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# Report

## Segregation Analysis in Shwachman-Diamond Syndrome: Evidence for Recessive Inheritance

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**Shwachman-Diamond syndrome is a rare disorder of unknown cause. Reports have indicated the occurrence of affected siblings, but formal segregation analysis has not been performed. In families collected for genetic studies, the mean paternal age and mean difference in parental ages were found to be consistent with the general population. We determined estimates of segregation proportion in a cohort of 84 patients with complete sibship data under the assumption of complete ascertainment, using the Li and Mantel estimator, and of single ascertainment with the Davie modification. A third estimate was also computed with the expectation-maximization (EM) algorithm. All three estimates supported an autosomal recessive mode of inheritance, but complete ascertainment was found to be unlikely. Although there are no overt signs of disease in adult carriers (parents), the use of serum trypsinogen levels to indicate exocrine pancreatic dysfunction was evaluated as a potential measure for heterozygote expression. No consistent differences were found in levels between parents and a normal control population. Although genetic heterogeneity cannot be excluded, our results indicate that simulation and genetic analyses of Shwachman-Diamond syndrome should consider a recessive model of inheritance.**

Shwachman-Diamond syndrome (MIM 260400) is a rare disorder characterized by exocrine pancreatic dysfunction and hematological and skeletal abnormalities. An array of associated but variably expressed features also occur (Aggett et al. 1980; Ginzberg et al. 1999). At early presentation, the syndrome is distinguished from cystic fibrosis by the constellation of clinical findings and occurrence of normal sweat electrolytes. We collected clinical data and blood samples from families with affected members from around the world, to increase understanding of disease pathophysiology and to define the basic defect(s). Families were ascertained from Australia, Canada, the United States, South and Central America, Great Britain, and mainland Europe. On the basis of clinical experience (Mack et al. 1996), together with the compiled data from the international set (Ginzberg et al. 1999), we adhered to diagnostic criteria of both exocrine pancreatic dysfunction and hematological abnormalities to select families for genetic analysis.

Parental ages were initially evaluated to determine whether Shwachman-Diamond syndrome may be secondary to a sporadic dominant mutation. There was no such indication; at the time of birth of the proband, the mean paternal age (30.40 years) and the mean difference between parental ages (2.47 years) were not higher than those expected in a normal population (Modell and Kuliev 1990; Bender and Ford 1993; Moll et al. 1996; Tellier et al. 1996).

Formal segregation analysis has not previously been performed, because large collections of families had not been ascertained. We examined the hypothesis that Shwachman-Diamond syndrome is an autosomal recessive disease, using our cohort. Patients and their families were included for analysis if diagnosis was supported by documented clinical evidence (Ginzberg et al. 1999) and if the nuclear family structure was known. Seventy families with 84 patients (31 female, 53 male; median age 5.8 years; range 0.2–31.9 years) were suitable for anal-

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ysis (table 1). The majority of families had one affected child. There were 12 families with two and 1 family with three affected children, with nearly equal distribution by sex (15 female, 12 male). One family involved a consanguineous marriage. There were no data indicating that any of the parents were clinically affected.

Estimation of the segregation proportion must allow for the ascertainment bias introduced by sampling through affected offspring. Because the study group was not from a single population, it was difficult to estimate the ascertainment probability, that is, the probability of an affected individual being identified as a proband. We therefore used two standard methods for segregation analysis, making the two most extreme assumptions—of complete and of single ascertainment (Nicholas 1982). A third estimate was also computed by use of the expectation-maximization (EM) algorithm, an iterative numerical technique that is not restricted by assumptions of ascertainment (Lange 1997).

On the assumption of *complete* ascertainment—that is, all affected siblings were independently identified as probands—the Li and Mantel estimator (1968) for segregation proportion ($p$) was computed as $p_{LM} = (R - J_1)/(T - J_1)$, where $R$ is the total number of affected children, $J_1$ is the total number of families with a single affected child, and $T$ is the total number of offspring in the sample. The variance for this estimator is the reciprocal of a weighted sum of terms representing the contribution from families of different sizes. Weights tabulated by Li and Mantel (1968) were used to compute the variance and its square root, the standard error. A 95% confidence interval for $p_{LM} = p_{LM} \pm 1.96 \times SE$. The estimate for the segregation proportion was $p_{LM} = 0.30$ (95% confidence interval 0.18–0.42). A modified Li and Mantel approach proposed by Davie (1979) assumes incomplete ascertainment, because it is unlikely that all affected individuals are identified as probands. This estimator is computed as $p_D = (R - J)/(T–J)$, where $R$ is the total number of affected children, $J$ is the total number of families with a single proband, and $T$ is the total number of offspring in the sample. On the assumption of single ascertainment—that is, all families have exactly one proband—$J$ is simply the total number of families and the variance of $p_D$ is approximated by $(R - J)(T - R)/(T - J)^3$. For the Shwachman-Diamond families, $p_D = 0.18$ (95% confidence interval 0.10–0.27).

