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Single and multiple congenic strains for hydrocephalus in the H-Tx rat

Hazel C. Jones, Gin-Fu Chen, Baligh R. Yehia, Barbara J. Carter, Elizabeth J. Akins, and Logan C. Wolpin

Department of Pharmacology and Therapeutics, University of Florida, Gainesville, Florida 32610-0267, USA

Abstract

The H-Tx rat has fetal-onset hydrocephalus with a complex mode of inheritance. Previously, quantitative trait locus mapping using a backcross with Fischer F344 rats demonstrated genetic loci significantly linked to hydrocephalus on Chromosomes 10, 11, and 17. Hydrocephalus was preferentially associated with heterozygous alleles on Chrs 10 and 11 and with homozygous alleles on Chr 17. This study aimed to determine the phenotypic contribution of each locus by constructing single and multiple congenic strains. Single congenic rats were constructed using Fischer F344 as the recipient strain and a marker-assisted protocol. The homozygous strains were maintained for eight generations and the brains examined for dilated ventricles indicative for hydrocephalus. No congenic rats had severe (overt) hydrocephalus. A few pups and a significant number of adults had mild disease. The incidence was significantly higher in the C10 and C17 congenic strains than in the nonhydrocephalic F344 strain. Breeding to F344 to make F.H-Tx C10 or C11 rats heterozygous for the hydrocephalus locus failed to produce progeny with severe disease. Both bicongenic and tricongenic rats of different genotype combinations were constructed by crossing congenic rats. None had severe disease but the frequency of mild hydrocephalus in adults was similar to congenic rats and significantly higher than in the F344 strain. Rats with severe hydrocephalus were recovered in low numbers when single congenic or bicongenic rats were crossed with the parental H-Tx strain. It is concluded that the genetic and epigenetic factors contributing to severe hydrocephalus in the H-Tx strain are more complex than originally anticipated.

Introduction

Hydrocephalus is dilatation of the cerebral ventricles associated with an excess of cerebrospinal fluid (CSF). In humans, congenital hydrocephalus occurs at a rate of 0.5–1.5 per 1000 births. About 50% of cases are acquired and the pathology is identifiable. For the remainder, there is no recognizable cause and there may be a genetic origin. There is abundant evidence for genetic causes in human hydrocephalus (reviewed in Jones et al. 2004), and characterization at the molecular level will greatly increase understanding of the disease and lead to new therapies. Animal models of inherited disease provide the opportunity to identify some potential genetic causes in humans.

The H-Tx rat strain, first described in 1981 (Kohn et al. 1981), has inherited disease with an onset in late gestation and a frequency of 30%–40% (Jones et al. 2000b). The first sign of ventricular dilatation occurs at 17–18 days gestation and is associated with an abnormality in the cerebral aqueduct (Jones and Bucknall 1988). Pups with severe hydrocephalus are detected

at birth by a domed head and die at 4–6 weeks of age. The initial events in the brain that lead to hydrocephalus are not well understood. Recently, however, it was demonstrated that ventricular dilatation is preceded by underdevelopment of the subcommissural organ (SCO), an ependymal secretory gland in the dorsal cerebral aqueduct, and is associated with a reduction in immunostaining of the secretory glycoprotein (Somera and Jones 2004). This is consistent with other evidence for a link between SCO function and hydrocephalus in that many animal models have an SCO deficiency (Pérez-Fígares et al. 2001).

