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FAM161A and TTC8 are Differentially Expressed in Non-allelic Early Onset Retinal Degeneration

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Abstract

Ciliary genes *FAM161A* and *TTC8* have been implicated in retinal degeneration (RD) in humans and in dogs. The identification of *FAM161A* and *TTC8* mutations in canine RD is exciting as there is the potential to develop novel large animal models for RD. However, the disease phenotypes in the dog and the roles of abnormal genes in disease pathology have yet to be fully characterized. The present study evaluated the expression patterns of *FAM161A* and *TTC8* during normal retinal development in dogs, and in three non-allelic, early onset canine RD models at critical time points of the disease: RCD1, XLPRA2 and ERD. Both genes were differentially expressed in RCD1 and ERD, but not in XLPRA2. These results add evidence to the hypothesis that a) mutations in many retinal genes have a cascade effect on the expression of multiple, possibly unrelated genes and b) a large number and wide range of genes probably contribute to RD in general.

27.1 Introduction

Progressive Retinal Atrophy (PRA) is the term used for a group of inherited retinal diseases that is characterized by degeneration of the retina, ultimately resulting in loss of vision. Rod photoreceptor (PR) responses are typically lost first, followed by cone PR responses (Parry 1953). Bilateral and symmetrical changes are observed in the fundus, including a hyper-reflective tapetum in the early stages, followed by attenuation of blood vessels, pigmentary changes and atrophy of the optic nerve head (Miyadera et al. 2012). PRA is considered the canine homologue of the retinitis pigmentosa (RP) group of diseases in man, and most of the genes that have been implicated in PRA to date have also been implicated in human retinal degeneration (RD).

Recently the ciliary genes *FAM161A* (family with sequence similarity 161, member A) and *TTC8* (tetratricopeptide repeat domain 8) have been reported to be causally associated with two distinct forms of PRA in the Tibetan Spaniel and Golden Retriever breeds, respectively (Downs and Mellersh 2014; Downs et al. 2014). Both genes have also been implicated in human RD: *FAM161A* in RP (Bandah-Rozenfeld et al. 2010; Langmann et al. 2010), and *TTC8* in RP and Bardet-Biedl Syndrome (Ansley et al. 2003; Riazuddin et al. 2010). While

these discoveries may enable the study of new canine models for human RD, the models have yet to be established and adequately characterized.

While retinal tissues from dogs homozygous for *FAM161A* or *TTC8* mutations was not available for study, retinas were available from three non-allelic canine models: RCD1 (Suber et al. 1993; Ray et al. 1994), XLPRA2 (Zhang et al. 2002) and ERD (Goldstein et al. 2010). To this end, we evaluated the expression of *FAM161A* and *TTC8* throughout normal retinal development, and in the three disease models, and compared the expression with that of PR-specific genes.

27.2 Materials and Methods

27.2.1 Tissue Samples

Retinal tissue was obtained from age-matched normal and mutant dogs as described previously (Genini et al. 2013). Mutant dogs comprised three canine models for early onset RD: rod cone dysplasia 1 (RCD1), X-linked progressive retinal atrophy 2 (XLPRA2) and early retinal degeneration (ERD). In all three models retinal development begins normally, but abnormalities soon develop, and there is progressive and relatively fast PR degeneration (Supplementary Figure 27. S1, reproduced from Genini et al. 2013). PR loss and the decrease in outer nuclear layer thickness is more rapid and aggressive in RCD1, and slightly delayed in XLPRA2 (Acland and Aguirre 1987; Farber et al. 1992; Beltran et al. 2006). ERD is characterized by abnormal development and degeneration of PR cells, as well as concurrent photoreceptor mitosis that generated new hybrid rod/S-cone cells (Berta et al. 2011). Tissues were obtained from normal, RCD1 and XLPRA2 animals at 3, 5, 7 and 16 weeks of age (3 biological replicates/time-point/group) and from two ERD mutants at 6.4 weeks, three at 8.3/9.9 weeks and two at 11.9/14.1 weeks of age (labelled 6, 9 and 13 weeks, respectively, for analysis).

