Heterogeneity of genetic modifiers ensures normal cardiac development

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SUPPLEMENTAL MATERIALS

Supplemental methods

Segregation analyses

Two segregation models are examined by comparison of their predicted and observed incidences of a defect in the F1 and F2 crosses. Statistically significant deviation from the model was determined in a χ^2-test by $P < 0.05$.

The segregation analysis models assume that a defect may occur in animals with the *Nkx2-5*$^{+/-}$ genotype in combination with homozygosity at one or more modifier loci. Model M1 postulates two loci *A* and *B*. C57Bl/6 and FVB/N carry the recessive, susceptibility alleles A^- and B^-, respectively. The penetrance of a defect in an *Nkx2-5*$^{+/-}$ animal are p_A for the $A^{-/-}B^+$ genotype, p_B for $A^+B^{-/-}$, and p_{AB} for $A^{-/-}B^{-/-}$. If no epistatic interaction or linkage exists between *A* and *B*, then the penetrance p_{AB} for the double homozygote $A^{-/-}B^{-/-}$ equals the sum of p_A and p_B. Assuming no epistatic interaction in this model, p_A and p_B are estimated from the incidences in the C57Bl/6 strain and the F2 backcross to FVB/N. The expected incidences in the F2 intercross and backcross to C57Bl/6 are calculated from equations derived from the model (Supplemental Table). Inclusion in the model of additional loci does not alter its predictions because the total effect of susceptibility alleles from one strain reduces to a single term; for example, given *n* loci at which C57Bl/6 carries the susceptibility alleles, $p_A = \sum p_{A,n}$.

Model M2 postulates multiple loci, two of which interact. Here C57Bl/6 carries the recessive susceptibility alleles at two modifier loci *A* and *B*. FVB/N carries the recessive susceptibility allele at a third locus *C*. As in model M1, the penetrances of the single homozygotes are p_A, p_B, and p_C. The penetrance of the double homozygote $A^{-/-}B^{-/-}$ is

p_{AB}. The other double and triple homozygote combinations are ignored for simplicity. If p_A and p_B are negligible compared to p_{AB}, implying a synergistic interaction between *A* and *B*, then p_{AB} would approximate the incidence of the defect in C57Bl/6. The incidences attributed to the $A^{-/-}B^{-/-}$ and $C^{-/-}$ genotypes in the F2 backcross to C57Bl/6 and intercross are derived as shown (Supplemental Table).

Supplemental Table

Cross	**Model**	
	M1	M2
C57Bl/6	p_A	F: $p_A + p_B + p_{AB}$ S: p_{AB}
F1	0	0
Intercross	F: 1/16 x ($3p_A + 3p_B + 4p_{AB}$) S: 1/4 x ($p_A + p_B$)	F: 1/16 x ($3p_A + 3p_B + p_{AB} + 4p_C$) S: 1/16 x ($p_{AB} + 4p_C$)
C57Bl/6 backcross	½ x p_A	F: 1/4 x ($p_A + p_B + p_{AB}$) S: ¼ x p_{AB}
FVB/N backcross	½ x p_B	1/2 x p_C

The expected incidence of a defect in a particular cross is defined by a set of equations given by the segregation model. The penetrance terms p_A, p_B, p_C, and p_{AB} are estimated from the incidences in the C57Bl/6 strain or FVB/N backcross, depending upon the model and term. F, full equation in which all terms described in the model are stated. S, simplified equation in which assumptions regarding the interaction term p_{AB} are made, as explained in the Supplemental Methods.