Inheritance of Guttural Pouch Tympany in the Arabian Horse

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Abstract

The objective of the present study was to analyze the mode of inheritance of guttural pouch tympany (GPT) using pedigrees of Arabian horses. Complex segregation analyses were employed to test for the significance of nongenetic transmission and for monogenic, polygenic, and mixed monogenic-polygenic modes of inheritance. Horses affected by GPT comprised 27 Arabian purebred foals. Of these 27 animals, 22 were patients at the Clinic for Horses, School of Veterinary Medicine Hannover, Hannover, Germany, between 1994 and 2001 and 5 Arabian foals were from stud farms. Information on the pedigrees of these patients allowed us to classify the affected foals into four families with a total of 276 animals. The regressive logistic model analysis took into account the nonrandomness of the pedigrees through multiple single ascertainment correction. The complex segregation analysis showed that, among all other models employed, a polygenic and a mixed monogenic-polygenic model best explained the segregation of Arabian foals with GPT. Models including only nongenetic distributions and monogenic inheritance could be significantly rejected. This is the first report in which a genetic component could be shown to be responsible for GPT in horses.

The equine guttural pouch is a large, air-filled diverticulum of the auditory tube that occupies a large area in the caudal portion of the head (Freeman 1980; Hawkins 1992; McCue et al. 1989). Other mammals that possess a diverticulum of the auditory tube include Tapiridae, Rhinoceritidae, Hydracoida, some species of microchiropterans, and the South American forest mouse (Baptiste 1998). The guttural pouch of the horse is the largest of any mammal. The strikingly unusual shape and size of the equine guttural pouch has led to many theories on additional functions besides those known for the auditory tube. There is increasing evidence for the hypothesis that guttural pouches have evolved as a brain-cooling mechanism. Respiratory air can enter the guttural pouch to ventilate and cool blood in the internal carotid arteries destined for the brain (Baptiste 1998; Baptiste et al. 2000).

An abnormal inflation of one or both guttural pouches with air can occur in foals, but is rare in older horses (Gaughan and Debowes 1993; Hardy 1991; Knottenbelt and Pascoe 2000). This pathological condition is called guttural pouch tympany (GPT), and surgical treatment is necessary for affected foals to recover from this disease. GPT can sometimes be life-threatening without appropriate veterinary treatment. Foals surgically treated for GPT have a good prognosis for recovery and to achieve their full performance later in life (McCue et al. 1989; Ohnesorge et al. 2001).

The cause of GPT is unknown. It is believed that GPT develops when air cannot leave the guttural pouch through the pharyngeal orifice. However, there is no information on the pathogenesis of GPT. A possible explanation for the development of GPT may be the presence of a mucosal flap of abnormally large size at the pharyngeal orifice. Another reason could be a functional failure of the pharyngeal orifice, trapping air in the guttural pouch (McCue et al. 1989; Tate et al. 1995). In both cases, the mucosal flap acts as a one-way valve that lets air enter the guttural pouch, but then traps the air there (Barber 1999).

Fillies are more often prone to GPT than colts. The ratio of fillies to colts given in the literature varies between 4:1 and 2:1 (Gaughan and Debowes 1993; Hawkins 1992; McCue et al. 1989; Ohnesorge et al. 2001). GPT is observed in several breeds, including standardbred trotters, English thoroughbred, Arabian, quarter horse, appaloosa, paint horse, and American saddle horse (Deen 1988; McCue et al. 1989; Ohnesorge and Deegen 1995; Tate et al. 1995; Tetens et al. 1994). At the Clinic for Horses, School of Veterinary Medicine Hannover, 51 foals affected by GPT were treated between 1994 and 2001. Of these 51 cases, 24 foals descended from Arabian horses, and for 22 Arabian foals, pedigree information could be collected at the stud farms where the foals were born. The other foals were progeny of
several other horse breeds and therefore were not used for this analysis. A striking feature of the Arabian foals with GPT was that some of these animals were from the same stud and some of these foals were full or half sibs. Thus a possible genetic influence on the occurrence of GPT was suspected.

