CASE REPORT

Microdeletion 11q13.1.q13.2 in a Patient Presenting with Developmental Delay, Facial Dysmorphism, and Esophageal Atresia: Possible Role of the GSTP1 Gene in Esophagus Malformation

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BACKGROUND: Esophageal atresia is a major congenital malformation characterized by a complete interruption of the esophageal continuity. It is frequently observed in associations and syndromes. As an isolated finding, it has a multifactorial etiology whose genetic factors are poorly known. Recently, the GST family, especially the GSTM1 null genotype (but not the GSTP1 polymorphism I105V), has been associated with esophageal atresia. These enzymes play a role in phase II detoxification of xenobiotics. Here we present the clinical and molecular findings observed in a patient suggesting that the loss of the GSTP1 allele might predispose to this malformation. CASE: We describe a patient presenting with esophageal atresia associated with developmental delay and facial dysmorphism, whose mother used tobacco and alcohol during the first 2 months of her pregnancy. Microdeletion/microduplication analysis was performed using comparative genomic hybridization and a 180K Agilent array. It detected a de novo 2 Mb chromosome 11q13.1.q13.2 deletion. CONCLUSION: The deleted chromosomal segment includes the GSTP1 gene. We hypothesize that the deletion of one GSTP1 allele (an isoform highly expressed in embryonic tissues), associated with specific environmental factors, such as tobacco and alcohol, could cause the esophageal atresia observed in our patient. Birth Defects Research (Part A) 97:463–466, 2013. © 2013 Wiley Periodicals, Inc.

INTRODUCTION

Esophageal atresia (EA) is a major congenital malformation characterized by a complete interruption of the esophageal continuity with or without a communication between the esophagus and the trachea. It affects 1:4,220 live births (Nassar et al., 2012). Its mode of inheritance and pathophysiology are complex (Jacobs et al., 2012). In approximately 50% of the cases, this anomaly is associated with other malformations, mainly gastrointestinal atresia or stenosis, anomalies of the urinary tract, and heart defects. EA could be part of different conditions, such as the VACTERL (vertebral, anal, cardiac, tracheo-esophageal, renal, and limb) association and chromosomal abnormalities (trisomies 21,18, and 13; deletions 22q11.2, 13q13., 16q24.1, 17q21.3.q23). In approximately 23% of the cases (Pedersen et al., 2012) it is observed in monogenic disorders such as the CHARGE, Feingold, Rogers, and Opitz syndromes or Fanconi anemia (Brunner and van Bokhoven, 2005; Felix et al., 2009, Genievieve et al., 2011).

As an isolated malformation, EA is considered to have a multifactorial etiology, although both the environmental and the genetic factors involved are poorly known (de...
The environmental factors include maternal diabetes and phenylketonuria, exposure to methimazole and diethylstilbestrol, exogenous sex hormones, alcohol, smoking, infectious diseases, and occupational exposure to pesticides (de Jong et al., 2010; Genievie et al., 2011). The higher concordance rate observed in monozygotic when compared to dizygotic twins highlights the role of genetic factors in the development of isolated EA (Schulz et al., 2012). Studies of foregut development in animal models suggest that candidate genes, such as the Wnt2, Wnt2b, and Barx1 genes, might be involved in isolated EA observed in humans (Jacobs et al., 2012).

The glutathione S-transferases (GST) are a family of enzymes that play an important role in phase II detoxification of xenobiotics, including alcohol and tobacco (Raijmakers et al., 2001). They catalyze the conjugation of reduced glutathione on a wide variety of toxic compounds, converting them in less biologically active and more easily excreted molecules (Ali-Osman et al., 1997). In adults, these enzymes play an important role against oxidative stress and DNA damage (Chen et al., 2012; Liu et al., 2006). Several studies underline the important role of the GSTM1, GSTT1, and GSTP1 isoforms in the pathogenesis of some environmentally related diseases such as cancer, respiratory disease, and lung function deficits in children (Filonzi et al., 2010). Nevertheless, the role of GST as a source of DNA protection in the embryonic tissues is still unclear (Raijmakers et al., 2001). Some authors suggest that there is evidence that the disruption of these genes could play a role in birth defects, such as oral clefts (Shi et al., 2008), heart defects (Cresci et al., 2011), and hypospadias (van der Zanden et al., 2012), by increasing the susceptibility of the embryonic tissues to environmental factors, especially tobacco (Filonzi et al., 2010; Shi et al., 2007).

