Understanding the Molecular Basis of Polydactyly

To the Editor:

The development of the vertebrate limb is dependent on 2 signaling centers: the apical ectodermal ridge, which provides essential growth factors, and the zone of polarizing activity, the source of the Sonic hedgehog protein.1 The Sonic hedgehog protein instructs cells of their position and thereby determines their fate along the anteroposterior limb axis.2 Sonic hedgehog regulates both digit number and identity.3

Sonic hedgehog regulates digit formation largely by preventing cleavage of the Gli3 transcription factor to a repressor form.3 The Hox genes regulate digit pattern downstream of Sonic hedgehog and Gli3. The Hox genes can also convert the Gli3 repressor into an activator of Sonic hedgehog.3

In addition, intraflagellar transport proteins have recently been shown to be required for both Gli activator and repressor function,4 and Gli proteins are insensitive to Sonic hedgehog in the absence of intraflagellar transport proteins. Finally, other proteins such as bone morphogenetic protein (BMP), fibroblast growth factors, MSx, Wnt, and Alx play significant roles.

Sonic hedgehog, a secreted protein, is expressed by the cells that make up the zone of polarizing activity.7 Ectopic expression of Sonic hedgehog is a major cause of polydactyly. Multiple polydactylous phenotypes previously reported in chicken and mice have been attributed to abnormal expression patterns of Sonic hedgehog.8

During early limb bud development, Sonic hedgehog expression is limited to the posterior domain of the limb bud, where it generates the zone of polarizing activity. The posterior portion of the limb bud expresses high levels of Sonic hedgehog and is thought to play a key role in determination of digit number and digit identity. Ectopic expression of Sonic hedgehog in the anterior limb bud causes digit duplication in the same region. Accordingly, ectopic expression of Sonic hedgehog in the anterior limb bud has been implicated in the development of polydactyly.7,8

Regulation of Gli3 by Sonic hedgehog signaling (and vice versa) is of general functional importance during embryonic development.6 Gli3 protein is a member of the Gli family of transcription factors, which function as a repressor of multiple Sonic hedgehog targets.3 Gli3 is strongly expressed in developing limb buds. Lack of Gli3 in both mice and humans causes polydactyly.9,10 Mice with mutations created at the Gli3 gene have polydactyly.11 Spontaneous mutations affecting Gli3 cause polydactyly; the limbs of Gli3-deficient embryos are polydactylous.6 In a recent study, homozygous mice with even a small deletion at the Gli3 locus developed 1 or 2 extra digits.12

Mice lacking both Sonic hedgehog and Gli3 develop polydactyly, suggesting a crucial role for the Gli3 gene in digit number determination.13-15 Gli3 protein processing to generate the Gli3 repressor is inhibited by Sonic hedgehog protein.12 Gli3 expression precedes the onset of Sonic hedgehog transcription and is normally restricted to the posterior early limb bud, where it plays an important role in the prepatterning of the skeletogenic mesenchyme.6 A polydactylyous radiation-induced mutant mouse was created in which the Sonic hedgehog protein was expressed at approximately 20% of wild-type limb buds.16 Correspondingly, Gli3 expression was increased. The polydactyly was due to an increased concentration of the Gli3 repressor form because of lowered Sonic hedgehog signaling.

During limb development, Hox proteins are considered among the most important factors regulating digit number and identity.17 Gli3 protects the apical ectodermal ridge from the deleterious effects of Hox genes.1 A recent study showed that Hox genes can also produce polydactyly independent of Sonic hedgehog and Gli3.18

Inactivation of intraflagellar transport proteins results in the development of polydactyly.4 Intraflagellar transport is an active event in which cargo is transported along microtubules by motor proteins.6 Intraflagellar transport proteins are required for the formation and maintenance of flagella and cilia.4 Mice with intraflagellar transport mutations exhibit polydactyly in all 4 limbs.4 Intraflagellar transport function is crucial in the control of both the positive and negative transcriptional activation of Gli proteins, and essential for Sonic hedgehog ligand-induced signaling cascade.8 These proteins are required for the efficient cleavage of Gli3 into a repressor form.5

Several BMPs are expressed in the apical ectodermal ridge. Apical ectodermal BMPs delimit the boundaries of the apical ectodermal ridge by preventing adjacent nonridge ectodermal cells from becoming apical ectodermal ridge cells, thereby negatively modulating apical ectodermal ridge activity and preventing polydactyly.19 Bone morphogenetic protein-7 deficient mice show polydactyly.20 Because BMP-7 is expressed at high levels in the interdigital mesenchyme, which normally undergoes programmed cell death, loss of BMP-7 likely allows for survival of these cells. These surviving cells can then give rise to extra digits.21

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REFERENCES


The Dilemma of the Wedding Band

To the Editor:

I read with interest the article “The Dilemma of the Wedding Band” (http://www.orthosupersite.com/view.aspx?rid=36001) in the February 2009 issue of ORTHOPEDICS. The authors presented their results with no statistical significance between infection rates of orthopedic surgical procedures and the wearing of a plain metal wedding ring. They suggested that the infection rates remained unchanged when a wedding ring was worn.