The objectives of this study were to analyze the importance of genetic influences on the occurrence of GPT in the Arabian horse, and if a genetic component was found, to test for the mode of inheritance using complex segregation analysis. In addition, the effects of inbreeding and sex were tested for significance.

Materials and Methods

Diagnosis and surgical treatment of foals affected by GPT was performed at the Clinic for Horses between 1994 and 2001. Of the 24 cases in Arabian foals, pedigree information for 22 Arabian foals could be collected at the stud farms where these foals were born. No pedigree information was available for two Arabian foals. The pedigree included ancestors, offspring, half and full sibs, as well as sex and birth dates of relatives for up to six generations. In total, using the pedigree and disease status information from the stud farms, there were an additional 5 animals affected by GPT as foals, 144 animals reported as unaffected by GPT, and 105 animals with unknown GPT status; the data collected allowed us to build four pedigrees with 3 to 9 affected foals, including the 22 foals that had been patients at the Clinic for Horses and the 5 cases of GPT that were not patients of the clinic. For these five cases, veterinary protocols were collected to confirm the report of the owner. The 27 foals with GPT (22 patients at the Clinic for Horses and 5 foals from stud farms) descended from 19 sires and 22 dams. There were five sires and four dams with more than one affected foal. A survey of the four pedigrees that include the 27 affected foals is given in Table 1. The four pedigrees are shown in Figure 1.

Table 1. Survey of pedigrees used for complex segregation analysis of GPT in Arabian foals

<table>
<thead>
<tr>
<th>Family</th>
<th>No. of foals with GPT (females)</th>
<th>No. of full/half sibs with GPT</th>
<th>No. of unaffected animals</th>
<th>No. of animals with unknown status for GPT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8 (7)</td>
<td>2/3</td>
<td>31</td>
<td>33</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>7 (5)</td>
<td>—/2</td>
<td>51</td>
<td>27</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>9 (6)</td>
<td>2/—</td>
<td>49</td>
<td>27</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>3 (1)</td>
<td>2/—</td>
<td>13</td>
<td>18</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>27 (19)</td>
<td>6/5</td>
<td>144</td>
<td>105</td>
<td>276</td>
</tr>
</tbody>
</table>

Figure 1. Pedigrees for the four families including the 27 Arabian foals affected by GPT. ◆ = unaffected female; □ = unaffected male; ● = affected female; ■ = affected male; ○ = unknown status for GPT; * = animal used for ascertainment correction; RP = animal duplicated in the graphical representation of the pedigree.

Statistical Methods

The influences of sex and inbreeding coefficient on the incidence of GPT in the affected foals studied here and their contemporary full and half sibs with known status for GPT were tested using the GENMOD procedure of SAS version 8.2 (SAS Institute 2002). The generalized linear model including both factors as fixed effects is as follows:
where \( y_{ijk} \) is the observed status for GPT (0 = unaffected, 1 = affected by GPT) of the \( ijk \)th foal and \( e_{ijk} \) represents the random residual effects. We used a probit link function for the binomial dependent variate. There were a total of 57 male foals, 8 of which were affected by GPT; the corresponding numbers for females were 76 and 19, respectively. The effect of inbreeding was divided into three classes: the inbreeding coefficient was 0 to less than 6% in 43 animals, 6% to less than 12% in 58 animals, and 12–34% in 32 animals.

