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INVITED EDITORIAL
A New Twist: Some Patients with Saethre-Chotzen Syndrome Have a Microdeletion Syndrome

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One of us (E.H.Z.), as a fellow with Dr. Roy Breg, published a paper regarding a child who had unicoronal synostosis, unilateral ptosis, short stature, microcephaly, simian crease, developmental delay, and a ring chromosome 7 (Zackai and Breg 1973). Twenty-five years later, after reading Johnson and colleagues' paper in this issue of the *Journal* (Johnson et al. 1998), I think I have been given an explanation of my patient's findings. Using a combination of techniques, including analysis of microsatellite markers, FISH, and Southern blot analysis, Johnson et al. have determined that a significant proportion of patients with Saethre-Chotzen syndrome have deletions in chromosome 7p21.1 that encompass the *TWIST* gene. Furthermore, patients with large (megabase-sized) deletions in this region have significant learning difficulties in addition to the clinical features of Saethre-Chotzen syndrome, which suggests that such mutations define a new microdeletion syndrome. The significance of this finding is twofold: It both refines the molecular tools for diagnosis of Saethre-Chotzen syndrome and provides an explanation for at least some of the phenotypic variability that can confound clinical diagnosis of this disorder.

The Saethre-Chotzen story, however, goes back to the early 1930s. In 1931 Norwegian psychiatrist Haakon Saethre described a disorder consisting of craniosynostosis; low frontal hairline; facial asymmetry; deviated nasal septum; vertebral column defects; brachydactyly; clinodactyly of the fifth finger; partial soft-tissue syndactyly of the second and third fingers and second, third, and fourth toes; and a large pigmented nevus of the back (Saethre 1931). In addition to the proband, the mother and maternal half-sister exhibited turricephaly and similar syndactyly. Saethre also described a sporadic case with craniosynostosis, low frontal hairline, bilateral ptosis, bilateral hallux valgus, brachydactyly, and soft-tissue syndactyly involving the second and third toes. In 1932, the German psychiatrist F. Chotzen reported a similar combination of anomalies in a father and his two sons (Chotzen 1932). Additional findings in this family included hypertelorism, esotropia, proptosis, high arched and narrow palate, facial asymmetry, growth retardation, short stature, conductive hearing defect, unilateral cryptorchidism, and mental retardation.

The striking pattern of anomalies described by Saethre and Chotzen, however, has been the subject of much confusion clinically, since many cases have been reported as Crouzon syndrome, pseudo-Crouzon syndrome, and simple craniosynostosis. In fact, in one classification, patients described by Saethre and Chotzen were nosologically separated from each other. Pantke et al. (1975), in a landmark paper based on findings in six kindred with 31 affected individuals and an analysis of well-documented cases from the literature, defined the syndrome clinically as characterized by craniosynostosis, low-set frontal hairline, parrot-beaked nose with deviated septum, ptosis of the eyelids, strabismus, refractive error, tear duct stenosis, dystopia canthorum, brachydactyly, and abnormal dermatoglyphic patterns (simian crease). They emphasized paying close attention to minor skeletal anomalies, including anomalies of the spine, the hands (brachydactyly, clinodactyly, and syndactyly), and the feet (hallux valgus and syndactyly). The syndrome follows an autosomal-dominant mode of transmission with complete penetrance and variable expressivity.

The history of Saethre-Chotzen syndrome follows a modern molecular genetics road map—through linkage studies and translocation identification in patients with the Saethre-Chotzen phenotype—leading to positional cloning, identification of a candidate gene, and then mutations in that gene. The locus for Saethre-Chotzen had been mapped to the chromosome 7p21-p22 region by linkage analysis (Brueton et al. 1992; Lewanda et al. 1994*b*; van Herwerden et al. 1994). Case reports of

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patients with the Saethre-Chotzen phenotype and apparently balanced translocations involving 7p21-7p22 supported the localization to this region (Reardon et al. 1993; Reid et al. 1993; Lewanda et al. 1994*a*; Rose et al. 1994; Tsuji et al. 1994; Wilkie et al. 1995). This suggested that the gene could be identified by positional cloning. After its localization to chromosome 7p21 (Bourgeois et al. 1996), the human *TWIST* gene became a candidate for Saethre-Chotzen syndrome. Further support for this candidate gene came from the finding that mice heterozygous for a *Twist* null mutation exhibited cranial and limb defects (El Ghouzzi et al. 1997*b*; Bourgeois et al. 1998). Heterozygous mutations in the coding region of the *TWIST* gene were identified in some, but not all, patients with Saethre-Chotzen syndrome (El Ghouzzi et al. 1997*a*, 1997*b*; Howard et al. 1997; Rose et al. 1997; Paznekas et al. 1998). The *TWIST* gene product is a basic helix-loop-helix transcription factor that dimerizes to form a bipartite DNA binding groove. Since heterozygous mutations in the gene, many of which lead to premature termination of protein translation, are believed to cause haploinsufficiency or complete loss of function of one copy, the normal function of the *TWIST* protein is believed to be critically sensitive to dosage.

