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# Phenotypic consequences of genetic variation at hemizygous alleles: Sotos syndrome is a contiguous gene syndrome incorporating coagulation factor twelve (FXII) deficiency

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**Purpose:** We tested the hypothesis that Sotos syndrome (SoS) due to the common deletion is a contiguous gene syndrome incorporating plasma coagulation factor twelve (FXII) deficiency. The relationship between FXII activity and the genotype at a functional polymorphism of the *FXII* gene was investigated. **Methods:** A total of 21 patients including those with the common deletion, smaller deletions, and point mutations, and four control individuals were analyzed. We examined FXII activity in patients and controls, and analyzed their *FXII* 46C/T genotype using direct DNA sequencing. **Results:** Among 10 common deletion patients, seven patients had lower FXII activity with the 46T allele of the *FXII* gene, whereas three patients had normal FXII activity with the 46C allele. Two patients with smaller deletions, whose *FXII* gene is not deleted had low FXII activity, but one patient with a smaller deletion had normal FXII. Four point mutation patients and controls all had FXII activities within the normal range. **Conclusion:** FXII activity in SoS patients with the common deletion is predominantly determined by the functional polymorphism of the remaining hemizygous *FXII* allele. Thus, Sotos syndrome is a contiguous gene syndrome incorporating coagulation factor twelve (FXII) deficiency. ***Genet Med* 2005:7(7):479–483.**

***Key Words:*** *Coagulation factor XII, Contiguous gene syndrome, FXII functional polymorphism*

Sotos syndrome (SoS, OMIM no. 117550), first reported by Sotos et al. in 1964, is characterized by overgrowth, specific craniofacial features, developmental delay, and advanced bone age, sometimes associated with microdeletion of chromosome 5q35.[1–8] The causative gene for SoS is *NSD1*, but about half of the cases, especially of Japanese ethnicity, harbor a deletion encompassing *NSD1* rather than a point mutation in this gene. An ≈2-Mb common microdeletion predominates, and we and others have demonstrated that there are low-copy repeats (proximal SoS-PREP and distal SoS-DREP) where the breakpoints for the common deletion cluster.[6,9,10] The SoS common deletion results from nonallelic homologous recombination (NAHR) utilizing the flanking LCRs as substrates, suggesting that SoS is a condition resulting from DNA rearrangement due to genomic region-specific architecture, also known as a genomic disorder.[6,9–11] When the genomic interval involved in the DNA rearrangement includes multiple genes that are also dosage-sensitive, this is termed a contiguous gene syndrome (CGS) and independent endophenotypes may occur.[11–13]

We encountered an individual with SoS who had low coagulation factor twelve (FXII; also known as Hageman factor) activity in plasma.[14] FXII is a member of the contact factors for blood coagulation such as factor IX, prekallikrein, and high molecular weight kininogen.[15] In FXII deficiency, a mild bleeding tendency has been reported,[16–18] and others have shown that low levels of FXII increased the risk of thrombosis.[19–22] The relationship between FXII activity and risk of coronary artery disease is still unclear.[23–25] Overall, the

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significance of abnormal FXII activities, especially FXII deficiency, remains unknown, but it is generally thought to have minimal clinical consequences.

Several factors influence the levels of FXII activity. Missense or nonsense mutations of *FXII* lead to its low levels in plasma.[26–33] The activity of this factor is also strongly determined by a common polymorphism within the *FXII* gene (46C/T) (RefSNP rs1801020) (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs = 1801020); with the 46T allele, the translation efficiency of *FXII* is reduced.[34,35]

*FXII* maps to 5q35.3 ≈106 Kb telomeric to *NSD1* and within the common SoS microdeletion region (Fig. 1). Herein, we demonstrate that the FXII activity of individuals with SoS is determined by the 46C/T polymorphism in the context of the type of *NSD1* mutation. If deletion is the mutational mechanism, then the hypoactive 46T hemizygous allele of *FXII* can be further exacerbated and result in deficiency. The complex phenotype of SoS suggests deletion of more than one gene may influence ultimate clinical expression and that this condition is a CGS incorporating FXII deficiency as a feature.

