Right Ventricular Hypertrophy Secondary to Pulmonary Hypertension Is Linked to Rat Chromosome 17 Evaluation of Cardiac Ryanodine Ryr2 Receptor as a Candidate

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Background—Fischer 344 (F344) rats are relatively resistant to hypoxia-induced right ventricular (RV) hypertrophy compared with the Wistar-Kyoto (WKY) strain. These 2 strains were used to examine the genetic basis for the differential response.

Methods and Results—Male F2 offspring from an F344 × WKY intercross were exposed to hypoxia (10% O2) for 3 weeks, and pulmonary artery pressure and cardiac chamber weights were measured. Genomic DNA was screened by use of polymorphic microsatellite markers across the whole genome (excluding the sex chromosomes). A quantitative trait locus (QTL) for RV weight was identified on rat chromosome 17 (lod score 6.5) that accounted for 22% of the total variance of RV weight in the F2 population and was independent of pulmonary artery pressure. The peak was centered over marker D17Rat41, close to Chrm3, with a 1-lod support interval of 5 cM. Comparison of homologous regions in mice and humans suggested that Ryr2, the cardiac isoform of the ryanodine receptor, colocalizes with our QTL. A panel of somatic cell hybrids and fluorescence in situ hybridization mapped Ryr2 close to the gene Chrm3 within our QTL. [3H]Ryanodine binding to cardiac membranes from the parental strains showed a 21% reduction in Bmax in the WKY compared with the F344 strain, with no difference in Kad.

Conclusions—These data provide the first demonstration of a QTL linked to the RV response to hypoxia-induced pulmonary hypertension. The Ryr2 receptor gene lies within this QTL and merits further investigation as a candidate for this differential RV response. (Circulation. 2001;103:442-447.)

Key Words: genetics ▪ hypertrophy ▪ hypoxia

The normal adult pulmonary circulation is a low-pressure, low-resistance system with little or no resting vascular tone. The thin walls of pulmonary vessels and the relatively thin-walled right ventricle (RV) (compared with the left ventricle [LV]) reflect this low hemodynamic stress. Pulmonary artery pressure is increased by chronic hypoxia (eg, in patients with obstructive airway disease or people living at high altitude), and this leads to RV hypertrophy and premature death.1,2

The initial rise in pulmonary artery pressure during exposure to hypoxia is due to vasoconstriction. Chronic hypoxia leads to structural remodeling of pulmonary vessels and RV hypertrophy, features that are common to a number of species, including the rat.3 A number of pathological abnormalities have been described in the pressure-overloaded hypertrophied RV, which can impair myocardial hypertrophy, including polymerization of microtubules in cardiac myocytes4 and stimulation of interstitial collagen deposition.5 However, the biochemical processes involved in the hypertrophic myocardial response to pressure overload remain unclear.

In the present study, we report the differential susceptibility of the heart to pressure overload of 2 inbred strains of rats, the development of which has allowed us to perform a genetic study that implicates the cardiac ryanodine Ryr2 receptor in the hypertrophic response of the RV to hypoxia-induced pulmonary hypertension.

Methods

Animals and Genetic Crosses

Inbred Wistar-Kyoto rats (WKY, Charles River, UK) and Fischer 344 (F344) rats (Olac, UK) were used. Reciprocal mating of the...
TABLE 1. Phenotypic Differences Between F344 and WKY Strains

<table>
<thead>
<tr>
<th>Strains</th>
<th>Condition</th>
<th>BW, g</th>
<th>PAP, mm Hg</th>
<th>BP, mm Hg</th>
<th>RV, mg</th>
<th>LV, mg</th>
<th>RV/LV</th>
<th>Hct, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F344 (n=12)</td>
<td>Normal air</td>
<td>290 (240–321)</td>
<td>16 (14–17)</td>
<td>121 (107–130)</td>
<td>139 (115–145)</td>
<td>536 (458–559)</td>
<td>0.25 (0.24–0.27)</td>
<td>46 (45–47)</td>
</tr>
<tr>
<td>F344 (n=23)</td>
<td>Hypoxia</td>
<td>238* (210–260)</td>
<td>27* (25–28)</td>
<td>112 (102–133)</td>
<td>207* (194–225)</td>
<td>533 (491–555)</td>
<td>0.41* (0.36–0.44)</td>
<td>70* (69–71)</td>
</tr>
<tr>
<td>WKY (n=12)</td>
<td>Normal air</td>
<td>250 (248–310)</td>
<td>15 (14–15)</td>
<td>91† (89–106)</td>
<td>179† (163–217)</td>
<td>599 (532–678)</td>
<td>0.31† (0.28–0.34)</td>
<td>45 (44–46)</td>
</tr>
<tr>
<td>WKY (n=28)</td>
<td>Hypoxia</td>
<td>270 (255–290)</td>
<td>38† (36–39)</td>
<td>78*† (70–89)</td>
<td>417*† (383–460)</td>
<td>765*† (714–796)</td>
<td>0.52*† (0.51–0.59)</td>
<td>71* (69–71)</td>
</tr>
</tbody>
</table>

Data are median (interquartile range). BW indicates body weight; PAP, pulmonary artery pressure; BP, systemic blood pressure; and Hct, hematocrit.

