F2-FAMILIES FROM EUROPEAN AND CHINESE BREEDS AS SUITABLE MODEL FOR THE MAPPING OF DISEASE RESISTANCE IN SWINE.

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Introduction

Detecting and improving disease resistance by traditional phenotype selection can hardly separate environmental and genetic effects. Thus the aims of modern genomic approaches are to shift the selection criteria from phenotypically expressed disease status to allele status at the DNA level and to get insight into the molecular mechanisms of resistance/susceptibility against certain infectious dieseases. Mapping of disease resistance loci by linkage analysis needs to adress three major requirements: a) an informative disease model and a suitable inbred line, i.e. founder populations differing as much as possible in their inherited degree of resistance against the disease of interest and in their marker phenotypes, b) informative disease records, gained under a highly standardized environment, c) linkage maps, moderately populated with informative markers.

Our current disease models are dealing with the *Pseudorabies Virus (PrV)*, *Sarcocystis miescheriana* and *Salmonella typhimurium*. European breeds like Pietrain or Large White and the chinese Meishan breed as founders, and their F2-crosses have proved a suitable and informative animal model. The present work describes significant differences in resistance/susceptibility between founder breeds in the three disease models, the status of phenotypic and genetic evaluation of the F2-crossbreds, and the way to the mapping of disease resistance loci as QTLs.

Material and Methods

Animals and pretesting: Purebred chinese Meishan pigs were available from a herd of the University of Stuttgart-Hohenheim. They were compared with commercial Large White or Pietrain pigs to check for suitability regarding potential disease models in clinical studies (PrV: Federal Research Center for Virus Diseases of Animals [BFAV] in Tuebingen; S. miescheriana: experimental station "Unterer Lindenhof" of the University of Stuttgart-Hohenheim; S. typhimurium: Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig).

F2-families: Significant differences have been found between european and chinese breeds for all three models; so, F2-families were set up to detect responsible disease resistance loci as QTLs by linkage analysis, starting with PrV and S. miescheriana.

F2-crossbreds were produced at the experimental station "Unterer Lindenhof". Litters were housed and fed under standardized conditions.

Challenge, sampling and investigation: At an age of around 12 weeks, piglets were challenged at the BFAV (*PrV*) or the Dep. of Swine Diseases in Giessen (*Sarcocystis*), rsp.

All F2-pigs were clinical examined and sampled to describe onset, course, degree and outcome of disease as accurately as possible.

Statistical analysis: Variance of clinical, haematological and clinical-chemical traits have been analysed with the Statistical package for Social Sciences (SPSS/Pc).

In the case of *Sarcocystis*, heritabilities for these traits have been evaluated with the CVE-version 4.2.5 (Groeneveld).

Genotyping: 110 microsatellite markers were selected from the public maps, based on their position, ease of scoring, and informativity. Markers were evenly spaced on the 18 porcine autosomes and the pseudoautosomal region of the X-chromosome to give maximal marker-intervals of less than 40 cM. 85 of them were established for genome-wide linkage mapping in PrV. In case of Sarcocystis, establishing of amerkers and genotyping has just started.

Linkage and QTL-analysis (PrV): Linkage was analysed with the software package CRIMAP. Clinical and genotyping data were combined, and QTL-analysis was done according to an interval mapping strategy with a monolocus regression analysis (Haley et al., 1994).

Results

Significant differences in resistance/ susceptibility between founder breeds were obvious in all three disease models.

PrV: The PrV model showed significant differences in resistance/susceptibility to PrV between european Large White and chinese Meishan pigs. After intranasal challenge with a highly virulent PrV-strain, all pigs developed clinical signs, e.g. fever from days 3 to 7 p.i. All purebred Large White pigs, all F1 and 75% of the F2 generation developed neurological symptoms at days 5 to 7 p.i. and died or had to be euthanized. The purebred Meishan pigs and 25% of the F2-pigs did not develop any signs of neurological disorder and reconvalescended until days 8 or 9 p.i..

Rise in temperature in F_2-animals started two days p.i.. At day three, two groups of different temperature response could be distinguished: one showing a quick rise, reaching temperatures of about 41°C, and a second group rising slowly and staying beyond 40.5°C. Temperature profiles and the appearence/non-appearance of neurological symptoms were not correlated.

QTLs (fig. 1) for appearence/non-appearence of neurological symptoms were found on chromosomes SSC9, SSC5 and SSC6. Together they explained 84% of the F2-response. Further significant QTLs, associated with immune-dependent rise in body-temperature have been found on chromosomes SSC2, 4, 8, 10 and 11 (Reiner et al., 2002b).

