Development of Hydronephrosis Inbred Strain Mouse, ICR/Mlac-Hydro

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Abstract: Hydronephrosis was accidentally observed when autopsied the outbred ICR mice in routine health monitoring program. Previously, a low incidence of mild hydronephrosis was observed mostly in male mice and found only on the right kidney. Selective inbreeding of this mutant led us to the establishment of a colony of inbred hydronephrosis mice. After inbreeding for 20 generations, a surprisingly high incidence of hydronephrosis was encountered and we finally obtained a strain of mice with a very high incidence of severe hydronephrosis affected on both sides of kidney and in both sexes as the process of inbreeding has been increased. Phenotypes determination, genetic segregation test crosses and histological study of kidneys were also performed.

Key words: Hereditary hydronephrosis, kidney disease, development of inbred mouse strain, autosomal recessive gene, pathological characteristic

INTRODUCTION

Hydronephrosis (HN) has been defined by many investigators as distension of the renal pelvis, renal calyces and diverticula of varying degrees. It can also be determined by a gross lesion of the kidneys. The dilatation of the pelvis and calyces was readily apparent on histopathological examination with atrophy of the renal parenchyma secondary to obstruction the urine outflow in most animals.

Observation of HN in laboratory mice has long been reported elsewhere for many years ago. Some have described strains of rats with unilateral right HN (Astarabadi and Bell, 1962; Sellers et al., 1960) whereas others have reported variably right or left side unilateral HN or bilateral HN (Warner, 1971; Lozzio et al., 1972). HN in STR/N inbred strain mice (Silverstein et al., 1961), NZC inbred mice (Warner, 1971; Collins et al., 1972), DDD inbred mice (Nakajima et al., 1983), C57BL/KsJ inbred mice (Lamont, 1991) and C57BL/6J-Mu inbred mice (Takano et al., 1973) were also reported. Almost of these strains are spontaneously mutation originate from inbred mice.

Since, researchers have been observed spontaneous HN in the ICR outbred mouse stock in routine health monitoring program. Researchers aim to develop a colony of inbred mice with congenital, persistent, bilateral HN suitable for such biomedical science studies by selective inbreeding for 20 generations. Subsequently, we purposed the lesion in this colony has been stable over several generations of inbreeding with a very high incidence of HN kidneys. Phenotypes determination, genetic segregation test crosses and histological study of kidneys were also performed.

MATERIALS AND METHODS

Housing: The animals were maintained in a clean conventional room. The room temperature was maintained at 22±2°C by air-conditioners. A cycle of 12 h artificial light and 12 h of darkness was maintained. Mice were housed in polycarbonate cages. The animals were fed ad libitum and provided a commercial mouse pellet diet and chlorinated water. The wood-shaving was used as bedding and were autoclaved before used. Animal studies were in agreement with Mahidol University policy for the care and use of animals for scientific purposes and approved by the institutional committee on ethics.

Method of inbreeding: The colony was initially started from 10 pairs of ICR outbred mice. All pups of first litter were killed and autopsied for observed affected kidneys. The pups from the second litter of the pairs which produced some hydronephrotic pups in their previous litters were used as the breeders for the first generation, beginning with brother and sister full-sib mating program.
In further subsequent generations, mating always conducted with the pups from the second litters after confirming the presence of HN among the pups of their first litter.

**Autopsy:** HN has been defined by many investigators as dilated renal pelvis and calyces of varying degree. HN can be determined by a longitudinal section of the kidneys in autopsy program. The dilatation of the pelvo-calyceal system was readily apparent on this cut section.

**Histological studies:** Longitudinal sections of normal, mild and the severe type hydronephrotic kidneys were fixed in buffered formalin. These specimens were embedded in paraffin, sectioned at 4 μ. The slides were stained with Hematoxylin and Eosin (H and E).

**Grading of hydronephrosis:** The degree of HN was scored as normal, mild and severe. Cases ranged from complete absence of HN (normal) through, varying degrees of pelvic dilation ranging from a suspicion mild to severe HN.

