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ORIGINAL ARTICLE

Sex-specific quantitative trait loci linked to autoresuscitation failure in SWR/J mice

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Autoresuscitation (AR) is a highly conserved response among mammals, which allows survival from transient extreme hypoxia. During hypoxia, bradycardia, and hypoxic gasping develop after a brief period of hyperactivity. Normally, AR occurs if oxygen is restored during the gasping period where an initial heart rate increase is rapidly followed resumption or eupneic breathing. Humans and other mammals can survive multiple immediately repeated AR. A defective AR capacity has been implicated in Sudden Infant Death Syndrome. We had reported earlier that inbred strains of mice such as BALB/cJ could survive a characteristic number of immediately repeated AR trials, but that SWR/J mice failed to AR from a single hypoxic episode. We now report that strains closely related to SWR/J, FVB/N and SJL/J exhibit partial resuscitation defects relative to BALB/cJ or other mouse strains, establishing a genetic basis for variation in AR failure. The AR trial phenotype of BALB/cJ × SWR/J intercross F_1 and F_2 mice was consistent with BALB/cJ dominance and a discrete number of loci. Genome-wide mapping conducted with 60 intercross F_2 animals linked two loci to the number of AR trials survived, including one sex-specific locus with male expression, consistent with the observed 50% male bias for Sudden Infant Death Syndrome in humans. A locus carried on SWR/J chromosome 10 seems to be particularly important in AR failure and was confirmed in a partial consomic line. These results establish a genetic basis for AR failure phenotype in mice, with relevance to Sudden Infant Death Syndrome.
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Introduction

Autoresuscitation (AR) is a mechanism that allows mammals to survive transient periods of hypoxia. When animals are deprived of oxygen, they will stop breathing and then exhibit bradycardia and gasping. Normal breathing resumes if air is restored during the gasping period (AR). Evidence suggests that infants who die for no apparent reason during the first year of life (Sudden Infant Death Syndrome; SIDS) often have a defective AR mechanism (Poets *et al*., 1999; Sridhar *et al*., 2003). The SWR/J infant mouse model is believed to be a particularly good animal model for SIDS in that they fail to autoresuscitate after brief exposure to severe hypoxia. It is unclear to what extent variation in resuscitation ability is due to variation in genes or environment.

Mice, like most mammals, can autoresuscitate after exposure to brief severe hypoxia. Most strains of mice can successfully autoresuscitate if re-exposed to hypoxia immediately after the first trial. Repeated hypoxic episodes eventually result in failure to autoresuscitate after several successful trials. An inbred strain of Swiss mice (SWR/J) has a developmental window (19–22-days old) during which they fail to autoresuscitate. Before and after this, AR is effective in producing recovery. As most human SIDS deaths occur during a narrow developmental window, the genetic defects of the SWR mouse model may be relevant to SIDS in humans (Jacobi and Thach, 1989).

SWR/J mice exhibit AR failure whereas other unrelated strains tested autoresuscitate as expected, suggesting a genetic basis to variation in AR. Our hypotheses were (1) Mouse strains closely related to SWR/J will have a similar AR phenotype; (2) that F_1 and F_2 intercross animals between SWR/J and a normal strain (BALB/cJ) will vary in their AR response and that this variation will segregate with discrete regions of the genome (quantitative trait loci; QTLs); (3) BALB/c mice that are consomic for the SWR/J chromosome 10 QTL region, will fail to autoresuscitate after less trials than the recipient BALB/cJ strain.

Materials and methods

The mice were placed in the barometric plethymograph at the start of the experiment. Humidified 97% N_2–3% CO_2 was then flushed in at the onset of hypoxic seizures

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as indicated by rapid twitching of the hind limbs (Jacobi and Thach, 1989). A measure of 30 ml of O_2 was injected into the chamber to give a concentration of 21% for adults and 35 ml for weanlings, with the volumes having been determined earlier to give an O_2 concentration in the chamber of ~21 % (Jacobi and Thach, 1989). All animals were tested between 19 and 22 days of age.

Animals from six different inbred strains of both sexes, NOR/LtJ ($N=13$), MA/MyJ ($N=11$), BALB/cJ (Brunton *et al*., 1979), SJL/J ($N=8$), FVB/NJ ($N=16$), and SWR/J ($N=15$), were tested for the number of ARs survived based on the protocol described above. Three of these strains, SJL/J, FVB/NJ, and SWR/J are closely related to one another whereas the others represent strains spread throughout the remainder of the inbred mouse strain geneology (Festing, 1998). The number of AR trials survived was also determined in a total of 16 F_1 and 60 F_2 animals, 28 from the intercross SWR/J × BALB/cJ and 32 from the reciprocal intercross BALB/cJ × SWR/J, the female partner listed first.