For hypoxia, *P<0.01 vs normal-air group within same strain; †P<0.01 vs F344 normal air group; and ‡ P<0.01 for hypoxic WKY vs hypoxic F344.

Results

Hypoxia-Induced Pulmonary Hypertension and RV Hypertrophy

Adult WKY and F344 rats have similar pulmonary artery pressures at rest when breathing air, but WKY have a lower systemic blood pressure and a greater cardiac mass (Table 1). Exposure to hypoxia (Fio 2 10%) for 21 days was associated with an increase in mean pulmonary artery pressure and RV weight in both strains but significantly more in the WKY strain. LV mass also increased more in the WKY strain despite a further fall in systemic blood pressure (Table 1). However, the LV measurement included the septum, which hypertrophies with the RV. Histology of the hypertrophied ventricles shows that the main cellular change is myocyte hypertrophy rather than proliferation of nonmyocyte cells.
excessive collagen deposition (data not shown). The rise in hematocrit was the same in the 2 strains, making it unlikely that the strain difference is due to variation in oxygen sensing. We examined the following phenotypes in a WKY F344 intercross: (1) RV weight, (2) RV/body weight ratio, (3) RV weight/LV weight ratio, (4) LV weight alone, and (5) pulmonary artery pressure. Because the septum hypertrophies as part of the RV response, expressing RV as a ratio of LV+septum weight reduced the interstrain differences in RV hypertrophy. For these reasons and because the animals were all studied at the same age, we focused on uncorrected RV weight.

F1 (n=27) and male F2 (n=130) animals from a WKY×F344 intercross showed an intermediate rise in pulmonary artery pressure and RV mass compared with the parental strains (Figure 1). The average of the phenotypic variances of the 3 nonsegregating generations (WKY, F344, and F1) was used to estimate the environmental variance of RV weight. The proportion of the total F2 variance that was due to genetic variation, expressed as heritability, was calculated according to the following formula: (total F2 variance−environmental variance)×100/total F2 variance; the result was found to be 64%. Comparison of the variances in the segregating generations with those in the nonsegregating generations indicated the involvement of at least 3 or 4 genes in the interstrain difference of the hypertrophic response of the RV.

**Linkage of RV Weight to Rat Chromosome 17 QTL**

A genetic linkage map for each of the 20 rat autosomes was constructed with genotypic data from the male F2 rats and a panel of 157 polymorphic microsatellite markers (the X and Y chromosomes were not screened). The best map order for the markers was determined by multipoint linkage analysis, and the genetic distances between markers were calculated by using the Haldane mapping function. We estimate that our markers cover 99% of the rat autosomes on the basis of the recent reevaluation of the genome size, with an average intermarker distance of 20 cM.

A genetic linkage map for rat chromosome 17 was generated with the data from 15 markers for all 130 F2 progeny. Using the Mapmaker/QTL program, we found a QTL on rat chromosome 17 that had a major affect on RV weight (Figure 2). This QTL was supported by a lod score of 6.5, accounted for 22% of the total variance of RV weight in the F2 population, and was independent of pulmonary artery pressure (lod score 1.6). The peak was centered over the marker D17rat41, close to Chrm3, with a 1-lod support interval of 5 cM. The linkage persisted when RV weight was expressed as a ratio of body weight (lod score 4.5) to LV+septum weight (lod score 3.7).

The results of 1-way ANOVA of the cosegregation of RV weight with D17Rat41 are shown in Table 2. This locus appears to affect RV weight in a dominant fashion, inasmuch as the F2 animals homozygous for the WKY (WW) or F344 (FF) allele had the largest and smallest mean RV weights,
respectively, and the mean values for the heterozygotes (WF) were indistinguishable from those of the homozygous F344 group. Rats homozygous for the WKY allele at the marker D17Rat41 have a RV weight $52.4$ mg greater than those homozygous for the F344 allele ($P < 0.001$).

No other QTLs for RV weight and no QTLs for LV weight or pulmonary artery pressure were identified in this cross that met our stringent criteria (see Linkage and Statistical Analysis) (Figure 3). Even though the maximal distance between markers was never $>31$ cM, it is possible that we may have missed genetic factors with an autosomal-recessive or sex-linked pattern of inheritance or autosomal-dominant loci of small effect.

