recent health problem might submerge recall of an earlier diagnosed *birth defect,* as they note.

Second, the query asked only about a diagnosis in the *first year of life.* A mother might be aware of a defect but believe it was diagnosed later in life, subsequent to the end of the first year. (This factor, incidentally, may also account for some of the few false positive replies especially with regard to positional defects of the legs. Some cases in controls may have been diagnosed after the first year [e.g., after the child began to walk] and thus not be in the authors' registry. Or, some mothers may have correctly recalled the presence of a problem, but not that the age of formal medical diagnosis was after one year of age.)

In any event, the methods used by the study are not representative of those used in eliciting family histories in genetic clinics, the usual source of recurrence-risk estimates.

Third, *neither* the study *nor* the control populations are representative of those families in which a recurrence of a defect has occurred. This is pertinent because a mother with an earlier child with a malformation is far more likely to recall the presence of a malformation in a later child, and recall it more accurately, than a mother with no such earlier affected child.

For the reasons above, the applicability of the results of the study to *recurrence*-risk estimates appears remote. (This is not to deny the value of validating the diagnosis of a defect reported in a history.)

On a separate matter, the "cases" of the study included stillbirths and live births, but the "controls" only live births, undermining the strict comparability of the two groups. It is quite unlikely there was as good ascertainment of significant defects, at least of internal organs, in "stillbirths" as in the live births in the same population group. (Non-autopsied stillbirths would dilute the rates here.) Thus the true "sensitivity" of the investigation for defects in stillbirths is likely to be significantly lower than the 56% Rasmussen et al. estimated on the basis of defects *of which they had knowledge.*

The differences in ascertainment and diagnosis of defects in live-borns and stillborns are so vast that the results on these categories should always be presented separately. And the precise definition of stillbirth used should always be specified because of the many different definitions of this term in current use (see, e.g., Hook 1982).

ERNEST B. HOOK

References

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Rasmussen SA, Mulinare J, Khoury MJ, Maloney EK (1990) Evaluation of birth defect histories obtained through maternal interviews. Am J Hum Genet 46:478–485

Am. J. Hum. Genet. 47:742–743, 1990

Reply to Dr. Hook

We thank Dr. Hook for his insightful comments. In response to his first criticism, we had to limit our question to birth defects diagnosed in the first year of life because the maternal responses were compared with data from the Metropolitan Atlanta Congenital defects Program (MACDP) registry, which ascertains only birth defects recognized in the first year of life. We agree that this limitation may be responsible for some of the differences between maternal responses and registry data; however, we believe this restriction is likely to produce more false positives, which represent a small number in our study (about 2% of controls gave a false positive response). We consider the scenario depicted by Dr. Hook, in which the mother is aware of the presence of a defect but believes that it was diagnosed after the first year of life and therefore does not mention it, to be less likely.

Dr. Hook also warns that both our study and control populations are unlikely to represent families with a recurrence since these families would be more likely to accurately recall a birth defect. We are unaware of any evidence to oppose or support his statement; however, we look forward to the availability of more data in this area.

Dr. Hook also notes that data on stillbirths and live births should be presented separately. Actually, we did present separately the overall sensitivity and specificity for live births and stillbirths (fetal death at >20 weeks gestation or >500 grams) (see Rasmussen 1990, table 2) and the difference between the two groups was not statistically significant because of the small number of stillbirths in our data set. For this reason, we did not present sensitivity and specificity for live births and stillbirths for the 66 individual defect categories.

Dr. Hook's final point is that stillbirths are likely to be poorly ascertained by MACDP and that the true sensitivity among stillbirths is probably lower than what

we report. In fact, stillborn infants with birth defects are ascertained by MACDP using multiple sources of ascertainment. Therefore, the recording of stillbirths is probably as complete as can be in any population. For the sensitivity among stillbirths to be significantly lower than 56%, however, one has to assume (1) that MACDP missed a large number of stillbirths and (2) that unascertained stillbirths have a much poorer sensitivity than ascertained ones. We have no data to either support or refute this argument.

We appreciate the interesting points made by Dr. Hook and are pleased to have the opportunity to respond to his concerns.

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Reference

Rasmussen SA, Mulinare J, Khoury MJ, Maloney E (1990) Evaluation of birth defects histories obtained through maternal interviews. Am J Hum Genet 46:478–485

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Identical Point Mutations in the Factor VIII Gene That Have Different Clinical Manifestations of Hemophilia A

To the Editor:

Screening 483 hemophilia A patients by Southern blot technique and direct DNA sequencing, we found two missense mutations in the *Taq*I restriction site of exon 24 within the factor VIII gene. Both mutations are G-to-A (CGA-to-CAA) substitutions at codon 2209 (for amino acid numbering, see Gitschier et al. 1986), which converts arg-to-gln. One patient (HP17) was affected with severe hemophilia (FVIII:C <1 %; FVIII:Ag not determinable, because of prophylactic substitution therapy), whereas the other 21-year-old hemophiliac (HP16) showed a moderate form of HA (FVIII:C 7%; FVIII:Ag 130%). It is surprisingly that patient HP16 developed an anti–factor VIII antibody. The same mutation was also found by three other laboratory groups: Bernardi et al. (1988) described two nonrelated cases that they classified as severe hemophilia A (Bernardi et al. 1989), Youssouffian et al. (1988) detected two further G-to-A transitions at amino acid codon 2209 in two severely affected patients (FVIII:C and FVIII:Ag < 1%), and Levinson et al. (1990) found the same mutation by screening two other unrelated patients, one having a moderate and the other having a moderately severe form of hemophilia A.

In addition, we identified a second type of missense mutation in the *Taq*I site of exon 26 within the factor VIII gene of a severely affected hemophiliac (HP13: FVIII:C < 1%). This mutation at codon 2307 turned out to be a G-to-T substitution (CGA-to-CTA) resulting in an arg-to-leu change. Determination of FVIII:Ag in this patient was not feasible because of prophylactic substitution therapy. In contrast to our finding, Inaba et al. (1989) described the same mutation in a patient having a mild form of the disease (FVIII:C 2%; FVIII:Ag 4%).

In case of the moderate hemophiliac described by Levinson et al. (1990) the mutation occurred de novo in one of the grandpaternal germ cells. Another de novo mutation occurred in the family of patient HP16 (maternal germ cells), presented in our study.

It is of great interest that identical mutations lead to different FVIII activities, which, in turn, account for different clinical manifestations of hemophilia A. Considering the substitution of codon 2209, we found it highly improbable, since the patients are unrelated, that in all six cases the severe form of hemophilia is caused by the same additional mutation within the factor VIII gene. The probability of the same mutation in position 2209 together with a second mutation that is different in each patient is also very unlikely. In contrast, a second mutation might be a plausible explanation for the severe form of hemophilia A in the case of the codon 2307 mutations, as this type of mutation, thus far, has been detected only in these two individuals.

Likewise, we cannot exclude the possibility that a second "positive" mutation occurred in the factor VIII gene of the patients having mild hemophilia (codon 2209 and 2307). This could be a type of mutation concerning the protein C cleavage site, with the result that the patients would show a higher level of FVIII:C, thereby preventing cleavage of factor VIII by protein C (Fulcher et al. 1987).

On the other hand, factor VIII protein interacts with many other proteins during its processing and within the coagulation cascade. These proteins could also contain amino acid polymorphisms which cause a different clinical severity of hemophilia A.