The H-Tx colony is fully inbred and maintained by breeding from nonhydrocephalic littermates. Quantitative trait locus (QTL) mapping was performed with a backcross experiment in which H-Tx rats were bred with Fischer F344 rats, a nonhydrocephalic strain, and the N_1 offspring backcrossed to the H-Tx hydrocephalic strain. The backcross generated 1500 N_2 offspring of which 12.3% had severe hydrocephalus and a further 5% had a mild form that was detected after examination of the fixed brains (Jones et al. 2000b, 2001b). A genome-wide scan was performed on all the hydrocephalic pups and on a subset of the non/hydrocephalic pups using 110 DNA markers (SSLPs). Linkage analysis demonstrated three loci with significant linkage on Chromosomes 10, 11, and 17 and three more loci with suggestive linkage on Chromosomes 4, 9, and 19 (Jones et al. 2001a, 2001b). Hydrocephalus loci on Chromosomes 10 and 11 were most frequently associated with the heterozygous genotype (one allele from each strain), whereas the hydrocephalus locus on Chromosome 17 was predominantly associated with the H-Tx homozygous genotype. In addition to genetic loci on several different chromosomes, there is evidence that epigenetic factors affect the frequency of hydrocephalus (Jones et al. 2002). A disadvantage for QTL mapping is that the minimum chromosomal interval containing the locus is relatively large, up to 30 cM, and contains hundreds of genes. Additional strategies are needed to increase the resolution and reduce the interval to about 1 cM (Darvasi and Pisante-Shalom 2002; Glazier et al. 2002). One such method is the analysis of congenic strains, created when the chromosomal region containing a locus is transferred onto the genome of an unrelated strain by repeated backcross breeding.

The aim of this study was to examine the function of three significant loci on Chromosomes 10, 11, and 17 by generating three congenic strains. Since this strategy did not produce rats with severe hydrocephalus, the three strains were recombined to make bi- and tricongenic rats. Although mild hydrocephalus was observed, the severe hydrocephalus phenotype was recovered only when single and bicongenic rats were bred back to the parental H-Tx strain.

Materials and methods

Rats

The H-Tx rat, an inbred strain H-Tx/hcj, maintained at the University of Florida, has severe hydrocephalus in 30% of pups. H-Tx rats used for this experiment were bred under Specific Pathogen Free (SPF) conditions but moved to conventional housing for congenic strain breeding. Fischer F344 rats (F344/NHsd, Harlan) were used for the recipient strain. Rats were paired at 11 weeks of age. Pups were examined at birth for overt hydrocephalus and again at 10 days of age when they were sexed. Pups were weaned at 21 days and given an ear punch unique to each litter. All rat procedures were approved by the University of Florida Animal Care and Use Committee and followed the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985).

Breeding scheme to generate single congenic strains

Congenic strains were generated by the marker-assisted protocol outlined by Markel et al. (1997) and Wakeland et al. (1997). In all, four backcrosses (N_2 – N_5) to the nonhydrocephalic F344 strain were performed and at each stage the best rats were selected, after genotyping, for

the next generation breeding. The N₅ rats were then intercrossed to create three homozygous congenic strains depicted as F.H-Tx C10, F.H-Tx C11, and F.H-Tx C17 and hitherto called C10, C11, and C17 strains.

Breeding scheme to generate bi- and tricongenic strains

In order to reconstruct the H-Tx hydrocephalic genotype, bi- and tricongenic strains were generated according to the scheme in Fig. 1. Single congenic rats with the homozygous genotype were bred together as follows: C10 × C17 and C11 × C17. Rats heterozygous at the same two loci were intercrossed and homozygous progeny selected for the bicongenic strains. In addition, heterozygous C10, C11 rats were created by breeding C10 × C11 homozygous congenic rats for use in further tests as described below. For tricongenic rats, the progeny from each C10 × C17 and C11 × C17 cross were crossed with each other and offspring genotyped and selected for heterozygosity on Chrs10 and 11 and either homozygosity or heterozygosity on Chr17 (Fig. 1). These first-generation tricongenic rats were intercrossed to make second-generation rats. From the second generation, rats homozygous at all three loci were intercrossed to make the homozygous tricongenic strain that could be maintained by interbreeding. Homozygous tricongenic rats were bred to C17 single congenic rats to create the genotype most likely to reproduce the phenotype, heterozygous on Chrs 10 and 11 and homozygous on Chr 17.

Test crosses

In order to determine if the severe hydrocephalus phenotype can be recovered, some single and bicongenic rats were backcrossed to the H-Tx parental strain. All pups were sacrificed at 0–21 days and the brains removed for examination.

