Supplementary note 2 for “Mutations in retinoic acid induced 1 (RAI1) associated with Smith-Magenis syndrome phenotype in patients without a detectable 17p11.2 deletion”, Slager et al., 2003

**Fluorescence in situ hybridization (FISH) analysis of patient chromosomes**

FISH was performed on patient metaphase chromosomes isolated and prepared using standard cytogenetic protocols. FISH probes were created using BAC, PAC, or cosmid DNA by using a commercially available nick translation kit to incorporate Spectrum Green or Spectrum Orange dUTP by following manufacturer instructions (Vysis, Inc., Downers Grove, IL). Probe DNA (100ng BAC and 180ng cosmid) was precipitated, hybridized to metaphase spreads and washed per manufacturer recommendations (Vysis Inc.). Slides were counterstained using Vectashield antifade with DAPI (Vector Labs, Burlingame, CA). Analysis of the FISH experiments was carried out on a Zeiss Axioplan2 microscope and photographed with a Hamamatsu black and white camera using Zeiss AxioVision software version 2.0.

**FISH analysis of nondeletion patients.**  

*a*. FISH analysis for SMS129 showing no deletion with a green test probe (BAC CITC-40I23, indicated by white arrows) containing the PEMT2 gene and a red chromosome 17q control probe (BAC RP11-314M5).  

*b*. FISH analysis for SMS129 showing no deletion with a green test probe from the chromosome 17 specific cosmid library (c62F2, indicated by white arrows) which contains the FLII gene and the red chromosome 17q control probe. These data are similar for SMS156 and SMS159, as well. None of these patients carry 17p11.2 deletions detectable by FISH.
**Triplet repeat analysis**

RAI1 is a large, complex protein which contains CAG and TCC trinucleotide repeats. The variable CAG repeat within exon 2 has been reported to correlate to the age of onset of spinocerebellar ataxia type 2\(^1\). The normal numbers of CAG repeats has been published to range between 10-18\(^1,2\); expanded alleles have not been reported. Patient SMS129 carries 12 CAG repeats on both alleles, SMS159 has 13 repeats, and we were unable to determine the precise repeat number for SMS156, as this individual has different numbers of repeats on the normal and mutant alleles.
