SNP genotyping and association: Genomic DNA isolated from blood samples of 100 half-sibling horses (cases HOARSI 3, 4; $N = 43$ and controls: HOARSI 1; $N = 57$) were screened for SNPs in all eight exons and their flanking regions; this identified 17 SNPs (Tables S1 and S2).

To study the combined effect of the SNPs, haplotypes were reconstructed (MERLIN) and the paternal haplotype (HP1 and HP2) was identified for each horse. HP1 (23223233322133221) was significantly more prevalent than HP2 (23121431322133221) in the RAO-affected individuals. The prevalence of HP1 among the 43 RAO-affected horses was 60.4% versus 39.6% in the 57 control horses ($P = 0.000193$). The odds ratio for horses with paternal HP1 was 4.98 times more likely to have RAO than horses with HP2.

As the analysed horses were paternal half-sibs, the LD on the paternal chromosomes is expected to be long, and consequently, mapping resolution is very limited. Therefore, we also tested the association at all 17 SNPs separately for the paternal and maternal alleles. None of the markers showed an association for the maternal alleles ($P > 0.05$).

Therefore, we conclude that the association of the paternal alleles is most likely caused by the extensive LD that is to be expected among paternal half-sibs. The fact that the maternal alleles are not associated indicates that *IL21R* is probably not causally involved in RAO.

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References

1 Ramseyer A. *et al.* (2007) *J Vet Intern Med* **21**, 149–56.
2 Swinburne J. E. *et al.* (2009) *Mamm Genome* **20**, 504–15.
3 Klukowska-Rötzler J. *et al.* (2011) *Anim Genet*, DOI: 10.1111/j.1365-2052.2011.02277.x.
4 Fröhlich A. *et al.* (2007) *Blood* **5**, 2023–31.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1. *IL21R* PCR primers used in this study.

Table S2. SNPs in the equine *IL21R* gene analysed in this study.

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Identification of a sex-linked marker for channel catfish

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Source/description: Fifty-three additional markers (EST and BAC end sequence) linked to the sex-determining region (U6, LG-14)[1–3] in a previous catfish linkage map were genotyped using sexually mature progeny generated by an F_1 channel catfish (*Ictalurus punctatus*) | blue catfish (*I. furcatus*) hybrid backcrossed for two generations to channel catfish (F_2-3 ♂ | Kansas Ch-606 ♀). Thirty-two males and 32 females from this family were sexed based on external genitalia. Blood samples were then collected for genotyping.

PCR conditions: PCR was performed in a 5-μl reaction mixture containing 1 μl of 50 ng/μl genomic DNA (Gentra Puregene kit), 0.5 μl of 10 | PCR buffer, 0.2 μl of 50 mM $MgCl_2$, 0.4 μl of 2.5 mM dNTP's, 0.2 μl of 10 pmol/μl forward primer (with tailed primer 5′GAGTTTTCCCAGTCACGAC3′ added at the 5′ end), 0.3 μl of 10 pmol/μl reverse primer, 0.1 μl of 1 pmol/μl primer label IRD700 and 0.05 μl of 5 U/μl of Platinum Taq polymerase. A touchdown PCR profile was performed as described by us previously.[1]

Sex-linked marker screening: Of the 53 screened markers, 25 markers showed evidence of linkage with sex. These markers were then screened using 20 sex-known, pond-run individuals each from two strains [Marion (MC) and Auburn | Rio Grande channel catfish (AR)]. Marker *AUEST0678* (forward primer: 5′ACATCGCTTTGAGAAGC TGC3′; reverse primer: 5′GTGAA TGTGAGACTAACAGGAGG3′) demonstrated 100% genotyping accuracy in correctly identifying the sex of the screened samples of both strains. Analysis of this marker was expanded to two additional strains [Tishomingo (TM) and Goldkist | Forks Albino (GF) channel catfish]. The same specific banding pattern was observed in all four tested strains (20 individuals each). One shared band (~212 bp) was found in both males and females, while a 205-bp product was observed only in male channel catfish (Fig. 1a).

Utility of AUEST0678 *marker in juvenile fish:* Forty individual fingerlings (size 7.5–17 cm) were sexed based on external characteristics and the presence of immature gonads. Whereas external sexing corresponded with internal anatomy in 70% of individuals, genotyping using the *AUEST0678* marker provided 100% accuracy, with banding patterns identical to those observed previously (Fig. 1b).