DNA extraction and testing

Pups for genotyping were anesthetized with halothane (2.5% in N₂O:O₂, 2:1 by volume) at 12–16 days of age. They were given a unique identifier at the base of the tail with a tattoo machine, 1 cm of tail was removed, and bleeding was prevented with silver nitrate sticks. The tissue was frozen at –80°C until extraction to recover genomic DNA. Genome-wide polymorphic DNA markers (SSLPs), informative for the cross between H-Tx and F344 strains, were identified previously (Jones et al. 2000b). In addition, more polymorphic markers were identified for Chr10, 11, and 17 to obtain a denser coverage for these chromosomes containing the hydrocephalus loci. Markers for these chromosomes were mapped in the backcross experiment used for linkage analysis (Jones et al. 2001a, 2001b, 2004). Amplification of the DNA repeats by PCR and electrophoretic separation on 5% agarose gels were performed according to the methods published previously (Jones et al. 2000b).

Genotyping and progeny selection

At each backcross, progeny were tested in two stages: first, to select rats that were H-Tx–F344 heterozygous on the chromosome containing the locus and, second, to select from this group rats that had the greatest F344 DNA elsewhere on the genome. The best progeny were bred to F344 for the next backcross. At each generation, the number of markers required for testing decreased as the introgression proceeded. Residual heterozygosity from the scan performed on the first two backcrosses with 63 markers was detected at the N₄ generation using an additional 62 SSLP markers. The two marker sets together gave a genome coverage of 90%. Additionally, the number of markers on the chromosomes containing the loci was increased to refine the interval and ensure it was as large as, or larger than, the LOD ± confidence interval as shown by MAPMAKER (Jones et al. 2001b, 2004). After four backcrosses, the heterozygous N₅ rats were intercrossed, the progeny genotyped, and the best rats selected to make the first-generation homozygous congenic lines. For the bicongenic and tricongenic strains, initial genotyping was

to select rats with the required genotype using a marker at the peak for the locus. Subsequent genotyping was performed to define the length of the congenic interval.

Hydrocephalus expression: Cerebral ventricle analysis—All rats were examined outwardly for hydrocephalus as pups. In addition, the brains were removed from all rats at sacrifice throughout the experiment. For ventricle analysis, the rats were sorted into two age groups: pups younger than 50 days sacrificed after genotyping but not required for breeding, and adults older than 50 days that were sacrificed after breeding. In order to make a comparison between congenic rats, the parental strains and their hybrid, 508 H-Tx rats, 90 F344 rats, and 35 N₁ rats (H-Tx × F344), including pups and exbreeders, were treated in the same way. Brains were placed in formalin, sliced at 1-mm intervals, and the slices examined under a binocular microscope for signs of dilated ventricles. Rats with no visible ventricles on slices 1-mm thick were designated nonhydrocephalic. Brains from rats with visible ventricles were measured according to a method described previously (Jones et al. 2001a) and the hydrocephalus severity determined from the ratio of ventricle width-to-brain width. Previous studies have shown that rats with overt hydrocephalus had the severe form and the severity ratio was greater than 0.4, whereas the majority of rats with the nonovert form or mild hydrocephalus had a severity ratio less than 0.4.

Histological examination—H-Tx rats with hydrocephalus have an underdevelopment of the subcommissural organ (SCO, Somera and Jones 2004). Hence, it was important to know whether single congenic strain rats show the same trend. Homozygous congenic pups at 0–1 days of age from each of the C10 ($n = 10$), C11 ($n = 8$), and C17 ($n = 8$) strains were sacrificed, together with three heterozygous C11 pups at the same age. The pups were perfused intravascularly with saline followed by Bouin's fixative, and the brains were excised, cryoprotected, and frozen for sectioning at 20 μ m on a cryostat. Coronal sections containing the aqueduct and SCO were immunostained with the antibody to Reissner's fiber glycoprotein (AFRU, Rodríguez et al. 1984). The aqueduct was examined for signs of narrowing or nonpatency and the dorsal regions, including the SCO, were studied for immunostaining. Quantification of the immunostained areas was performed on the brains of four rats from each strain, and ventricular dilatation was measured on sections of cerebral cortex as described previously (Somera and Jones 2004). The brains from nonhydrocephalic Fischer F344 pups were used as controls for the congenic strains.