27.2.2 qRT-PCR

Unlabeled primers were used to detect all isoforms of *FAM161A* (Fwd: GCTGAAAGCTGCCCCACTTGGAAC and Rev: TCAAGGAGGAAGACGGCCCTAAATC) and *TTC8* (Fwd: ACTCATGTGGAAGCCATTGCATGC and Rev: AGCTGCCTCTTCTTCGTTTTTCAGC). Primers and fluorescently labelled probes described previously (Komaromy et al. 2010) were used to detect *RHO* and *OPN1LW*. TaqMan assays (Applied Biosystems) were used to detect *ARR3* (Cf03460116_m1), *SAG* (Cf02628845_m1) and *GAPDH* (Hs02786624_g1). The preparation of cDNA and qRT-PCR reactions were conducted as described previously (Genini et al. 2013), using the 7500 real-time PCR machine and detection software (v2.0.1, Applied Biosystems).

27.2.3 qRT-PCR Analysis

The C_T values of the genes were normalized against those of *GAPDH*. The ratios and fold change (FC) were calculated using the C_T method (Livak and Schmittgen 2001) for 3, 5 and 7 week normal versus 16 week normal; RCD1 and XLPRA2 versus age-matched normal; and ERD at 6, 9 and 13 weeks versus normal at 5, 7 and 16 weeks. An unpaired t-

test was applied, and p-values were controlled using the Benjamini & Hochberg (BH) step-up false discovery rate (FDR) procedure to determine whether any differences observed were statistically significant ($p < 0.05$). Samples with $p < 0.05$ and $FC > \pm 2$ were considered differentially expressed (DE).

27.3 Results

27.3.1 Expression in normal retina

Expression levels of all 6 genes in normal retinal development (3, 5 and 7 weeks) was compared with normal adult retina at 16 weeks (Acland and Aguirre 1987). The expression levels of cone genes, Arrestin (*ARR3*) and L/M-Opsin (*OPN1LW*) and rod gene S-Antigen (*SAG*), did not change significantly throughout development (Figure 27.1). however, the other rod gene analyzed, rhodopsin (*RHO*), was DE (-2.3 fold) at 3 weeks, and then increased from 5 weeks to levels that were not significantly different than at 16 weeks. Expression of *FAM161A* and *TTC8* was highest at 3 and 5 weeks of age, followed by decreased expression to the levels in the 16 week retina; only *TTC8* was DE ($+2.6$ fold) at 3 weeks compared with 16 weeks.

27.3.2 Expression in disease models

Expression levels of all six genes in mutant retina at all ages was compared with age-matched normals (Figure 27.2 and Supplementary Fig 27.2), and DE genes/models/time-points identified. In all models *RHO*, *ARR3* and *SAG* showed a general trend of down-regulation compared with age-matched normal. *RHO* was DE in one or more stages of all three models, *ARR3* in RCD1 and ERD, and *SAG* in RCD1 only. Conversely, *OPN1LW* was not DE in any disease. *FAM161A* and *TTC8* have similar expression patterns to one another in each model (Figure 27.2 and Table 27.1). In RCD1 both genes are down-regulated throughout, with *FAM161A* DE at 7 and 16 weeks (-2.1 and -2.5 fold, respectively), and *TTC8* at 3 weeks (-2.1 fold). In XLPR2, neither gene is DE. In ERD, only *TTC8* is DE at 13 weeks ($+4.1$ fold), although the up-regulation of *FAM161A* is also statistically significant ($+1.6$ fold).

27.4 Discussion

In this study we examined the expression of *FAM161A* and *TTC8*, along with selected rod- and cone-specific genes throughout normal retinal development, and in three canine RD models, RCD1, XLPR2 and ERD.