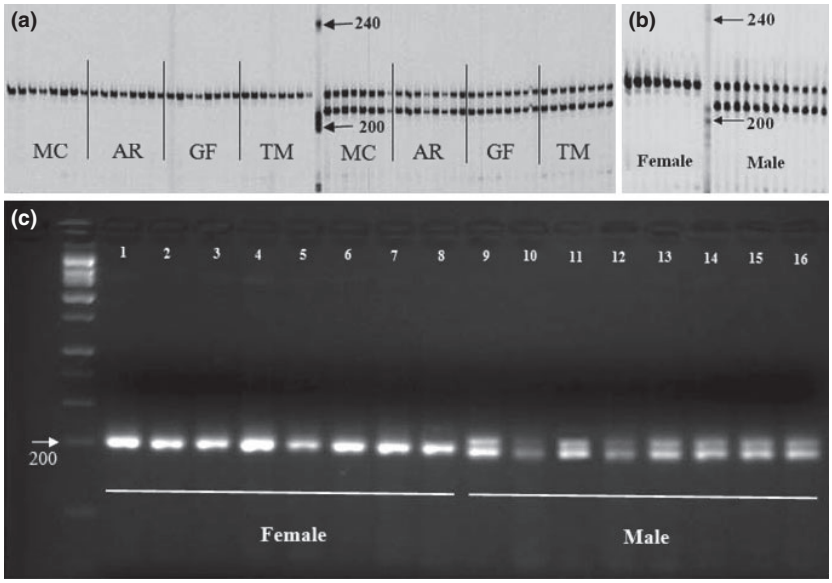

Figure 1 The AUEST0678 marker allows accurate sex-typing in channel catfish. (a) Representative females (left) and males (right) of four different strains of channel catfish running on 7% denaturing polyacrylamide gel with size marker indicated in the centre of the gel; (b) eight female and 12 male sacrificed, sex-verified Marion channel catfish fingerlings running on 7% denaturing polyacrylamide gel; (c) female (lane 1–8) and male (lane 9–16) channel catfish analysed on 3% agarose gel stained with ethidium bromide. Note that on a denaturing polyacrylamide gel, the longer autosomal allele ran slower than the shorter Y-chromosomal allele, as expected; however, on a non-denaturing agarose gel, the two PCR products (6 bp different in length) were detected as a single faster-migrating band, whereas the heteroduplexes formed between them were detected as a slower-migrating band.

Quick and economical way of sex genotyping: Using the same PCR protocol as above (without a tailed primer and label) and running the PCR products on a 3% agarose gel stained with ethidium bromide allowed the visualization of the sex-specific banding pattern (Fig. 1c). Amplification of both autosomal and Y-chromosomal bands in males resulted in the presence of two PCR products that share sequence homology except that the Y-chromosomal band was shorter. As a result, heteroduplexes of the two molecules formed during PCR[4] allowed for the detection of two bands: the slower-migrating band corresponded to the heteroduplexes, while the faster-migrating band corresponded to the two original DNA segments. Because these two DNA fragments were just 6 bp different in length, they were detected as a single band on a 3% agarose gel. Therefore, this simple heteroduplex analysis using agarose gels is a convenient way for sexing channel catfish.

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References

1 Kucuktas H. *et al.* (2009) *Genetics* **181,** 1649–60.
2 Lu J. *et al.* (2011) *Nucleic Acids Res* **39,** 815–21.
3 Waldbieser G. C. *et al.* (2001) *Genetics* **158,** 727–34.
4 Glavac D. & Dean M. (1995) *Hum Mutat* **6,** 281–7.

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Development of EST-SSRs in Japanese scallop (*Mizuhopecten yessoensis*) from cDNA libraries

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Source/description: The Japanese scallop, *Mizuhopecten yessoensis*, is a cold-water shellfish and an important economic aquaculture species with high nutritive and economic value, distributed widely along the coastline of the northern islands of Japan, the northern part of the Korean Peninsula and the Sakhalin and the Kuril Islands.[1] It was introduced to China from Japan in the 1980s and has become an important economic aquaculture species on the northern coastline of China. Some microsatellite loci have been reported for the Japanese scallop.[1–3] However, more polymorphic microsatellite markers are needed to address several important questions related to the biogeography, population structure and genetic linkage map. In this paper, 21 EST-SSR loci were developed from previously published cDNA libraries[4,5] and were used to assess the genetic diversity in a domestic population of Japanese scallop.

Development of microsatellite: All SSRs were obtained by screening ESTs sequences that were generated from two cDNA