Results

Single congenic strains

Three single homozygous congenic strains were obtained after four backcross generations followed by intercrossing. Genotyping was performed on 255 N₂ progeny. From these, rats were selected to be the founders for the next cross for each congenic strain. The number of rats bred, their percentage heterozygosity, and thenumber of progeny genotyped at each generation are shown in Table 1. At the N₅ generation, rats with these heterozygous regions were excluded from further breeding. N₅ rats were tested with an expanded group of markers ($n = 11$ or 12) on the chromosome containing the hydrocephalus locus in order to redefine the H-Tx (HF) interval. N₅ rats were intercrossed to produce rats homozygous on the chromosome of interest and the three strains designated F.H-Tx C10, C11, and C17.

Characterization of the single congenic strains

Congenic interval—The maximum LOD score for the locus on Chr 10 is near the telomeric end between markers D10Rat136 and 135 at 108 cM (Fig. 2a). F.HTx C10 congenic rats were homozygous for the H-Tx genotype from marker D10Rat207 at 90 cM to the last marker mapped, D10Rat2 at 115 cM. This region contained the confidence interval LOD ± 1 as defined

by MAPMAKER.QTL, for the locus. On Chr 11, the maximum LOD score is also near the telomeric end at marker D11Rat46 at 60 cM. F.H-TxC11 congenic rats were homozygous for the H-Tx genotype between D11Rat4 at 46 cM and D11Rat50, the last marker mapped at 65 cM (Fig. 2b). Again, this congenic region contained the confidence interval for the locus. The maximum LOD score for the Chr 17 locus was found near the center of the chromosome at marker D17Mit4 at 34 cM. However, the region between the peak and the end of the chromosome also had a significant LOD score, suggesting that there may be more than one locus for hydrocephalus on Chr 17. For this reason, two congenic strains were generated: one that spanned only the peak that was H-Tx homozygous genotype between D17Rat79 at 24 cM and D17Rat62 at 46 cM, and a second that was longer and extended to the last marker, D17Rat154 at 56 cM (Fig. 2c). Both congenic intervals contained the confidence interval for the locus at D17Mit4.

Expression of hydrocephalus—During construction of the congenic strains, only two rats had severe hydrocephalus and these were in the progeny of the first backcross ($N_1 \times F344$). Among the single congenic rats, there were no rats with severe hydrocephalus but there were some with mild disease and most of these were in the older age group, older than 50 days. The percentage among rats over 50 days was 14.4%, 10.2%, and 17.4% for C10, C11, and C17 rats, respectively (Fig. 3a). This compares with 22.8% for H-Tx parental strain rats and 22.2% for N_1 rats (H-Tx $\times$ F344) and 2.0% for Fischer F344 rats (Fig. 3a). There were no significant differences in the incidence of mild hydrocephalus between the different congenic strains and between the two C17 strains. Only one out of 51 adult F344 rats had mildly dilated ventricles. The C10 and C17 strains had a significantly higher incidence of mild hydrocephalus than the F344 nonhydrocephalic strain, $p < 0.05$ and 0.01 , respectively, but the incidence in the C11 strain was not significantly different from F344. Both the parental H-Tx rats and the hybrid N_1 rats had a higher incidence than F344, $p < 0.01$. The incidence of mild disease in the younger than 50 days group was very low: 1.0%, 1.3%, and 0.5% for the C10, C11, and C17 strains, respectively (Fig. 3a). The percentage of younger rats with mild hydrocephalus in the H-Tx strain was higher at 5.0%, although this was not significant. No F344 rats younger than 50 days old had mild disease.

Hydrocephalus is most frequently associated with the heterozygous genotype for the Chr 10 and Chr 11 loci. In order to examine the possibility that heterozygous C10 and C11 rats were more likely to have hydrocephalus, the rats generated at the N_3 to N_5 stages were studied (Table 1). None of these had severe hydrocephalus and the incidence of mild disease was low. In addition, homozygous congenic C10 rats and C11 rats were crossed to Fisher F344 to recreate the heterozygous genotype and the progeny examined ($n = 65$ for C10 and $n = 59$ for C11). None of these had either severe or mild disease, confirming that the incidence in pups was not increased when the locus became heterozygous.

