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Correspondence

MUTATIONS IN SALL4 IN MALFORMED FATHER AND DAUGHTER POSTULATED PREVIOUSLY DUE TO REFLECT MUTAGENESIS BY THALIDOMIDE

To the Editor:

In 1994, W.G. McBride wrote a letter to the Editor entitled "Thalidomide may be a mutagen" (McBride, 1994). We write to bring important developments related to this hypothesis to the attention of the readers of *Birth Defects Research (Part A): Clinical and Molecular Teratology*. Kohlhase et al. (2003) have identified and reported mutations in the *SALL4* gene in Case 2 and her father, who were described in Dr. McBride's letter (McBride, 1994). We will review the original hypothesis, this new information, and other related suggestions for clinicians.

To put this finding in perspective, McBride (1994) described two families in which the affected parent had been exposed during gestation to thalidomide. A child of each thalidomide-exposed parent had been born with the same pattern of limb malformations that the parent had. In Case 1, the child had, "... no thumbs and only two digits on both hands. She has severe malformations of both legs, and the left leg is much shorter than the right. Both feet taper to one toe, neither of which has nails. Her father was born in 1960 with malformations of both hands and both legs .... His mother ... said that she had taken six to eight thalidomide tablets. Her father had no thumbs or digits on the right hand but has a thumb and one digit on the left hand and has normal forearms."

In Case 2, "... the father has bilateral malformations of the forearm and hand and also suffers from left-sided deafness. His daughter also has malformations of both forearms and hands." Dr. McBride suggested that "The birth of these children raises the possibility of thalidomide being a human mutagen" (McBride, 1994)

The editors of the journal (*British Medical Journal*) asked Dr. Andrew Read, a medical geneticist at St. Mary's Hospital at the University of Manchester, to comment on these two cases presented by Dr. McBride. He noted, "... if thalidomide ... had a second, independent activity as a mutagen there would be no reason why it should specifically produce mutations leading to limb malformations. Mutagens attack genes at random .... I think ... the two affected children have genetic syndromes .... Case 1 seems to have split hand deformity .... Case 2 has a different condition, involving reduction of the whole arm and shoulders, probably the Holt-Oram syndrome .... The grandparents are reported as unaffected, which suggests that a new mutation has occurred at some point in each pedigree .... Since each father was exposed to thalidomide in utero, it is quite possible that the father's malformations were caused by thalidomide—or maybe a combination of a genetic predisposition and the teratogen" (Read, 1994).

Three aspects of the original hypothesis and these new findings should be noted and are reviewed here: 1) absence of the thumb and hypoplasia of the radius (as in Case 2) are features of many disorders; 2) mutation analysis is now possible for many hereditary malformations; and 3) the expected effects of mutagenesis.

Regarding the nonspecific nature of the phenotype in Case 2, Andrew Poznanski, the radiologist who characterized the full spectrum of the skeletal effects of the Holt-Oram syndrome, reminded us over 30 years ago that "thumb changes and other radial defects are not specific for the Holt-Oram syndrome" (Poznanski et al., 1970). Absence/hypoplasia of the thumb and radius in a parent and child can be features of several autosomal dominant conditions, including Holt-Oram syndrome (OMIM# 142900), Okihiro syndrome (OMIM# 607323), and Stiles-Dougan syndrome (Stiles and Dougan, 1940; Holmes and Borden, 1974), among others.

Before concluding that the child (Case 2) had Holt-Oram syndrome, it would help to know if the phenotype included more of the expected phenotypic features: abnormalities in the shoulder, such as hypoplasia and bipartite ossification of the scaphoid (Hurst et al., 1991), and abnormal ossification of carpal bones, such as the persistence of an extra carpal bone, the os centrale, which fuses normally by eight weeks gestation with the scaphoid carpal bone. Since most individuals with Holt-Oram syndrome have a heart malformation, such as atrial and ventricular septal defects (Basson et al., 1997; Li et al., 1997), it would be more complete to present the clinical cardiac findings, as well as the findings by echocardiography.

Because the father of Case 2 had hearing loss, the clinical evaluation should have included a search for other features of Okihiro syndrome, such as Duane anomaly of eye movement and kidney anomalies, in addition to the preaxial forearm anomalies and hearing loss.