**Localization of Ryr2 to Rat Chromosome 17**

Inspection of the mouse and human genetic maps homologous to our QTL on rat chromosome 17 suggested that Ryr2, the gene encoding the cardiac form of the ryanodine receptor, is in the region of interest. The rat Ryr2 gene was first assigned to this rat chromosome by using a panel of 15 (rat×mouse) somatic cell hybrids segregating rat chromosomes. A 0.76-kb rat Ryr2 cDNA probe detected 6 fragments when rat genomic DNA was digested with the restriction enzyme PstI (5.0, 4.2, 3.5, 3.0, 2.6, and 1.7 kb) (data not shown). The 2 most intense bands (5.0 and 3.5 kb), as well as the 2.6- and 1.7-kb bands, were easily distinguishable from the bands obtained with digested mouse genomic DNA (8.9, 8.1, 5.8, 4.2, 4.0, and 2.8 kb). The 4 unique rat fragments segregated together with rat chromosome 17 (data not shown), as did other genes previously assigned to this chromosome, eg, Chrm3 (17q12.1) and Tpl2 (17q12.1-q12.2). To confirm the position of Ryr2 within rat chromosome 17, FISH was performed with the use of a biotin-labeled 289-bp rat Ryr2 cDNA probe. Double spots (2 labeled sister chromatids) were found only on rat chromosome 17 (Figure 4). The fluorescent signals were located in the distal half of 17q, namely, in the region 17q12.2-q12.3. Because the human homologues CHRM3, RYR2, and TPL2 are separated (CHRM3 and RYR2 map to human chromosome 1,17,18 whereas TPL2 maps to human chromosome 1019), the most likely order of the rat genes on rat chromosome 17 is as follows: 17qcentr-Chrm3-Ryr2-Tpl2-17qtel.

**Ryanodine Binding Studies**

To explore the role of Ryr2 in our rat cross, we looked for evidence of a difference in functional expression of this channel in the 2 strains. Ryr2 is the predominant form of the ryanodine receptor in myocardium. Therefore, we performed ligand-binding studies with the use of [3H]ryanodine and homogenates of the RV containing sarcoplasmic reticular...
membranes. There was a significant 21% reduction in B_max for ryanodine binding to cardiac membranes from normal WKY compared with F344 animals, with no significant difference in K_d (Figure 5).

**Discussion**

QTLs contributing to the regulation of adult cardiac mass and blood pressure have been reported on several rat chromosomes, including 1, 3, 10, 11, and 17. In these cases, the cardiac mass phenotype may be acting as a surrogate for the pressure load. On the other hand, genetic loci for cardiac mass that are independent of the influence of blood pressure have been reported on several rat chromosomes, including 1, 3, 20, 21, and 8. The latter QTL was close to the Edn1 and Drd1A genes on the short arm of rat chromosome 17 (17p14), at least 40 cM away from the QTL we describe in the present study. It is important to emphasize that none of the above studies provided substantive evidence of functional differences or genetic sequence variation implicating a particular gene in the control of cardiac mass.

Genetically determined variation in the cardiovascular response to hypoxia is well documented in animals and humans, but few attempts have been made to elucidate the genetic basis of the differential response to hypoxia. In the only study published to date, a locus on rat chromosome 1 cosegregates with RV weight and pulmonary artery systolic pressure in a (fawn-hooded × F344)F1 × fawn-hooded backcross. We did not detect a significant QTL on rat chromosome 1 in the present study.

Several lines of evidence make an abnormality of Ryr2 receptor activity an attractive candidate for the differential hypertrophic response observed between the WKY and F344 strains. This receptor plays an essential role in excitation-contraction coupling by releasing Ca^{2+} ions from the sarcoplasmic reticulum after stimulation by calcium ions entering through the dihydropyridine receptor. Furthermore, perturbations of Ryr2 function have already been linked with myocardial hypertrophy. In pressure overload–induced cardiac hypertrophy in several species, including the rat, there is a reduction of receptor density and Ryr2 mRNA that correlates with a decrease in Ca^{2+} transient. However, it has been unclear whether these changes are primary events or a consequence of the hypertrophic process. In support of the former is the observation that administration of FK-506 (tacrolimus), an immunosuppressant that binds to FKBP12.6, has been associated with the development of hypertrophic cardiomyopathy in children. FKB12.6 itself is known to bind to and modulate channel gating of the Ryr2 receptor. Knockout mice lacking either FKB12 or Ryr2 die during embryogenesis, principally because of disruption of heart tube development.

In conclusion, we have described segregation and linkage data that implicate a gene on rat chromosome 17 in the hypertrophic response of the RV to hypoxia-induced pulmonary hypertension. We have also presented the first evidence that Ryr2 is in the vicinity of the QTL, and we clearly demonstrate a difference between F344 and WKY in the binding of [3H]ryanodine to myocardial homogenates. Significantly, these differences in binding are evident before exposure to hypoxia. It is of considerable interest that our previous study examining the genetic factors controlling normal cardiac mass in these 2 strains identified a QTL on rat chromosome 3 but not on chromosome 17, highlighting the differences between the genetic mechanisms controlling normal cardiac growth and pathological hypertrophy. It is interesting to speculate that the greater cardiac hypertrophy in the WKY strain results from an attempt of cardiac myocytes to compensate for a lower abundance of ryanodine receptors compared with those in the myocardium of F344 rats. If true, mechanisms to improve cardiac Ryr2 activity and increase contractile efficiency may, paradoxically, reduce the stimulus to hypertrophy and the potential to develop cardiac failure.

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References


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