The authors compared the surgical outcomes before and after wearing the wedding ring (987 vs 1140 procedures). A large sample size increases external validity (generalizability) and power of a study.1-4 However, learning curve may affect the results. With time, the skill of a surgeon will improve. It is generally accepted that experienced hands reduce surgical complications.5-7 The unchanged infection rate may be linked to the surgeon’s skill. Moreover, intervention types and sample (patient) characteristics were not mentioned. It is unknown whether these factors were comparable between the no-ring and ring groups. If not, the findings of this study should be interpreted cautiously.

Albeit required by the journal’s instructions to authors, the article contained no ethical documentation on human patient protection (ethics committee approval and obtaining patient consent). The authors presented their analysis as a retrospective cohort study.

Governing bodies such as the US Food and Drug Administration regulate experiments on drugs and other medical devices and products, but not surgical researches. The boundary between minor modifications and more prominent or extensive alterations of a surgical technique is usually unclear. A surgeon may change his or her practice more permanently based on experience with an individual patient. Case series can therefore be unrecognized as research until they are presented or published. The nuances of experimental, investigatory, and innovative surgery are still insufficiently explored.8-12

Innovative research is defined as a case series that provides clinical parameters and routine follow-up data as study outcomes without a written protocol. It may encompass no more than a placebo effect, and may be costly, time consuming, and dangerous to humans.10-13 Innovative reports are usually strictly personal because they present a case series from talented surgeons in a well-equipped environment. It may therefore distribute danger to other practitioners who lack appropriate training and elaborate equipment. In this way, the journal would become the media of harm to humans.9-13 Human patient protection is therefore crucial for human research (research involving human patients, human tissue, or identifiable personal data).11-13

The US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research defines the research objectives as “to test a hypothesis, permit conclusions to be drawn, and thereby to develop or contribute to generalize knowledge.”10 Hence, case series or any retrospective cohort studies are
considered as a subset of human research, necessitating the strict adherence to the ethical requirements and peer review.\textsuperscript{11,12} Without ethical approval, an ethical question in the study by Stein and Pankovich-Wargula would have arisen: Increased microbial load has been well known when a wedding ring is worn. Should they risk their patients on the known postoperative infections? Clinical research must be transparent (publicly available trial registration and result reporting), and it must not be mixed with routine practice and then later reported as a retrospective study. Physicians frequently take on a dual role as investigators. The authors should merely point out that mixing those roles requires caution, because the central goal of a physician is to serve the best interests of their patients, while that of investigators is to seek truth. These 2 goals are not always compatible.\textsuperscript{11,12,14,15}

According to the Declaration of Helsinki, informed consent is not all the same. In clinical practice, the consent is information about study results that are considered as standard in the medical community. Conversely, the aim of the consent in research practice is to describe the research hypothesis, methods, and outcomes that may be somewhat unknown. The nature of the consent, despite the same terminology used, is significantly different. It is therefore difficult for a physician-scientist to explain these differences of practice is to describe the research hypothesis, methods, and outcomes which may be somewhat unknown. The nature of the consent, despite the same terminology used, is significantly different. It is therefore difficult for a physician-scientist to explain these differences of consent.\textsuperscript{15} Furthermore, patient permission given under unfair or undue pressure is not consent.\textsuperscript{16} To appropriately differentiate between the physician’s role as a clinician and investigator, the American Medical Association suggests that informed consent be obtained by someone other than the treating physician.\textsuperscript{17}

In the study by Stein and Pankovich-Wargula, it is unclear whether the authors received the ethical approval before commencing the project, whether the patients (or more correctly, the subjects) were well informed, and who gave the informed consent. Foramite Pitak-Arnopp, MD Leipzig, Germany

**References**


**Reply:**

The study in question was a review of patient experience from 1998 to 2002. It was strictly a retrospective study based on deidentified patient data provided by our institution. The Institutional Review Board does not require consent for study of deidentified retrospective data. In addition, this data review was performed in 2005, years after the patient experience.


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