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Supernumerary Digital Flexion Creases: An Additional Clinical Manifestation of Alagille Syndrome

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Alagille syndrome (AGS; OMIM 118450) is a complex dominantly inherited multisystem disorder involving the liver, heart, eyes, facies, skeleton, and other systems. Criteria for the clinical diagnosis have been established as the presence of bile duct paucity on liver biopsy in association with three of five major clinical findings (cholestasis, butterfly vertebrae, posterior embryotoxon, congenital heart disease, and facial features). *Jagged1* has been identified as the AGS disease gene. *Jagged1* is a large gene, with no mutational hot spots, making molecular testing difficult at this time. Other clinical features would prove helpful in establishing the diagnosis in the absence of molecular confirmation. Supernumerary digital flexion creases have been identified in 16/46 (35%) of AGS probands examined through the Alagille Syndrome Diagnostic Center at the Children's Hospital of Philadelphia. Although digital abnormalities have been noted in AGS in the past, including short distal phalanges and fifth finger clinodactyly, digital crease abnormalities have never before been reported. Supernumerary digital creases have been reported in less than 1% of the general population. The presence of extra and missing digital creases in individuals with normal joint anatomy, their occurrence in several syndromes, and mouse in situ expression studies indicate that genetic factors contribute to digital crease formation. However, these factors are poorly understood. Hypotheses regarding the origin of flexion creases are discussed. The identification of supernumerary digital creases in one-third of AGS probands provides another diagnostic aid and allows for speculation of the role of *Jagged1* in the molecular development of digital crease patterns.

KEY WORDS: supernumerary digital flexion creases; Alagille syndrome

INTRODUCTION

Alagille syndrome (AGS; OMIM 118450) is a complex dominantly inherited multisystem disorder involving the liver, heart, eyes, face, and skeleton. Alagille et al. [1975] defined clinical criteria for the syndrome as the histological finding of bile duct paucity on liver biopsy in association with three out of the following five major clinical features: cholestasis, butterfly vertebrae, posterior embryotoxon, congenital heart disease, and facial features. Other systems are also involved in AGS and these minor clinical criteria include renal abnormalities and intracranial bleeding [Emerick et al., 1999]. *Jagged1* (*JAG1*) has been identified as the disease gene in AGS [Li et al., 1997]. *JAG1* is a large gene (26 exons, 36 kb) and over 200 different mutations have so far been described in individuals with AGS with no mutational hot spots. The size of the gene and number of mutations seen makes mutational analysis technically difficult and it is therefore available on a research basis only. Genetic analysis is not feasible in daily clinical practice and is of limited value in the immediate diagnosis of AGS. In this context, the identification of additional clinical features is useful to aid diagnosis in the absence of molecular confirmation.

Digital abnormalities have been described in AGS in the past, namely, short distal phalanges and fifth-finger clinodactyly [Riely et al., 1979; Rosenfield et al., 1980].

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Here we describe digital crease abnormalities, which have never before been reported in AGS. Although literature on the subject is scarce, supernumerary digital creases of the fifth finger have been reported in less than 1% of the general population [Komatz et al., 1978]. In studies of individuals with sickle cell anemia, supernumerary digital flexion creases of the distal interphalangeal joints were noted in 24–90% of individuals with sickle cell anemia and in 7–11% of the control populations studied [DeJong and Platou, 1967; Zizmor, 1973]. This may indicate that prevalence rates of supernumerary digital flexion creases may vary among different populations. The digital flexion creases represent the location of firmer attachment of the skin to the underlying structures and correspond to regions where accelerated growth does not take place. Their name reflects the functional relationship of these creases to the flexion movements of the fingers but does not necessarily relate to their developmental origin.

Abnormalities in the digital flexion creases have been described in a number of syndromes. A reduced number of digital flexion creases has been seen in the fetal alcohol syndrome [Jones and Smith, 1973], Down syndrome [Plato et al., 1973], trisomy 18 [Hodes et al., 1978], and in several disorders involving digital joint flexion limitations. Supernumerary digital creases have been described in partial deletions of chromosome 1q [Watson et al., 1986], partial trisomy of 14q [Raoul et al., 1975], partial trisomy of 13q [Schinzel et al., 1976], cerebro-oculo-facio-skeletal syndrome [Lurie et al., 1976], and in sickle cell disease [DeJong and Platou, 1967; Zizmor, 1973]. Individuals with Larsen syndrome have been reported to have both supernumerary and reduced numbers of digital flexion creases [Latta et al., 1971; Dallapiccola and Capra, 1973].

