2.6% of the X chromosome (Ross et al. 2005). Consistently, our sequence analysis and the data reported by Ross et al. (2005) exclude an intrachromosomal segmental duplication within the region of interest.

Other mechanisms underlying deletions, such as unequal crossover, have been previously described in the Xp22 region and lead to a high deletion frequency in X-linked ichthyosis (Shapiro et al. 1989; Yen et al. 1990). Interestingly, with a prevalence of 1 in 6,000 males, X-linked ichthyosis shows one of the highest incidences of deletions observed so far. Ninety percent of all patients exhibit deletion of the steroid sulfatase gene that results from homologous recombination between repetitive S232-like VNTR sequences that reside in the gene's flanking region (Yen et al. 1990; Li et al. 1992). Furthermore, approximately two-thirds of the patients suffering from DMD or Becker muscular dystrophy display deletions of one or many exons in the 2.5-Mb region encompassing the *DMD* gene (Blonden et al. 1989; Muntoni et al. 2003). Two deletion hotspots were found, one residing in the central part of the *DMD* gene and the other in the 5′ end of the gene, and screening of these two regions identified ~98% of all deletions. A detailed analysis of the more distal clustering of breakpoints in intron 44, however, has shown that the breakpoints are actually spread over a large intron of 240 kb (see the Leiden Muscular Dystrophy Pages). A real clustering at the sequence level, therefore, does not

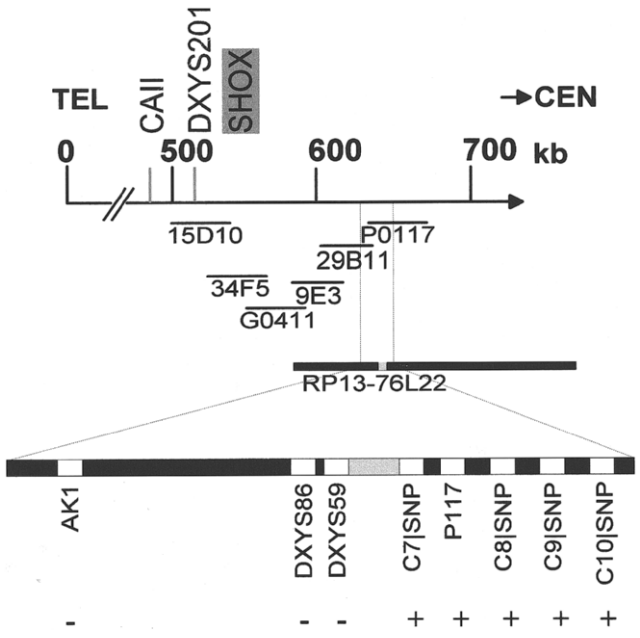

Figure 3 Results of SNP analysis of the critical breakpoint region of the overlap of cosmid clones P0117 and 29B11. This region—at ~655–678 kb from the telomere on the X chromosome and at 57–80 kb on the BAC clone RP13-76L22 (GenBank accession number AL683871.15) (on the basis of the UCSC Genome Browser human May 2004 assembly and NCBI build 35)—harbors the VNTR AK1 (655,357–656,210 bp), as well as the microsatellite marker *P117* (672,153–675,202 bp). SNPs within AK1, *DXYS86* (*P131*), *DXYS59* (dbSNP accession number rs6644571 at 666,749 bp), C7/SNP (dbSNP accession numbers rs1016964 at 671,526 bp and rs3995647 at 671,472 bp), and C8/SNP (dbSNP accession numbers rs2399945 at 676,936 bp and rs2399946 at 676,787 bp), as well as the few as-yet-unpublished SNPs within C9/SNP and C10/SNP, were checked for heterozygosity in seven families (patients LWD1, LWD6, LWD7, LWD10, LWD15, LWD16, and ISS30). The breakpoint of ~5 kb (indicated in gray) could be defined by the heterozygosity of the SNPs within C7/SNP and the absence of heterozygosity of SNPs within DXYS59. *SHOX* maps to 555,079–582,318 bp, AK1 to 655,357–656,210 bp, *DXYS86* to 665,323–665,477 bp, *DXYS59* to 666,696–666,875 bp, C7/SNP to 671,103–671,885 bp, *P117* to 672,153–675,202 bp, C8/SNP to 676,618–676,192 bp, C9/SNP to 679,663–681,329 bp, and C10/SNP to 683,363–684,552 bp (from the telomere).