DNA was extracted from the tail clips of the 60 F_2 animals and processed with Qiagen kits (QIAamp DNA kit, Valencia, CA, USA). Ninety-one microsatellite loci, polymorphic between BALB/cJ and SWR/J, were scored. Loci were spaced evenly across the entire genome, at an average interval of 19.3 cM (see Appendix 1). Only 2% of the microsatellite genotypes remained unresolved.

Differences among mouse strains, among the parental, F_1 and F_2 hybrids, and between the chromosome 10 congenic strain and the BALB/cJ recipient strain were evaluated using ANOVA (Sokal and Rohlf, 1995) with the number of AR trials survived as the dependent variable and strain as the independent variable. Overall genetic variation among strains is measured as the ratio of between-strain variance to total phenotypic variance. This is referred to as the broad-sense heritability (H^2; 14). Pairwise strain differences were tested using Tukey's Honestly-Significant-Difference Test (Sokal and Rohlf, 1995). Means and standard errors reported here are the least squares means and associated standard errors produced by the ANOVA model.

The means and variances of the parental, F_1 and F_2 populations were used to estimate the effective number of factors (n_e) responsible for differences between the parental strains (Lynch and Walsh, 1998). The effective number of factors is the number of freely segregating loci, all with the same additive effect, that would result in the same differences in parental means and segregation variance (difference between F_1 and F_2 trait variances) as observed here. It is usually an underestimate of the true number of loci responsible for parental strain differences.

QTL analysis was performed using the software R/qtl 1.08–56 (Broman *et al*., 2003). The Haldane mapping function was used and genotypes imputed every 1 cM. Separate significance thresholds for the autosomes and the X chromsome were generated using the 'scanone' and 'scantwo' functions with 4000 permutations (Broman *et al*., 2006). Mapping was performed using the Haley–Knott regression method (Haley and Knott, 1992; Broman *et al*., 2003). We tested for a single QTL on a chromosome, a sex-specific single QTL on a chromosome, and two interacting QTL on the same chromosome or on separate chromosomes. Finally, a test for epistatic interactions between loci identified as having a significant effect on the phenotype and the rest of the genome were performed (Cheverud, 2000).

The QTL identified on chromosome 10 was used to create a partial consomic strain by moving SWR/J's chromosome 10 into the BALB/cJ background (BALB/cJ-Chr10$^{SWR/J}$). Standard speed congenic techniques were used selecting positively on SWR/J genotypes for chromosome 10 and negatively on SWR/J genotypes across the rest of the genome. Genotypes were obtained from a fixed whole genome panel of 768 mouse SNPs. The 768 SNP panel, which uses the Illumina genotyping platform, was provided by the Mutation Mapping and Developmental Analysis Project (MMDAP; Brigham and Women's Hospital, Harvard Medical School). It has an average SNP density of 3 Mb across autosomes and 7 Mb across chromosome X. Two-hundred and seventy-two of these SNPs were polymorphic between BALB/cJ and SWR/J and they were distributed, on average, every 9 Mb across the genome. After six generations of backcrossing, pups were tested for AR. The mother of these pups was consomic for chromosome 10 but still carried about 13% donor genome on other chromosomes. None of these donor regions had shown evidence for QTL effects in the F_2 intercross.

Results

The average number of immediately repeated transient hypoxic episodes survived by the six inbred strains of mice is provided in Figure 1 and Table 1. The broad-sense heritability is 58% indicating a strong, highly significant (Prob. $=2\times10^{-11}$) genetic effect on strain differences. SWR/J mice failed to autoresuscitate from a single hypoxic period when oxygen was restored. SJL/J, a close relative of SWR/J, does not autoresuscitate significantly more than SWR/J (Prob. $=0.46$) surviving an average of 2.2 trials. FVB/NJ, another close relative of SWR/J, survives 4.5 trials but is only significantly different from BALB/cJ and SWR/J, the two most extreme strains. NOR/LtJ is quite similar to FVB/NJ surviving an average of 4.8 trials and only being significantly different from SWR/J. BALB/cJ and MA/MyJ survive 7.2 and 6.8 trials, respectively. BALB/cJ

