Fibroblast Growth Factor Receptor 2 IIIb Invalidation—A Potential Cause of Familial Duodenal Atresia


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Background/Purpose: Duodenal atresia (DA) occurs in 1 in every 6,000 live births and represents a significant surgically correctable cause of intestinal obstruction in the neonate. Familial or congenital DA has been reported, implying that at least some cases of DA are the result of genetic, heritable abnormalities. The genes controlling duodenal development are incompletely understood. Fibroblast growth factor receptor 2IIb (Fgfr2b) is known to play a critical role in the development of multiple organ systems including other gastrointestinal tract (GIT) structures. This study shows the key role of Fgfr2b in normal duodenal development and the pathogenesis of DA.

Methods: Wild type (Wt) and Fgfr2b<sup>−/−</sup> embryos were harvested from timed pregnant mothers at stage E18.5 and were analyzed for duodenal phenotype.

Results: Inactivation of Fgfr2b results in DA. DA is present in the Fgf2b<sup>−/−</sup> mutants with a 35% penetrance. The duodenal phenotype of the Fgf2b<sup>−/−</sup> mutants ranges from normal to a mucosal web, type I, and type III atresia.

Conclusions: Fgfr2b is a critical regulatory gene in the development of the duodenum. Fgfr2b invalidation (Fgfr2b<sup>−/−</sup>) results in a reproducible, autosomal recessive duodenal atresia phenotype with incomplete penetrance and a variable phenotype.

INDEX WORDS: Fibroblast growth factor receptor 2IIb, duodenal atresia, congenital duodenal atresia, familial duodenal atresia, intestinal atresia, gastrointestinal development.

DUODENAL ATRESIA (DA) is a significant clinical problem facing the neonatal patient population. The diagnosis may be made by prenatal ultrasound scan or postnatally with bilious vomiting and the classic “double bubble” sign on abdominal radiograph. DA requires urgent surgical intervention.

Classically, there are 2 theories to explain the pathogenesis of intestinal atresia. Tandler proposed an error of revacuolization of the solid cord of intestine in 1900. Fifty-five years later, Louw and Barnard<sup>2</sup> implicated late intrauterine vascular accidents as the cause of midgut intestinal atresia. However, there remains no specific teratogen or genetic factor identified as a causal agent of either theoretic mechanism of development. It is certainly conceivable that teratogens or genetic factors could account for an error in the process of recanalization of the intestine or an error of vascular patency or development. Clinical reports have suggested a genetic cause of congenital duodenal atresia with an autosomal recessive inheritance. There also is an association with Down’s Syndrome.<sup>3-14</sup>

The fibroblast growth factors (Fgf) act through tyrosine kinase transmembrane receptors.<sup>15</sup> The Fgf receptor gene family has genetic linkage to skeletal dysplasias and autosomal dominant craniosynostosis syndromes.<sup>16</sup> However, the Fgf receptor family has not been implicated in gastrointestinal malformation in humans. Therefore, we have sought to evaluate the importance of Fgf receptor 2IIb (Fgfr2b) in the development of the duodenum.

MATERIALS AND METHODS

A Cre-mediated excision was used to generate mice lacking Fgfr2b on the C57B1/6 murine strain background as described by DeMoerlooze et al.<sup>10</sup> The Fgfr2b<sup>−/−</sup> (−/−), indicates homozygous deletion of Fgfr2b gene) mutant embryos are viable until birth at which time they have respiratory failure secondary to lung agenesis.<sup>16,17</sup> Wild-type (Wt) C57B1/6 littermates were used as controls. All Wt (n = 20) and Fgfr2b<sup>−/−</sup> (n = 20) embryos evaluated were the result of timed matings. No statistical analysis was required for the interpretation of these data. Embryos at E18.5 (E indicates embryonic day 18.5, or 18.5 days’ postconception) were removed from the uterus of pregnant mothers. The gastrointestinal tract (GIT) was dissected and photographed as fresh samples. These specimens then were preserved in 4% paraformaldehyde, embedded in paraffin, and sectioned sagittally at 8 μm using a microtome (Leica, McBain Instruments, Chatsworth, CA). The sections were mounted and processed using standard H & E staining, and photomicrographs were taken. Animals were used under Institutional Animal Care and Use Committee Animal Care Protocol 32-02.
RESULTS

Homzygous deletion of Fgfr2b results in duodenal atresia with a 35% penetrance and a variable phenotype. This phenotype is inherited in autosomal recessive fashion as part of a constellation of severe developmental defects.