Severity of the hydrocephalus—The mean severity ratio for the congenic rats with mild hydrocephalus was not significantly different for the three groups: 0.22 ± 0.02 , 0.21 ± 0.02 , and 0.22 ± 0.02 for C10, C11, and C17 strains, respectively (Fig. 3b). Because there was only one F344 rat with very mild ventricular dilatation, severity = 0.11, statistical comparisons could not be made. Taking all congenic rats as a combined group, the distribution of the severity ratios was compared to a group of 194 rats from the H-Tx strain, most of which had severe disease (Fig. 3c). Although there is some overlap, the two groups are quite distinct, with congenic rats having a severity of 0.42 or less and most of the H-Tx rats having a severity greater than 0.42.

Histological examination of brains—In a previous study it was shown that H-Tx pups with potentially severe hydrocephalus at 0–1 days of age have an abnormal cerebral aqueduct and hypoplasia of the subcommissural organ, whereas H-Tx pups without ventricular dilatation

and Fischer F344 pups do not have these features (Somera and Jones 2004). In this study, C10, C11, and C17 congenic rats all appeared to be completely normal when compared to the same region in F344 rats. None had aqueducts that were narrowed or closed (nonpatent). Furthermore, the histologic appearances of the SCO and of immunostaining for the SCO glycoprotein were also normal, as depicted by measurement of the staining areas. In addition, the dilatation of the lateral ventricles was not significantly different among the four groups of pups. Hence, the congenic rats do not have any of the aqueduct or SCO features associated with severe hydrocephalus as seen in the parental H-Tx strain.

Bicongenic rats

Both heterozygous and homozygous bicongenic rats and also a combination of hetero- and homozygous rats were generated.

Expression of hydrocephalus—Among the heterozygous bicongenic rats, none of the younger than 50 day rats had ventricular dilatation, but 26% rats older than 50 days had mild hydrocephalus. Among the mixed homozygous heterozygous group, 13.0% had mild hydrocephalus. Taking all bicongenic rats together, the frequency of mild hydrocephalus in adults was 18.5%. This was not significantly different than the frequency for single locus rats.

Severity of hydrocephalus—The severity of ventricular dilatation for bicongenic rats was not significantly different from that for the single congenic strains, mean = 0.18 ± 0.01 for all bicongenic rats and 0.21 ± 0.02 for all single congenic rats.

Tricongenic rats

C11 and C17 rats used to generate the tricongenic strain had congenic intervals that were longer than for the single congenic strains, but they still encompassed the confidence intervals for the loci. The C10 rats had the same congenic interval as the founders for the single strain. Tricongenic strain rats were derived from two rats with H-Tx homozygous genotype that covered the lower third of Chr 10 and most of Chrs 11 and 17. These were intercrossed and the offspring provided rats that made five third-generation pairs and two fourth-generation pairs, at which stage the experiment was terminated. The congenic intervals are illustrated in Fig. 4. The introgressed H-Tx chromosomal sections covered the regions containing the loci more than adequately.

Expression of hydrocephalus—No tricongenic rats had severe hydrocephalus. Among the tricongenic rats homozygous at all three loci (20 adults and 76 pups), there was one adult and one pup with mild disease. In order to generate rats with the most favorable genotype for hydrocephalus, six tricongenic rats were bred to C17 rats and 55 pups were generated. None of these had severe or mild hydrocephalus.

Crosses with the parental H-Tx strains

Heterozygous congenic rats at the N₅ stage were bred to H-Tx. Out of 90 pups from C10 × H-Tx, one had severe hydrocephalus. This pup died before tissue was taken for DNA. Out of 55 pups from C11 × H-Tx, three had severe hydrocephalus and were heterozygous at the peak marker for the locus. Out of 60 pups from C17 × H-Tx, none had hydrocephalus. First-generation homozygous congenic rats were crossed with H-Tx rats, creating pups that were all homozygous at the locus. No pups from the C10 × H-Tx or from the C11 × H-Tx pairs had either form of hydrocephalus. However, with the C17 × H-Tx rats there was one pup with severe disease.

