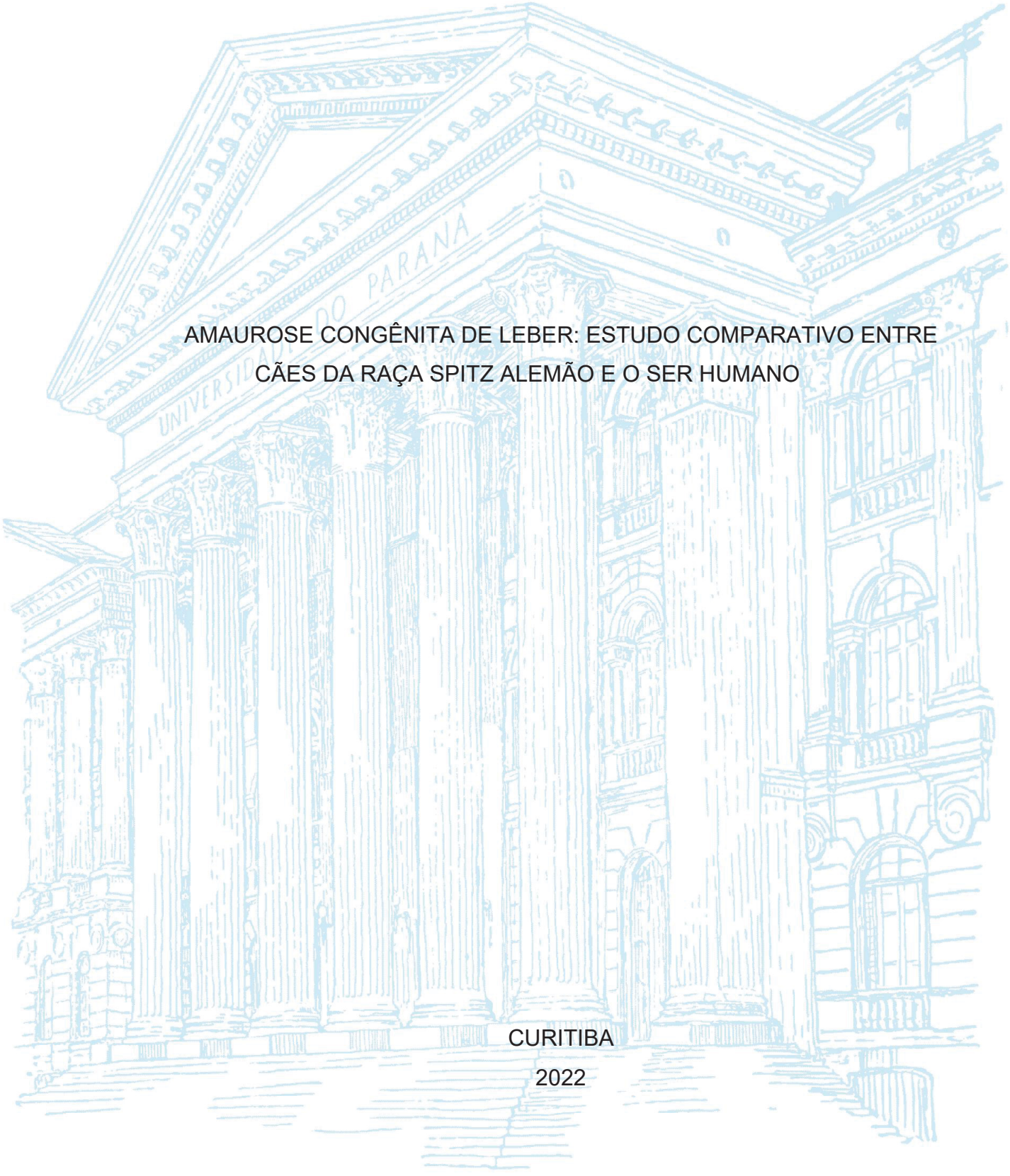
UNIVERSIDADE FEDERAL DO PARANÁ

BIANCA LUIZA VALDUGA GUARESCHI



AMAUROSE CONGÊNITA DE LEBER: ESTUDO COMPARATIVO ENTRE CÃES DA RAÇA SPITZ ALEMÃO E O SER HUMANO

CURITIBA

2022

BIANCA LUIZA VALDUGA GUARESCHI

AMAUROSE CONGÊNITA DE LEBER: ESTUDO COMPARATIVO ENTRE CÃES DA RAÇA SPITZ ALEMÃO E O SER HUMANO

Dissertação apresentada ao curso de Pós-Graduação em Ciências Veterinárias, do Setor de Ciências Agrárias, da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ciências Veterinárias com ênfase em Oftalmologia.

Orientador: Professor Dr. Fabiano Montiani-Ferreira

CURITIBA

2022

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS AGRÁRIAS

Guareschi, Bianca Luiza Valduga
Amaurose congênita de Leber: estudo comparativo entre cães da raça Spitz Alemão e o ser humano = GUCY2D-associated retinopathy: a comparative study between german Spitz dogs and humans / Bianca Luiza Valduga Guareschi. – Curitiba, 2022.
1 recurso online: PDF.

Dissertação (Mestrado) – Universidade Federal do Paraná, Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciências Veterinárias.
Orientador: Profª Dr. Fabiano Montiani-Ferreira

1. Doenças congênitas. 2. Terapia gênica. 3. Oftalmologia. 4. Cães. I. Montiani-Ferreira, Fabiano. II. Universidade Federal do Paraná. Programa de Pós-Graduação em Ciências Veterinárias. III. Título.

Bibliotecária: Ana Camila Quaresma Moura CRB-9/2212

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIÊNCIAS AGRÁRIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIAS VETERINÁRIAS - 40001016023P3

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS VETERINÁRIAS da Universidade Federal do Paraná foram convocados para realizar a avaliação da defesa de qualificação de Mestrado da aluna **BIANCA LUIZA VALDUGA GUARESCHI** intitulada: **AMAUROSE CONGÊNITA DE LEBER: ESTUDO COMPARATIVO ENTRE CÃES DA RAÇA SPITZ ALEMÃO E O SER HUMANO**, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

CURITIBA, 19 de Julho de 2022.

Assinatura Eletrônica
20/07/2022 11:22:12.0
FABIANO MONTIANI FERREIRA
Presidente da Banca Examinadora

Assinatura Eletrônica
20/07/2022 11:40:57.0
HELOISA HELENA ABIL RUSS GIACOMETTI
Avaliador Externo (HELOISA RUSS OFTALMOLOGIA)

Assinatura Eletrônica
20/07/2022 21:46:14.0
MARIANA VALLIM SALLES
Avaliador Externo (UNIVERSIDADE FEDERAL DE SÃO PAULO)

Dedico essa dissertação a minha família. Meu esposo, pais, avós, tios e tias os quais sempre me incentivaram nos estudos.