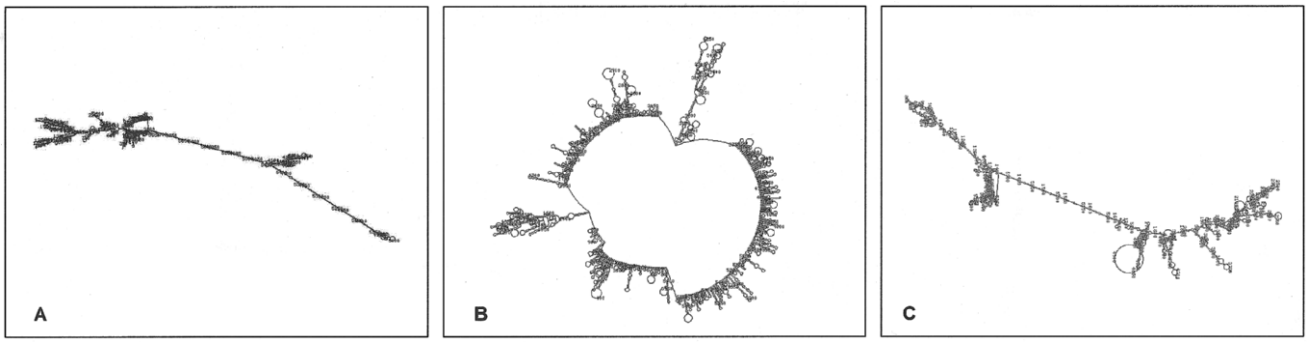

Figure 4 Secondary-structure prediction, made by use of Mfold analysis, of the sequence harboring the hotspot region (*B*) and the 6-kb sequences distal (*A*) and proximal (*C*) to it. The free-energy values for each secondary-structure prediction are −902 kcal/mol (*A*), −617.9 kcal/mol (*B*), and −677.8 kcal/mol (*C*). High DNA bending and an extensive secondary-structure formation are observed only in the hotspot region (*B*).

exist. With a prevalence of 1 in 3,500 males (i.e., 1 in 7,000 in the total population), the two deletion hotspots reach a frequency of ~1 in 11,000 individuals in the population (Bogdanovich et al. 2003).

The presence of a deletion hotspot in 73% (95% CI 56%–85%) of patients with short stature and with a *SHOX* deletion suggests a prevalence of this breakpoint of ~1:2,000 in the total population (95% CI 1:2,350–1:3,570), which is based on the assumption that the disorder found in most people with short stature is idiopathic. This implies that the recurrent deletion breakpoint identified in this study is rather frequent in the heterogeneous group of patients with short stature and, furthermore, may represent the most frequent known deletion breakpoint leading to disease.

Acknowledgments

We are indebted to all the clinicians and patients who worked closely with us. This work was performed with the support of the University of Heidelberg Medical Faculty and of Eli Lilly and Company. We thank Dr. Martijn Breuning, Beatrix Startt, and Anne Jordan for helpful comments.

Web Resources

The accession number and URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/ (for *DXYS59* [accession number rs6644571], C7/SNPs [accession numbers rs1016964 and rs3995647], and C8/SNPs [accession numbers rs2399945 and rs2399946])

GenBank, http://www.ncbi.nih.gov/Genbank/ (for RP13-76L22 [accession number AL683871.15])

Leiden Muscular Dystrophy Pages, http://www.dmd.nl/DMD_deldup.html

Mfold, http://www.bioinfo.rpi.edu/applications/mfold/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nih.gov/Omim/ (for *SHOX*, LWD, and ISS)

UCSC Genome Bioinformatics, http://www.genome.ucsc.edu/

References

Antonarakis SE, Krawczak M, Cooper DN (2000) Disease-causing mutations in the human genome. Eur J Pediatr 159 Suppl 3:S173–S178

Ballabio A, Bardoni B, Carrozz R, Andria G, Bick D, Campbell L, Hame B, Ferguson-Smith MA, Gimelli G, Fraccaro M, Maraschio P, Zuffard O, Guioli S, Camerino G (1989) Contiguous gene syndromes due to deletions in the distal short arm of the human X chromosome. Proc Natl Acad Sci USA 86:10001–10005