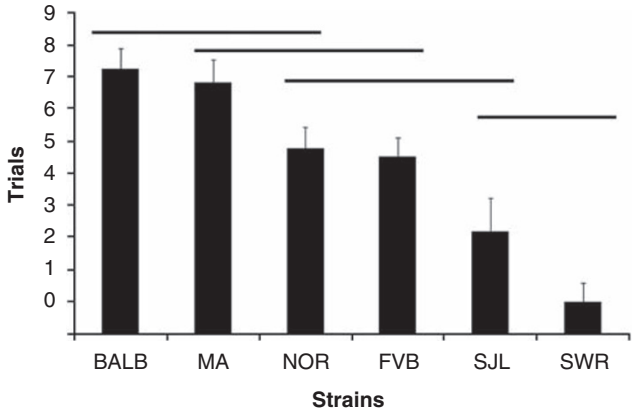

Figure 1 Mean number of autoresuscitation trials survived by various inbred mouse strains. FVB and SJL are close relatives of SWR. Horizontal bars over the columns indicate sets of strains that are not significantly different from one another using pairwise tests and correcting for multiple comparisons. Whiskers indicate one standard error above the mean.

survives significantly more trials than FVB/NJ, SJL/J and SWR/J whereas MA/MyJ only survives significantly more trials than the last two strains.

SWR/J was crossed with BALB/cJ to examine the genetic basis of differences in AR ability (see Figure 2; Table 1). Autoresuscitation failure is dominant in this cross as the F_1 hybrids (average 6.25 trials) are not significantly different from BALB/cJ (Prob. = 0.74) but are highly significantly different from SWR/J (Prob. = 4×10^{-6}). The F_2 hybrids survived significantly fewer trials (4.0 trials) than either the BALB/cJ parent strain (Prob. = 0.001) or the F_1 hybrids (Prob. = 0.03) and significantly more trials than SWR/J (Prob. = 1×10^{-5}). The effective number of factors separating the parental strains is 3.73 so there are probably at least 3–4 loci responsible for the strain differences reported here.

Table 1 Least squares mean, standard error, and sample size for the different mouse strains and hybrid populations

Strain	*Mean*	*s.e.*	N
BALB/cJ	7.31	0.77	13
MA/MyJ	6.82	0.71	11
NOR/LtJ	4.77	0.65	13
FVB/NJ	4.50	0.59	16
SJL/J	2.20	1.05	5
SWR/J	0.00	0.60	15
F_1	6.25	0.69	16
F_2	4.03	0.36	60
F_2 male	3.18	0.60	28
F_2 female	4.78	0.56	32
Consomic	2.29	0.59	7

Consomic refers to the BALB/cJ-chr10$^{SWR/J}$ partial consomic strain.

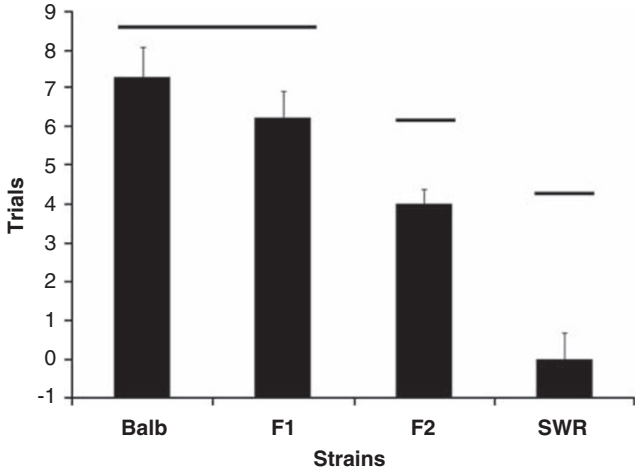

Figure 2 Mean number of autoresuscitation trials for parental strains and F_1 and F_2 hybrids. Horizontal bars over the columns indicate sets of strains that are not significantly different from one another using pairwise tests and correcting for multiple comparisons. Whiskers indicate one standard error above the mean.

Linkage of AR was examined in the F_2 generation. Sex differences in AR are borderline significant ($P = 0.055$) in the F_2 animals with males surviving an average of 3.17 trials and females 4.78 trials. Mapping is carried out both jointly, pooling the sexes, and in each sex separately. A locus 0.6 cM proximal to marker *D10Mit113* was found to affect both males and females and account for 21% of the phenotypic variance (genome-wide corrected $P = 0.07$; see Table 2). The dominance ratio (dominance genotypic value to additive genotypic value ratio; d/a) is 0.93, which indicates nearly complete dominance of the BALB/c allele. This locus is more strongly significant when just males are included in the analysis (genome-wide corrected $P = 0.02$; 42% of variance; see Table 2). In the male-specific analysis the dominance effect is not significant (Prob. = 0.41). The QTL identified on chromosome 10 is near *D10Mit113*, at 66.9 Mb. The confidence interval runs roughly from 51.7 to 82.1 Mb. We refer to this QTL as *Auto10.1*.

