

# Genetic Modifier(s) of Embryonic Lethality in Type IIA Procollagen Deficient Mice

□□ Tang<sup>1</sup>, SYY Wong<sup>1</sup>, AWL Leung<sup>1</sup>, PC Sham<sup>2</sup>, KSE Cheah<sup>1</sup>, YQ Song<sup>1\*</sup>

1. Department of Biochemistry, University of Hong Kong, Hong Kong SAR, China

2. Department of Psychiatry, University of Hong Kong, Hong Kong SAR, China

\*email: songy@hku.hk

## Discovery of Genetic Modifier(s) in Type IIA Procollagen Deficient Mice

*Col2a1* encodes type II procollagen, the major cartilage matrix protein. During embryogenesis *Col2a1* is differentially transcribed to give IIA mRNA containing an exon (exon 2) and IIB mRNA, which lacks this exon. We produced IIA procollagen deficient mice by deleting exon 2 of *Col2a1* and observed that mice homozygous for the IIA null mutation (*IIA*<sup>-/-</sup>) display complex congenital malformations, ranging from heart malformations and head truncation, resulting in prenatal lethality to near normal phenotype, depending on the strain background of the mice. Upon backcrossing (6 generations) to C57BL background an increasing proportion of the *IIA*<sup>-/-</sup> mice die in the prenatal period with complex congenital malformations, e.g. heart defects. These observations led us to hypothesize that the phenotypic variability in *IIA*<sup>-/-</sup> mice were related to differences in the genetic background and therefore the complement of one or more modifying loci.

## Characterization of mode of inheritance of the modifier(s)

Three congenic mouse lines backcrossed over 7 generations to different genetic backgrounds (129/sv; C57BL; ICR) are therefore established to characterize the inheritance model of the modifier(s) and hence identify its physical location in the genome. Our segregation analysis shows that there is a significantly underrepresentation of *IIA*<sup>-/-</sup> offspring from a C57BL *IIA*<sup>+/-</sup> intercross (Chi Square test: p-value=0.0137, df=2), however, the ratio appears to be normal for offspring from 129/sv *IIA*<sup>+/-</sup> and ICR *IIA*<sup>+/-</sup> intercross. This data would be consistent with an inheritance model with one or a set of recessive modifier gene(s) in the C57BL mice interacting with the *IIA*<sup>-/-</sup> genotype and results in prenatal lethality.

## Mapping of physical location of the modifier(s)

Having understood the characteristics of the modifier(s), we designed a mapping panel to identify its physical location by first crossing ICR *IIA*<sup>+/-</sup> x C57BL *IIA*<sup>+/-</sup> to obtain F1. Taking the advantage of the recessive and lethal behavior of the modifier, genotype of the surviving *IIA*<sup>-/-</sup> F1 would be limited to heterozygous at the modifier locus, intercrossing the *IIA*<sup>-/-</sup> F1 would generate a *IIA*<sup>-/-</sup> F2 population with segregating modifier alleles. Mice in F2 with both recessive alleles would be expected to have died before birth. Therefore, markers, and hence candidate regions, would be significantly deviated from the expected Mendelian ratio. Using this mapping strategy (illustrated in Figure 1), we genotyped 135 markers from the whole genome for 10 families (with 17 F1 and 175). Two markers on chromosome 7 and 8 show significant association with the lethality phenotype with

p-value =  $2.95e-013$  and 0.03 respectively in a binomial exact test. Fine mapping is being carried out for the identification of candidate genes in these two regions.

### **Towards understanding the dynamics in development**

Type IIA procollagen deficient mice is a very good genetic resources for study of dynamic regulatory mechanism in development. Identification of modifier genes would provide insight into genes that underlie diverse developmental and physiological processes, and enhance our understanding of individual risk and variation in response to environmental factors. By combining familial linkage analysis, gene expression profiling, and computer methodologies, we are going to identify a novel dynamic regulatory network in early development.