

A Cluster of Sulfatase Genes on Xp22.3: Mutations in Chondrodysplasia Punctata (CDPX) and Implications for Warfarin Embryopathy

Chondrodysplasia punctata (CDP) refers to a group of skeletal dysplasias characterized by abnormal calcium deposition in regions of enchondral bone formation. This results in the "stippling" of epiphyses, which tends to disappear within the first few years of life. One type of chondrodysplasia punctata is X-linked recessive (CDPX). It is characterized by aberrant bone mineralization, severe underdevelopment of nasal cartilage, and distal phalangeal hypoplasia. The authors demonstrated that some of these patients have an inherited deficiency of a novel sulfatase (arylsulfatase E, or ARSE). However, not all patients with the clinical syndrome have this defect. Other patients have a recessive form of CDP. CDPX shows remarkable phenotypic similarities to 2 well-characterized disease entities involving vitamin K metabolism: warfarin embryopathy and a congenital metabolic error of vitamin K epoxide reductase deficiency. Warfarin embryopathy is caused by the administration of warfarin, an anticoagulant drug, during a critical period of pregnancy: the sixth through ninth weeks. The vitamin K epoxide reductase deficiency disease, also known as pseudowarfarin embryopathy, is a rare autosomal recessive disorder affecting the recycling of vitamin K. By extensively analyzing DNA from overlapping yeast artificial chromosome clones that spanned the critical Xp22.3 region, Franco et al identified 3 adjacent genes that encoded previously unrecognized sulfatase enzymes. Because of predicted structural similarities to arylsulfatases A, B, and C, the novel sulfatase genes were named ARSD, ARSE, and ARSF. The authors concluded that mutations of the ARSE gene account for many cases of CDPX and that the phenotype results from reduced ARSE enzyme activity. Warfarin probably produces a CDPX-like syndrome because it inhibits ARSE activity. The authors demonstrated a significant decrease of ARSE activity and postulated that ARSE activity is

inhibited by warfarin. Patients with CDPX had demonstrably deficient ARSE activity. The ARSE gene is mutated in some cases of CDPX. Intriguingly, the congenital deficiency of vitamin K epoxide reductase, the enzyme recycling vitamin K epoxide to vitamin K, produces an identical picture. The striking similarities among CDPX, warfarin embryopathy, and vitamin K epoxide reductase deficiency phenotypes and the evidence that warfarin inhibits ARSE suggest that these disorders are due to abnormalities in the same metabolic pathway but are of different etiologies.

Franco B, et al. *Cell* 1995;81:15-25.

Editor's comment: *This paper begins to tie together a number of loose ends for biochemists interested in the arylsulfatase family of enzymes, clinicians interested in sorting out the different forms of CPDX and related conditions, and for geneticists interested in the ancestry of the pseudoautosomal region of the X chromosome, which is where not only the gene for ARSE but also the genes for ARSC and ARSD exist. The patients themselves have underdevelopment of nasal cartilage and distal phalangeal hypoplasia, as well as short stature.*

William A. Horton, MD

2nd Editor's comment: *The saying that if it looks like an elephant and walks like an elephant, then it is an elephant may apply to elephants but does not apply to patients with CPD. Drugs obviously can induce enzymatic deficiencies identical to those induced by genetic mutations or the absence of genes.*

Robert M. Blizzard, MD

Trisomy 18, Molecular Studies, Parental Origin and Cell Division in the Extra Chromosome 18 Material

Trisomy 18, or Edwards syndrome, was first described in 1960. It is the second most common autosomal trisomy. Individuals with trisomy 18 present with characteristic facial features, growth retardation, severe mental retardation, clenched hands with

overlapping fingers, and renal and cardiac anomalies. Trisomy 18 has an incidence of 0.18% in all clinically recognized pregnancies and, like other autosomal trisomies, is associated with advanced maternal age. The majority of pregnancies with trisomy 18 abort spontaneously, and only 5% survive to birth. The mean survival after birth is 1 to 3 months, and 95% of those born alive die within the first year of life.

The gene or genes responsible for the trisomy 18 phenotype are not known. While the features of trisomy 18 are most often associated with duplication of the entire chromosome, there are a number of cases in which individuals with a partial duplication of chromosome 18 present with the same or similar features. An effort to identify the regions of chromosome 18 that are critical in producing the phenotype was reported by Boghosian-Sell et al, who analyzed 6 patients with partial duplications of chromosome 18. Fluorescent in situ hybridization with DNA-specific probes to chromosome 18 was used to determine the precise duplication in these patients. The clinical features and the extent of the duplication were compared with 4 previously reported partial trisomy 18 patients. This

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permitted identification of the regions of chromosome 18 that may be responsible for the clinical features of trisomy 18. They concluded that the critical region lies between 18q12.1-18q21.2 and 18q22.3.

The parental origin of the additional chromosome as well as the cell division leading to trisomy 18 are important for understanding the etiology of trisomy 18 (Fisher et al).