FAM161A exists in two isoforms formed by alternative splicing of exon 4, and is expressed in multiple tissues, including the retina (Langmann et al. 2010). It localizes to the basal body, connecting cilium and centriole, and associates with the microtubule network during mitosis (Di Gioia et al. 2012; Zach et al. 2012). *TTC8* also exists in two main isoforms. One isoform, containing exon 2A, is expressed exclusively in the retina (Riazuddin et al. 2010), while the isoform lacking exon 2A is expressed in multiple ciliated tissues. Both isoforms localize to ciliated structures such as connecting cilium, centrosomes and basal bodies (Ansley et al. 2003). Our finding that maximum expression of *TTC8* and *FAM161A* occurs at 3 and 5 weeks in normal retina, and is followed by reduced expression at 7 and 16 weeks

suggests that both of these genes are required for both the development and maintenance of the retina. This is similar to previously reported results for *FAM161A* expression in mice (Langmann et al. 2010).

Similar expression patterns, for the most part, were reported for *RHO*, *SAG*, *ARR3* and *OPN1LW* using the same models and time-points in a previous study (Genini et al. 2013). The main difference was observed with *SAG*, which was DE in the late stages of ERD and XLPRA2 in the Genini study, but not here. Two factors may account for these differences: 1) this study was conducted and cDNA generated separately from that of Genini et al, resulting in probable variations in template concentration; 2) the small number of tests in this study will have impacted the BH-controlled p-value. *FAM161A* and *TTC8* are DE at one or more time-points in the RCD1 retina, while neither are DE in XLPRA2. The difference in expression between RCD1 and XLPRA2 could be due to the comparatively greater severity of the former. Interestingly, we observed that *FAM161A* and *TTC8* are significantly up-regulated at 13 weeks in ERD, compared with 16 weeks normal. *FAM161A* binds to microtubules and undergoes redistribution during mitosis (Zach et al. 2012) and is thought to play a role in the structural composition, maintenance and function of the connecting cilium (Karlstetter et al. 2014). *TTC8* is thought to be associated with ciliary biogenesis or function (Ansley et al. 2003). Characteristic of ERD is concurrent PR apoptosis and mitosis (Berta et al. 2011). It is therefore not entirely unexpected to observe the up-regulation of *FAM161A* and *TTC8* in this disease, especially given the pivotal role of the microtubule network in mitosis.

We have characterized *FAM161A* and *TTC8* expression throughout the development of the normal retina, as well as in three non-allelic RD models. Both genes are DE in two of these models, providing further evidence that degeneration of the retina is likely caused by aberrant expression of multiple genes, not only by the mutant gene.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 27.1.

RNA expression changes in developing normal retina at 3, 5 and 7 weeks compared to young adult (16 weeks). A double asterisk indicates significant differential expression and error bars represent standard deviation of biological triplicates.

Fig. 27.2.

RNA expression changes in RCD1 and XLPRA2 at 3, 5, 7 and 16 weeks versus age-matched normal, and in ERD at 6, 9 and 13 weeks versus normal at 5, 7 and 16 weeks, respectively. An asterisk indicates statistical significance, a double asterisk differential expression and error bars represent standard deviation of biological triplicates/duplicates.

Table 27.1

Fold change of *TTC8* and *FAM161A*. Differentially expressed genes ($FC > \pm 2$, $p < 0.05$) are in **bold**.

		ERD	RCD1	XLPR2
3 wks	<i>FAM161A</i>	-	-1.1	-1.0
	<i>TTC8</i>	-	-2.1	-1.8
5/6 wks	<i>FAM161A</i>	-3.1	-1.4	-1.2
	<i>TTC8</i>	-4.2	-2.1	-1.4
7/9 wks	<i>FAM161A</i>	-1.3	-2.1	-1.6
	<i>TTC8</i>	-1.3	-1.6	+1.0
16/13 wks	<i>FAM161A</i>	+1.6	-2.5	-1.2
	<i>TTC8</i>	+4.1	-1.2	+1.1