Recently, a study of the GST family activity and its relation with EA showed an association of this disorder with the GSTM1 genotype, but surprisingly failed to show a significant association with the GSTP1 polymorphism (II05V), known to be the most important GST enzyme during fetal development (Raijmakers et al., 2001).

Here we report on a patient with a microdeletion involving the GSTP1 gene, suggesting that a relationship might exist between a low activity of this enzyme and his EA phenotype.

CASE REPORT

The subject is a 1-year-old boy, the first child of non-consanguineous parents. During the first 2 months of her pregnancy, the mother used tobacco and alcohol. Fetal ultrasound showed polyhydramnios and a possible EA. He was born preterm (34 4/7 weeks), by Cesarean section, with a birth weight of 1345 g, length of 40.5 cm, and Apgar scores 6/8. Soon after birth, EA without a tracheo-esophageal fistula was confirmed, and a gastrostomy/espagostomy was performed the first day of life. He was discharged from the hospital after 31 days. The patient had developmental delay; at 7 months of age he did not have complete control of his head.

Complementary studies, including abdominal ultrasound, brain magnetic resonance imaging, ophthalmologic evaluation, and a G-banded karyotype, were normal. Echocardiogram disclosed a patent foramen ovale. The physical examination at 7 months of age showed: weight 7 kg (5th percentile), length 64 cm (<5th percentile), occipital frontal circumference 44.5 cm (50th percentile), a large open anterior fontanelle, frontal bossing, ocular hypertelorism (inner canthal distance 2.7 cm and outer canthal distance 7.5 cm), downsplaying palpebral fissures, horizontal nystagmus, and normal male genitalia and limbs.

As the patient presented with developmental delay, a major malformation, and some dysmorphic facial features, an array comparative genomic hybridization (array-CGH) was requested.

METHODS

Array-CGH

Array-CGH was performed using the Agilent Human Genome CGH Microarray kit 180K (Agilent Technologies, Santa Clara, CA), hybridized according to the manufacturer’s protocols.

For the location of genes in the deleted genomic segment, the University of California Santa Cruz (UCSC; http://genome.ucsc.edu/) database and the Database of Genomic Variants (http://decipher.sanger.ac.uk) database; second, a search in the PubMed database for an association between each of the 95 gene symbols and the following entries (EA or esophagus) was done; and, finally, using the Mouse Genome Informatics (MGI) (http://www.informatics.jax.org) database a search for an association between each of the 95 gene entries and an abnormal digestive tract phenotype was performed.

RESULTS

The array-CGH showed a de novo 2 Mb chromosome 11 deletion (array 11q13.1.q13.2 65,265,478-67,229,716)x1dn [hg18]). It was confirmed using fluorescence in situ hybridization (FISH) analysis.

Based on the fact that it was a de novo event, and two patients with similar deletions were described previously in the DECIPHER database, the deletion was considered pathogenic. Recently, a third patient showing a microdeletion in the same chromosome band was described (Floor et al., 2012).

The deletion found in our patient could be larger if one considers the segment between the first and last probes on the array (chr11:65,265,469 and chr11:67,330,919). However, as this extended region could not be assessed precisely, we decided to consider only the genes included in the region where only one signal was observed.

Databases were searched in an effort to find a candidate gene that might be related to the malformation observed in our patient. The following results were obtained: no match for EA and chromosome 11; in the PubMed database only one match for EA and one gene,
namely the GSTP1 gene, out of the 95 deleted genes; and no match for a specific gene and EA in the MGI database. But with a more extensive search for an abnormal digestive tract phenotype, an association between the TBX10 gene and oral cleft came out.

Despite the fact that mutations in TBX1, TBX4, and TBX5 have been associated with EA, no association between TBX10 and esophagus or foregut was observed. In mouse models, gain of function mutations of the TBX10 gene have been associated only with oral cleft (Bush et al., 2004).

In the three patients described with an overlapped deletion, none showed EA. Conversely, all of them showed developmental delay, hypotelorism, and frontal bossing, as observed in our proband. One of them presented with an oral cleft. Interestingly, only in this last patient did the deletion encompass the GSTP1 gene.

Thus, the GSTP1 gene was the only gene deleted in the region that showed some connection with the malformation observed in our patient.