AGRADECIMENTOS

Primeiramente, agradeço a Deus, minha luz e força diária, que ilumina meu caminho e abre as portas possíveis conforme Seus propósitos em minha vida.

Agradeço à minha família: pais, irmãs, tios, tias e avós, que me acompanham desde a infância, sempre incentivando meus estudos e minha trajetória profissional.

Agradeço ao meu orientador, Professor Dr. Fabiano Montiani-Ferreira, pela oportunidade e pelo crescimento científico, tanto pessoal quanto para a comunidade. Através de seus olhos, consigo enxergar um amor imenso e uma dedicação pela ciência e pela pesquisa, que são essenciais para que, um dia, possamos ajudar muitas pessoas e animais por meio de tratamentos de saúde e cura de doenças graves, como as distrofias hereditárias de retina. Enfim, meu professor, orientador e brilhante pesquisador.

Agradeço também aos meus exemplos profissionais na área da oftalmologia: Dr. João Guilherme Oliveira de Moraes, Dra. Heloisa Russ, Dra. Juliana Sallum e Dra. Mariana Vallim, pela disponibilidade em me apoiar com conhecimento científico e pelo fornecimento de casos que enriqueceram os projetos científicos. Admirar vocês, tanto como pessoas quanto como profissionais, me motiva a sempre dar o meu melhor para cada paciente ou colega que posso ajudar.

Agradeço às minhas adoráveis cachorrinhas Amora e Sashimi, que enchem meu coração de amor pelos animais, tornando este projeto ainda mais encantador.

Por fim, agradeço ao meu marido, José Pedro Konrad Bezerra, por estar ao meu lado neste projeto e vibrar comigo a cada passo e conquista. Ele nunca mede esforços para me ajudar e sempre compreende minha ausência, muitas vezes inevitável devido aos estudos e ao trabalho.

Se não houver frutos, valeu a beleza das flores; se não houver flores, valeu a sombra das folhas; se não houver folhas, valeu a intenção da semente. (Henrique de Souza Filho, 1977)

RESUMO

Esta tese de dissertação visa avaliar semelhanças e diferenças entre pacientes acometidos por mutação no gene *GUCY2D* relacionado a amaurose congênita de Leber (LCA) e um grupo de cães da raça *Spitz Alemão* afetados por retinopatia progressiva hereditária relacionada a mutações no mesmo gene, com o objetivo final de determinar a viabilidade desse grupo de cães como modelo animal para estudos de terapia gênica. Este é um estudo observacional retrospectivo que revisa dados de prontuários médicos, testes genéticos e exames oftalmológicos dos grupos estudados, avaliando dados como idade, testes genéticos, fotografias de fundo de olho, melhor acuidade visual corrigida (BCVA), autofluorescência do fundo, tomografia de coerência óptica (OCT), eletrorretinografia (ERG) e campo visual. O Grupo 1 consistiu de 13 pacientes, enquanto o Grupo 2 consistiu de 16 cães. Em ambos os grupos, os sinais clínicos se desenvolveram em idade precoce, e o nistagmo estava presente em vários indivíduos, assim como a redução severa da acuidade visual. Anormalidades nas imagens de fundo foram muito sutis em ambos os grupos. Registros de ERG ausentes a severamente reduzidos foram observados em ambos os grupos. O exame de OCT de mácula do grupo de pacientes diagnosticados com LCA apresentou redução da espessura da retina com alteração em retina externa. Esses mesmos achados são similares ao grupo dos cães, os quais também apresentaram descolamentos focais de retina neurossensorial. O modelo animal de cão da raça *Spitz Alemão* afetado por mutação no gene *GUCY2D* apresenta semelhanças importantes e preservação estrutural retiniana em relação à perda funcional, podendo ser um bom candidato à terapia gênica para LCA.

Palavras-chave: *GUCY2D*, amaurose congênita de Leber, modelo animal, terapia gênica, cães Spitz Alemães.

ABSTRACT

This master's thesis aims to evaluate similarities and differences between patients affected by mutation in the *GUCY2D* gene causing Leber's congenital amaurosis (LCA) and a group of *German Spitz* dogs with a form of hereditary retinopathy caused by a mutation in the same gene, with the ultimate aim of determining the prospect of this group of dogs as an animal model for gene therapy studies. Observational retrospective study with review of data from medical records, genetic testing and ophthalmological examinations of the groups studied, evaluating data such as age, genetic testing, fundus photography, best corrected visual acuity (BCVA), fundus autofluorescence, optical coherence tomography (OCT), electroretinogram (ERG) and visual field. Group 1 consisted of 13 patients while group 2 consisted of 16 dogs. In both groups, clinical signs developed at an early age and nystagmus were present in several individuals as well as severe visual acuity reduction. Abnormalities in fundus images were very subtle in both groups. Absent to severely reduced ERG tracings were observed in both groups. The macula OCT scan diagnosed with LCA showed reduced retinal thickness with changes in the outer retina layer. These same findings are similar to the group of dogs, which also showed focal neurosensory retinal detachments. The *German Spitz* dog model affected by mutation in *GUCY2D* gene shows important similarities and retinal structural preservation in relation to functional loss and may be a good candidate for gene therapy for LCA.

Keywords: *GUCY2D*, Leber's congenital amaurosis, animal model, gene therapy, Spitz German dogs.

LISTA DE ABREVIATURAS OU SIGLAS

AD	- Autosomal dominant
AR	-Autosomal recessive
BCVA	- Best corrected visual acuity
CEUA	- Committee on the Ethical Use of Animals
CRD	- Cone-rod dystrophy
FAF	- Autofluorescence
ERG	- Electroretinogram
LCA	- Leber's congenital amaurosis
HM	- Hand movements
LP	- Light perception
NRD	- Neurosensory retinal detachment
OCT	- Optical coherence tomography
ONL	- Outer nuclear layer
PRA	- Progressive retinal atrophy
RD	- Retinal dystrophy
RPE	- Retinal pigment epithelium
UFPR	- Federal University of Paraná
VF	- Visual field

LISTA DE SÍMBOLOS

© - copyright

® - marca registrada

SUMÁRIO

1 INTRODUCTION 16
2 MATERIALS AND METHODS 17
3 RESULTS 19
4 DISCUSSION 29
REFERENCES 32

1 INTRODUCTION

The *GUCY2D* gene is responsible for the production of the retina-specific enzyme guanylate cyclase 1 present in the outer segment, which is responsible for photoreceptor recovery in phototransduction. The enzyme's function is decreased with reduced levels of cytoplasmic calcium levels (Ca 2+)[1].

