Table 1

Genotyping Data for SW575 Trio

Subject	Allele Size (bp)		
	SHOX-CA	*DXYS233*	*DXYS234*
SW575	138	282	246
Father	140	274	246
Mother	138,152	274,282	246

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A Second Recombination Hotspot Associated with *SHOX* Deletions

To the Editor:

We read with interest "Identification of a Major Recombination Hotspot in Patients with Short Stature and *SHOX* Deficiency" (Schneider et al. 2005). We have characterized 30 unrelated subjects—from kindreds with Leri-Weill dyschondrosteosis (LWD [MIM 127300]) ascertained from U.S. and Canadian clinics focused on genetics, pediatric endocrinology, and orthopedic hand surgery—on the basis of short stature and/or Madelung wrist deformity (Ross et al. 2001). Patients with karyotypic abnormalities were excluded. *SHOX* [MIM 312865] deletions were identified by FISH with cosmids LLNOYCO3′M′34F5 and/or LLNOYCO3′M′15D10 (Rao et al. 1997), as described elsewhere (Wei et al. 2001), by genotyping the *SHOX*-CA microsatellite marker (Belin et al. 1998), located at nucleotides 540504–540660 of the human X chromosome (May 2004; hg17) assembly (UCSC Genome Browser), or by a commercial diagnostic test for homozygosity of multiple intragenic SNPs (SHOX-DNA-Dx [Esoterix Endocrinology]). Deletions were characterized as follows.

We genotyped probands and available parents for pseudoautosomal markers *DXYS233* and *DXYS234*, respectively, located at nucleotides 868388–868748 and 1711448–1711779 of the X chromosome (hg17), by capillary electrophoresis by use of fluorescent-labeled primers selected from the GDB Human Genome Database. Markers that showed two alleles of distinct size were scored as "not deleted." Markers that showed only one size allele were scored as "deleted" (hemizygous) if inspection of the pedigree revealed noninheritance of a parental allele or as "uninformative" if homozygosity could not be excluded. Table 1 shows representative genotyping data for proband SW575 and her parents. It is apparent that this proband inherited null alleles of *SHOX-CA* and *DXYS233* from her father, which implies a deletion encompassing both these markers (deletion of *SHOX* was confirmed by FISH; data not shown). *DXYS234* was uninformative in this kindred.

We also generated human-hamster somatic-cell hybrid clones that retained the deleted X chromosome but not the other human sex chromosome, for 11 probands or their first-degree relatives, and we mapped the deletions by STS content mapping (table 2), using PCR assays designed from publicly available pseudoautosomal sequence. All PCRs gave the expected product from a positive control (X-only hybrid GM06318) and from probands' genomic DNA and no product from hamster DNA. Finally, we mapped the deletion breakpoint proximal to *DXYS234* in one proband, by FISH, with BAC RPCI3-431I1, near the pseudoautosomal boundary (Ross et al. 2000).

Our results (table 3) differed markedly from those reported by Schneider et al. (2005). *DXYS233* was deleted in 17 (65%) of 26 of our informative cases, as compared with 6 (18%) of 33 cases reported by Schneider et al. (2005). By contrast, a similarly small proportion of deletions encompassed *DXYS234* in our sample (3/27; 11%) and that of Schneider et al. (2005) (4/31; 13%), inferred from their figure 1 (*DXYS234* maps just proximal to *ANT3*). Our genotyping and STS content-map-

Table 2

STS Content-Mapping Data for Hybrids

	STS Position on the X Chromosome Sequence[a]												
Proband	401599 to 401819	413340 to 413779	516318 to 516686	521289 to 521370	549537 to 549738	565237 to 565698 (*SHOX* exon 3)	597765 to 598234	868388 to 868748 (*DXYS233*)	1000510 to 1001758	1093641 to 1093815	1182168 to 1182351	1415973 to 1416498	1499109 to 1499293
316	+	+	+	−		−	−	−	−	−	−	+	+
325	+	+	−	−		−	−	−	−	−	−	+	+
368	+	−				−		−	−	−	−	−	+
378	+	+	+	+	−	−	−	−	−	−	−	+	+
447	−	−			−	−	+	+	+	+		+	+
467	+	−			−	−	−	+	+	+	+	+	+
507	+	+	+	+	−	−	−	−	−		−	+	+
575	+	+	+	+	−	−	−	−	−	−	−	+	+
598	+	+	−	−	−	−		−	−	−	−	+	+
617	+	+	+	+	−	−	−	−	−	−	−	+	+
619	+	+	+	+	−	−	−	−	−	−	−	+	+

Note.—Deleted intervals are shaded in gray.

[a] From the human (May 2004; hg17) assembly (UCSC Genome Browser).