Heritability was estimated using a linear residual maximum likelihood (REML) model and a Bayesian analysis employing a threshold model. The additive genetic effect of the animal, including all additive genetic relationships of the available pedigrees, was added to the model outlined above. The numerator relationship matrix included 697 animals. We included here other animals without known status for GPT to increase the information for the relationship matrix, but these additional ancestors could not be used to establish larger families for the affected foals in the segregation analysis. These animals were determined from the stud book records. The estimation of heritability in the linear model was performed using VCE4 (Groeneveld 1998); the beta version of the program MTGSAM (Van Tassell and Van Vleck 1996) was used for the threshold model analysis. The model was as follows:

\[
y_{ijkl} = \mu + \text{sex}_i + \text{inbreeding coefficient}_j + a_{kl} + e_{ijkl},
\]

where \( a_{kl} \) is the random additive genetic effect of the animal distributed with zero mean and variance \( \text{var}(a) = A\sigma^2_a \), where \( A \) constitutes the relationship matrix. The heritability estimate \( h^2 \) is given by \( h^2 = \sigma^2_a / (\sigma^2_a + \sigma^2_e) \), where \( \sigma^2_a \) is the additive genetic variance and \( \sigma^2_e \) is the residual variance.

The pedigrees were analyzed employing class A regressive logistic models (Bonney 1986). The REGD procedure of SAGE version 3.0 (SAGE 1997) was used to test the mode of inheritance for GPT in Arabian horses. Multiple single ascertainment correction was applied because the pedigrees were not randomly sampled. In each pedigree, ascertainment correction was performed for the probands, that is, the foals affected by GPT through which the pedigree entered the analysis. GPT was treated as a dichotomy-dependent variable, with unaffected animals coded 0 and affected animals coded 1. A complete grid search for starting values was used to validate the results obtained by different models. Hypotheses tested for the mode of inheritance were as follows: for a single phenotypic distribution (\( \mu \)) without any genetic component; for two phenotypic distributions (\( \mu_0 \)) attributed to the effect of the sex; monogenic inheritance with one gene locus and two alleles in Hardy-Weinberg equilibrium; polygenic inheritance; mixed major gene inheritance with a polygenic component and an independently segregating major gene locus with two alleles.

The polygenic component was taken into account for regressive familial effects. These were estimated using logistic regressions of the GPT status of offspring on the phenotype of sire and dam. In addition, the effect of the mating partner (spouse) was used to allow for a correlation among mating partners. So the phenotype of one specific mating partner of each couple was always regressed on the phenotype of the other mate. The mating partners were included in our analysis, as there were loops between mating partners in the pedigrees which would otherwise have been omitted. SAGE version 3.0 also allows the generation of additional sibship effects. A sib-sib correlation was not used because it is very unlikely that GPT in one foal influences the expression of GPT in other full sibs.

Likelihood ratio tests were used to evaluate the goodness-of-fit of the model to the data. Therefore a saturated model was defined with no restrictions on parameters used in the model. The likelihood ratio test statistic compares a specific null hypothesis (\( H_0 \)) defined by a restricted model against a saturated (most general) model. The test statistic is given by the difference of the log-likelihoods multiplied by \(-2\). The ratio of log-likelihoods asymptotically follows a chi-square distribution, and significance levels can be obtained by using this distribution. Degrees of freedom are given by the difference of independently estimated parameters for the models compared. The information criterion of Akaike (AIC) was used as an additional measure to choose the sparsest model with the best fit to the data. AIC is given by the log-likelihood multiplied by \(-2\) plus two times the number of independently estimated parameters. The model with the smallest AIC fits the best with a minimum number of parameters, but all hypotheses that cannot be rejected against the most general model using the likelihood ratio test must also be considered as possible. The AIC criterion can be used to differentiate between alternative models with different numbers of parameters to be estimated if more than one hypothesis is accepted which was tested against the most general model. Nevertheless, the AIC criterion cannot be used to exclude a hypothesis if the model was not rejected against the most general model by using the likelihood ratio test.

**Results**

The analysis of variance (ANOVA) showed that the effects of sex and inbreeding coefficient were not significantly different from zero (Table 2). The fillies exhibited an affection rate of 23.7% with a 95% confidence interval (CI) ranging from 15.1% to 34.5%, and the colts had a rate of 11.8% with a 95% CI ranging from 5.3% to 22.6%. Foals with an inbreeding coefficient between 6% and 12% had a frequency of 23.2% (95% CI 13.7–35.5%) for GPT, whereas animals with a lower (0–6%) or higher (12–34%)
inbreeding coefficient had a frequency of 19.9% (95% CI 10.1–33.9%) and 10.2% (95% CI 3.2–24.5%), respectively.