Additionally, a locus with effects on males only was identified on chromosome 12 at marker *D12Mit20* (genome-wide corrected $P = 0.04$; 45% of variance; see Table 2). This locus has a significant genotype by sex interaction (Prob. = 0.012) indicating sex differences in allelic effects. The male-specific locus on chromosome 12 is overdominant ($da = 5.21$), the heterozygotes surviving more trials than either homozygote. This QTL is located at 115.6 Mb, which is the most distal marker on the chromosome. The confidence interval runs proximally to about 101.9 Mb. No significant effects were found in females alone. This locus is referred to as *Auto12.1*.

We also performed a scan for loci with epistatic interactions with *Auto10.1* and *Auto12.1*. This scan was performed jointly for both sexes because there were not enough data to support separate two-locus scans in either sex alone. Results indicate epistatic interaction between the middle of chromosome 17 (*Auto17.1e*) with chromosome 10, and the distal end of chromosome 4 (*Auto4.1e*) with chromosome 12 (see Table 3 and Figure 3). The interaction between *Auto10.1* and *Auto17.1e* includes negative additive by additive (aa = −1.55; $P = 0.016$) and dominance by dominance (dd = −4.26; $P = 0.008$) interactions and accounts for an additional 13.5% of the phenotypic variance relative to *Auto10.1* alone. The interaction between *Auto12.1* and *Auto4.1e* includes a negative additive by additive (aa = −1.52; $P = 0.022$) and positive additive by dominance (ad = 3.49; $P = 0.002$) interaction and accounts for an additional 17% of the variance.

A model including the two main effect QTLs (*Auto10.1* and *Auto12.1*) and the main effects of the two epistatic

Table 2 QTL mapping results: 'a' is the additive genotypic value (half the difference between the two homozygotes), 'd' is the dominance genotypic value (deviation of the heterozygote from the midpoint of the two homozygotes), CI provides the genome coordinates of the confidence interval for the QTL

Chr	*Position (cM)*	*Sex*	*LOD*	*Corrected genome-wide probability*	*5% chromosome-wise threshold*	*5% genome-wise threshold*	a	d	R^2	*CI (Mb)*
10	23	Both	3.45	0.07	1.85	3.65	1.77	4.64	0.23	51.7–82.1
10	24	Male	4.43	0.0215	1.83	3.91	2.64	0.75	0.48	61.1–98.1
12	51.3	Male	4.09	0.0385	1.79	3.91	−0.78	4.07	0.49	101.9–end

Abbreviation: QTL, quantitative trait loci.

Table 3 Genotypic values, standard errors, and probabilities associated with the two-locus models for epistatically interacting QTLs: '*a*' is the additive genotypic value, '*d*' is the dominance genotypic value, 'aa' is the additive by additive epistasis, 'ad' is the additive by dominance epistasis, 'da' is the dominance by additive epistasis, 'dd' is the dominance by dominance epistasis

	Constant	*Auto10.1*		*Auto17.1e*		*Epistasis*			
		a	d	a	d	*aa*	*ad*	*da*	*dd*
Genotypic values	**2.87**	**2.09**	**3.42**	−1.06	1.59	**−1.55**	−1.05	1.12	**−4.26**
Standard errors	0.62	0.62	0.94	0.62	1.10	0.06	1.10	0.94	1.53
Probabilities	0.000030	0.001430	0.000640	0.091760	0.153670	0.015550	0.343910	0.236570	0.007590
	Constant	*Auto12.1*		*Auto4.1e*		*Epistasis*			
		a	d	a	d	*aa*	*ad*	*da*	*dd*
Genotypic values	**3.48**	**−1.92**	1.24	1.11	0.14	**−1.52**	**3.49**	−1.95	0.59
Standard errors	0.65	0.65	1.08	0.65	1.08	0.65	1.09	1.08	1.68
Probabilities	0.000002	0.004672	0.254889	0.092656	0.896724	0.022816	0.002235	0.075574	0.727934

Significant values are in boldface.