Furthermore, when heterozygous C10, C11 bicongenic rats were bred to H-Tx, one pup had severe hydrocephalus. When C10, C17 homozygous bicongenic rats were crossed with H-Tx,

two pups had hydrocephalus. These experiments indicate that the congenic rats do carry the loci for hydrocephalus despite the finding that the phenotype is not expressed in the single or multicongenic strains. Furthermore, it was noted that, with one exception, the hydrocephalic pups were produced from a cross between a congenic male and a female H-Tx rat and not from the reciprocal cross.

Discussion

Previously, we have shown that phenotypic expression of congenital hydrocephalus in H-Tx rats is controlled by a combination of genetic and epigenetic factors (Jones et al. 2000b, 2001a, 2001b, 2002). QTL mapping from a backcross to Fisher F344 rats identified possible hydrocephalus loci on 6 different chromosomes (Jones et al. 2001a). Of these, only three on Chrs 10, 11, and 17 reached a LOD score required for full significance in the first analysis. For two of these, Chr 10 and 11 loci, the hydrocephalic phenotype was preferentially, but not exclusively, associated with a heterozygous genotype (one allele from each strain), whereas for the Chr 17 locus the hydrocephalus was preferentially associated with two alleles from the H-Tx strain, the homozygous genotype. All three loci appear to contribute to the expression of the phenotype in that the percentage of backcross progeny with hydrocephalus increased with the number of hydrocephalus loci present, but none was essential (Jones et al. 2001a, 2004). The aim of this study was to characterize the function of each locus alone and in combinations through the production of single and multicongenic strains and to identify the contribution of each locus to the expression of the trait. It was also hoped that this strategy would enable the loci to be fine mapped through the generation of congenic recombinants. The strategy of “speed congenics” has been used successfully in rats to map blood pressure loci (Deng et al. 2001; Jeffs et al. 2000).

Expression of severe hydrocephalus

The congenic strains differ from the recipient nonhydrocephalic strain only by the genomic region containing the selected interval. There was no severe hydrocephalus associated with any of the three loci, either singly or in combination. Hence, the stated aim of the study, functional characterization of individual loci, was not achieved. Furthermore, when the C10 or C11 single congenic rats were crossed with F344 rats to make the locus heterozygous, no severe hydrocephalus occurred. One reason for the lack of phenotype might be the relatively low penetrance of the severe form of the disease in the parental H-Tx strain (33%) and in the backcross progeny (12%). Nevertheless, it was anticipated that the overall number of rats generated in this experiment was sufficiently large to produce some rats with the hydrocephalic phenotype. Another possible reason for the absence of severe disease could be that genetic factors elsewhere on the genome are essential for expression. Since this study commenced, a fourth locus with a significant LOD score was identified on Chromosome 9 (Jones et al. 2004). As with the loci in this study, however, the Chr 9 locus also was found not to be essential for hydrocephalus. A previous study identified further possible loci on Chrs 4 and 19, but these were only suggestive for hydrocephalus (Jones et al. 2001a).

There is evidence that epigenetic factors are needed for phenotypic expression in that the frequency of hydrocephalus in the H-Tx strain is influenced by whether or not the female is suckling a previous litter during pregnancy (Jones et al. 2002). The onset of ventricular dilatation occurs in late gestation and the number of affected pups increased if the dam was pregnant from a *postpartum* mating. It was hypothesized that the maternal nutritional or hormonal status could be interacting with genetic factors to cause the increase in frequency *in utero*. The H-Tx maternal influence could explain the birth of severely hydrocephalic pups that occurred when congenic rats were crossed with the parental strain. Another possibility for the absence of phenotype is that there are alleles on the F344 genome outside the congenic regions

that protect against or oppose the function of loci from the H-Tx strain. The consequence could be that the phenotype is altered to the late-onset mild form of the disease. Whether the results would have been more conclusive if the reverse experiment had been performed, i.e., with hydrocephalus-sensitive H-Tx as the recipient strain, cannot be determined.