The additive genetic and residual variance in the linear model amounted to \( \sigma_a^2 = 0.03 \) and \( \sigma_e^2 = 0.078 \), respectively, resulting in a heritability estimate of \( h^2 = 0.274 \pm 0.232 \). The large standard error of the heritability estimate is due to the small number of animals with known disease status for GPT. The corresponding estimates for the additive genetic and residual variance and the heritability in the threshold model were \( \sigma_a^2 = 5.38 \), \( \sigma_e^2 = 1 \), and \( h^2 = 0.492 \pm 0.284 \), respectively.

The likelihood ratio test statistic rejected the models which accounted for only one phenotypic distribution (\( \mu_1 \) model) or the sex effect (\( \mu_2 \) model) (Table 3). Models accounting for monogenic inheritance were not suited for the pedigrees analyzed here. The polygenic and major gene models were not rejected in favor of the most general model and fitted the data significantly better than the environmental models.

Table 3. Complex segregation analysis using regressive logistic models and ascertainment correction for occurrence of GPT in Arabian horses

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>(-2 \ln L)</th>
<th>df</th>
<th>AIC</th>
<th>(\chi^2)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated model</td>
<td>39.28</td>
<td>16</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(\mu_1) model</td>
<td>134.17</td>
<td>2</td>
<td>136.17</td>
<td>94.89</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>(\mu_2) model</td>
<td>132.74</td>
<td>2</td>
<td>136.74</td>
<td>93.46</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Monogenic models</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant gene effect</td>
<td>131.79</td>
<td>5</td>
<td>141.79</td>
<td>92.51</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Recessive gene effect</td>
<td>130.97</td>
<td>5</td>
<td>140.97</td>
<td>91.69</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Arbitrary gene effect</td>
<td>130.65</td>
<td>7</td>
<td>144.65</td>
<td>91.37</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Polygenic model</strong></td>
<td>45.43</td>
<td>7</td>
<td>59.43</td>
<td>6.15</td>
<td>.725</td>
</tr>
<tr>
<td><strong>Mixed models</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant major gene effect</td>
<td>45.41</td>
<td>10</td>
<td>65.41</td>
<td>6.13</td>
<td>.410</td>
</tr>
<tr>
<td>Recessive major gene effect</td>
<td>45.41</td>
<td>10</td>
<td>65.41</td>
<td>6.13</td>
<td>.410</td>
</tr>
<tr>
<td>Arbitrary major gene effect</td>
<td>45.41</td>
<td>12</td>
<td>69.41</td>
<td>6.13</td>
<td>.190</td>
</tr>
</tbody>
</table>

\(-2 \ln L\), \(-2 \log\)-likelihood; df, degrees of freedom; AIC, information criterion of Akaike; \(\chi^2\), compares the model tested against the most general (saturated) model.

The comparisons between the model with only phenotypic distributions and the models accounting for monogenic, polygenic, or mixed monogenic-polygenic inheritance revealed that the polygenic and mixed monogenic-polygenic models differed significantly, whereas the models with monogenic inheritance could not explain significantly more variance than the environmental model (Table 4).