Mutations in the gene coding for the retina-specific enzyme guanylate cyclase 1 exhibit two phenotypes: autosomal recessive (AR) inheritance causing type I Leber's congenital amaurosis (LCA) and autosomal dominant (AD) inheritance causing cone-rod dystrophy (CRD)[2]. Among the most severe and earliest congenital dystrophies of childhood, LCA represents approximately 9% of all patients[3] and *GUCY2D* mutations are causal in 10-20% of those cases[2]. CRD starts in the first decade of life and affects the macula. The main symptom is visual impairment with central scotoma. There are 13 genes responsible for non-syndromic CRDs, one of these is *GUCY2D* mutations (approximately 25% of cases) restricted to exon 13 encoding the dimerization domain of guanylate cyclase[2,4,5].

A condition analogous to LCA in humans was recently identified as an important cause of vision loss in domestic canines carrying a *GUCY2D* mutation and exhibiting a form of progressive retinal atrophy (PRA)[6]. Anatomical similarities with the human eye, especially size and a retinal area with a high density of cones destined for high visual acuity, can make dogs a superior animal model for vision studies compared to rodents[7,8].

The present study compares a group of *German Spitz* dogs, which exhibit an early onset hereditary retinopathy, with a group of human patients affected by mutation in the same gene and express the LCA phenotype. The goal of the study is to observe similarities and differences that may help in future translational ophthalmology investigations, including gene therapy research.

2 MATERIALS AND METHODS

This study was based on a review of medical records and ophthalmologic examinations of thirteen human patients (group 1) affected by mutation in the *GUCY2D* gene and at the Institute of Ocular Genetics, São Paulo, SP, Brazil. The second comparative study (group 2) was composed of sixteen *German Spitz* dogs affected by genetic mutation in the *GUCY2D* gene and with phenotypic expression of an early onset PRA at the Veterinary Hospital of the Federal University of Paraná (UFPR), Curitiba, PR, Brazil. Three human patients with an AD mutation of the *GUCY2D* gene and a CRD phenotype were excluded from the group 1 sample, leaving only patients affects by the AR phenotype.

In reviewing the medical records of human patients, the following information was considered: sex, age, genetic test (alleles and variant when available), clinical history, best corrected visual acuity (BCVA) improvement using the Snellen chart, and ophthalmologic examination. BCVA was considered extremely compromised when without light perception (LP), LP and hand movements (HM). Fundus photographs were analyzed and categorized by the same ophthalmologist with emphasis on the presence of arteriolar attenuation, venous attenuation, optic disc pallor and retinal macular changes. Some patients had also undergone additional tests such as fundus autofluorescence (FAF), electroretinogram (ERG), optical coherence tomography (OCT) (Heidelberg Engineering©, USA) and visual field testing (Humphrey® 24-2, Zeiss, USA).

The records of 16 *Spitz German* dogs diagnosed with PRA at the Veterinary Hospital of the UFPR were reviewed. The following data were evaluated: sex, age at diagnosis, type of genetic inheritance, visual acuity (cotton ball test and obstacle avoidance in photopic and scotopic conditions), presence of nystagmus, and genetic test results when present. Fundus photography was analyzed by the same ophthalmologist for the following parameters: presence of blood vessel attenuation, presence or absence of neurosensory retinal detachment (NRD), the area of the tapetum, presence or absence tapetal hyperreflectivity, accumulation of pigment spots in the retina and optic disc pallor. Dogs that were submitted to OCT had images evaluated by the same individual for total retinal thickness of the ventral retina in relation to the dorsal and thickness of the outer nuclear layer (ONL), and the presence or absence of NRD. ERGs was also performed and evaluated.

This is a retrospective descriptive study and was approved by the Ethics Committee of the Health Sciences UFPR (54931221.4.0000.0102) on April 6, 2022 and approved by the Committee on the Ethical Use of Animals (CEUA) of the Sciences of the UFPR- Brazil (050/2021) on October 15, 2021.

3 RESULTS

Group 1 consisted of ten human participants (5 females, 5 males) affected by the *GUCY2D* autosomal recessive gene mutation. Their ages ranged from 4 to 55 years. Human patients were identified as 01 to 10. Each individual is described with the respective genetic and ocular findings (Table 1).

TABLE 1 – GUCY2D AUTOSOMAL RECESSIVE VARIANTS, CLINICAL AND OPHTHALMOLOGICAL FINDINGS.

ID	Allele 1	Allele 2	Sex	Age (yo)	BCVA (Snellen table)	History and Eye Exam	Macular alteration
01	c.1956+1G>A (paternal)	c.1245delT (maternal)	M	10	No LP OU	3-month-old nystagmus (sister of G11)	Foveal hyper-autofluorescence
02	c.2620G>A (p.Glu874Lys)	c.1A>G (p.Met1?)	F	17	20/60 OU	Phenotype suggestive of Cone Dystrophy	Foveal hyper-autofluorescence
03	c.1245delT (p.Phe415LeufsX73)	c.2598G>C (p.Lys866Asn)	M	4	HM OU	Nystagmus and ET	No
04	c.1052A>G (p.Tyr351Cys)	c.1052A>G (p.Tyr351Cys)	F	55	20/80 OD - 20/70 OS	Night blindness since childhood and myopia	Hypertrophy of foveal RPE OD, foveal hyper-autofluorescence OS
05	c.1972C>T (p.His658Tyr)	c.1972C>T (p.His658Tyr)	M	37	HM OU	Consanguineous parents, keratoconus and corneal leucoma OS	Bull's eye OU, ring hypo-autofluorescence around fovea
06	c.1957-2A>G	c.1957-2A>G	M	14	HM OD-LP OS	Consanguineous parents	No
07	c.1343C>A (p.Ser448X)	c.1343C>A (p.Ser448X)	M	10	No LP OU	Consanguineous parents, low VA and nystagmus from birth, hyperopia	No
08	c.1956+1G>A (paternal)	c.1245delT (maternal)	F	8	LP OU	Nystagmus, strabismus (brother of G02)	Foveal hyper-autofluorescence
09	c.1343C>A (p.Ser448X)	c.1957-2A>G	F	4	LP OU	Nystagmus, strabismus, low VA since 2 months	-
10	c.2302C>T (p.Arg768Trp)	c.767A>C (p.Gln256Pro)	F	23	20/150 OU	Change in macular brightness	Foveal RPE atrophy, foveal hyper-autofluorescence with some hypo-autofluorescence areas OU