The affected kidneys had been phenotyped and scored for HN severity by defined as degree of renal pelvic dilation. In mild hydronephrotic kidneys, the swelling size of kidney was not very apparent but a dilatation of pelvocalyceal region was presence.

In extreme cases, swelling kidneys were usually marked and varying in size according to the degree of severity, the urine like fluid was replaced in the areas of grossly loss tissue. The fluid was spilling out from the swelling kidney when a cut section was made and the kidney was collapsed down due to the great loss of supporting tissues. In some severe cases, almost all tissues were loss and left the kidney as a transparent sac filled with yellow colored fluid (Fig. 1).

**Phenotyping:** Phenotypes determination for the Gpi-1, Trf, Es-1, Mup-1 and Hbb loci was carried out by using cellulose acetate electrophoresis (Hoffman, 1984).

**Segregation:** Genetic segregation tests were done between crosses HN inbred mice (F20) and free HN ICR inbred mice (F20) inter-crosses between F1 mice of the two parental strains and reciprocal back-crosses between F1 mice and their two parental strains mice.

**Statistical analysis:** ANOVA was used as a tool to detect the differentiation of quantitative variable among generation at the significance level of p<0.05.

**RESULTS AND DISCUSSION**

**Incidence of hydronephrosis:** Incidence of HN and inbreeding performances of HN mice through completion of inbreeding process from F0-F20. A direct relationship was observed between severity and incidence of HN and inbreeding process because % of HN in males and females, overall % HN in both sexes and on both kidneys were relatively increased as generation of inbreeding increased. But in contrast to this, the average litter size of the animals was decreased in later generations (from 13-8) due to inbreeding process. In addition, Table 1 shows that incidence of HN increased significantly with generation increased gradually in % of incidence of both sexes, overall incidence and in % incidence in both kidneys.

**Histological findings:** Histological findings were shown in Fig. 2. The pelvocalyceal region was found dilated in both mild and severe type hydronephrotic kidneys. In most severe cases, the papillary tissues became atrophy and were hanging in the lumen of dilated pelvis area. Gross tissues were loss in medulla and the urine like fluid replaced in these areas. The trapped-fluid compressed the medulla tissues and may causes renal tubules collapsed. The trapped fluid in pelvis region also changed the appearances of kidney shape largely distended and swelling.

**Phenotyping**

**Determination of phenotypes:** Phenotypes for Gpi-1, Trf, Es-1, Mup-1 and Hbb loci of two inbred mouse strains, HN and Free-HN (F20-21) were determination by cellulose acetate electrophoresis. The electrophoresis was carried out in two inbred mice strains, HN and Non-HN strain of F20-21 in 5 loci. Both of these inbred lines were originally derived from outbred ICR strain mice. The two lines were separated into hydronephrotic and free-hydronephrotic lines since, early generations. Selective inbreeding program was started in the late 1992. They were in F20-21 generations by the time this electrophoresis was done. Among the tested loci, a polymorphism found in Gpi-1
Table 1: Incidence of hydrenephrosis mice among 0-20 generations

<table>
<thead>
<tr>
<th>Gen (F)</th>
<th>Av. Lit. size</th>
<th>HN in male (%)</th>
<th>HN in female (%)</th>
<th>Overall % HN</th>
<th>HN on right (%)</th>
<th>HN on both (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>12.0001-3</td>
<td>73.4002</td>
<td>26.8001</td>
<td>39.6002</td>
<td>87.0001-3</td>
<td>13.0001-3</td>
</tr>
<tr>
<td>5-9</td>
<td>7.6001</td>
<td>82.000</td>
<td>18.000</td>
<td>45.6002</td>
<td>49.0001</td>
<td>51.2001</td>
</tr>
<tr>
<td>10-15</td>
<td>7.8000</td>
<td>84.700</td>
<td>15.300</td>
<td>56.2002</td>
<td>35.0006</td>
<td>65.2006</td>
</tr>
<tr>
<td>16-20</td>
<td>7.8000</td>
<td>99.000</td>
<td>15.000</td>
<td>93.0002</td>
<td>36.0001</td>
<td>64.0001</td>
</tr>
</tbody>
</table>

