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Report

The 28-kb Deletion Spanning D15S63 Is a Polymorphic Variant in the Ashkenazi Jewish Population

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D15S63 is one of the loci, on chromosome 15q11-q13, that exhibit parent-of-origin dependent methylation and that is commonly used in the diagnosis of Prader-Willi or Angelman syndromes (PWS/AS). A 28-kb deletion spanning the D15S63 locus was identified in five unrelated patients; in each of them the deletion was inherited from a normal parent. Three of the five families segregating the deletion were reported to be of Jewish Ashkenazi ancestry, and in the other two families the ancestral origin was unknown. To determine whether the 28-kb deletion is a benign variant, we screened for the deletion in 137 unselected Ashkenazi individuals and in 268 patients who were referred for molecular diagnosis of PWS/AS, of whom 89 were Ashkenazi and 47 were of mixed origin (Ashkenazi and non-Ashkenazi Jews). In the control group, three individuals were carriers of the deletion; among the patients, three were carriers, all of whom were Ashkenazi Jews. There was no significant difference between the control group and the Ashkenazi patients, indicating that the deletion is not a cause of PWS- and AS-like syndromes. The frequency of the 28-kb deletion in the Ashkenazi population was 1/75. Since methylation analysis at the D15S63 locus may lead to misdiagnosis, we suggest the use of *SNRPN*, either in a PCR-based assay or as a probe in Southern hybridization, as the method of choice in the diagnosis of PWS/AS.

Prader-Willi syndrome (PWS [MIM 176270]) and Angelman syndrome (AS [MIM 105830]) are caused by a deficiency of imprinted gene expression in, respectively, the paternally and maternally inherited chromosome 15q11-q13 (Nicholls et al. 1998). The clinical diagnosis of these two syndromes is based on differential methylation in the PWS/AS region; currently, the two loci that are used for the methylation assay are the *SNRPN* gene and the D15S63 locus (American Society of Human Genetics/American College of Medical Genetics Test and Technology Transfer Committee 1996). The methylation status at the D15S63 locus is analyzed by Southern hybridization using methylation-sensitive restriction enzymes and hybridization with the PW71B probe (Dittrich et al. 1992). Recently, a 28-kb deletion spanning D15S63 was detected, which led to misdiagnosis of either PWS or AS (Buiting et al. 1999). The deletion was identified in five unrelated patients, three of whom were reported to be of Ashkenazi Jewish ancestry; the chromosomes bearing the deletion had common alleles, suggesting a founder mutation. Among 1,000 unrelated German individuals the deletion was not detected. We further investigated the 28-kb deletion to determine whether it is a benign polymorphism in the Ashkenazi Jewish population or a mutation that has a clinical effect on its carriers.

Two groups of individuals were analyzed: 137 unrelated and unselected healthy individuals of Ashkenazi origin (a control group) and a group of 268 patients referred for molecular diagnosis of either PWS or AS in whom the clinical diagnosis could not be confirmed. The origin of the patients is described in table 1.

The 28-kb deletion was analyzed in the control group by PCR-based assay (Buiting et al. 1999). Three of the 274 alleles in the control group had the 28-kb deletion; the deletion was confirmed by Southern hybridization using *Cfo*I+*Bgl*II and PW71B as probes

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Table 1

Ethnic Origin of the Patients and the 28-kb Deletion

Origin	No. of		
	Individuals	Alleles	Deletion Alleles
Control group:			
Ashkenazi individuals	137	274	3
Patients:			
Ashkenazi Jews	89	178+47=225	3
Jews of mixed origin (Ashkenazi and non-Ashkenazi)	47		0
Sephardic/Oriental Jews	62	124+47=171	0
Jews of unknown origin	40	80	0
Arabs	30	60	0
Total	268	536	3

(Lerer et al. 1994). In the three individuals the deletion was on the paternally derived chromosomes. In the patient group, the methylation analysis was performed, by Southern hybridization (*Cfo*I+*Bgl*II/PW71B), as part of the molecular diagnosis. Among the patients, 3 of the 225 alleles of Ashkenazi origin had the 28-kb deletion; two patients, one each from families 63 and 221, have been described elsewhere (Buiting et al. 1999). In two patients the deletion was on the paternally derived chromosomes, and in one it was on the maternally derived chromosome. In all three patients the methylation status of the *SNRPN* was normal (Buiting et al. 1999). None of the non-Ashkenazi Jewish patients (171 alleles) or the Arab patients (60 alleles) had the 28-kb deletion.

The frequency of the 28-kb deletion mutation was 1.1% in the control group of Ashkenazi Jews and 1.3% in the patients of Ashkenazi origin; the difference between these samples is not statistically significant ($\chi^2 = 0.059$; $P = .81$). It is therefore suggested that the deletion is a benign variant and is not a cause of syndromes such as PWS and AS. In both the patient group and the control group, the frequency of the 28-kb deletion variant in the Ashkenazi population was 1/75 (.0133 [95% confidence interval .0061–.0289]). In the non-Ashkenazi Jewish population, if the deletion exists, its frequency is much lower. As already mentioned by Buiting et al. (1999), this polymorphism is a pitfall in the diagnosis of PWS and AS and, if the methylation assay using the PW71B probe is considered, a positive diagnosis should be confirmed by alternative methods (Abeliovich 2000), especially in Ashkenazi Jewish patients. This observation illustrates the advantage of the differential methylation assay at the *SNRPN* gene, since it is not affected by the polymorphic deletion of 28 kb. It is therefore recommended that the method of choice in the diagnosis of PWS/AS be based on *SNRPN*, in either a Southern hybridization (Glenn et al. 1996) or PCR-based assay (Kosaki et al. 1997; Zeschnigk et al. 1997). The *SNRPN* assay is also preferable for the analysis of fetal tissues such as chorionic villi or amniocytes (Glenn et al. 2000).

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Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for PWS [MIM 176270] and AS [MIM 105830])

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