Belin V, Cusin V, Viot G, Girlich D, Toutain A, Moncla A, Vekemans M, Le Merrer M, Munnich A, Cormier-Daire V (1998) SHOX mutations in dyschondrosteosis (Léri-Weill syndrome). Nat Genet 19:67–69

Binder G, Ranke MB, Martin DD (2003) Auxology is a valuable instrument for the clinical diagnosis of SHOX haploinsufficiency in school children with unexplained short stature. J Clin Endocrinol Metab 88:4891–4896

Blaschke RJ, Rappold GA (2000) SHOX: Growth, Léri-Weill and Turner syndromes. Trends Endocrinol Metab 11:227–230

Blonden LA, den Dunnen JT, van Paassen HM, Wapenaar MC, Grootscholten PM, Ginjaar HB, Bakker E, Pearson PL, van Ommen GJ (1989) High resolution deletion breakpoint mapping in the DMD gene by whole cosmid hybridization. Nucleic Acids Res 17:5611–5621

Bogdanovich S, Perkins K, Krag TOB, Khurana TS (2003) Therapeutics for Duchenne muscular dystrophy: current approaches and future directions. J Mol Med 82:102–115

Cooper DN, Ball EV, Krawzcak M (1998) The human mutation database. Nucleic Acids Res 26:285–287

Cormier-Daire V, Belin V, Cusin V, Viot G, Girlich D, Toutain A, Moncla A, Vekemans M, Le Merrer M, Munnich A (1999) SHOX gene mutations and deletions in dyschondrosteosis or Léri-Weill syndrome. Acta Paediatr Suppl 88:55–59

Cormier-Daire V, Huber C, Munnich A (2001) Allelic and nonallelic heterogeneity in dyschondrosteosis (Léri-Weill syndrome). Am J Med Genet 106:272–274

Ellison JW, Wardak Z, Young MF, Robey PG, Laig-Webster M, Chiong W (1997) PHOG, a candidate gene for involvement in the short stature of Turner syndrome. Hum Mol Genet 6:1341–1347

Falcinelli C, Iughetti L, Percesepe A, Calabrese G, Chiarelli F, Cisternino M, De Sanctis L, Pucarelli I, Radetti G, Wasniewska M, Weber G, Stuppia L, Bernasconi S, Forabosco A (2001) SHOX point mutations and deletions in Leri-Weill dyschondrosteosis. J Med Genet 39:E33

Flanagan SF, Munns CF, Hayes M, Williams B, Berry M, Vickers D, Rao E, Rappold GA, Batch JA, Hyland VJ, Glass IA (2002) Prevalence of mutations in the short stature homeobox containing gene (SHOX) in Madelung deformity of childhood. J Med Genet 39:758–763

Grigelioniene G, Eklof O, Ivarsson SA, Westphal O, Neumeyer L, Kedra D, Dumanski J, Hagenas L (2000) Mutations in short stature homeobox containing gene (SHOX) in dyschondrosteosis but not in hypochondroplasia. Hum Genet 107:145–149

Grigelioniene G, Schoumans J, Neumeyer L, Ivarsson A, Eklof O, Enkvist O, Tordai P, Fosdal I, Myhre AG, Westphal O, Nilsson NO, Elfving M, Ellis I, Anderlid BM, Fransson I, Tapia-Paez I, Nordenskjold M, Hagenas L, Dumanski JP (2001) Analysis of short stature homeobox-containing gene (SHOX) and auxological phenotype in dyschondrosteosis and isolated Madelung deformity. Hum Genet 109:551–558

Hernandez-Martin A, Gonzales-Sarmiento R, De Unamuno P (1999) X-linked ichthyosis: an update. Br J Dermatol 141: 617–627

Huber C, Cusin V, Le Merrer M, Mathieu M, Sulmont V, Dagoneau N, Munnich A, Cormier-Daire V (2001) SHOX point mutations in dyschondrosteosis. J Med Genet 38:323–351

Klein S, Zenvirth D, Sherman A, Ried K, Rappold GA, Simchen G (1996) Double strand breaks on YACs during yeast meiosis may reflect meiotic recombination in the human genome. Nat Genet 13:481–484

Klink A, Wapenaar M, van Ommen GJ, Rappold G (1993) AK1 detects a VNTR locus in the pseudoautosomal region. Hum Mol Genet 2:339