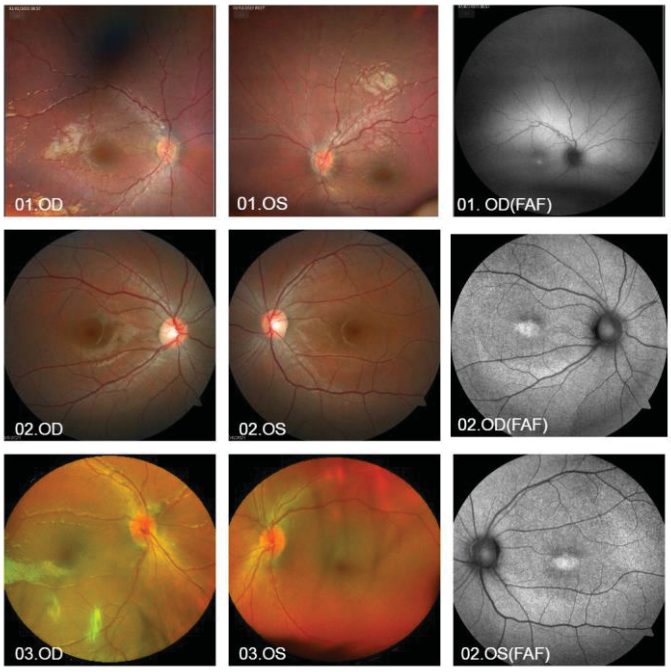
SOURCE: The author (2022).

NOTE: AR: autosomal recessive; WT: wild type; F female, M male, yo years old, BCVA best corrected visual acuity, OU both eyes, LP light perception, HM hand movements, OD right eye, OS left eye, VA visual acuity, XT exotropia, ET esotropia; RPE retinal pigment epithelium.

SUBTITLE: Details of the 10 patients affected by AR mutation in the GUCY2D gene and hereditary retinal dystrophy. Note the alleles affected. Patient 09 did not have complete data.

Patient 05 presented with keratoconus and two patients had strabismus (03 and 08). Extreme low BCVA was seen in seven patients (70%) and nystagmus was described in five patients (50%). A history of consanguinity was described in three patients (30%). Twenty percent of patients presented with arteriolar attenuation (04 and 05) and two patients presented with increased arteriolar tortuosity (patients 01 and 08, who are siblings) (Figure 1).

FIGURA 1 – OPHTHALMOLOGICAL FINDINGS OF PATIENTS 01-03 IN GROUP 1.



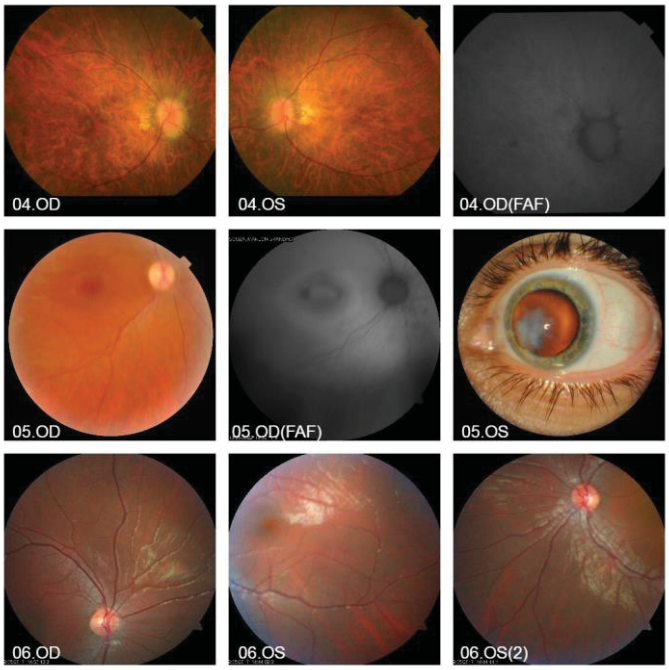
SOURCE: The author (2022).

SUBTITLE: 01.OD and 01.OS. Fundus photographs showing increased arteriolar tortuosity are present OU. 01.OD(FAF). Auto fluorescence examination showing hyper-autofluorescence OD. 02.OD and OS. Color fundus photography without notable abnormalities. 02.OD(FAF) and OS(FAF). Auto fluorescence examination of the same patient showing hyper-autofluorescence OU. 03.OD and OS. Color fundus photography without alterations. OU both eyes, OD right eye, OS left eye.

Venous attenuation and optic disc pallor were not observed in any of the patients.

In patients evaluated with an autofluorescence exam, all of them exhibited change with the presence of hyper-autofluorescence (patients 01, 02, 04, 06, 08 and 10). Fundus photos are available (Figure 2 and 3).

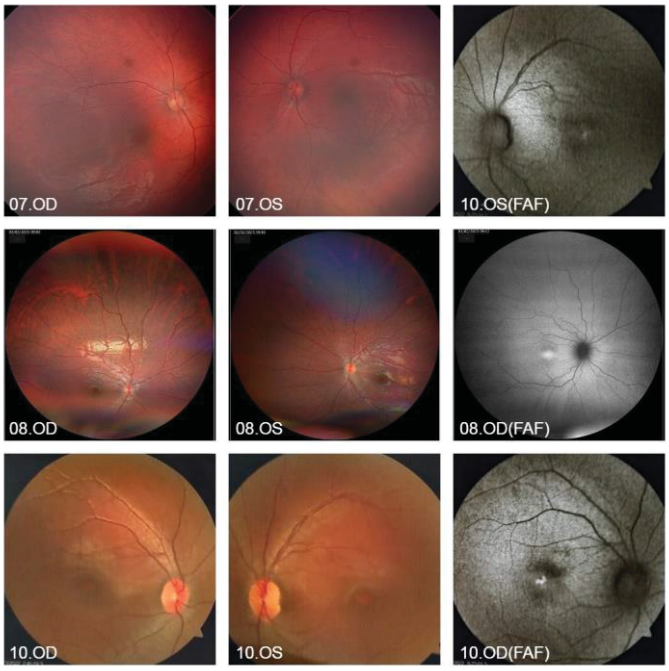
FIGURA 1 – OPHTHALMOLOGICAL FINDINGS OF PATIENTS 04-06 IN GROUP 1.



SOURCE: The author (2022).

SUBTITLE: 04.OD and 04.OS. On the color fundus photography foveal RPE hypertrophy/atrophy OD, choroidosis and arteriolar attenuation OU. 04.OD(FAF). In the auto fluorescence focal hypo-autofluorescence OD. 05.OD. On the color fundus photography, a maculopathy pattern in Bull's eye OD and arteriolar attenuation. 05. OD(FAF). In the auto fluorescence hyper-autofluorescence surrounded by hypo-autofluorescence surrounded by retinal mottling OD. 05.OS. In the photo of the anterior segment showing keratoconus and corneal leukoma in OS. 06.OD, OS and OS (2). On the color fundus photography without alterations. RPE retinal pigment epithelium. OU both eyes, OD right eye, OS left eye.