The presence of extra and missing digital creases in individuals with normal joint anatomy and their occurrence in several syndromes indicate that genetic factors contribute to digital crease formation. However, these factors are poorly understood. Mouse in situ studies to determine the expression pattern of *JAG1* in the limb bud provide further evidence in support of the genetic contribution to digital crease development. The hypotheses regarding the origin of digital creases are discussed below.

The identification of supernumerary digital creases in AGS probands described in this study provides another diagnostic aid for this entity and allows for speculation of the role of *JAG1* in the molecular development of digital crease patterns.

MATERIALS AND METHODS

Clinical Examination

Clinical examination of 46 individuals with AGS and their parents have been performed through the Alagille Syndrome Diagnostic Center (ASDC) at the Children's Hospital of Philadelphia. Complete dysmorphology exams were performed on all individuals, including dermatoglyphics and digital and palmar crease analysis. Dermatoglyphic, palmar, and digital crease patterns were also examined concomitantly on individuals referred to the general genetics clinic at the Children's Hospital of Philadelphia, as well as on all unaffected family members of the probands in this study.

Genetic Analysis

Mutational analysis and/or deletion testing by fluorescence in situ hybridization (FISH) was performed for *JAG1* in all individuals enrolled in the study.

Mouse In Situ Studies

Tissue collection and preparation. CD1 mouse embryos (Charles River Laboratory) were harvested from timed matings at 10.5 and 12.5 days postcoitum (d.p.c.). Embryos for sectioning were fixed overnight in 4% paraformaldehyde, dehydrated, and embedded in paraffin wax. Embryos to be used for whole mount in situ hybridization were fixed overnight in 4% paraformaldehyde, dehydrated, and stored in methanol.

Section in situ hybridization. Sense (control) and antisense (test) riboprobes were transcribed from linearized DNA templates in the presence of ^{35}[S]UTP using an in vitro transcription kit (Roche, Indianapolis, IN). Probe templates were as follows. Mouse *JAG1* probe: EST (expressed sequence tag) containing 2.2 kb of mouse *Jag1*, including the polyA tail and 3′ UTR, cytoplasmic domain, and part of the EGF repeats (Research Genetics (Huntsville, AL)) [Loomes et al., 1999]. The antisense template was linearized with *Eco*RI and transcribed with T3 polymerase. The sense template was linearized with *Not*I and transcribed with T7 polymerase. The general protocol for section in situ hybridization and probe labeling is essentially as in Sassoon and Rosentha [1993] and Wilkinson and Nieto [1993].

Whole mount in situ hybridization. Mouse embryos were fixed overnight in 4% paraformaldehyde and taken through a series of washes. The general protocol for whole mount in situ hybridization is essentially as in Rosen and Beddington [1993] and Wilkinson and Nieto [1993] with some additional modifications. Probe templates were as described above. Digoxigenin-labeled sense and antisense riboprobes were transcribed from linearized DNA templates using an in vitro transcription kit (Roche, Indianapolis, IN).

RESULTS

Clinical Examination

We have identified supernumerary digital flexion creases in 16/46 (35%) of AGS probands examined through the Alagille Syndrome Diagnostic Center at the Children's Hospital of Philadelphia (Fig. 1).

The supernumerary digital creases were found almost always, but not exclusively, in the middle phalanges (between the proximal and distal interphalangeal joints). They were found to be present unilaterally, bilaterally, and on anywhere from 1 to all 10 digits. Altered digital crease patterns were not appreciated in unaffected parents of the probands or in 250 patients seen in the general genetics clinic of one of the authors