ANOVA p-value: 0.015, 0.045, 0.010, 0.000, 0.002, 0.008

Table 2: Genetic test crossings for hydrenephrosis gene

<table>
<thead>
<tr>
<th>Type of crossing HN</th>
<th>No. of cross</th>
<th>No. of born</th>
<th>Av. Lit. size</th>
<th>No. with animal HN</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parental crosses (Hm/hm x +/+)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male HN (F19) x Female free HN</td>
<td>4</td>
<td>56 (F1)</td>
<td>14.0</td>
<td>0/56</td>
<td>0.0</td>
</tr>
<tr>
<td>Male HN (F18) x Female free HN</td>
<td>5</td>
<td>36 (F1)</td>
<td>12.0</td>
<td>0/36</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Intermediate crosses (hm/+ x hm/+)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1 x F1</td>
<td>12</td>
<td>133</td>
<td>15.5</td>
<td>25/133</td>
<td>18.7</td>
</tr>
<tr>
<td><strong>Reciprocals Back Crosses (B/C) (Parental x F1) (B/C 1 +/+ x bm/bm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male free HN x Female F1</td>
<td>4</td>
<td>18</td>
<td>72.0</td>
<td>0/72</td>
<td>0.0</td>
</tr>
<tr>
<td>Female free HN x Male F1</td>
<td>9</td>
<td>11.8</td>
<td>87.0</td>
<td>0/87</td>
<td>0.0</td>
</tr>
<tr>
<td>B/C (2) bm/hm x bm/bm</td>
<td>10</td>
<td>10.0</td>
<td>100.0</td>
<td>36/100</td>
<td>36.0</td>
</tr>
<tr>
<td>Female HN x Male F1</td>
<td>12</td>
<td>10.3</td>
<td>118.0</td>
<td>61/118</td>
<td>51.7</td>
</tr>
</tbody>
</table>

Av. Lit. size: Average Litter size, HN and hm: Hydrenephrosis

Fig. 2: Severity grading of hydrenephrosis (mild to severe) and intact kidney, macroscopically and microscopically with H and E stained; a) In normal kidney, a normal texture of renal tubules is found at papillary region; b) In mild type hydrenephrosis kidney, tissues lost are found at papillary and renal pelvis areas. Renal fluid in the enlarge region are trapped and cause the pelvocalyceal dilatation and made tubules collapsed; c) In severe type of hydrenephrosis kidney, grossly loss of medullary and papillary tissue was observed. Trapped fluid made the tubules and tissues compressed with flattened epithelia and the appearance of kidney largely distended.

locus between the two strains as Gpi-1 a for HN strain and b for Free-HN strain but the rest were similar to its original ICR mice.

**Genetic test crosses:** Genetic test cross was shown in Table 2. Reciprocal crosses between two parental strains, HN inbred (F19) and Free-HN inbred strain (F18) were mated. Among 72 F1 weaning from these crosses, none was found hydrenephrotic kidney. In F1 intercrosses, out of 133 weaning of these crosses, 25 (18.79%) were found positive HN in their kidneys. In reciprocal back-crosses between F1 animals and Free-HN parental strain mice, a total of 159 animals produced, none of them were found hydrenephrotic kidney.

The other reciprocal back-crosses between F1 mice and HN parental mice, a considerable number of mice with hydrenephrotic kidneys were observed. Back-crosses between hydrenephrotic male mice (F20) and the female
mice from F1 group, produced 36.0% (36/100) while back-crosses between hydronephrotic female mice (F20) and male mice from F1, produced 51.75% (61/118).