Trisomy 18 occurs because of nondisjunction during cell division (Antonarakis). Nondisjunction occurs during meiosis when a homologous pair of chromosomes has failed to separate during the first meiotic division or when the double-stranded chromosome has failed to separate into single-stranded chromatids at the second meiotic division. Nondisjunction also can occur during mitotic somatic cell division. The result of nondisjunction is an abnormal number of chromosomes (aneuploidy) for a specific chromosome. Other trisomies associated with nondisjunction include trisomy 21 and trisomy 13.

The analysis of inherited DNA markers, restriction fragment length polymorphisms (RFLPs), and microsatellite repeat polymorphisms has allowed for the tracking of the parental origin and thereby the identification of the mechanism(s) leading to the additional chromosome 18 in individuals with trisomy 18 (Antonarakis; Sherman). In the majority of cases, the additional chromosome is maternal in origin and occurs during the second meiotic division. Recently, Fisher et al documented that in 63 cases of trisomy 18, the maternal chromosome was duplicated in 61. Both paternal cases were attributable to a postzygotic mitotic error. Of 54 maternal cases identifiable for testing, 16 were attributable to an error in the first meiotic division, 35 were due to a second meiotic error, and 3 were the result of a postzygotic mitotic error. Of the cases due to first meiotic

error, one third lacked recombination, which apparently made them prone to nondisjunction. All maternal errors were associated with advanced maternal age; however, only the examples of nondisjunction in second meiosis were calculated to be statistically significantly increased because of maternal age.

Antonarakis SE. *N Engl J Med* 1991;324:872-876.

Boghossian-Sell L, et al. *Am J Hum Genet* 1994;55:476-483.

Fisher JM, et al. *Am J Hum Genet* 1995;56:669-675.

Editor's comment: *New molecular techniques allow the tracking of genes and chromosomes in such a way as to give important clues to the mechanisms causing disease. In the case of trisomy 18, just as in Down syndrome, only part of the chromosome seems to produce the abnormal phenotype seen when present in triplicate. Probably lots of the chromosome 18 is either active or not important because only a few bands on the long arm seem to be required. Since the area of the chromosome producing the phenotype has been narrowed, it seems likely to expect that the specific gene(s) will soon be identified. The DNA markers also allow determination that chromosomal errors can occur at many different times. In the case of trisomy 18, maternal second meiosis (while the egg sits waiting to ovulate) seems to be the most vulnerable time for things to go wrong. However, if the chromosomes have not undergone recombination (crossover), they may malsegregate during meiosis I. At this point in time, it is hard to predict how these errors can be prevented, but it is important to know when they occur.*

Judith G. Hall, MD

Thanatophoric Dysplasia (Types I and II) Caused by Distinct Mutations in Fibroblast Growth Factor Receptor 3

Achondroplasia (ACH) is the most common of the chondrodysplasias. Thanatophoric dysplasia (TD) is the most common of the neonatal lethal skeletal dysplasias. Homozygous ACH and TD are comparably lethal. The clinical and radiographic features of the ACH and TD entities are similar except that heterozygous ACH is less severe. Classic features of both syndromes include micromelic shortening of the limbs; relative macrocephaly with frontal bossing; reduced height of the vertebral bodies; poor cellular proliferation and column formation in the cartilaginous growth plates of the long bones; and shortened ribs, resulting in a reduced thoracic cavity and a bell-shaped abdomen.

Based primarily upon specific radiologic differences, newborns with TD have been classified as having either type I or type II. Those with TD type I have curved, short (telephone receiver-shaped) femora with or without cloverleaf skull deformity. Those with type II TD have relatively longer and straighter femora and the cloverleaf skull deformity is constant.

When mutations of the fibroblast growth factor receptor 3 (*FGFR3*) gene were identified in ACH, the search was on for *FGFR3* mutations in TD. The highest levels of expression of *FGFR3* are in the cartilage growth plates and central nervous system; lower levels of expression are seen in the lung, intestine,

and kidney. Because of the striking phenotypic similarities between homozygous ACH and TD and because of recent evidence demonstrating an important role for FGFRs in skeletal development, the investigators extensively analyzed *FGFR3* in individuals with TD to determine if mutations in this gene cause one or more forms of this severe skeletal dysplasia. In the present paper, 22 out of 39 TD type I patients harbored amino acid substitutions in the *extracellular* domain of *FGFR3* at codon 248. In contrast, a heterozygous mutation of codon 650 in all 16 cases of TD type II was found. All of these had a lysine in the intracellular tyrosine kinase domain of the receptor replaced by glutamic acid. None of the TD mutations were identified in normal individuals. Moreover, no mutations were detected in parental DNA from 3 sets of parents tested (1 set from a TD type I patient and 2 sets from TD type II patients). This demonstrates the sporadic nature of the mutations.

Tavormina PL, et al. *Nature Genet* 1995;9:321-328.

Editor's comment: *This paper settles several long-standing debates regarding TD and ACH. FGFR3 is involved in both instances. Different heterozygous mutations are responsible at the FGFR3 locus to produce TD types I and II and ACH. An*