FIGURA 3 – OPHTHALMOLOGICAL FINDINGS OF PATIENTS 07-08-10 IN GROUP 1.

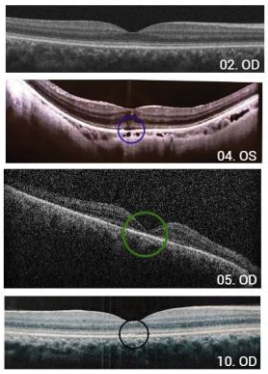


SOURCE: The author (2022).

SUBTITLE: 07.OD and 07.OS. On the color fundus photography without alterations OU. 10. OD(FAF) and OS(FAF). In the auto fluorescence foveal hyper-autofluorescence with some hipo-autofluorescence areas OU. 08.OD and OS. On the color fundus photography increased arteriolar tortuosity are present OU. 08. OD(FAF). In the auto fluorescence hyper-autofluorescence. 10.OD and OS. On the color fundus photography foveal RPE atrophy OU. RPE retinal pigment epithelium, OD right eye, OS left eye.

Patient 02 presented with normal OCT of the macula and optic nerve (Figure 4). The OCT of the macula of patients 05 showed foveal outer retinal atrophy, patient 04 had foveal focal outer retinal defect and extrafoveal outer retinal rarefaction, and patient 10 had foveal outer retinal irregularity (Figure 4).

FIGURA 4 – MACULA OCT SCANS GROUP 1.

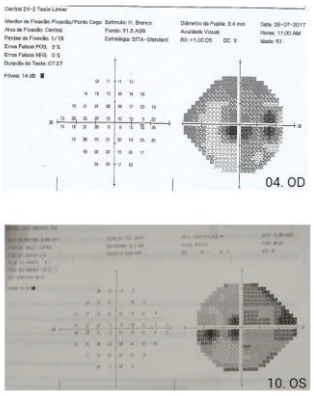


SOURCE: The author (2022).

SUBTITLE: 02. OD. The macula OCT scan of patient 02 shows an unaltered inner, outer retinal architecture and choriocapillary. 04. OS. The macula OCT scan of patient 04 shows a reduced reflectivity of the RPE and the ellipsoid zone with point interruption in the fovea (blue circle). 05. OD. The macula OCT scan of patient 05 indicates an absence of the ellipsoid zone foveal and perifoveal RPE (green circle). 10. OD. The macula OCT scan of patient 10 displays foveal outer retinal irregularities (black circle). OCT optical coherence tomography, RPE retinal pigment epithelium, OD right eye, OS left eye.

Patients 03, 09, and 10 presented an ERG exam with absence of cone and rod responses. Patient 04 and 10 presented with VF with significance reduction macula sensitivity and decreased sensitivity in all quadrants (Figure 5).

FIGURA 5 – VISUAL FIELD EXAMS OF GROUP 1.



SOURCE: The author (2022).

SUBTITLE: VF exams of patient 04 and 10 exhibits a significant reduction in sensitivity macula and decreased sensitivity in all quadrants. VF visual field.

Group 2 consisted of 16 German Spitz dogs (6 females, 10 males) affected by early onset retinopathy caused by the AR mutation in the *GUCY2D* gene, with ages ranging from 1.5 to 36 months. The dogs were numbered 1-16, and their age at diagnosis, fundus photography, ERG, and OCT, as well as results of genetic testing, are reported (Table 2).

TABLE 2 – GUCY2D AUTOSOMAL RECESSIVE VARIANTS, CLINICAL AND OPHTHALMOLOGICAL FINDINGS.

ID	Gender	Age at diagnosis/Fundus photography (m)	ERG age (m)	OCT age (m)	Genetic testing
1.	M	3	3		
2.	M	3	3	32	
3.	M	3	3	36	
4.	F	5	5		
5.	F	22	9		
6.	M	9	9		
7.	M	3/9	3	15	
8.	M	3/8	4	8	c.1598_1599insT homozygosis (p.Ser534GlufsTer20)
9.	F	3/7	4	8	c.1598_1599insT homozygosis (p.Ser534GlufsTer20)
10.	M	3	4	13	
11.	M	4	4		
12.	M	15	15		
13.	F	2	2	4	
14.	F	2	2		
15.	F	2	2		
16.	M	1,5	3		

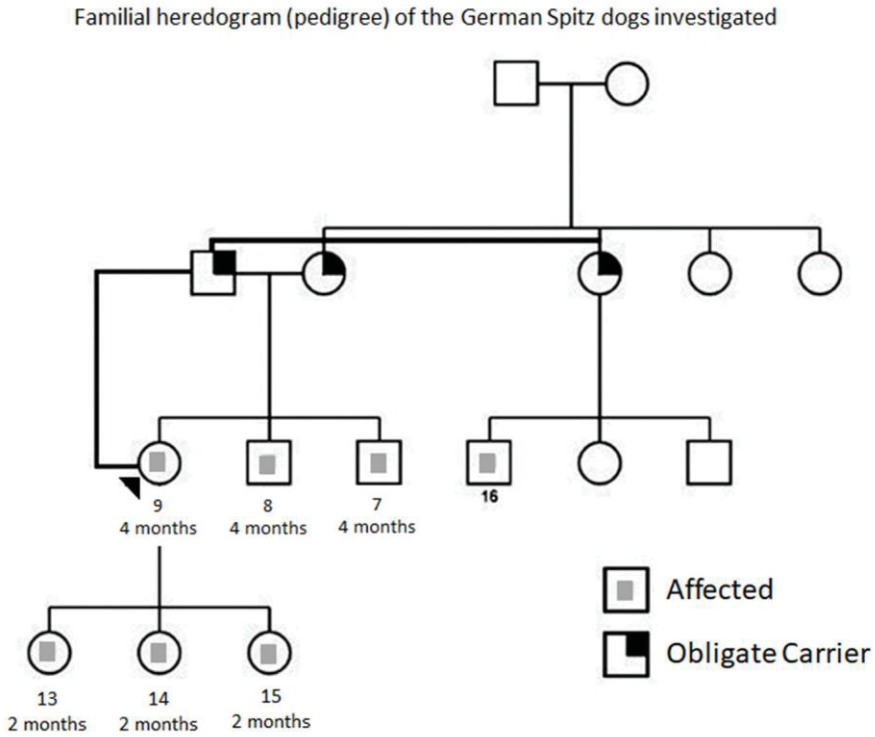
SOURCE: The author (2022).

NOTE: F female, M male, m months, OCT optical coherence tomography, ERG electroretinography.

SUBTITLE: Details of the dogs affected by a mutation in the GUCY2D gene, sex, age at diagnosis/Fundus photography, ERG and OCT. The patient 12 did not have complete data.