## METHODS

### Clinical subjects

We enrolled a total of 21 SoS patients (17 males and four females; median age, 5 years, ranging from 9 months to 29 years old), with deletion of *NSD1* comprising 17 cases (Table 1). Of these deletions cases, 10 have a de novo common microdeletion (previously described as deletion "A"[6] Cases nos. 1–10), three cases had smaller deletions of almost identical sizes without deleting *FXII* (nos. 11–13) (Fig. 1),[6,14] and the size of the deletion in four individuals (nos. 14–17) was not determined. The remaining four patients with SoS (nos. 18–21, all male; median age 4 years old, ranging from 3 to 29 years) have intragenic mutations of *NSD1*.[3,6,36] Four additional individuals were analyzed, the parents of a "small" deletion case,[6] and the parents of a common deletion case.

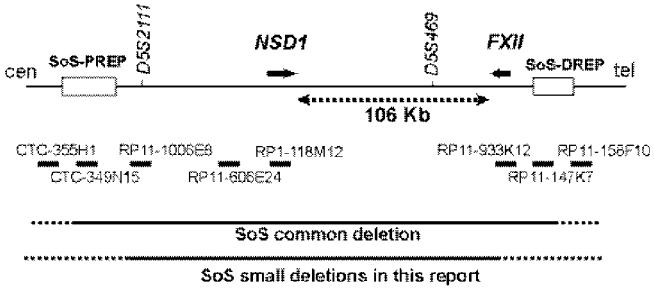


**Fig. 1.** Physical map detailing the position of *NSD1* and *FXII* in 5q35.3. Two microsatellite genetic markers, *D5S2111* and *D5S469*, map to this region. The distance between the 3′ end of *NSD1* and the 3′ end of *FXII* is ≈106 Kb. The low copy repeats that mediate the common deletion, proximal SoS-PREP and distal SoS-DREP copies are shown by open rectangles. Bold lines indicate large genome insert bacterial artificial chromosome (BAC) and P1 artificial chromosome (PAC) clones that were used to determine the deletion size.[6] The RP11-933K12 clone contains the entire *FXII* gene. The genomic intervals for the SoS common deletion and smaller deletions are shown by horizontal straight lines under BAC/PAC clones with dashed line representing the nondeleted portion of the genome.

**Table 1**
SoS cases with information on assayed FXII activity levels and the *FXII* 46C/T genotype

| Case Nos. | *NSD1* mutation | *FXII* genotype | FXII activity (%) | Reference |
|---|---|---|---|---|
| 1 | com del | 46T | 39 | 6 |
| 2 | com del | 46T | 34 | 3 (Pt 3) |
| 3 | com del | 46T | 30 | 6 |
| 4 | com del | 46T | 46 | 6 |
| 5 | com del | 46T | 42 | 6 |
| 6 | com del | 46T | 28 | 3 (Pt 5) |
| 7 | com del | 46T | 48 | 3 (Pt 19) |
| 8 | com del | 46C | 88 | 6 |
| 9 | com del | 46C | 86 | 6 |
| 10 | com del | 46C | 107 | 6 |
| 11 | sml del | 46T/T | 37 | 14 |
| 12 | sml del | 46T/T | 38 | 3 (Pt 9) |
| 13 | sml del | 46C/C | 81 | None |
| 14 | del | 46T/(T) | 19 | None |
| 15 | del | 46T/(T) | 34 | None |
| 16 | del | ND | 27 | None |
| 17 | del | ND | 57 | None |
| 18 | pm | 46T/C | 87 | 6 (SoS 98) |
| 19 | pm | 46C/C | 59 | 36 |
| 20 | pm | 46C/C | 91 | 36 |
| 21 | pm | 46T/C | 85 | 8 (SoS 147) |
| Father of 2 | None | ND | 93 | None |
| Mother of 2 | None | ND | 115 | None |
| Father of 11 | None | T/T | 62 | 14 |
| Mother of 11 | None | T/C | 102 | 14 |

com del, common deletion; sml del, small deletion; pm, point mutation; ND, not determined.

### Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed on all small deletion SoS cases (nos. 11 [ref 14], 12, 13) to determine if the *FXII* gene was deleted. Two bacterial artificial chromosome (BAC clones) containing the entire *FXII* gene were used in this study (RP11-933K12, CTB-22D11; UCSC Genome Browser, May 2004 Assembly, http://genome.ucsc.edu/cgi-bin/hgGateway). Probe DNA was labeled with Spectrum Orange-11-UTP (Vysis, Downers Grove, IL) by nick translation, and after hybridization, the signal was detected using fluorescence photomicroscopy.

### Laboratory analysis of FXII

FXII activity (normal range: 50–150%) was evaluated with an automatic analyzer (standard clotting time assay using specific plasma without FXII, and activated partial thromboplastin time).

### Genotyping of the *FXII* 46C/T variant

The *FXII* 46C/T polymorphism was analyzed by direct sequencing using ABI PRISM BigDye Terminators version 3.1 Cycle Sequencing Kit (PE Applied Biosystems).

After amplifying genomic DNA, the PCR products were sequenced using an ABI3100 autosequencer.

## RESULTS

Plasma factor XII activity levels were obtained for all subjects and controls. *FXII* genotypes at the 46C/T functional polymorphism were determined for all individuals in which DNA was available; 19 out of 21 patients and two out of four controls (Table 1). The distribution of FXII activity as a function of mutational status is shown in Figure 2. With the exception of one reported case,[14] no individual in this study had any known complications of bleeding, heart disease, or venous thrombosis.

The mean activity of FXII in SoS individuals with any *NSD1* deletion (nos. 1–17) was 49.5% (standard deviation 25.5%) (Table 1). Ten SoS cases had a common microdeletion (nos. 1–10) and of these, seven had low FXII activities, whereas the remaining three cases (nos. 8–10) showed activities within the normal range (86%–107%) (Table 1). As a result of genotyping of the 46C/T functional polymorphism in *FXII* for the SoS patients with the common deletion, seven patients with low FXII activities had the 46T variant that is associated with reduced FXII activity,[34,35] whereas the three SoS patients with normal levels all had the 46C variant on the remaining hemizygous allele (Fig. 3).

Three SoS cases harbored smaller-sized deletions (nos. 11–13) and FISH analysis was performed to determine if the *FXII* gene was deleted. In all three instances, the distal breakpoints were localized in the region between BAC clones RP1-118M12 and RP11-933K12. The latter clone containing *FXII* was not deleted (Fig. 1),[3,14] suggesting an intact *FXII* locus on the deletion chromosome. The assayed FXII activities of these three cases were low except for one (no. 13) who is homozygous for the 46C variant.

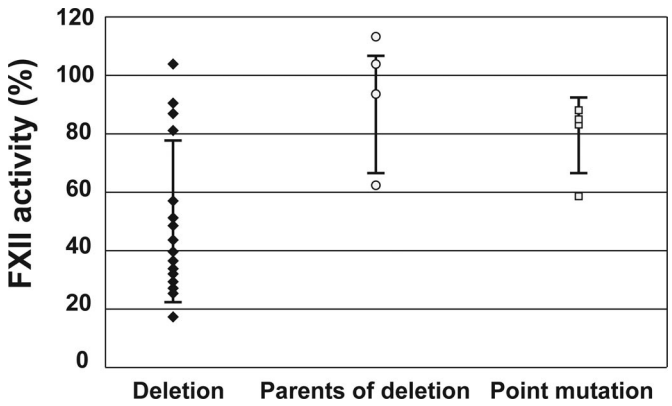


**Fig. 2.** Distribution of FXII activity in plasma for individuals with three different genotypes at the *NSD1* locus: heterozygous genomic deletion, wild type, and heterozygous point mutation. Black diamonds (left), white circles (middle), and white squares (right) show FXII activity of deletion patients, parents of deletion patients, and patients with *NSD1* intragenic point mutations, respectively. The averages and standard deviations for each group are 49.5 ± 25.5 (deletion), 93.0 ± 22.6 (parents of deletion representing the wild type), and 80.5 ± 14.5 (point mutation).