DISCUSSION

Microarrays are important molecular tools to uncover copy number variations associated with multiple congenital anomaly syndromes. A microdeletion/microduplication may contain a gene that could be directly related to a specific malformation, revealing important information in order to elucidate the genetic factors of complex disorders, such as isolated congenital anomalies.

The patient described here presented with a major gastrointestinal malformation, developmental delay, and facial dysmorphism, associated with a 11q13.1q13.2 microdeletion.

In this chromosomal region, the GSTP1 gene is a potential candidate gene for EA. The esophagus is a foregut-derived structure. The separation into the ventral respiratory and the dorsal esophageal parts occurs at four-week gestation. The mechanism responsible for EA is not well understood (Jacobs et al., 2012).

Toxic products can cause DNA damage and consequently cause malformations during the embryonic period. Numerous substances taken by a pregnant woman are known to cause malformations in the fetus, such as alcohol, smoking, and drugs (Wlodarczyk et al., 2011). The toxic substances consumed by the mother may affect organs exposed to the amniotic fluid. During the first weeks of gestation, the detoxification role of the placenta is not fully developed and, in order to avoid the entrance of toxic products in fetal circulation, these substances are excreted in the amniotic fluid (Rajmakers et al., 2001).

Therefore, the GST family could exert a scavenging effect on the toxic substances that escape the placental barrier. Rajmakers et al. (2001) showed that, among the different GST isoforms, the GSTP1 one shows the higher expression in the embryonic and fetal tissues, especially the tissues exposed directly to the amniotic fluid such as the esophagus.

Animal models of Gstp1/2 knockout mice fail to show a higher prevalence of birth defects when compared to wild type (Henderson et al., 1998). However, the Gstp1/2 knockout mice are generally used as a model of adult response to xenobiotics in studies of tumorigenesis or drug metabolism (Henderson and Wolf, 2011). In these studies, the function of the mouse gene is very similar to the human ortholog GSTP1. The loss-of-function of Gstp1/2 enhances the DNA damage leading to a higher risk of developing skin and lung cancer. It is also involved in the inflammatory response and damage caused by tobacco smoke (Henderson and Wolf, 2011). A brief review of the literature did not show any study of these knockout mice exposed to xenobiotics during the embryonic life. Therefore, no inference can be made about the possible birth defects related to a low activity of Gstp1/2.

Polymorphisms of the GST genes, causing a lower enzymatic activity, have been implicated as one of the genetic factors predisposing to the development of complex disorders. Specifically, the GSTM1 null alleles leading to reduced catalytic activity and inefficient detoxification in tissues exposed to the amniotic fluid could be a factor altering proliferation/apoptotic pattern taking place during the gut–trachea separation (Filonzi et al., 2010). Interestingly, in the same study, the GSTP1 polymorphism I105V, known to have a lower enzymatic activity, did not show a positive association with EA.

One may hypothesize that in the study of Filonzi et al. (2010) the enzymatic level of GSTP1 did not reach the threshold required to cause the malformation. In our patient, the mother used alcohol and tobacco during the first 2 months of her pregnancy. The presence of only one copy of the GSTP1 gene could reduce the activity to such a level that the detoxification of these products in contact with the esophagus is impaired, causing the atresia. Therefore, we favor the hypothesis that the deletion of one GSTP1 allele, namely the isoform that has a higher expression in embryonic tissues, associated with specific environmental factors, such as tobacco and alcohol, could cause EA.

Of the three patients presenting with overlapping deletions, only the patient with a cleft palate had a deletion of the GSTP1 gene. This could be considered as further evidence for the disruptive role of the GSTP1 gene in organs exposed to the amniotic fluid. We could speculate that the exposure to xenobiotics during the embryonic period could lead to oral cleft or EA.

A weakness of the present study is that we did not have the opportunity to genotype the isoforms, both in the patient and his mother. The GSTP1 I105V polymorphism occurs in 38% of individuals in specific populations. If present in our proband or his mother, it would result in a much lower activity of GSTP1, enhancing the possibility of DNA damage and foregut malformation. Moreover, the fact that the null alleles of the GSTM1 and GSTT1 genes are frequent in different populations (56% and 18%, respectively), including the Caucasians as our proband, we cannot rule out that our patient and/or his mother also might carry one or both of these genotypes. This in turn would amplify the toxic effects of the xenobiotics (Shi et al., 2007).

This is the first clinical report associating the deletion of one allele of the GSTP1 gene with EA. Further studies of microdeletions involving this specific gene will be important to confirm this association.

REFERENCES


