SHORT COMMUNICATION

Assignment of Human Erythroid δ-Aminolevulinate Synthase (ALAS2) to a Distal Subregion of Band Xp11.21 by PCR Analysis of Somatic Cell Hybrids Containing X;Autosome Translocations

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The erythroid-specific (ALAS2) and housekeeping (ALAS1) genes encoding δ-aminolevulinate synthase have recently been mapped to chromosomes Xp21.1→q21 and 3p21, respectively. The erythroid-specific gene is a candidate for mutations resulting in X-linked sideroblastic anemia. Analysis of DNA from hybrid clones containing translocations in the region Xp11.21→Xq21.3 permitted the finer localization of the ALAS2 gene with respect to other loci and breakpoints within this region. These studies localized the ALAS2 gene to the distal subregion of Xp11.21 in Interval 5 indicating the following gene order: Xpter–OATL2–[L62-3A, Xp11.21; A62-1A-4b, Xp11.21]–(ALAS2, DXS323)–[B13-3, Xp11.21; C9-5, Xp11.21]–(DXS14, DXS429)–DXS422–(DXZ1, Xcen). Thus, the reported linkage of acquired sideroblastic anemia and sideroblastic anemia with ataxia to Xq13 presumably results from genes other than ALAS2.

δ-Aminolevulinate synthase [Succinyl-CoA:glycine *C*-succinyltransferase (decarboxylating); EC 2.3.1.37, (ALAS)] catalyzes the first committed step in heme biosynthesis. The erythroid-specific gene (ALAS2) has been mapped to the X chromosome (2) and localized to Xp21.1→q21 (3). This localization makes ALAS2 a candidate gene for mutations resulting in X-linked sideroblastic anemia (2, 3), consistent with the decreased ALAS activity commonly found in patient's bone marrow (1). Indeed, the first report of a mutation of ALAS2 in a case of pyridoxine-responsive X-linked sideroblastic anemia (Cotter *et al.*, in press) demonstrates the association between ALAS2 and this disease. However, Raskind *et al.* (7) reported the linkage of X-linked sideroblastic anemia with ataxia to Xq13, and Dewald *et al.* (4) found chromosomal rearrangements involving Xq13 in selected patients with idiopathic acquired sideroblastic anemia. In this report, a somatic cell hybrid panel of X;autosome translocations has permitted a finer localization of ALAS2 to the specific subregion Xp11.21.

The somatic cell hybrid panel used to dissect the region Xp21.1→Xq21.3 consisted of the following translocation clones: A2-4, Xp21.1→Xqter; DUA-1CsAzB, Xpter→Xp11.22; L62-3A, Xpter→Xp11.21; A62-1A-4b, Xp11.21→Xqter; A48-1Fa, Xcen→Xqter; A63-1A, Xq11.2→Xqter; A68-2A, Xq13→Xqter; W4-1A, Xq13→Xqter; A50-1Acl.3A, Xq21.3→Xqter (6) and B13-3, Xp11.21→Xqter; C9-5, Xp11.21→Xqter (5). None of these hybrid lines contained an intact X chromosome as assessed by polymerase chain reaction (PCR) analysis of the steroid sulfatase gene (STS) on Xp22.32 and the phosphoglycerate kinase gene (PGK1) on Xq13.3 ((8, 9); Yen, GenBank Accession No. M16505). The PCR primer pair for STS (5′-TAATGA CGTATACATAGGCATCATTTCA-3′ and 5′-CCA-CATTGTTGAATTGAGTCACGATAG-3′) amplified a 335-bp fragment, and the PCR primers for PGK1 (5′-CTTAGCATTTTCTGCATCTCCACTTG-3′ and 5′-CATGCTGAGTAGTGAAACAGTGACA-3′) amplified a 312-bp product. PCR primers for ALAS2 (5′-GCCGCCGAATTCAAACTTGAATTTTCATG-3′ and 5′-GCCGCCGAATTCGCCCTTCTGTACTGTTT-3′), amplified a 333-bp fragment encompassing exon 4 and flanking intronic sequences (Bishop *et al.*, unpublished). The 100-μl PCR reaction mixture contained 1 μg of DNA, 10 μl of 10× *Taq* polymerase buffer, 50 μ*M* of each dNTP, 100 pmol each of the sense and antisense oligonucleotides, and 2.5 units of *Taq* polymerase (Promega, Madison, WI). The reaction mixture was incubated at 94°C for 6 min, then 30 cycles of PCR amplification were performed with denaturation at 94°C for 1 min, primer annealing at 60, 68, and 54°C for the STS, PGK1, and ALAS2 primers, respectively, for 1 min, and extension at 72°C for 2 min.

