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Invited Editorial

Priming the Search for *HOX* Mutations

The article by Kosaki et al. (p. 50) illustrates the power of the ever-expanding DNA sequence databases in greatly accelerating the development of tools for genomic mutation discovery. In this case the authors have developed coding sequence PCR primer sets for a large, highly conserved group of transcription factors, the *HOX* genes, whose full mutation spectrum in humans has not been elucidated. Due to their broad and important biological roles, it is anticipated that the availability of these sets will facilitate the identification of mutations or polymorphisms in *HOX* genes in a wide variety of phenotypes.

HOX gene mutations in humans associated with specific defects have been described for only three of the 39 known genes, *HOXD13*, *HOXA13*, and *HOXA11*. Except for the newly described mutation in human *HOXA11*, this subject has been reviewed (Innis, '97; Veraksa et al., '00; Goodman and Scambler, '01). All of these human syndromes are associated with heterozygous *HOX* mutations that are inherited in an autosomal dominant pattern with almost complete penetrance. Two spontaneous coding sequence *Hox* mouse mutants have been identified (Mortlock et al., '96; Johnson et al., '98), and many of the *Hox* genes have been "knocked out" in mice via homologous recombination in embryonic stem cells. Mice carrying heterozygous, engineered null mutations for these genes often exhibit incomplete penetrance, which has been attributed in large part to functional overlap of HOX proteins to the growth and allocation of mesenchyme (Davis and Capecchi, '96; Fromental-Ramain et al., '96; Zakany et al., '97; Greer et al., '00). Stochastic variables and background genetic effects may also play a role and phenotypic differences with humans may reflect the nature of the mutations or variation between species. A brief glance at the human conditions caused by these mutations, as well as the available mouse models illustrates the future of *HOX* mutation searches.

HOXD13 mutations in synpolydactyly involve an expansion of an endogenous homopolymeric alanine repeat with increasing severity related to increased expansion length, and mice heterozygous for *Hoxd13* alanine expansions are very similar (Muragaki et al., '96; Akarsu et al., '96; Goodman et al., '97; Johnson et al., '98). A different, and distinct, limb malformation occurs with human intragenic *HOXD13* deletions, suggesting that HOXD13 alanine expansions act through a gain-of-function (Goodman et al., '98). *Hoxd13* engineered null mice exhibit malformations distinct from human intragenic *HOXD13* deletion, suggesting that the roles of the genes in mice may differ in comparison to humans (Dolle et al., '93; Davis and Capecchi, '96). It is also possible that differences in this comparison result from some remaining function of mutant human HOXD13 proteins or unforeseen experimental consequences of engineered null alleles in mice.

Sporadic or familial point mutations and alanine tract expansion of *HOXA13* have been reported in hand-foot-genital syndrome (Mortlock and Innis, '97; Goodman et al., '00). A constitutional deletion of 7p14-p15 causing a loss of the entire *HOXA* cluster including *HOXA13* results in a phenotype similar to hand-foot-genital syndrome in addition to other anomalies (Devriendt et al., '99). Therefore, except for a patient with a missense mutation in the homeodomain, which may lead to a gain-of-function (Goodman et al., 2000), haploinsufficiency of *HOXA13* function is sufficient to observe hand-foot-genital syndrome. Similar defects to human *HOXA13* haploinsufficiency were observed in the mouse mutant *Hypodactyly*, however, this mutation is a simultaneous loss and gain of function and is more severe than the engineered *Hoxa13* knockout (Mortlock et al., '96; Fromental-Ramain et al., '96; Post and Innis, '99; Post et al., '00).

A unique combination of radio-ulnar synostosis and amegakaryocytic thrombocytopenia has been reported for heterozygous *HOXA11* homeodomain mutations in humans (Thompson and Nguyen, '00). Whether or not there are other skeletal manifestations in these patients remains to be determined, however, mice with engineered null alleles of *Hoxa11* have a dissimilar skeletal phenotype and have not been reported to have thrombocytopenia (Small and Potter, '93). These data suggest that the human mutation may not be simply a null allele or that the function of *HOXA11* in humans may be different compared to mice.