The genetic inheritance observed is AR for all dogs as shown in the partial pedigree analysis (Figure 6).

FIGURA 6 – PEDRIGREE OF THE GROUP 2.



SOURCE: The author (2022).

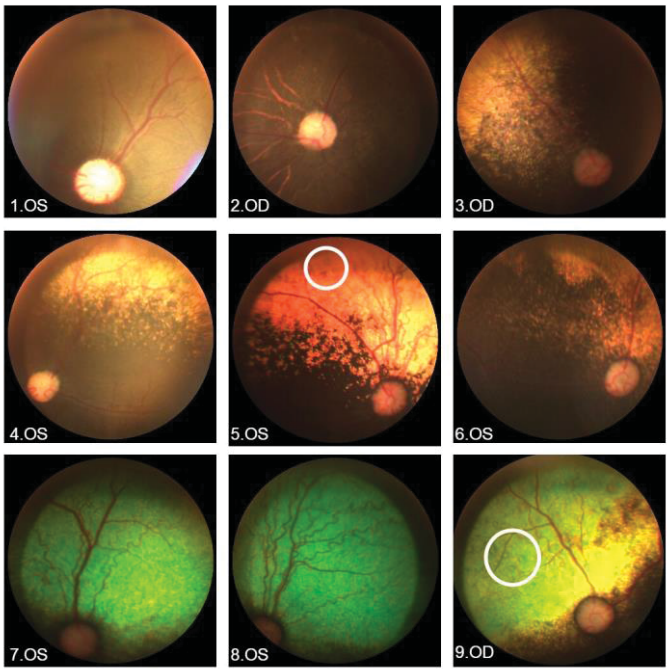
SUBTITLE: In this partial heredrogram of group 2, an autosomal recessive inheritance pattern is present, including unaffected and obligate carrier parents and their offspring of affected and unaffected phenotypes.

Two of the affected dogs underwent genetic testing and a c.1598_1599insT spontaneous frameshift homozygosis mutation was found in exon 7 of the *GUCY2D* gene, which produces the p.Ser534GlufsTer20 protein. The remainder of the dogs have the same phenotypic condition and most are related to each other. Due to the unlikely chance of new mutation based on being same breed, consanguinity, and presenting with a similar clinical pattern, they were considered homozygous for the same genetic mutation.

All dogs failed the cotton ball test and obstacle avoidance in photopic and scotopic conditions in the exam room, and thus were considered to have low visual acuity. Oscillatory nystagmus was present in 13 of 16 dogs (81.25%). In all dogs, there was no response in scotopic and photopic ERGs exam through one year of age.

Fundus photography (Figure 7 and Figure 8) showed slight arteriolar attenuation in 53.84 % (seven dogs) and increased tortuosity in 30.76% (four dogs). Pink optic nerves and retinal veins of normal caliber were present in all dogs. Three of the dogs had slight pigmentary mobilization and some degree of choroidosis (2, 13, 14). The area of the tapetum was reduced or hypoplasic in 53.84% and completely absent in 30.76%. Hyperreflectivity of the tapetum was observed in 61.53% (eight dogs).

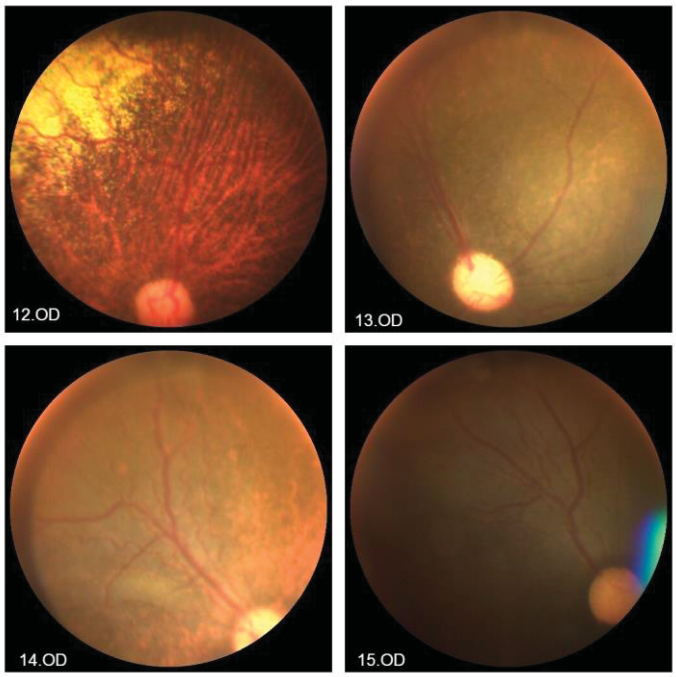
FIGURA 7 – OPHTHALMOLOGICAL FINDINGS IN GROUP 2.



SOURCE: The author (2022).

SUBTITLE: 1.OS. Arteriolar attenuation and absent tapetum. 2.OD. Arteriolar attenuation, hypoplastic tapetum, slight pigmentary mobilization and choroidosis. 3.OD. Mild arteriolar attenuation and hypoplastic tapetum with hyperreflectivity. 4.OS. Mildly affected with hypoplastic tapetum with hyperreflectivity. 5.OS. Increased arteriolar tortuosity, hypoplastic tapetum with hyperreflectivity and presence of neurosensory retinal detachment (circle). 6.OS. Arteriolar attenuation, hypoplastic tapetum with hyperreflectivity. 97. OS. Increased arteriolar tortuosity and tapetal hyperreflectivity. 8.OS. Increased arteriolar tortuosity and tapetal hyperreflectivity. 9. OD. Arteriolar attenuation, hypoplastic tapetum with hyperreflectivity and presence of neurosensory retinal detachment (circle). OS left eye, OD, right eye.

FIGURA 8 – OPHTHALMOLOGICAL FINDINGS GROUP 2.