The variability of expression and occasional mild phenotype seen in Saethre-Chotzen make this one of the more difficult craniosynostosis syndromes to diagnose clinically. Now that the molecular era is upon us, the confusion surrounding this syndrome is beginning to dissipate. Many patients whose conditions were previously diagnosed with a variety of labels, including Saethre-Chotzen syndrome (von Gernet et al. 1996; Muenke et al. 1997; Rose et al. 1997; Golla et al. 1997; Paznekas et al. 1998), turned out to have the newly defined fibroblast growth factor receptor 3 (*FGFR3* [Pro250Arg]) mutation (Muenke et al. 1997), and there has been much made of the "heterogeneity of Saethre-Chotzen." We agree with Johnson et al. (1998) when they suggest classifying patients by genotype rather than phenotype and suggest avoiding semantic arguments as to whether there is genetic heterogeneity for Saethre-Chotzen syndrome or whether patients were previously misdiagnosed.

The ability to diagnose Saethre-Chotzen syndrome at the molecular level has brought back into the fold a group of patients thought by some to be part of a separate syndrome. Carter et al., in 1982, described a distinct entity of craniosynostosis with a "Saethre-Chotzen-like" facies and bifid hallux and suggested it be called Robinow-Sorauf syndrome (MIM 180750) in recognition of its prior description (Robinow and Sarouf 1975). Cohen (1986), however, felt that the family described by Robinow and Sorauf represented the Saethre-Chotzen syndrome (MIM 101400). Reardon and Winter (1994) and Shidayama et al. (1995) noted that the hallux reduplication was present in some members and not in other members of families with classical Saethre-Chotzen syndrome. Our group has identified *TWIST* mutations in three families with classical Saethre-Chotzen syndrome in which a bifid hallux was present in some members of each family (Zackai et al. 1998). We feel that the limb findings in Robinow-Sorauf syndrome represent part of the clinical spectrum of the *TWIST* mutation and that it is not a distinct entity.

Johnson et al. (1998) have now applied what was learned about the *TWIST* mutations to a series of eight patients with the clinical diagnosis of Saethre-Chotzen syndrome, six with bicoronal synostosis and two with unicoronal synostosis. As expected, coding mutations were found in *TWIST*, however, in only five of the eight patients. The remaining three patients had no etiologic diagnosis.

Johnson and coworkers then reasoned that, in some patients, deletion of the whole *TWIST* gene, not detectable cytogenetically, might account for Saethre-Chotzen. The detection of microdeletions involving *TWIST* was performed by a combination of assays, including (1) patient/parent genotype analysis with a newly identified polymorphic marker 7.9 kb downstream of the *TWIST* stop codon, (2) Southern blot analysis of genomic DNA, and (3) FISH analysis using a cosmid clone spanning the *TWIST* gene.

In two patients, a deletion in 7p21.1 was suspected on the basis of inheritance of only the maternal allele for the CA repeat marker and multiple microsatellite markers flanking the *TWIST* gene. Deletion of the *TWIST* gene was confirmed in these patients through FISH analysis by using the 45-kb cosmid clone spanning the gene. The deletions in these patients ranged in size between 3.5 Mb and 10 Mb and were associated with significant learning difficulties. In a third patient without learning difficulties, Southern blot analysis indicated a smaller (~3.0 Mb) deletion encompassing the *TWIST* gene. Thus, in this series of eight clinically diagnosed Saethre-Chotzen patients, all were found to have mutations in the *TWIST* gene. Three (37.5%) of the eight deletions could not be picked up by sequence analysis of the gene. Thus, deletions account for a sizable group of patients with Saethre-Chotzen syndrome in this series.

As part of the same study, Johnson et al. (1998) analyzed 43 patients with craniosynostosis for whom no specific diagnosis could be determined. None of these patients had other known mutations in *FGFR1, FGFR2,* or *FGFR3* that can cause craniosynostosis. (As an aside, it is interesting and humbling to see such a large number of patients in whom no molecular diagnosis has been found to date. We had a similar experience after testing 82 patients, only 46 (56%) of whom had a detectable mutation; Zackai et al. 1998.) One of the patients of

Johnson et al. had a point mutation in the *TWIST* gene, whereas another, who also had learning difficulties, had a deletion associated with an apparently balanced translocation (7;8(p21;q13)). In the former, the diagnosis of Saethre-Chotzen became fairly obvious retrospectively. There was no molecular diagnosis made in the remaining 41 patients. Thus, the clinical utility of such analysis in a series of patients whose conditions are undiagnosed is quite low.