The EM algorithm exploits observed data to estimate expected values of missing data, in this case the ascertainment probability. By use of the imputed values for missing data, maximum likelihood estimates of parameters are calculated. The EM algorithm can be used to compute estimates of the segregation proportion ($p$) and the ascertainment probability ($\pi$) by iterating through a series of expectation ($E$) and maximization ($M$) steps until both converge (Lange 1997). We obtained intervals for the EM estimate of the segregation proportion by using a bootstrapping method (Efron and Tibshirani 1993), which involved taking repeated samples (typically 1,000) from the observed data to create a distribution of values for a specific estimate. We computed a 95% bootstrap interval estimate by taking the 2.5th and 97.5th percentiles of the bootstrap distribution. For the segregation analysis, $p_{EM} = 0.18$ (95% bootstrap interval 0.12–0.30).

**Table 1**

**Shwachman-Diamond Families for the Segregation Analysis**

| No. of Affected Sibs per Family | No. of Families | No. of Families with Unaffected Sibs = | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 |
| 1 | 57 | 16 | 27 | 10 | 4 |
| 2 | 12 | 8 | 4 | 0 | 0 |
| 3 | 1 | 1 | 0 | 0 | 0 |

NOTE.—The total number of affected sibs is 84, and the total number of unaffected sibs is 63.

All three estimates of the segregation proportion were consistent with a recessive mode of inheritance, because all of the interval estimates included the value $p = 0.25$. The close agreement of $p_D$ and $p_{EM}$ suggests that an assumption of complete ascertainment was not warranted, that is, $p_{LM}$ appeared inflated because the chance of ascertainment was indeed increased for multiplex families.

The calculated segregation ratios confirm the initial classification of Shwachman-Diamond syndrome as an autosomal recessive disorder, as suggested by the occurrence of affected siblings in early case descriptions and literature surveys (Shmerling et al. 1969; Aggett et al. 1980). There are, however, features with our patient collection that warrant comment. Our collection suggests higher numbers of affected males in the total number of probands. The numbers of males and females are essentially equal in our multiply affected families, so we currently attribute the overall difference to a diagnostic bias rather than to differential penetrance. Indeed, this conclusion is supported by the analysis of the phenotypic manifestations of the patients in our collection, in which it appears that females with poor growth but relatively mild disease are less likely to be investigated (Ginzberg et al. 1999). Although our patient numbers are low, we have also calculated segregation ratios by sex, under the assumption of incomplete ascertainment by Davie (as above, data not shown). No difference was detected between males and females. We have recently identified a second family with related parents, but the overall incidence of consanguineous matings leading to disease appears to be low. This may suggest that disease allele

frequency is not as rare as anticipated from the overall low number of reported cases, but this cannot be adequately addressed unless a true disease incidence can be determined. Finally, a case of Shwachman-Diamond syndrome reported with a de novo translocation, t(6;12)(q16.2;q21.2) (Masuno et al. 1995), raised the possibility that the phenotype may arise from the loss of a gene or its abnormal expression at the affected chromosomal regions. This is, however, the only known case of constitutional rearrangement, and exclusion mapping by linkage analysis has indicated that neither breakpoint region is commonly involved in our patient cohort (Goobie et al. 1999).

Physiological evidence for affected and carrier phenotypes had been suggested, with the detection of decreased neutrophil chemotaxis in patients and intermediate levels in parents predicting homozygote and heterozygote expression, respectively (Aggett et al. 1979). We have not assessed chemotaxis in our families, since subsequent reports have revealed inconsistent decreases in chemotaxis (Komiyama et al. 1985; Repo et al. 1987) and variation with the methods used, leading to concerns of test interpretation and reliability.

It is recognized that pancreatic acinar function can improve with age in a proportion of patients and that a normal serum trypsinogen value in older patients does not preclude occurrence of disease (Mack et al. 1996); however, anecdotal reports also describe symptoms of malabsorption in parents of patients with Shwachman-Diamond syndrome. To evaluate whether heterozygote expression of exocrine pancreatic disease phenotype is detectable, we assayed parental serum trypsinogen as a measure of exocrine pancreatic function (Moore et al. 1986). Serum samples were available for 127 parents. Parental trypsinogen levels were measured (mean 34.7 $\mu$g/liter; standard deviation 10.3; range 8.2–64.2 $\mu$g/liter) and compared with serum samples of 100 anonymous unpaid blood donors, with general linear modeling used to detect age effects. Serum trypsinogen values showed a statistically significant but minimal increase with age for both the parents of patients with Shwachman-Diamond syndrome and controls; however, there were no significant differences between the groups. Parental serum trypsinogen data from our cohort does not generally support heterozygote expression of exocrine pancreatic dysfunction. The few parents ($n$ = 3; 2 female) with values below the lower value of the normal control range of 16.7 $\mu$g/liter (i.e., 8.2, 13.7, and 14.9 $\mu$g/liter) were from singleton families with no other features supportive of the disease phenotype. They remain of interest from the perspective of mutation and disease manifestation.

In conclusion, segregation analysis performed on a cohort of 84 patients with complete sibship data supported an autosomal recessive mode of inheritance. Mean paternal age and mean difference in parental ages were consistent with those expected in the general population. There were no overt signs of disease in the adult carriers (parents), and the use of serum trypsinogen as a measure of exocrine pancreatic dysfunction did not reveal consistent heterozygote expression of the defect. Although we cannot exclude genetic heterogeneity, our results do indicate that simulation and family analyses of Shwachman-Diamond syndrome should consider a recessive model.

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## Electronic-Database Information

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

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for Shwachman-Diamond syndrome [MIM 260400])

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