Sequencing of the Janus kinase 2 gene in cats with primary erythrocytosis

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BACKGROUND

- Feline primary erythrocytosis (PE) is a rare myeloproliferative disease that results in the excessive production of red blood cells, independent of erythropoietin concentrations ^[1]
- Polycythaemia vera in humans and canine PE may represent homologous disorders to feline PE
- Polycythaemia vera in 80-90% of affected humans is due to a mis-sense variant (c.1849G>T; p.V617F) in exon 14 of the Janus kinase 2 gene (*JAK2*), ^[2] with many of the remainder having variants in exon 12 of *JAK2* ^[3]
- Variants in canine *JAK2* exon 14 have been described in one dog with PE, out of five dogs evaluated in a previous study ^[4]. A test for this variant is now commercially available.

OBJECTIVE

- Partially sequence *fJAK2* from cats with PE to establish if similar variants account for the feline disorder

RESULTS

- DNA was successfully purified from all cats
- Sequencing of fragments amplified from control cats confirmed accuracy of the primers at targeting *fJAK2*
- Usable sequence data were obtained for two of the three cats with PE for exon 12, and for all three cats for exon 14
- No variants were detected within either exon 12 or exon 14 of *fJAK2* of the cats with PE
- Single nucleotide polymorphisms were identified within the flanking intronic sequence; however, these are of unknown significance

Aligned raw sequencing data over exon 14 of *fJAK2* with the nucleotide corresponding to the site of the mis-sense variant (c.1849G>T) found in human cases of polycythaemia vera highlighted

Example of a heterozygous, single nucleotide polymorphism; in this case it is 'silent' (i.e. it does not change the encoded amino-acid sequence)

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METHODS

- DNA was extracted from archived, excess whole blood from three cats diagnosed with PE and two control cats (healthy, blood donors)
- Primers were designed to amplify the entirety of exons 12 and 14 of *fJAK2* (corresponding to the sites of common variants described in humans and dog) along with associated fragments of intronic sequence
- Conventional PCRs amplified fragments ranging from 535 to 921bp in size
- Amplified fragments were purified and sequenced (DNA Sequencing & Services, www.dnaseq.co.uk)
- Sequences were aligned in MacVector v15.5.4

CONCLUSIONS

- Causative variants in *JAK2* commonly associated with polycythaemia vera in human patients were not detectable in exons 12 and 14 of *fJAK2* amplified from whole blood DNA obtained from three cats with PE
- This could reflect alternative causative variants or suboptimal cell populations as the DNA source (i.e. the DNA purified from whole blood might not reflect the genetic complement of the myeloproliferative population, as most red blood cells are anuclear in circulation)
- Future studies could include amplification and sequencing of bone marrow aspirates from cats with PE

REFERENCES

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