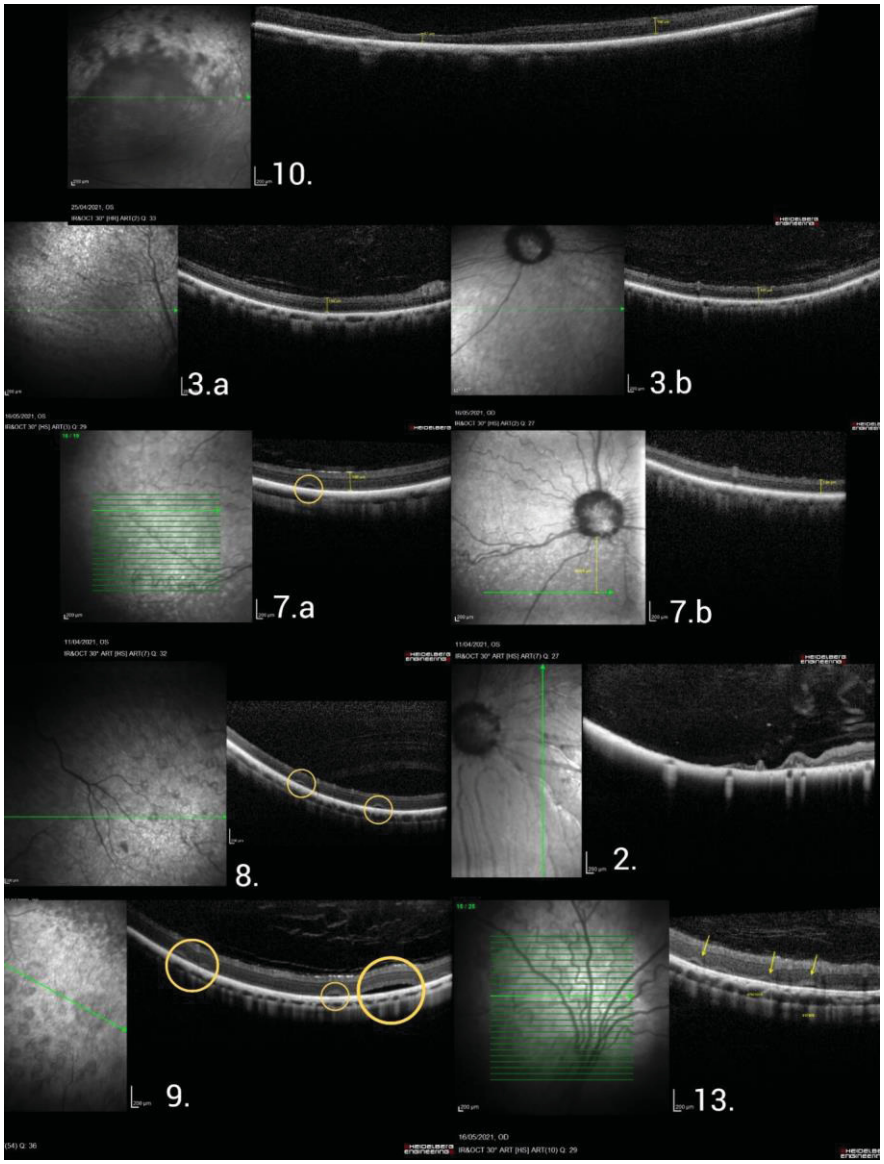


SOURCE: The author (2022).

SUBTITLE: 12.OD. Arteriolar attenuation and increased tortuosity, tapetum hypoplasic and hyperreflexivity associated choroidosis were present in OD. 13.OD. Arteriolar attenuation, tapetum absent, slight pigmentary mobilization and choroidosis were present in OD. 14.OD. Tapetum absent, slight pigmentary mobilization and choroidosis were present in OD. 15.OD. Tapetum absent in OD. OS left eye, OD, right eye.

Seven dogs (2, 3, 7-10 and 13) had OCT exams, in which focal NRD (Figure 9) were observed in 4 (7-9, 13) and reduction of the ventral retina in relation to the dorsal retina in all cases. An additional three dogs had focal NRDs detected in fundus photography (1, 4 and 5).

FIGURA 9 – MACULA OCT SCANS FROM RESPECTIVELY LABELED DOGS IN GROUP 2.



SOURCE: The author (2022).

SUBTITLE: 10. Local atrophy of the outer retina with collapse of the inner retina reducing the thickness to 87 µm. 3.a and 3.b. Ellipsoid zone rarefaction and measurements of dorsal retinal thickness (3.a) 154 µm relative to ventral retinal thickness (3.b) of 147 µm. 7.a and 7.b. Ellipsoid zone rarefaction, focal detachments of the neurosensory retina (NRD) (yellow circle) and measurements of dorsal retinal thickness (7.a) 186 µm relative to ventral retinal thickness (7.b) of 134 µm. 8. Presence of two focal NRDs (yellows circles). 2. Complete atrophy of all the ventral retinal layers in relation to the dorsal. 9. Presence of four focal NRDs (yellow circles). 13. Ellipsoid zone rarefaction and five focal NRD (yellow arrows). OCT, optical coherence tomography; NRD, detachment of the neurosensory retina.

OCT scans (dogs and age at the time of the exam present in Table 2) of dogs 3, 7, 10, and 14 showed an ONL medium thickness of 42 µm, 47.5 µm, 56 µm, and 64.5 µm respectively, with ellipsoid zone atrophy in one of the dogs (10) and ellipsoid zone rarefaction in dogs 3, 7, and 13.

4 DISCUSSION

A total of 144 mutations have been reported in the *GUCY2D* gene, of which 127 (88%) have been associated with LCA and 13 (9%) cause CRD[9]. The variants found in group 1 were AR with phenotypic expression of LCA, and our results correspond to those published in relation to the prevalence of the AR phenotype. All the gene variants related to the *GUCY2D* gene in humans described in this retrospective study have been previously identified[10].

Consanguinity is present in more than 10% of patients with hereditary retinal dystrophy (RD), and more specifically in LCA it accounts for 9% of cases, and in early RD it accounts for 2%[3]. Consanguineous marriages increase the risk of AR dystrophies, which is prevalent in South Asian countries where the practice is most common, as well as in the Pakistani population[11,12]. The present study showed a higher inbreeding rate of 30% in group 1.

Reported rates nystagmus are between 78.6% until 100% and severe visual impairment is a predominant feature in LCA[13,14]. The results of this study found a 50% prevalence of nystagmus, lower than previously published data. Contrarily, we found a higher percentage of patients with severely impaired BCVA (70%) in patients with LCA associated mutation in the *GUCY2D* gene in the no LP, LP or HM compared to previously published studies (57%)[15]. Comparing the visual acuity of group 1 with group 2, 100% of dogs had severe low visual acuity, failing both the cotton ball test and avoidance of obstacles in photopic and scotopic conditions. This finding support similar phenotypic expression of severe visual impairment due to a recessive mutation in the *GUCY2D* gene.

Some patients of group 1 showed RPE alteration, a finding that has been previously related to mutation of this gene[16]. Color fundus photography exams in group 1 showed subtle alterations, predominantly associated with arteriolar attenuation. As already well documented in the literature, the dissociation between function and morphology is very striking in patients with LCA and was confirmed in the present study[17]. The present study also found subtle changes to the fundus photographs of in group 2 that were better visualized on OCT as subtle outer retina changes, similar to group 1. The fundus image abnormalities included slight changes in arteriolar attenuation, pigmentary mobilization, choroidosis, and possible variations in the tapetal reflectivity. The presence of NRD and hyperreflectivity of the tapetum,

characteristic of the canine species and common in cases of progressive retinal atrophy, is a major difference due to the absence of tapeta in humans.