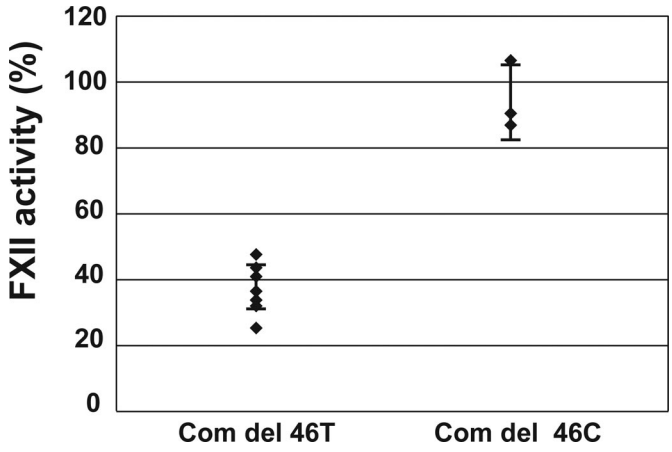


**Fig. 3.** Distribution of FXII activity in common deletion individuals with the *FXII* 46T or 46C polymorphism. The black diamonds indicate the value of FXII activity for individuals with the 46T and 46C polymorphism, respectively. The averages and standard deviations of each group are 38.1 ± 7.8 and 93.7 ± 11.6.

Of the four patients with SoS who harbored a point mutation in *NSD1* (nos. 18–21), the mean FXII activity was 80.5% (standard deviation 14.5%); an affected patient and his affected father were included in this group (nos. 19 and 20, respectively).[36] The FXII activities for four control individuals [the parents of a small deletion patient (nos. 11),[14] and of a common deletion patient (nos. 2; Table 1)] were determined and none demonstrated FXII deficiency.

## DISCUSSION

In this report, we examined the relationship between FXII activity, a functional polymorphism in the *FXII* gene, and Sotos syndrome. The majority of the study population were male, but FXII levels are not known to be influenced by age or gender.[37] The mechanism of *NSD1* disruption among the individuals who were studied was deliberately skewed toward deletion cases, because the primary focus was to correlate the type of the deletion (common type "A" ≈2 Mb microdeletion vs. other types of deletion) with any disruption of the *FXII* gene, and subsequently, serum FXII activities. The mean FXII activity in all SoS individuals with deletion (including both common and smaller deletions) is statistically lower than that observed for SoS patients with *NSD1* point mutation, or for the control parents of SoS children ($t$ test, $P = 0.001$) (Table 1) (Fig. 2). The FXII activities in the parents (father and mother of patients of nos. 2 and 11) are greater than the activity of their children with deletion (Table 1) (Fig. 2). However, an interesting pattern emerged upon genotyping the 46C/T functional polymorphism of the *FXII* gene. In SoS patients with the common deletion, the predominant factor influencing FXII activities is the genotype at the hemizygous *FXII* allele (Fig. 3).

Three of the deletion cases with uncommon smaller deletions (nos. 11–13) were further studied by FISH with probes

encompassing the *FXII* region. In all three, the *FXII* gene was determined to be present, although two of the cases had FXII deficiency. The FXII activities and genotypes of cases nos. 11, 12, and 13 were 37% (46T/T), 38% (46T/T) and 81% (46C/C), respectively. Thus, the FXII activity was determined by the 46C/T functional polymorphism of the *FXII* gene similar to the situation in other individuals with two intact alleles.