Table 3

Deletions of Markers *DXYS233* and *DXYS234* by Ethnicity

	DXYS233			*DXYS234*		
Race/Ethnicity	No. Deleted	No. Not Deleted	No. Uninformative	No. Deleted	No. Not Deleted	No. Uninformative
White, not Hispanic (n = 23)	11	8	4	3	17	3
White, Hispanic (n = 7)	6	1	0	0	7	0
Total (n = 30)	17	9	4	3	24	3

ping results were concordant in cases in which both data were available. The proximal breakpoint in 8 (72%) of our 11 hybrids mapped to the same ~150-kb gap between contigs NT_086931 and NT_086933. Thus, we found a recombination hotspot several hundred kilobases proximal to the hotspot reported by Schneider et al. (2005) (fig. 1).

The reason for this discrepancy is unclear. One difference is the populations studied. Our population included seven Hispanic subjects, six (86%) of whom had deletions at *DXYS233,* whereas the population studied by Schneider et al. (2005) was European, predominantly German. However, 10/19 (53%) of our non-Hispanic subjects also had deletions at *DXYS233*. Phenotypic differences are unlikely to explain the discrepancy, since all of our subjects and 27 of the 33 subjects studied by Schneider et al. (2005) had LWD, for which the size of the deletion does not correlate with the severity of the phenotype (Schiller et al. 2000). There are also significant methodological differences between our studies: Schneider et al. (2005) mapped deletions principally by cosmid FISH, with fine mapping by SNP analysis of only seven families, whereas we used primarily microsatellite marker–segregation analysis and somatic cell hybrid STS content mapping. Our result is not likely to be due to false paternity, since we did not observe any nonparental genotypes. It is possible that either or both studies were confounded by segmental duplications within the pseudoautosomal region, which is known to be enriched in repeats (Ried et al. 1998). In fact, a recent genomewide survey of normal copy-number variation reported a polymorphic duplication at or near *SHOX* (Sharp et al. 2005). Further mapping of *SHOX* deletion breakpoints associated with LWD or idiopathic short stature (MIM 604271) in different populations and completion of the pseudoautosomal sequence may shed light on the nature and mechanism of recombination hotspots in this genomic region.

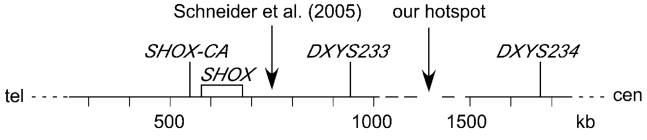

Figure 1 Diagram showing relative locations of *SHOX* gene (*box*), microsatellite markers, contigs (*horizontal lines*), and deletion breakpoint hotspots. Scale is numbered according to human (May 2004; hg17) assembly (UCSC Genome Browser).

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Web Resources

The URLs for data presented herein are as follows:

GDB Human Genome Database, http://www.gdb.org/ (for microsatellite markers *DXYS233* and *DXYS234*)

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

UCSC Genome Browser, http://genome.ucsc.edu (for STS markers at the Human [*Homo sapiens*] Genome Browser Gateway)

References

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 (Leri-Weill syndrome). Nat Genet 19: 67–69

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) Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. Nat Genet 16:54–63

Ried K, Rao E, Schiebel K, Rappold GA (1998) Gene duplications as a recurrent theme in the evolution of the human pseudoautosomal region 1: isolation of the gene *ASMTL*. Hum Mol Genet 7:1771–1778

Ross JL, Roeltgen D, Kushner H, Wei F, Zinn AR (2000) The Turner syndrome–associated neurocognitive phenotype maps to distal Xp. Am J Hum Genet 67:672–681

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

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 Leri-Weill syndrome. Eur J Hum Genet 8:54–62

Schneider KU, Sabherwal N, Jantz K, Röth R, Muncke N, Blum WF, Cutler GB Jr, Rappold G (2005) Identification of a major recombination hotspot in patients with short stature and *SHOX* deficiency. Am J Hum Genet 77:89–96

Sharp AJ, Locke DP, McGrath SD, Cheng Z, Bailey JA, Vallente RU, Pertz LM, Clark RA, Schwartz S, Segraves R, Oseroff VV, Albertson DG, Pinkel D, Eichler EE (2005) Segmental duplications and copy-number variation in the human genome. Am J Hum Genet 77:78–88

Wei F, Cheng S, Badie N, Elder F, Scott C Jr, Nicholson L, Ross JL, Zinn AR (2001) A man who inherited his *SRY* gene and Leri-Weill dyschondrosteosis from his mother and neurofibromatosis type 1 from his father. Am J Med Genet 102:353–358

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