Table 4. Comparison of the \(\mu_1\) model with models accounting for polygenic or mixed monogenic-polygenic inheritance

<table>
<thead>
<tr>
<th>Hypothesis</th>
<th>(-2 \ln L)</th>
<th>df</th>
<th>(\chi^2)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu_1) model</td>
<td>132.74</td>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Polygenic model</td>
<td>45.43</td>
<td>7</td>
<td>87.31</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mixed models</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant major gene effect</td>
<td>45.41</td>
<td>10</td>
<td>87.33</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Recessive major gene effect</td>
<td>45.41</td>
<td>10</td>
<td>87.33</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Discussion

We were able to perform a genetic analysis for GPT in Arabian foals because the cases treated at the Clinic for Horses and collected at stud farms were not isolated, but were connected through their ancestors. According to Knottenbelt and Pascoe (2000), thoroughbred foals may be at a higher risk for GPT than foals of other breeds. Our present study showed that a genetic component significantly contributes to the development of GPT in Arabian foals. The segregation analyses revealed that models with only phenotypic distributions could be significantly rejected against models including polygenic or mixed monogenic-polygenic inheritance. However, the data did not provide evidence for a simple monogenic inheritance because this hypothesis could be rejected against the most general model. The large standard errors of the heritability estimates using the variance component approach can be explained by the large degrees of freedom necessary in the animal model for the parameter estimates and the small numbers of animals with observations. In contrast to the variance component estimation, the regressive models need fewer parameters for modeling the polygenic component and are therefore more robust in statistical tests.

According to the reports in the literature (Freeman 1990) and our data, GPT is a rare disease, and therefore our sampling scheme was efficient for pedigree analysis. The nonrandomness of the pedigrees analyzed was taken into account by the multiple single ascertainment correction. Neglecting the ascertainment bias may lead to inconsistent results if the study is repeated with an independently collected dataset. Furthermore, the results of the segregation analysis may be biased if ascertainment correction is omitted and may result in invalid conclusions.

Additional analyses using the monogenic model and with the transmission probability \(\tau_{AB}\) as an estimable parameter in the model showed that the estimates for \(\tau_{AB}\) were significantly different from \(\tau_{AB} = 0.5\). Therefore a monogenic segregation of GPT can obviously be excluded in our pedigrees. Apparently development of GPT seems to be under the control of more than a single gene. How many genes may contribute to the expression of GPT in Arabian foals cannot yet be determined, but we are now able to
predict breeding values for parents for the expected incidence of GPT in future progeny. Continued collection of data for all Arabian foals affected by GPT will be necessary to produce efficient selection against this disease and to lower the possibility for GPT in the Arabian horse population of Germany. Using these breeding values, the risk for a stallion or a broodmare to produce foals affected by GPT in a specific mating can be predicted, and thus recommendations can be made to avoid matings among parents that will transmit genes predisposing their offspring to GPT.

A very rough estimate on the incidence in the German Arabian horse population can be deduced from our data. At the Clinic for Horses, the number of Arabian foals affected by GPT was 22, and approximately 282 stud farms with an average of about three foals born per year are clients of the clinic. Given that our patients are a random sample of all German Arabian foals, a very rough estimate for the incidence of GPT in the German Arabian foal population is given by \( 22 / (282 \times 3 \times 8 \text{ years}) = 0.325\% \). A lower limit of the proportion of foals affected by GPT can be estimated using the relation between affected Arabian foals and the total number of Arabian foals registered between 1994 and 2001 in Germany. This estimate is \( 22 / 13,488 = 0.163\% \). These very roughly estimated proportions indicate that GPT may be a rare inherited disease in Arabian foals.

Another interesting point would be to use molecular genetic approaches to characterize genes or genome regions involved in the development of GPT. A combined approach using pedigree and marker information would be very useful for identifying quantitative trait loci for GPT and determining the gene loci involved in the expression of GPT. The chances of detecting such genome regions depend on the size of variance explained by the genetic component and how large an effect each gene locus has. The probability may increase the more rarely an inherited disease is observed in the total population and the more cases are available from segregating families and the larger the effects of a major gene on the occurrence of GPT are. In this case, carriers of the disease may harbor mutations in a gene that originates from one or only a few founder animals, and therefore their offspring may be well distinguished in the genome regions around the mutated gene from the other animals in the same population without this mutated gene. Identification of the genes responsible for GPT would be helpful to prevent this disease more efficiently and to gain more insight into the biological function and development of the guttural pouches.

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