Unilateral or bilateral HN is a relatively common finding in laboratory mice. It is usually regarded as an incidental finding, particularly if only 1 kidney is involved (Percy and Barthold, 2001). Previously, before ICR mice have been inbreeding, mild type HN were mostly observed on the right side kidneys in male mice. Rarely found in both kidneys or on left kidney alone or in female mice. After inbreeding, more percent of severely affected kidneys were observed and more incidence of both side affected kidneys in both sexes were also increased in the later generations. By this evidence, there is a marked relationship between the incidence and severity of HN and subsequent inbreeding process. Completion of 20 generations (F20), took about 5 years.

HN of the mice can be classified as an open hydronephrotic type which is the more common form contrast to obstructive type (Wallace and Spickett, 1967) because of unable to find the definite cause such as malformation of skeleton system and the obstructions along the urinary tract, especially indicated by hydro-ureter. Both sides of the kidney gradually developed hydronephrosis, there for the severity of lesion depended on age. By this reason, the affected animals showed no clinical abnormal signs because of the remaining renal reserve function. Similar to MRCH rats, an autosomal dominant trait for the bilirubin glucuronol transferase deficiency (Loizzo et al., 1972), notwithstanding the severity of renal damage, like that seen in animals with severe bilateral HN, the rat survive, grow and reproduce in obviously healthy condition.

The phenotype indicated the difference of a and b on Gpi-1 loci between two inbred mouse strains. Since, Gpi-1, Glucose phosphate isomerase 1 (multifunctional protein), act in the development of somatosensory and motoric neural structure (Repiso et al., 2008). Unfortunately there is no report the association between Gpi-1 and HN, unless Gpi deficiency related to nonspherocytic hemolytic anemia in human (Beutler et al., 1997) and neurological impairment (Repiso et al., 2008). However, this polymorphism could be useful for a Marker gene among the two strains in future genetic identification.

Genetic test cross revealed that the gene controlled the HN model is a recessive gene and gene interaction between HN and free HN animals is followed the Mendalian's trait of inheritance with the true expected values. Researchers therefore, concluded a bilateral HN-bearing ICR mice which are recessively inherited have been established by full-sibling mating. Several mouse HN models related to recessive gene have been reported and suggested that phenotype trait was controlled by polygenes, e.g., Bone morphogenetic protein (B mp4) (Miyazaki et al., 2000), Glial Derived Neurotrophic Factor (GDNF) and ret-K (Schuchardt et al., 1996), murine forkhead/winged helix gene (Kume et al., 2000) and Fox-2 (Favor et al., 1996) moreover with some effect of hormone, e.g., sex hormone and rennin angiotensin system, etc. (Goto et al., 1984; Horton et al., 1988; Susie et al., 1975; Taushi and Kanshara, 1996; McDill et al., 2006).

Generally, there are three types of experimental animal models for fetal renal disease including HN, base on spontaneous mutation, surgical model and knockout model (Peter, 2001). Hereditary and spontaneous mutation has been described in many strain, e.g., STR/N, NZC, C3H, C57BL, DDD and eph, mice (Cohen et al., 1970; Nakajima et al., 1983; Goto et al., 1984; Horton et al., 1988). Some of these models including the mice therefore raise the question of whether the appearance of HN may occur without a hydrodynamic obstruction. Although, this model has not been yet describes as showing spontaneous resolution as seen in the human, researchers yet consider that this inbred strain of HN mice would be a useful animal model for the urologists, pathologists, especially for the study of experimental HN. At least advantage of this model is its mechanistic precision and the ability to manipulate it's functionally that gradually developed in human being.

CONCLUSION

The results indicated that autosomal recessive gene controlled the inheritance of these non obstruction type hydronephrosis mice. In all cases did not appear to have had a marked effect on survival. These mice may accordingly be a suitable laboratory animal model in which to further explore the detailed process whereby this condition might be shared some pathological characteristic between man and animals.

REFERENCES