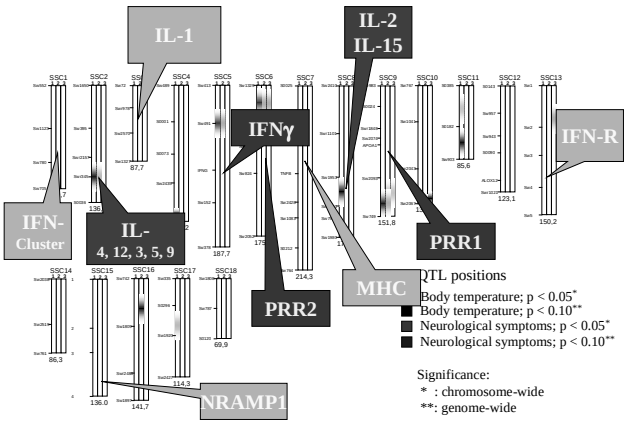

Fig. 1: Genome-wide mapping results for QTLs on body temperature and neurological symptoms in F_2-pigs after intranasally challenge with 10^5 pfu of the PrV-strain NIA3 at the age of 12 weeks. QTLs are indicated as marked areas on the chromosomes.

S. miescheriana: Significant differences appeared in clinical, serological, haematological and parasitological findings of Pietrain (PI) and Meishan (ME) founder piglets. after challenge with S. miescheriana. The major discriminating period post infection (p.i.) was between days 42 and 45 (Reiner et al., 2002a). Severity of signs was negatively correlated with specific immunoglobulin titres during the first 3 weeks p.i. and positively with the load of merozoites in muscle tissues, the latter being 20 times higher in PI than in ME. Sarcocystis-specific variances in F2-pigs showed significant shares of additiv-genetic variance, with moderately up to high heritabilities, supposing the existence of significant disease resistance loci in this model. Genotyping experiments for QTL-analysis have just been started.

S. typhimurium: The status of elucidation of the Salmonella-model still is at its beginning. After an oral challenge of purebred weaners, clinical diarrhoe scores decreased faster in Meishan piglets than in commercial hybrid piglets.

Discussion

PrV: QTLs found in this study point to gene effects on a) the appearance/non-appearance of neurological symptoms and b) QTLs for temperature course and thus immunological response after challenge with PrV. Major QTLs on SSC9 and SSC6 are linked with the loci PRR1 and PRR2. Both receptor proteins are involved in adsorption and penetration of the PrV to the cell in rodent models. Initiation of infection by alphaherpesviruses requires a cascade of interactions between different viral and cellular membrane components. Linked QTLs presented in our study stimulate to investigate these genes more specifically. Specific immunology against herpesviridae seems to be sustained by the Interleukin 12 (IL12) – INFg-pathway. The IL12-gene is located within an interleukin cluster on SSC2, close to a region associated with a QTL on temperature course p.i.. Further QTLs are linked with the INFγ-locus (SSC5).

Since our study elucidates genetic differences in resistance/susceptibility against PrV between Meishan and Large White pigs, these genetic diverse breeds are informative to elucidate the role of host defense against PrV in swine.

S. miescheriana: The present work describes clinical, clinical-chemical and haematological data, with definite clues for genetically determined differences in resistance/susceptibility against this protozoan parasite. The data highlight the suitability of this model to further analyse chromosomal regions, candidate genes and thus the molecular basis of host-parasite interaction.

S. typhimurium: More detailed studies are needed to define more precisely differences in resistance/ susceptibility against this very important pathogene and to examine if a specific F2-approach would have enough power to find associated disease resistance loci.

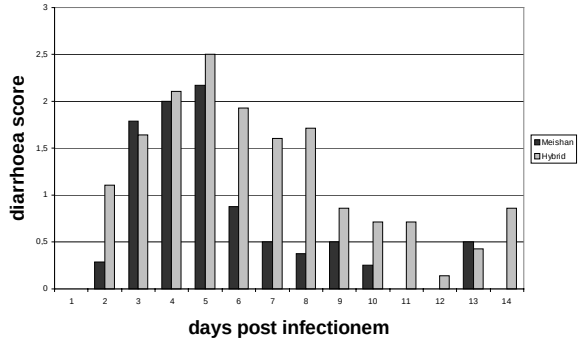

Fig. 2: Diarrhoea score of Meishan and Hybrid pigs after oral challenge with S. typhimurium.

Conclusion

Our current disease models are dealing with the *Pseudorabies Virus*, *Sarcocystis miescheriana* and *Salmonella typhimurium*. Elucidation status of the models is quite different, but all three models show that european breeds like Pietrain or Large White and the chinese Meishan breed make a suitable and informative animal model for disease resistance in differing kinds of host-parasite interactions. Exemplary, the PrV-model shows the way from clinical differences in resistance/ susceptibility against a specific disease towards disease resistance loci. Further research, including fine mapping of candidate genes and the evaluation of responsible gene variants may lead to a better understanding of pathogenesis and to a better prophylaxis based on less suceptible pigs.

Acknowledgements

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