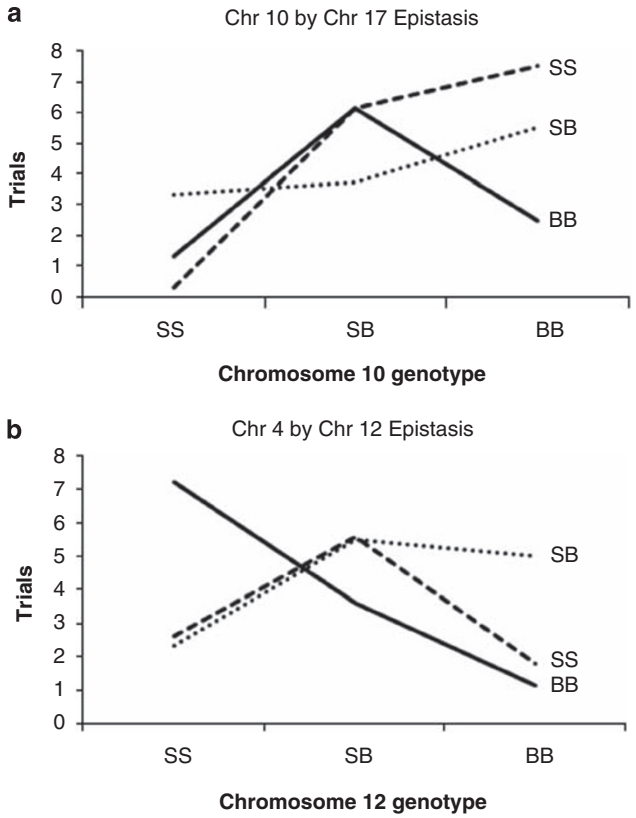

Figure 3 Epistatic interactions between *Auto10.1* and *Auto17.1e.* The figures show how the effects of genotypes at the epistatic loci change depending on the genotype at the main effect locus, and vice versa. (**a**) *Auto10.1* genotypes are along the horizontal axis and *Auto17.1e* genotypes are given to the right of the lines connecting *Auto17.1e* genotypes across *Auto10.1* genotypes. (**b**) Epistatic interactions between *Auto12.1* and *Auto4.1e* with *Auto12.1* genotypes along the horizontal axis and *Auto4.1e* genotypes identified to the right of the lines connecting them across the *Auto12.1* genotypes.

QTLs (*Auto4.1e* and *Auto17.1e*) accounts for 26% of the phenotypic variance in AR. The model accounts for a total of 53% of the phenotypic variance when the epistatic interactions between *Auto10.1* and *Auto17.1e* and between *Auto12.1* and *Auto4.1e* are included. Hence, epistasis accounts for 27% of the phenotypic variance, the same as the main effects alone.

Animals from the BALB/cJ-chr10$^{SWR/J}$ partially consomic line survived significantly fewer trials, 2.28 trials, than the BALB/cJ recipient strain (7.2 trials; see Table 1; Prob. $= 2 \times 10^{-6}$). This result shows that the AR phenotype has been transferred along with the selected region of chromosome 10 and confirms the mapping result obtained for chromosome 10. Although the consomic mean is not significantly different from the SWR/J parent (Prob. $= 0.50$), it is significantly greater than zero (Prob. $= 0.004$), suggesting that there are other factors responsible for the BALB/cJ—SWR/J strain differences in addition to chromosome 10.

Discussion

We found substantial genetic variation among inbred mouse strains for ability to repeatedly autoresuscitate, with a heritability of 58%. We confirm that mouse strain SWR/J fails to autoresuscitate even after a single episode of deprivation. Strains closely related to SWR/J, including SJL/J and FVB/NJ, only resuscitate a few times before expiry, whereas other strains, such as BALB/cJ and Ma/MyJ resuscitate repeatedly across trials. These results indicate that differences among strains in AR are likely to have a genetic basis that is shared, in part, among strains related to SWR/J. This relationship can be used along with haplotypes of the strains across QTL intervals to help fine-map the genes responsible for the QTL effect.

As BALB/cJ had the most robust AR phenotype, we intercrossed SWR/J and BALB/cJ to produce populations of F_1 and F_2 hybrids. The F_1 hybrids autoresuscitated nearly as well as the parental BALB/cJ animals showing significant heterosis because the F_1 hybrid mean was 2.6 trials higher than the midpoint of the two parental strains (3.6 trials), even though they autoresuscitated fewer times than the BALB/cJ strain. This indicates strong dominance in the BALB/cJ by SWR/J cross, which can be due to overdominance, where the heterozygote survives more trials than either homozygote, or directional dominance, where BALB/cJ alleles are regularly dominant to SWR/J alleles, at individual loci and/or dominance epistasis at pairs of loci. As expected under heterosis, the F_2 population regressed towards lower levels of AR (Falconer and Mackay, 1996).