How does this information impact human *HOX* mutation search strategies and interpretation? First,

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model system phenotypes are extremely valuable in predicting domains of effect, if not identical human malformations, associated with *HOX* gene mutations and in guiding gene searches. Those searches may also be guided by model system Hox expression data, e.g., available at the Mouse Genome Informatics database at The Jackson Laboratory, and allow investigators to actively consider alternative sites of malformation or alteration in physiological function. On the other hand, the phenotypic differences suggest caution in drawing conclusions about the genetic basis of a phenotype or in the prediction of a phenotype from a specific mutation. Humans appear to exhibit greater phenotypic effects and penetrance for presumed null mutations than do mice, however, we cannot exclude a bias of ascertainment in the cases that have so far been reported. If a substantial fraction of mice show no evident abnormalities when carrying heterozygous null mutations, then it seems quite possible that a fraction of the human population may also. In this case, some *HOX* loss of function mutations could be overlooked potentially leading to over-representation by gain-of-function single gene *HOX* mutation discoveries in the future. Given the degree of functional redundancy among HOX proteins in endogenous, overlapping domains as demonstrated in mouse models (Greer et al., '01), it will now be easier to screen multiple genes within paralogous groups in cases of malformations suggestive of *HOX* mutations. Although all human *HOX* gene mutations described so far affect the coding sequence, failure to identify a coding sequence alteration in suspect cases should prompt investigators to examine for larger rearrangements, as well as to sequence promoters, introns, and untranslated regions.

The *HOX* PCR primer sets will also be useful for identifying the full population spectra of HOX protein polymorphisms. Efforts to determine the relationship of any polymorphism to malformation parallel international efforts to associate SNP combinations with common disease risk. The contribution of environment to risk of individuals with certain Hox polymorphisms, or even clearly deleterious mutations, must also be examined, especially in light of recent findings relative to valproic acid, hyperthermia and diabetes in animal models (Jacobs et al., '98; Li and Shiota, '99; Faiella et al., '00). In addition, the utility of this comprehensive primer set extends beyond causes of malformations. Many Hox genes are expressed in adult organ systems including blood, genitourinary tract, gut, kidney and skin and their roles in these organ systems are not yet known. A broader role in leukemia and solid tumors in being investigated (Cillo et al., '99), and a disruption of hindbrain patterning via *HOX* mutation is an attractive hypothesis, although unproved, for autism spectrum disorders (Ingram et al., '00).

The absence of detectable *HOX* coding mutations in patients with highly suggestive patterns of defects could result from chromosomal (visible cytogenetically or submicroscopic) deletion. This was evident in the findings by Devriendt et al. ('99) who reported the occurrence of hand-foot-genital syndrome, velopharyngeal insufficiency and persistent patent ductus Botalli in a patient with a chromosomal deletion involving the entire *HOXA* cluster, and in the patient reported by Del Campo et al. ('99) with a *HOXD* cluster deletion, monodactylous limbs and abnormal genitalia. All patients should have high-quality karyotypes supplemented if possible with FISH or genomic marker data to define deletion endpoints. Such deletions or translocations, though rare, may offer valuable insight into the regulation of the *HOX* genes in various organ systems.

Other than coding mutations and large chromosomal rearrangements, mutations within promoters, enhancers or insulators could exert major phenotypic effects by changing the level, domain or timing of *HOX* expression in the embryo. HOX proteins ectopically expressed in regions more anterior or proximal than usual generally exert functional dominance over other *HOX* gene products, giving rise to malformations or "posterior" transformations of axial or limb structures (Duboule and Morata, '94). This interesting aspect of Hox function noted initially in *Drosophila* (termed phenotypic suppression), and subsequently in mice (posterior prevalence), is poorly understood at the molecular level. The mouse *Ulnaless* mutant is a well-known example of a vertebrate Hox regulatory mutation that alters the expression level in the autopod, as well as the domain for *Hoxd13* and *Hoxd12* to more anterior regions of the developing limb resulting in reduction deficiency (Peichel et al., '97; Herault et al., '97). Its molecular identity remains elusive even though it is tightly linked to the *Hoxd* cluster. Most likely conserved in humans, the site of disruption in *Ulnaless* would be a logical place to explore for mutations in humans with various segmental deficiencies, or perhaps polydactyly. Identification and characterization of enhancer and insulator elements in model systems would facilitate identification of such regulatory mutations in humans (Kmita et al., '00; Spitz et al., '01). In the context of HOX gene regulation, the influence of the *Polycomb* and *Trithorax* genes, as well as HOX cofactors, should also be considered in the evaluation of phenotype (see reviews Schumacher and Magnuson, '97; Mann and Affolter, '98; Veraksa et al., '00).

In summary, teratologists, physicians and molecular geneticists have long been cognizant of the likely existence of *HOX* mutations in the pathology of malformations. Given that mutations have been identified in three *HOX* genes, we look with anticipation to the others. The tools provided by Kosaki et al. bring us much closer to finding and learning more about the roles of HOX proteins in humans.

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