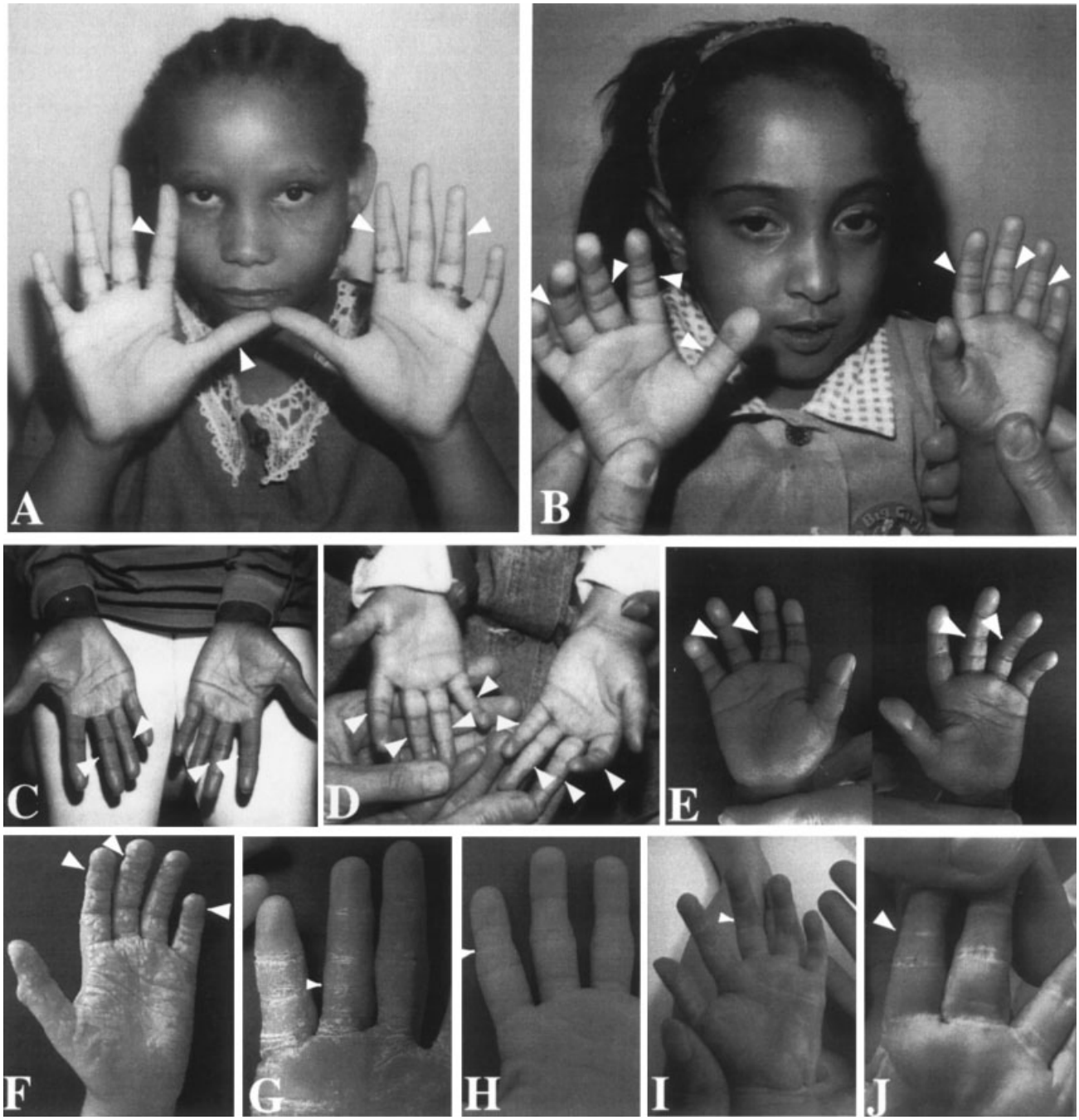

Fig. 1. Digital flexion creases in Alagille syndrome. Note characteristic facial features seen in **A** and **B**. Arrows indicate localization of supernumerary digital flexion creases. **C** and **D** represent a mother and daughter, respectively, both with Alagille syndrome and supernumerary digital flexion creases. A through **J** demonstrates the variable number and localization of the supernumerary creases on any given individual. Note the severe xanthomatous changes of the hand in the patient depicted in **F**.

(I.D.K.) during the period between July 2000 and June 2001. In the three probands that had an affected family member, the supernumerary creases were also present in those individuals (Fig. 1). The supernumerary digital creases appear to become less pronounced with age, making them more difficult to identify in adults.

Genetic Analysis

Genetic analysis revealed that 12/16 (75%) individuals with supernumerary digital creases had mutations in *JAG1* and 25/30 (83%) individuals without supernumerary creases were mutation-positive. These