Mapping results identified two main effect QTLs significant at the genome-wide 5% level in males, *Auto10.1* and *Auto12.1*. These loci jointly account for 27% of the phenotypic variance as indicated by the adjusted R^2 value when both sexes are included and 59% in the males alone. Although the sample size is small, these two QTLs are very strongly supported and have less than a 5% chance of being false-positive results. Although the percentages of phenotypic variance accounted for by these QTLs are undoubtedly positively biased because of the small size of the F_2 population (Beavis, 1994), their identity as genomic locations affecting AR is not in question because considerations of sample size were included in determining the appropriate significance thresholds. We also used the parental means and F_1 and F_2 variances to estimate the effective number of factors responsible for differences between the parental strains. This estimate was 3.73 indicating that there are probably at least 3–4 genes affecting AR survival segregating in the cross. Therefore, it is likely that there are additional loci responsible for the parental strain difference with effects too small to detect without a larger sample. Such loci remain to be discovered in a larger experiment.

Auto10.1 was further validated and replicated by the production of a partial consomic line with chromosome 10 transferred from SWR/J into a BALB/cJ background. However, the consmoic strain, unlike SWR/J does, on average, survive a couple of trials, suggesting that other genetic factors also contribute to the differences observed between the parental strains. However, it seems likely that *Auto10.1* is a major reason for the strain difference as it recapitulates nearly 70% of the original observed difference.

In the F_2 generation, females survived about 1.6 trials more than males, although this difference was not quite statistically significant. Furthermore, both of the main effect QTLs were stronger in males than in females, significantly so at *Auto12.1*. This indicates genetic variation in sexual dimorphism for the AR trait. These results are of particular relevance to SIDS in that this bias towards effects in males is consistent with the epidemiology of SIDS in humans, in which males comprise 60% of the SIDS cases.

Auto10.1 and *Auto12.1* were found to epistatically interact with chromosomes 17 and 4, respectively. These epistatic interactions served to mask the effects of *Auto17.1e* and *Auto4.1e* in the F_2 population. Inspection of Figure 3 indicates that there are no differences among genotypes at *Auto17.1e* when averaged over the genotypes at *Auto10.1*. However, *Auto17.1e* has an additive effect with the SWR/J allele promoting resuscitation in *Auto10.1* BALB/cJ homozygotes, underdominance in *Auto10.1* heterozygotes, and overdominance in *Auto10.1* SWR/J homozygotes. Likewise, the effects of *Auto4.1e* are not apparent when averaged over the *Auto12.1* genotypes (see Figure 3). Here, *Auto4.1e* has a strong additive effect with SWR/J dominant in the *Auto12.1* SWR/J homozygotes, no effect in *Auto12.1* heterozygotes, and an overdominant effect in *Auto12.1* BALB/cJ homozygotes. Epistatic interactions explained an additional 27% of the phenotypic variance in AR, arguing for their importance in determining variation in this mouse cross. Further confirmation of these interactions and a search for more interacting loci will require larger samples than reported here.

The comparison of the parental and hybrid generation means indicated that there was heterosis in the cross, although the F1 hybrid mean was not higher than that of the BALB/cJ parental strain. The QTL results support this conclusion with the dominance of the BALB/cJ allele at *Auto10.1* and the overdominance found at *Auto12.1*. We also noted dominance epistasis involving both these loci. Together, these effects are consistent with the level of heterosis seen in the cross.

Cardiac glycogen reaches a developmental nadir in 21-day-old mice. Significantly cardiac glycogen is very much lower in SWR mice compared with other strains (Despande *et al*., 1999). We have proposed that decreased cardiac glycogen is an important factor in the failure of AR in SWR mice. With onset of hypoxia, the capacity of the heart to function depends on anaerobic glycogenolysis, which depends, in turn, on availability of adequate glycogen stores. We searched for candidate genes within the confidence regions of the two main effect QTLs. There are 361 genes in the *Auto10.1* interval. The first gene in the *Auto10.1* region was at 51 742 237 (Vg ll) and the last is at 82 113 069 (Tdq).

We have found a candidate gene on chromosome 10. Serine threonine protein kinase encodes an enzyme that interacts with glycogen synthase kinase 3 in the regulation of glycogen synthesis in heart muscle (Biondi and Nebreda, 2003; Taegtmeyer, 2004).