Expression of mild hydrocephalus

Mild hydrocephalus was defined as hydrocephalus that was not overtly visible before the rats were sacrificed but that showed as relatively small dilatation of the ventricles on fixed brain slices. In H-Tx pups the percentage of rats with mild hydrocephalus was 5%, but when the brains of adult ex-breeder H-Tx rats were examined, 23% had mild ventricular dilatation. The same was true for the H-Tx $\times$ F344 N₁ rats. This mild form was almost completely absent from the Fisher F344 strain. Older congenic rats had 14%–17% with mild hydrocephalus. The question arises as to whether there is any analogy between the mild disease in older rats and the severe disease found in pups. There are some similarities in that the percentage incidence is lower for the single congenic rats than for the parental H-Tx strain or the N₁ hybrid and that it is absent from the F344 strain. The frequency of mild hydrocephalus was not significantly different between the congenic strains, and, with the exception of C11 rats, it was significantly higher than in the F344 rats. Overall, however, the number of rats expressing the mild phenotype was low, thus reducing the power of statistical testing. Thus, in two respects the expression of mild hydrocephalus in congenic rats behaves in a similar way to the expression of severe hydrocephalus as revealed previously in the backcross progeny (Jones et al. 2001a). On the other hand, the loci did not show any signs of being additive since the frequency was not increased in multicongenic strains.

Causes for congenital hydrocephalus

Hydrocephalus is a syndrome rather than a single disease and the most common cause is an obstruction of CSF flow. Many animal models of congenital hydrocephalus have an abnormality of the cerebral aqueduct, a narrow channel that conducts CSF from the third to the fourth ventricle (D'Amato et al. 1986; Jones et al. 1987; Jones and Bucknall 1988; Sasaki et al. 1983; Takeuchi et al. 1987, 1988; Wagner et al. 2003). In the H-Tx rat the onset of ventricular dilatation occurs at 17–18 days gestation and is associated with an abnormal cerebral aqueduct (Jones and Bucknall 1988). It was shown recently, however, that at 17 days gestation ventricular dilatation can occur without aqueduct obstruction and that reduction in the immunoreactivity of the glycoprotein in the subcommissural organ precedes both ventricular expansion and aqueduct closure (Somera and Jones 2004). This suggests that the pathogenesis is more complex than obstruction of the CSF through aqueduct closure. The primary events that lead to the underdevelopment of the subcommissural organ in the H-Tx strain are not known, although abnormalities at E16 at the foramen of Monro have been reported (Boillat et al. 2001) as have abnormal cortical neuronal proliferation and differentiation (Fukumitsu et al. 2000; Mashayekhi et al. 2002).

Surprisingly, targeted deletion or gain of a number of genes in mice has resulted in hydrocephalus (Homanics et al. 1995; Chen et al. 1998; das Neves et al. 1999; Bucher et al. 2000) to name but a few examples. This suggests that many genes can affect ventricular size. Recently, it was shown that the normal variation in ventricular size in mice is a heritable trait and that the QTLs are close to genes that have been implicated in hydrocephalus (Zygourakis and Rosen 2003). Clearly, the expression of hydrocephalus in H-Tx rats depends on a number of genetic and environmental factors. It requires new resources, similar to those proposed for the mouse, to enable the identification of the genes underlying such complex traits in rats (Threadgill et al. 2002). Despite the complexity of the genetics, the H-Tx rat has proved to be a very useful model for hydrocephalus research. It produces affected offspring consistently

from generation to generation by breeding from nonhydrocephalic littermates and has been used successfully for many nongenetic studies (Jones et al. 2000a).

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Fig. 1.