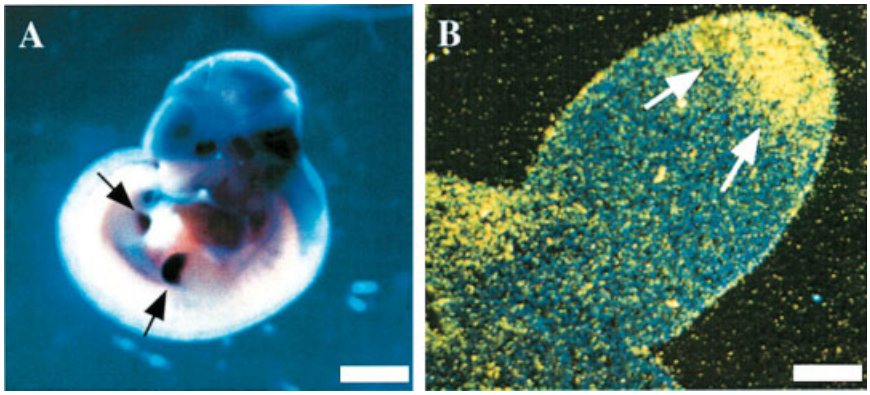

Fig. 2. *Jag1* is expressed in the mouse limb bud at 10.5 d.p.c. by in situ hybridization. **A:** Whole mount in situ hybridization using a probe for *Jag1* (positive hybridization indicated by purple stain) on a 10.5 d.p.c. mouse embryo shows expression in the developing forelimb and hindlimb (arrows). **B:** Section in situ hybridization on a 10.5 d.p.c. mouse embryo shows *Jag1* expression in the apical epidermal ridge and surrounding distal mesenchyme of the limb bud (arrows). Scale bar in A, 300 μM; in B, 45 μM.

numbers are not statistically different and are consistent with the reported mutation detection rates among individuals with clinical AGS [Krantz et al., 1998].

Mouse In Situ Studies

In situ studies of *JAG1* expression in the mouse have shown strong expression in the distal limb buds (Fig. 2). This has been of interest since no overt limb abnormalities have been conservedly identified in AGS (although short distal phalanges and fifth-finger clinodactyly have been reported).

DISCUSSION

Digital crease abnormalities have never before been reported in AGS. This study describes the presence of supernumerary flexion creases in 35% of an AGS population and provides a new aid to diagnosis. Supernumerary digital creases of the fifth finger have been reported in less than 1% of the general population [Komatz et al., 1978] and distal interphalangeal supernumerary creases have been reported to be as high as 11% in a control population for a study of patients with sickle cell disease [DeJong and Platou, 1967; Zizmor, 1973]. Supernumerary digital flexion creases were not identified in 250 patients referred for genetics consultation to one of the authors of this study (I.D.K.) over the past year, making the high incidence among this Alagille syndrome population unlikely to be due to random population variation. Furthermore, the high level of *JAG1* expression in the mouse limb bud supports the hypothesis of a genetic contribution to flexion crease development.

The digital flexion creases represent the location of firmer attachment of the skin to the underlying structures and correspond to regions where accelerated growth does not take place. Their name reflects the functional relationship of these creases to the flexion movements of the fingers but does not necessarily relate to their developmental origin. There has been some disagreement regarding the origin of digital (and palmar) creases. The first hypothesis is that hand creases develop secondary to flexion movements of the developing hand. This is supported by studies of abnormal crease patterns in malformed hands [Popich and Smith, 1970] and by the correspondence of palmar and digital creases to the location of underlying joints. It has been demonstrated that digital flexion creases begin to appear between 7 and 9 weeks of gestational age while spontaneous flexion of the digits does not occur until 11.5 weeks of gestation [Kimura and Kitagawa, 1986]. This would seem to refute the hypothesis that the flexion creases arise secondary to functional movements of the digits and hand.

The second hypothesis is that the flexion creases arise independently of fetal palm movement and are genetically determined. The presence of extra and missing digital creases in individuals with normal joint anatomy supports this. This hypothesis also seems more consistent with their temporal appearance during gestation and alterations in their normal pattern being associated with specific syndromes and exposures. It is also possible, however, that true flexion creases over a joint or flexion plane do require movement for normal development, but those supernumerary creases described here do not. Controversy still surrounds the discussion since arguments regarding the timing of the appearance of digital creases in relation to the onset of fetal hand movement are based on different definitions of flexion movement in utero. Stevens et al. [1988] also observed digital creases at 8 weeks of gestation. However, they compared reflex movements, which can be evoked at 8.5 weeks in the fetal hand. Kimura and Kitagawa [1986] had compared the appearance of creases with spontaneous movement, which occurs later at 11.5 weeks of gestation and therefore discounted the significance of movement in flexion crease development. Since Stevens et al. [1988] were using reflex movements as the comparison, which can be elicited earlier, at the time when digital creases first appear, they concluded that though genetic factors do probably play a role in digital crease development, fetal hand movement is necessary for normal crease pattern formation. The exact contribution of genetic factors remains unclear,

though the high level of *JAG1* expression seen in the mouse limb bud supports the concept of genetic predetermination.

The identification of supernumerary digital creases in over one-third of AGS probands provides another diagnostic aid for this entity and allows for speculation of the role of *JAG1* in the molecular development of digital crease patterns. These findings also support the hypothesis that digital flexion creases are genetically predetermined and develop independently of the flexion movements of the digits.

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