There are 274 genes identified in the chromosome 12 QTL region. The initial gene in this region is at 101 787 988 (*Kp56a5*) and the last at 115 595 970 (*Iqhv13–2*). There are two candidate genes in this region. Hexokinase-1 is among the genes we have identified on chromosome 12. Hexokinase-1 is crucial for phosphorylation of glucose and therefore is important in glycogen synthesis and also in anaerobic glycolysis (Voet and Voet, 2004). Hence, a defect in this gene could have a critical role contributing to decreased cardiac muscle glycogen as well as the decrease in anaerobic glycolysis essential for heart function during hypoxia (Beck *et al*., 2000). Another candidate gene in this region is 6-phosphofructokinase. The positive effect of this enzyme on glycogenolysis in perfused rat hearts has been documented (Vercesi and Focesi, 1975).

In summary, we have found 4 genomic regions (*Auto10.1, Auto12.1, Auto17.1e, Auto4.1e*) with effects on ability to repeatedly resuscitate in mice in a F_2 intercross between SWR/J and BALB/cJ. Mapping results were found to be stronger in males than in females, consistent with the male bias in SIDS cases. *Auto10.1* was replicated in a partial consomic line and seems to have a large effect on AR variation. Further fine mapping of this region will provide a tighter support interval for the QTL effect and a consequent reduction in the number of positional candidate genes.

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References

Beavis WD (1994). The power and deceit of QTL experiments: lessons from comparative QTL studies. In: Wilkinson DB (ed) *49th Annual Corn and Sorghum Research Conference*. American Seed Trade Association: Washington DC. pp 252–268.

Beck JA, Lloyd S, Hafezparast M, Lennon-Pierce M, Eppig JT, Festing MFW *et al*. (2000). Genealogies of mouse inbred strains. *Nat Genet* **24**: 23–25.

Biondi RM, Nebreda AR (2003). Signaling specificity of Ser/Thr protein kinases through docking-site-mediated interactions. *Biochem J* **372**: 1–12.

Broman KW, Sen S, Owens SE, Manichaikul A, Southard-Smith EM, Churchill GA (2006). The X chromosome in quantitative trait locus mapping. *Genetics* **174**: 2151–2158.

Broman KW, Wu H, Sen S, Churchill GA (2003). R/qtl: QTL mapping in experimental crosses. *Bioinformatics* **19**: 889–890.

Brunton LL, Hayes JS, Mayer SE (1979). Hormonally specific phosphorylation of cardiac troponin I and activation of glycogen phosphorylase. *Nature* **280**: 78–80.

Cheverud JM (2000). Detecting epistasis among quantitative trait loci. In: Wolf JB, Brodie ED, III and Wade MJ (eds) *Epistasis and the Evolutionary Process*. Oxford University Press: New York. pp 58–81.

Despande P, Khurana A, Hensen P, Wilkins D, Thach BT (1999). Failure of the autoresuscitation mechanism in weaning mice: significance of cardiac glycogen and heart rate regulation. *J Appl Physiol* **87**: 203–210.

Falconer DS, Mackay T (1996). *Introduction to Quantitative Genetics*. Longman Press: New York.

Festing M (1998). Origins and characteristics of inbred strains of mice. In: Lyon MF, Rastan S, Brown SDM (eds) *Genetic Variants and Strains of the Laboratory Mouse*. Oxford University Press: New York. pp 1537–1576.

Haley CS, Knott SA (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* **69**: 315–324.

Jacobi MS, Thach BT (1989). Effect of maturation on spontaneous recovery from hypoxic apnea by gasping. *J Appl Physiol* **66**: 2384–2390.

Lynch M, Walsh JB (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Inc.: Sunderland, MA.

Poets CF, Meny RG, Chobanian MR, Bonofiglo RE (1999). Gasping and other cardiorespiratory patterns during sudden infant deaths. *Pediatr Res* **45**: 350–354.

Sokal RR, Rohlf FJ (1995). *Biometry*. W H Freeman & Co.: New York.

Sridhar R, Thach BT, Kelly D, Henslee J (2003). Characterization of successful and failed autoresuscitation in Sudden Infant Death Syndrome (SIDS) and other infants dying sudden and unexpectedly. *Pediatr Pulmonol* **36**: 113–122.

Taegtmeyer H (2004). Glycogen in the heart- an expanded view. *J Mol Cell Cardiol* **37**: 7–10.

Vercesi AE, Focesi A (1975). On the mechanism of the glycogenolytic effect of dinitrophenol. Activity of phosphofructokinase in perfused hearts. *Cell Mol Life Sci* **31**: 771–772.