Identifying the precise molecular defect for a patient with a genetic disease is important. It allows one to begin to prognosticate regarding outcomes for patients with the same genotypes for the purpose of advising parents, surgeons, and educators involved in patient care. The finding that patients with both Saethre-Chotzen syndrome and learning difficulties have a different genotype (i.e., microdeletions presumably involving other genes) that accounts for the developmental component is important. The translation of this new information into the clinical setting has immediate implications.

First of all, it appears that the clinical criteria for Saethre-Chotzen syndrome are fairly reliable. Johnson et al. (1998) found facial asymmetry, low frontal hairline, and ptosis to be most helpful for identifying patients with Saethre-Chotzen syndrome in the absence of pathognomonic features such as 2, 3 syndactyly of fingers and duplicated halluces. In addition, we have found that prominent ear crura and small, posteriorly rotated ears can be helpful findings. Craniosynostosis in Saethre-Chotzen syndrome may be unicoronal or bicoronal; metopic suture fusion is found in some cases, but sagittal suture fusion is rare. It is useful to perform analysis first for the Pro250Arg mutation in *FGFR3* in cases of unicoronal synostosis in which other findings of Saethre-Chotzen syndrome are absent (Gripp et al. 1998*b*). Sequence analysis and analysis for deletions of the *TWIST* gene are indicated for patients with unicoronal or bicoronal synostosis and other findings suggestive of Saethre-Chotzen syndrome.

One important conclusion from the current work by Johnson et al. (1998) is that such analysis for *TWIST* gene mutations in clinical Saethre-Chotzen cases has a sensitivity approaching 100%. However, since their study excluded patients with mutations in *FGFR3,* analysis for this mutation as well should be considered in patients who test negative for a mutation in the *TWIST* gene. Patients with Saethre-Chotzen syndrome and learning difficulties should be screened by techniques that include analysis for deletions in 7p21.1 because of the additional implications for the patients and their families of a microdeletion syndrome. However, developmental status should not be used as a definitive criterion to clinically distinguish patients with Saethre-Chotzen syndrome from patients with *FGFR3* mutations, since we have found some patients with the latter to also have this finding (Gripp et al. 1998*a*).

Should we be screening all patients with isolated metopic suture fusion? In a study by Paznekas et al. (1998), 3/39 patients with Saethre-Chotzen syndrome had metopic suture fusion. In the present study by Johnson and colleagues, one patient with a point mutation and another patient with a deletion in the *TWIST* gene had metopic suture fusion. It is important to note that these latter two patients have other features diagnostic of Saethre-Chotzen syndrome as well. We identified a patient with Saethre-Chotzen syndrome and metopic suture fusion who had a 21-bp insertion in the *TWIST* gene before the DNA binding domain (Zackai et al. 1998). This patient also had other findings: bilateral syndactyly of fingers 3 and 4 and bilaterally duplicated halluces. Of the 43 patients with no clear diagnosis studied by Johnson et al. (1998), 5 had metopic suture fusion, but none had a point mutation or deletion of the *TWIST* gene or other mutations in *FGFR1, FGFR2,* or *FGFR3*. It seems unlikely, therefore, that a patient with isolated metopic suture fusion will have a mutation in the genes currently known to be involved in craniosynostosis.

As for sagittal-suture fusion, none of the patients who were found to have a point mutation in or deletion of the *TWIST* gene had sagittal-suture fusion. The original sample of 43 patients had 5 patients with sagittal-suture fusion, none of whom turned out to have mutations in *FGFR1, FGFR2, FGFR3,* or the *TWIST* gene. Thus, such analysis seems unwarranted in cases of isolated sagittal suture fusion at this time.

A *TWIST*-ing path led Johnson et al. from a known disease gene to the discovery of a new microdeletion or contiguous gene deletion syndrome. In 1986, Schmickel observed that phenotypes of many multisystem disorders resulted from the involvement of genes related to each other by their physical proximity on a chromosome rather than by their function. In Schmickel's words, "The contiguous gene syndromes are the experiments of nature that tell us about the organization of genes on chromosomes and where they are. These syndromes tell us where important developmental genes are located and molecular biology provides the means to explore those areas with genetic probes. If we know where a gene is, we can find out what is definitive and ultimately how it should work" (Schmickel 1986, p. 239). In the work of Johnson et al. (1998), failure to find *TWIST* mutations in some patients with Saethre-Chotzen syndrome suggests deletions of the gene. Large deletions were found in patients with Saethre-Chotzen syndrome and learning difficulties, and a new microdeletion syndrome was discovered. Clearly, it will be important to identify the genes surrounding *TWIST* that are responsible for the developmental component in these patients.

Electronic-Database Information

The URL for data in this article is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for Robinow-Sorauf syndrome [MIM 180750])

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