The FXII activities of the individuals with a deletion of unknown size (nos. 14–17) were either in the deficiency range or borderline low (57%). Unfortunately, the 46C/T functional polymorphism could not be analyzed because DNA was not available (with the exception of nos. 14 and 15). However, due to the Japanese ethnicity and the prevalence of the common microdeletion in this population, they are likely hemizygous for the *FXII* locus,[6] with FXII deficiency arising in a similar fashion as for cases nos. 1 through 7. The FXII activities of the individuals with *NSD1* intragenic mutations (nos. 18–21) were all within normal range, demonstrating that the *NSD1* intragenic mutation itself does not influence the FXII activities of these SoS patients.

The allele frequency of the 46C/T polymorphisms differs between ethnic populations. In Japan, the T allele (frequency 0.653) is more common than the C allele (ss4935759), whereas in France, the reverse is true (ss3856582) (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs = 1801020). The ethnic composition of our study population was predominantly Japanese, and among the common microdeletion cases, the frequency of the T allele (7 out of 10, or 0.7) (Table 1) was as expected. Further experimentation is necessary to determine if the prevalence of FXII deficiency in *NSD1* deletion cases of SoS in other ethnic groups is lower than what we report in this study, and reflective of differences in the proportion of that population that have the C versus the T allele.

Our results show that serum FXII activities may be a useful and convenient surrogate genetic marker to screen for the SoS common microdeletion. Other congenital anomaly syndromes also have hematological abnormalities. For example, Noonan syndrome is associated with deficiency of coagulation factor XI.[38–40] Reports vary on the prevalence of coagulation problems (from 20–74%) but, consistently, a clinically significant bleeding tendency has been noted. However, in contrast to SoS, the genetic basis for this hematological abnormality is unknown.

Recently, targeted deletion mice of *FXII* have been generated and analyzed.[41] Homozygous knockout mice are deficient in serum activities of this factor, whereas heterozygous knockouts have an intermediate activity. No increased incidence of bleeding tendency or venous thrombosis was observed. Thus, consistent with reports in humans, a clinically significant phenotype may not be associated with FXII deficiency.

Knowledge regarding the genetics of SoS has advanced rapidly since its first characterization 40 years ago. In the past few years, we have identified the etiologic gene (*NSD1*),[1–8] and further delineated the mechanistic basis behind its disruption (point mutation vs. microdeletion due to NAHR utilizing flanking LCRs as homologous recombination substrates).[6,9,10]

Recently, slight differences in clinical manifestations in SoS have been correlated to the type of *NSD1* alteration, with deletion individuals having more heart, brain, and genitourinary abnormalities.[42] Incorporating FXII deficiency with SoS, this study provides insight into the underlying genetics behind this phenotypic variability. First, the genomic deletion leads to the absence of the primary etiologic gene responsible for the syndrome. Depending on the size of the deletion and differences in breakpoint location, disruption of neighboring genes in the same region may contribute in part to any observed clinical differences. However, also responsible for the phenotypic variability are functional polymorphisms of the remaining allele in any genes now present in a hemizygous state. This study clearly demonstrates this phenomenon with SoS and the 46C/T polymorphism influencing serum FXII activities (Fig. 3).

This concept of genetic variation of the remaining hemizygous alleles influencing phenotype is similar to well-characterized examples.[13] In both Prader-Willi syndrome (PWS) and Angelman syndrome (AS), patients hemizygous for the *P* gene are usually hypopigmented (deletion vs. UPD cases) and unmasking of a recessive *P* allele can have a further effect on skin color.[43,44] Unmasking of a recessive deafness allele, in the *MYO15A* gene at the *DFNB3* locus, is associated with sensorineural deafness in a Smith-Magenis syndrome (SMS) patient with the common SMS deletion.[45,46]

We hypothesize that other genes[6,9,10] in the SoS common deletion interval in addition to *FXII* may influence the ultimate clinical phenotype. To examine this hypothesis further, chromosome-engineered animal models of SoS deletion may be useful.[47] These experiments, along with further studies of the genomic region deleted in SoS patients, are likely to promote further insights into the specific genes that contribute to the clinical phenotype of SoS.

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