Voet D, Voet JG (2004). *Biochemistry*, 3rd edn. John Wiley Sons, Inc: New York.

Appendix 1

Microsatellites scored in the Balb/cJ by SWR/J F2 intercross

Locus	*Location (cM)*	*Position (Mb)*	*Build 37*
D1Mit68	0.0	13,312,697	
D1Mit236	13.6	45,435,458	
D1Mit132	47.7	77,143,053	
D1Mit495	72.1	129,522,019	
D1Mit507	107.7	166,978,064	
D1Mit210	132.7	194,352,892	
D2Mit5	0.0	9,445,386	
D2Mit369	23.3	40,654,653	
D2Mit66	48.7	84,657,700	
D2Mit395	62.2	119,350,649	
D2Mit77	64.0	131,428,366	
D2Mit113	107.1	173,180,172	
D3Mit1	0.0	21,674,045	
D3Mit184	18.1	53,010,924	
D3Mit311	40.8	92,821,117	
D3Mit194	60.7	138,429,453	
D3Mit88	80.9	153,603,906	
D4Mit196	0.0	39,399,159	
D4Mit17	12.9	63,026,328	
D4Mit9	42.7	94,736,033	
D4Mit170	68.7	138,171,253	
D4Mit42	77.1	150,944,103	
D5Mit386	0.0	28,048,768	
D5Mit81	14.2	50,722,564	
D5Mit201	21.7	75,550,712	
D5Mit314	43.3	110,111,319	
D5Mit374	78.9	139,608,988	

Continued

Locus	*Location (cM)*	*Position (Mb)*	*Build 37*
D6Mit138	0.0	4,453,823	
D6Mit268	16.9	34,684,663	
D6Mit123	31.7	55,085,225	
D6Mit328	81.7	112,729,344	
D6Mit373	99.9	147,000,978	
D7Mit294	0.0	28,074,461	
D7Mit69	11.8	56,320,467	
D7Mit323	40.4	108,024,450	
D7Mit259	81.4	151,943,409	
D8Mit155	0.0	4,976,602	
D8Mit125	13.1	35,916,997	
D8Mit45	34.7	89,829,274	
D8Mit120	65.2	120,865,409	
D8Mit42	72.7	129,076,217	
D9Mit297	0.0	33,875,129	
D9Mit97	20.5	50,487,896	
D9Mit336	26.8	65,425,671	
D9Mit347	43.6	103,159,628	
D9Mit19	71.1	120,271,887	
D10Mit123	0.0	9,952,319	
D10Mit107	10.6	43,301,449	
D10Mit113	23.6	66,970,458	
D10Mit159	34.4	85,197,468	
D10Mit35	61.2	121,642,455	
D11Mit2	0.0	12,218,640	
D11Mit231	26.3	35,501,291	

Continued

Locus	*Location (cM)*	*Position (Mb)*	*Build 37*
D11Mit86	41.2	54,003,617	
D11Mit285	69.3	89,789,103	
D11Mit126	82.3	116,930,798	
D11Mit48	104.6	117,993,078	
D12Mit182	0.0	10,877,409	
D12Mit112	18.7	47,855,788	
D12Mit158	34.8	88,076,070	
D12Mit20	52.4	114,599,777	
D13Mit117	0.0	37,654,832	
D13Mit13	14.6	56,582,797	
D13Mit74	39.4	106,679,893	
D13Mit260	51.2	113,155,804	
D14Mit222	0.0	24,793,292	
D14Mit101	10.6	50,144,321	
D14Mit263	15.7	89,360,701	
D14Mit170	41.9	106,665,012	
D15Mit80	0.0	7,815,616	
D15Mit63	33.3	65,076,746	
D15Mit70	54.9	81,032,515	
D15Mit161	66.8	96,841,490	
D16Mit73	0.0	12,856,286	
D16Mit41	20.8	42,872,002	
D16Mit139	29.3	65,669,762	
D16Mit86	49.8	93,134,312	
D17Mit143	0.0	8,635,986	
D17Mit10	29.8	46,163,038	
D17Mit39	58.7	69,735,652	
D17Mit221	61.3	90,487,044	
D18Mit64	0.0	6,108,402	
D18Mit177	27.2	41,142,161	
D18Mit208	39.2	55,634,471	
D19Mit42	0.0	10,157,495	
D19Mit40	15.8	25,410,811	
D19Mit90	32.1	42,295,742	
DXMit187	0.0	12,789,527	
DXMit68	17.9	50,676,500	
DXMit172	51.3	119,197,077	
DXMit121	82.7	157,867,829	