Photomicrographs of the upper GIT show the phenotypic variability of DA in the Fgfr2b/H11002/H11002 mutant (Fig 1). The omentum, mesentery and surrounding abdominal organs were removed to illustrate intestinal anatomy. Wt duodenum is shown in Fig 1A as control. Mutants were found to have a grossly normal-appearing duodenum as shown in Fig 1B, in 65% of embryos (13/20). However, 2 of 4 of the specimens sectioned and stained with H & E showed mucosal web lesions without luminal patency on microscopic evaluation (data not shown). Type I (4 of 20) and III (3 of 20) DA were found in approximately 35% (7 of 20) of the Fgfr2b−/− mutants as shown in Fig 1C & D. Dilation of the proximal duodenum is evident, consistent with obstruction. The mesentery was grossly normal in all specimens evaluated both with and without DA. No type II DA (0 of 20) were encountered in this study.

DISCUSSION

DA and other GIT atresias represent an immediate and long-term surgical challenge that begins in the neonatal period and can continue for many years with short bowel syndrome, intestinal obstruction, or dependence on parenteral nutrition. Whereas our knowledge of incidence, treatment techniques, and long-term outcomes of DA is increasing, our knowledge of specific causal agents is unclear. It is likely that DA and other intestinal atresias are the final common pathway by which multiple genetic or teratogenic processes are manifest. The goal of this work is to identify specific genes involved in the normal and pathologic development of the GIT.

When Fgfr2b is invalidated, one developmental defect is DA with an incomplete penetrance and a variable phenotype inherited in an autosomal recessive pattern. The variable duodenal phenotype and autosomal recessive inheritance correlates with the descriptions of DA in humans. This finding implicates the Fgfr2b gene as a potential cause of heritable DA in humans. Because invalidation of Fgfr2b results in other lethal developmental defects, it is unlikely that a human fetus with a germ line invalidation of Fgfr2b would be viable. A more likely mechanism would involve the mutation of a local tissue specific transcription factor controlling Fgfr2b expression or misexpression of its associated ligand in the duodenum. This would result in downregulation of Fgfr2b in a localized distribution. It is also possible that DA could result from a somatic mutation early in development in which Fgfr2b is invalidated locally in the upper GIT in mosaic genetic pattern.

Fgfr2b is a receptor for FGFs1, 3, 7, and 10. These receptor ligand interactions have been proven to have a regulatory role in mesenchymal/epithelial cell interactions. There is a potential role for these genes in regulating either the process of recanalization of the GIT or in

Fig 1. Duodenal phenotype. (A) Wild type shown as control, E, esophagus; s, stomach; d, duodenum. Fgfr2b−/− mutants shown in (B, C & D). (B) Grossly normal duodenum. (C) Type I duodenal atresia with proximal dilation. (D) Type III duodenal atresia with proximal dilation and distal decompression. All specimens shown are E18.5.
their vascular development. We currently are evaluating these ligands to determine their role in duodenal development. Intestinal development is a complex process, the very basic components of which we are only beginning to understand. With growing potential for tissue engineering and gene therapy applications, it is increasingly important to understand the genetic and molecular mechanisms of both normal and abnormal development. This knowledge will prove valuable in developing screening tests, early diagnosis, and new potential treatments.

The invalidation of Fgfr2b is a causal factor in the pathogenesis of DA in mice and correlates well with the human manifestation of DA. It is interesting to note the DA in the Fgfr2b−/− has a reduced penetrance and variable phenotype and that no type II atresias were identified. Other major malformations found in the Fgfr2b−/− mutant, such as pulmonary agenesis occur with complete penetrance and uniform phenotype. Ongoing investigation of the mechanism by which invalidation of Fgfr2b results in DA will likely explain these interesting findings. Through the investigation of the Fgfr2b gene and others, we hope to better understand the complex process of duodenal development and the pathogenesis of duodenal atresia.

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