Breeding scheme to make bicongenic and tricongenic rats from single strain rats. Single congenic C10 or C11 rats were crossed to C17 rats to make heterozygous bicongenic rats (Stage I). The heterozygous rats were intercrossed and homozygous progeny were selected to make bicongenic strains (Stage II). To make tricongenic rats, C10, C17 and C11, C17 heterozygous bicongenic rats (Stage I) were crossed and progeny were selected with heterozygous genotypes at all three loci or heterozygous on C10 and C11 but homozygous on C17 (Stage II). These rats were intercrossed and progeny that were homozygous at all three loci were selected to make the tricongenic strains (Stage III). Some were crossed with C17 single congenic rats to make the most favorable genotype for hydrocephalus.

Single congenic strains

Fig. 2.

Chromosome maps of the three congenic strains. The scale on the left is the mapping distance along the chromosome in centimorgans (cM) with the telomeric end at the bottom. The position of the peak LOD score is depicted by the dashed arrow and the confidence limits are depicted by the solid arrow. The microsatellite markers used to map the locus are on the right of the chromosome and the vertical bar indicates the position of the congenic interval. Black fill indicates the H-Tx homozygous region, the checked fill indicates the region where there is recombination between H-Tx and F344, and the white fill is the F344 homozygous region. **a.** The F.H-TxC10 strain had congenic interval that extended from D10Rat207 to the last marker D10Rat2 and included the hydrocephalus locus between D10Rat 136 and D10Rat135. **b.** The F.H-TxC11 strain had a congenic interval from D11Rat4 to the last marker mapped and included the locus at D11Arb4. **c.** F.H-TxC17 strain 1 had a congenic interval from D17Rat 79 to D17Rat42 and included the locus peak at D17Mit4. For strain 2, The congenic interval was longer and extended to the last marker mapped D17Rat154.

Fig. 3.

a. The incidence of mild hydrocephalus in adult rats and in pups for the parental and congenic strains. The C10 and C17 congenic strains had significantly more adult rats with mild hydrocephalus than the Fischer F344 strain, $p < 0.05$ and 0.01 , as did the H-Tx strain and the N₁ rats, $p < 0.01$. Very few congenic pups had mild disease and none occurred in the N₁ or the F344 pups. **b.** The severity of hydrocephalus for all congenic strain rats. There was no significant difference among the groups. Data are means $\pm$ SEM. **c.** Frequency distribution graph of the hydrocephalus severity for all congenic rats and all H-Tx rats. Most congenic rats have a severity of less than 0.4 and most H-Tx rats have a severity of greater than 0.4. Some H-Tx rats have a severity of less than 0.4 and these tended to be the adult animals.

Tricongenic rats

Fig. 4.

Chromosome maps of C10, 11, and 17 for tricongenic rats. The chromosomes are labeled as described for Fig. 3. Note the congenic interval was much longer in tricongenic rats for Chrs 11 and 17. This is because the genetic interval of the founder rats was longer than in those used to found the single congenic strains.

Table 1

The generations and numbers of rats used to make the congenic strains F.H-TxC10, F.H-TxC11, and F.H-TxC17

Parentage	Rats selected to make generation	Percent heterozygosity of parents	Generation of progeny	Total progeny genotyped
$N_1 \times F344$	16	100	N_2	255
Chromosome 10				
$N_2 \times F344$	4♀ 7♂	31–44	N_3	61
$N_3 \times F344$	2♀ 2♂	8–16	N_4	61
$N_4 \times F344$	2♀ 2♂	1	N_5	67
$N_5 \times N_5$	4♀ 4♂	0	F.H-Tx C10	74
Chromosome 11				
$N_2 \times F344$	3♀ 3♂	26–40	N_3	65
$N_3 \times F344$	2♀ 2♂	8–15	N_4	60
$N_4 \times F344$	2♀ 2♂	2–6	N_5	90
$N_5 \times N_5$	7♀ 7♂	1	F.H-Tx C11	129
Chromosome 17				
$N_2 \times F344$	1♀ 3♂	41–44	N_3	62
$N_3 \times F344$	2♀ 2♂	13–18	N_4	62
$N_4 \times F344$	2♀ 2♂	1–6	N_5	56
$N_5 \times N_5$	6♀ 6♂	2	F.H-Tx C17	147

The percentage of residual heterozygosity was reduced with each generation and was completely eliminated at a later generation.