In the evaluation of the individual with absence/hypoplasia of the thumb and radius, whose mother reported having taken thalidomide tablets during pregnancy, the additional distinctive, common features of the thalidomide embryopathy should be noted as present or absent: abnormalities of many organ systems, including eyes, ears, kidneys, and limbs; and the distinctive abnormalities of the eye, including Duane syndrome, uveal coloboma, and abnormal lacrimation (crocodile tears) (Miller and Strömland, 1999). In their follow-up evaluation of 86 adults with thalidomide embryopathy, Miller and Stromland (1999) reported that 44% (of 84) had incomitant strabismus (usually Duane type), 7% had horizontal comitant strabismus, and 20% had abnormal lacrimation, such as tearing while eating or "crocodile tears."

*Correspondence to: Lewis B. Holmes, Genetics and Teratology Unit, Mass General Hospital for Children, Boston MA 02114. E-mail: holmes.lewis@mgh.harvard.edu
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Regarding mutation analysis, the diagnostic evaluation of a parent and child with hypoplasia of the thumb and radius can now include mutation analysis of the *TBX5* gene, in the case of Holt-Oram syndrome (Basson et al., 1997; Li et al., 1997) and the *SALL4* gene for individuals with the features of Okihiro syndrome (Kohlhase et al., 2002). This letter was prompted by the fact that Kohlhase et al. (2002) identified in Case 2 and her father mutations in the *SALL4* gene, which is part of the *SALL* gene family that encodes a related group of zinc finger transcription factors. Four *SALL* genes have been identified: *SALL1*, on chromosome 16q12.1, is associated with Townes-Brocks syndrome; mutations in *SALL4*, on chromosome 20q13.13–13.2, have been shown to cause Okihiro syndrome (Duane-Radial Ray syndrome, OMIM# 607323), an autosomal dominant condition characterized by anomalies of the radius, and Duane syndrome (abnormal eye movements due to aberrant innervation of the extraocular muscles).

There is also very strong logic against the concept that thalidomide exposure in the pregnancy with the father of Case 2 had been mutagenic. The reasons are:

a) As was noted by Read (1994) in his response to the initial hypothesis, if thalidomide is a mutagen, mutations should occur in several genes, not just mutations in the *TBX5* locus for Holt-Oram syndrome (Basson et al., 1997; Li et al., 1997), the *SALL4* locus for Okihiro syndrome (Kohlhase et al., 2002), or the P63 locus that causes split-hand/split-foot syndrome (which is the suggested diagnosis for Case 1) (van Bokhoven et al., 2001).

b) If the father's exposure during his mother's pregnancy occurred in the period of greatest sensitivity to thalidomide, i.e., days 20–36 after fertilization (Lenz and Knapp, 1962; Miller and Strömland, 1999), it would have caused, most likely, a mutation in only some of the cells in the exposed fetus. This mosaicism in the father would have been unlikely to produce a phenotype in the father that was similar to the phenotype of his unexposed daughter, who would be postulated to be affected by a germline mutation.

c) In addition to the expected characteristics of mutagenesis, it should be noted that the examination of 64 children born to one or two parents with the thalidomide embryopathy showed that none had any of the physical features of this phenotype; five of these children had been born to a father and mother who both had the thalidomide embryopathy (Strömland et al., 2002).

Having outlined the more logical diagnosis for Case 2 and her father, we asked ourselves if her father's exposure to thalidomide during gestation could have caused the mutations identified in *SALL4*. It seems very unlikely in view of the similarity of their phenotypes.

Furthermore, even when a fetus has had a well-established exposure to thalidomide in the period of greatest sensitivity, every exposed fetus is not damaged by this exposure. Kajii et al. (1973) reported four individuals with established exposure in this period who were examined carefully as infants, and two again at 11 years of age. No structural abnormalities were identified.

We should expect additional families to be reported in which the affected parent was exposed to thalidomide and has by chance an unrelated spontaneous mutation for a recognized autosomal dominant disorder. The fact that alleged victims from exposure to thalidomide in utero actually had hereditary disorders that shared some features of the thalidomide embryopathy was observed frequently by Widukind Lenz, the German pediatrician and geneticist who first reported the causal relationship between exposure to thalidomide and the occurrence of a distinctive embryopathy (Lenz, 1992).

To date, no human teratogen has been shown to be mutagenic. When this is alleged in the future, we hope the authors will consider the theoretical aspects of this hypothesis, provide more details from the clinical and radiographic examinations of the "affected" individuals, and incorporate mutation analysis, if available.

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Jürgen Kohlhase[1] and Lewis B. Holmes[2]

[1]Institute for Human Genetics and Anthropology
University of Freiburg
Freiburg, Germany
[2]Genetics and Teratology Unit
MassGeneral Hospital for Children
Boston, Massachusetts

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