As illustrated in Fig. 1, clones A2-4, and A62-1A-4b (lanes 2 and 4, respectively) were positive for the ALAS2 gene. In particular, clones L62-3A, C9-5, and B13-3, which were ALAS2-negative (lanes 5, 6, and 7, respectively), and ALAS2-positive clone A62-1A-4B localized ALAS2 to a narrow overlap region of Xp11.21 within Interval 5 (6). The remaining five clones with breakpoints in the region Xcen→q21.3 were all ALAS2-negative and are not shown in Fig. 1. PCR analysis of the hybrid clones with exon 10 ALAS2 primers gave identi-

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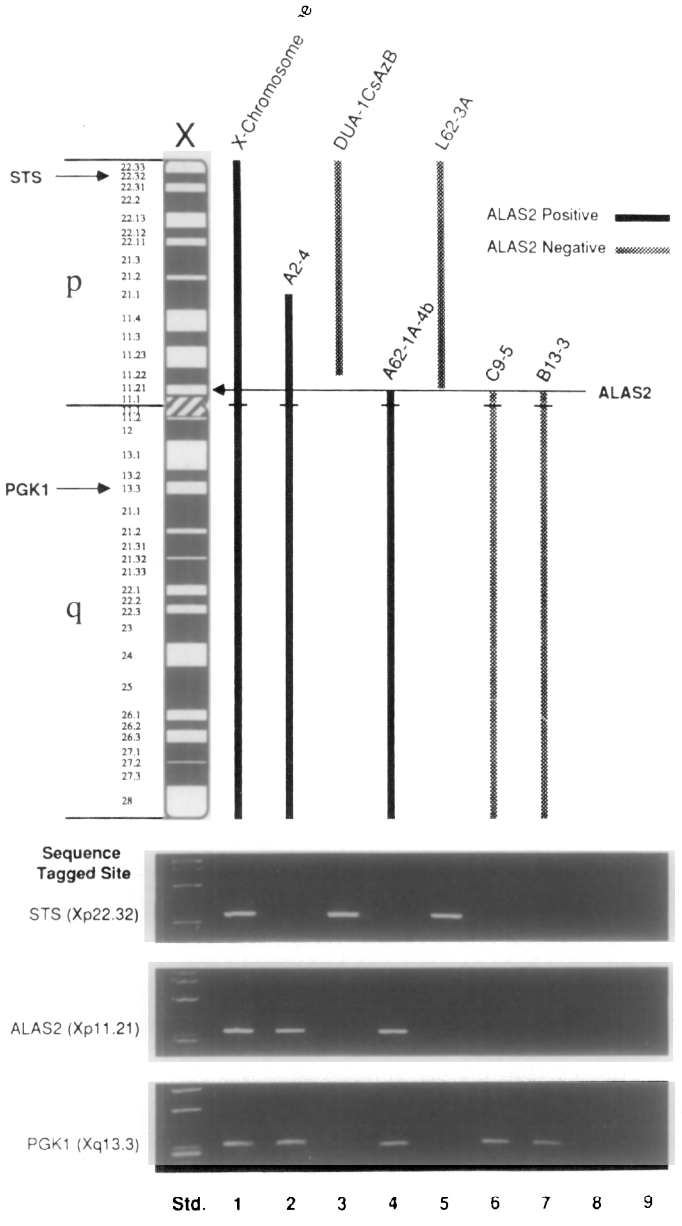

FIG. 1. Localization of ALAS2 to band Xp11.21 by PCR analysis of translocation hybrids. The three gel panels, showing ethidium bromide-stained 1.7% agarose gels, contain PCR products from the primer pairs for the STS, ALAS2, and PGK1 genes. The **Std.** lane contains ϕX174 DNA digested with *Hae*III. Lanes **1**, **8** and **9** contain human, Chinese hamster, and mouse genomic DNA, respectively. Lanes **2** through **7** contain DNA from the hybrid clones indicated in the diagram above the panels. The solid vertical bars indicate genomic DNA and hybrid clones positive for the ALAS2 gene, while the shaded bars indicate ALAS2-negative clones.

cal results (data not shown). PCR analyses of all hybrid clones with both Xp and Xq sequence-tagged sites demonstrated concordance with the published X chromosome breakpoints and the absence of complete X chromosomes (Fig. 1). These results confirm and extend the previous regional mapping (3) by confining the gene to a specific subregion of band Xp11.21.

Integration of these results with previous data on physical mapping, including Interval 5 of the pericentromeric region of the human X chromosome (5, 6), made it possible to refine the localization of ALAS2 with respect to other loci and breakpoints according to the order: Xpter–OATL2–[L62-3A, Xp11.21; A62-1A-4b, Xp11.21]–(ALAS2, DXS323)–[B13-3, Xp11.21; C9-5, Xp11.21]–(DXS14, DXS429)–DXS422–(DXZ1, Xcen). Thus, the ALAS2 locus and DXS323 are subregionally localized to the distal segment of Xp11.21, as defined by the incontinentia pigmenti-1 (IP-1) breakpoints within hybrid clones B13-3 and C9-5. The proximal segment of Xp11.21 presumably contains the IP-1 locus and additional loci as noted in Gorski *et al.* (5). The ordering of these loci relative to each other within Xp11.21 awaits further study. This mapping of ALAS2 to the distal subregion of Xp11.21 also suggests that genes other than or in addition to ALAS2 are involved in the X-linked and idiopathic-acquired sideroblastic anemias associated with Xq13.

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