Li XM, Yen PH, Shapiro LJ (1992) Characterization of a low copy repetitive element S232 involved in the generation of frequent deletions of the distal short arm of the human X chromosome. Nucleic Acids Res 20:1117–1122

Lichter P, Cremer T (1992) Human cytogenetics: a practical approach. Oxford University Press, Oxford

May CA, Shone AC, Kalaydjieva L, Sajantila A, Jeffreys AJ (2002) Crossover clustering and rapid decay of linkage disequilibrium in the Xp/Yp pseudoautosomal gene SHOX. Nat Genet 31:272–275

Morizio E, Stuppia L, Gatta V, Fantasia D, Guanciali Franchi P, Rinaldi MM, Scarano G, Concolino D, Giannotti A, Verrotti A, Chiarelli F, Calabrese G, Palka G (2003) Deletion of the SHOX gene in patients with short stature of unknown cause. Am J Med Genet A 119:293–296

Muntoni F, Torelli S, Ferlini A (2003) Dystrophin and mutations: one gene, several proteins, multiple phenotypes. Lancet Neurol 2:731–740

Rao E, Weiss B, Fukami M, Mertz A, Meder J, Ogata T, Heinrich U, Garcia-Heras J, Schiebel K, Rappold GA (1997*a*) FISH-deletion mapping defines a 270-kb short stature critical interval in the pseudoautosomal region PAR1 on human sex chromosomes. Hum Genet 100:236–239

Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, Muroya K, Binder G, Kirsch S, Winkelmann M, Nordsiek G, Heinrich U, Breuning MH, Ranke MB, Rosenthal A, Ogata T, Rappold GA (1997*b*) Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. Nat Genet 16: 54–63

Rappold GA, Fukami M, Niesler B, Schiller S, Zumkeller W, Bettendorf M, Heinrich U, Vlachopapadoupoulou E, Reinehr T, Onigata K, Ogata T (2002) Deletions of the homeobox gene SHOX (short stature homeobox) are an important cause of growth failure in children with short stature. J Clin Endocrinol Metab 87:1402–1406

Rappold GA, Klink A, Weiss B, Fischer C (1994) Double crossover in the human Xp/Yp pseudoautosomal region and its bearing on interference. Hum Mol Genet 3:1337–1340

Ross JL, Scott C Jr, Marttila P, Kowal K, Nass A, Papenhausen P, Abboudi J, Osterman L, Kushner H, Carter P, Ezaki M, Elder F, Wei F, Chen H, Zinn AR (2001) Phenotypes associated with SHOX deficiency. J Clin Endocrinol Metab 86: 5674–5680

Ross MT, Grafham DV, Coffey AJ, Scherer S, McLay K, Muzny D, Platzer M, et al (2005) The DNA sequence of the human X chromosome. Nature 434:325–337

Schiller S, Spranger S, Schechinger B, Fukami M, Merker S, Drop SL, Troger J, Knoblauch H, Kunze J, Seidel J, Rappold GA (2000) Phenotypic variation and genetic heterogeneity in Léri-Weill syndrome. Eur J Hum Genet 8:54–56

Shapiro LJ, Yen P, Pomerantz D, Martin E, Rolewic L, Mohandas T (1989) Molecular studies of deletions at the human steroid sulfatase locus. Proc Natl Acad Sci USA 86:8477–8481

Shears DJ, Vassal HJ, Goodman FR, Palmer RW, Reardon W, Superti-Furga A, Scambler PJ, Winter RM (1998) Mutation and deletion of the pseudoautosomal gene SHOX cause Léri-Weill dyschondrosteosis. Nat Genet 19:70–73

Spranger S, Schiller S, Jauch A, Wolff K, Rauterberg-Ruland I, Hager D, Tariverdian G, Troger J, Rappold, G (1999) Léri-Weill syndrome as part of a contiguous gene syndrome at Xp22.3. Am J Med Genet 83:367–371

Wapenaar MC, Petit C, Basler E, Ballabio A, Henke A, Rappold GA, van Paassen HMB, Blonden LAJ, van Ommen GJB (1992) Physical mapping of 14 new DNA markers isolated from the human distal Xp region. Genomics 13:167–175

Yen PH, Li XM, Tsai SP, Johnson C, Mohandas T, Shapiro LJ (1990) Frequent deletions of the human X chromosome distal short arm result from recombination between low copy repetitive elements. Cell 61:603–10

Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res 31:3406–3415