The ERGs were mostly undetectable but may maintain a residual rod response in some patients with *GUCY2D* associated LCA[17,18]. ERG scans of group 1 showed significant a- and b-wave reduction in humans, and in group 2 virtually no response to light stimulation of rods under scotopic and residual cone response in photopic conditions. ERGs are extremely compromised in both groups 1 and 2, compatible with the recessive mutation phenotype of the *GUCY2D* gene.

OCT scans show compromised integrity of the ONL and ellipsoid zone in patients with LCA[13,17]. Although other studies reported mild outer retinal involvement with progression over the years[15,16,17], group 1 had five patients who had OCT scans of the macula that showed greater outer retinal involvement than the previously published data. However, group 1 OCT scans ranged from apparent normality, outer retinal irregularities, focal ellipsoid zone atrophies, to complete outer retinal atrophy. Similar outer retinal changes were also found in group 2, including ellipsoid zone rarefaction, areas of outer retinal atrophy, and complete outer retinal atrophy in one dog (dog 10). Another study of APR in dogs associated with the *RPE65* mutation showed via OCT a reduction in total retinal thickness in the affected group, with the main reduction in the outer retina and an increase in the thickness of the RPE [19].

In normal dog eyes subjected to OCT, the total retinal thickness of the superior sector (dorsal) was 204 µm and the inferior (ventral) sector was 178 µm. Total retinal thickness was significantly greater in the superior sector compared to the inferior ($p = 0.010$)[20]. Another study of the ONL in dogs showed that it rapidly decreases in thickness between 4 and 12 weeks of age, and the average thickness of the ONL of the superior retina is 58.9 ± 4.6 µm, compared to the average thickness of the ONL of the inferior retina (52.4 ± 7.7 µm)[21]. Retinal OCTs of the dogs in group 2 showed a reduction in ventral retinal thickness relative to dorsal retinal thickness in the entire group, and reduction of ONL thickness in two dogs only. In addition, OCT scans and fundus photography identified retinal *bullae* (the same of NRD in human) which has also been observed in *Whippet* dogs affected with another form of inherited retinopathy [22]. Overall, the preservation of the ONL found in dogs in group 2 is similar to the OCT scans of group 1, in which the predominance of alterations is in the outer retina.

Patients with involvement of the macula with foveal hypo-reflectivity is not suggestive of fluid NRD, but rather is a degenerative process featuring photoreceptor loss and separation of the layers transiently for subsequent focal retinal atrophy at that location. This finding is similar to the NRD in dogs, the *bullae* and has been described previously in hereditary RD[23]. However, finding was not identified in any of the group 1 patients of the present study.

Another study involving 31 *German Spitz* dogs with a form of hereditary retinopathy, 58% of the affected dogs were male and the main fundoscopy findings were tapetal hyperreflectivity, diffuse vascular attenuation, hypopigmentation of the non-tapetal area, and pallor of the optic disc[24]. Three dogs affected by a different form of early onset hereditary retinopathy in the *Shih Tzu* breed showed similar findings [25]. In the present study, fundus photographs of group 2 showed very subtle changes similar to those already described in the literature. These data are also similar to those found on fundus photography of group 1, with six patients presented presenting very close to normal (or only showing abnormalities with FAF).

The limitations of this review was the absence of genetic evaluation of the entire group of dogs and the use a retrospective data. In addition, as inherent with all retrospective evaluations, review of the data could be more robust had greater continuity in the exams and better quality images been obtained.

This study demonstrates that the animal model of *German Spitz* dogs has clinical features similar to humans affected with LCA, these dogs show subtle changes in fundus photographs, retinal thickness reduction with changes in outer retina and important functional reduction determined by extreme visual impairment and reduction of a and b waves in ERG. Recently, a patent was published for gene therapy of the *GUCY2D* mutation associated with LCA, consisting of a recombinant adenovirus vector containing the nucleic acid for the production of guanylate cyclase. Restoration in a mouse model resulted in the improvement of the ERG by 45% for cones, and increased retinal organization on OCT compared to untreated mice [26]. Gene therapy in this group of dogs with a spontaneous mutation would be an excellent study for the treatment of variants in the *GUCY2D* gene in both dogs and potentially human patients.

REFERENCES

1. KOCH, K.-W.; STRYER, L. Controle de feedback altamente cooperativo da guanilato ciclase da haste da retina por íons de cálcio. *Nature* , v. 334, p. 64–66, 1998.

2. DUDA, T.; KOCH, K.-W. Doenças da retina associadas à guanilato ciclase do fotorreceptor. *Molecular and Cellular Biochemistry* , v. 230, n. 1-2, p. 129–138, 2002.

3. MOTTA, FL; MARTIN, RP; FILIPPELLI-SILVA, R.; SALLES, MV; SALLUM, JMF Frequência relativa de distrofias retinianas hereditárias no Brasil. *Scientific Reports* , v. 8, n. 1, p. 1–9, 2018.

4. HAMEL, CP Cone rod dystrofias. *Orphanet Journal of Rare Diseases* , v. 2, n. 1, p. 1–7, 2007.

5. JIANG, F.; XU, K.; ZHANG, X.; XIE, Y.; BAI, F.; LI, Y. GUCY2D mutations in a Chinese cohort with autosomal dominant cone or cone–rod dystrophies. *Documenta Ophthalmologica*, v. 131, n. 2, p. 105–114, 2015.

6. BORTOLINI, M.; WINKLER, P.A.; MORENO, J.C.D.; et al. Preliminary characterization of a novel form of progressive retinal atrophy in the German Spitz dog associated with a frameshift mutation in GUCY2D. *Veterinary Ophthalmology* [Internet], 2023 Mar 5 [cited 2023 Apr 16].

7. OCCELLI, L.M.; PASMANTER, N.; AYOUB, E.E.; PETERSEN-JONES, S.M. Changes in retinal layer thickness with maturation in the dog: an in vivo spectral domain optical coherence tomography imaging study. *BMC Veterinary Research*, v. 16, n. 1, p. 1–11, 2020.

8. MOWAT, F.M.; PETERSEN-JONES, S.M.; WILLIAMSON, H.; et al. Topographical characterization of cone photoreceptors and the area centralis of the canine retina. *Molecular Vision*, v. 14, p. 2518–27, dez. 2008.

9. SHARON, D.; WIMBERG, H.; KINARTY, Y.; KOCH, K.W. Genotype-functional-phenotype correlations in photoreceptor guanylate cyclase (GC-E) encoded by GUCY2D. *Progress in Retinal and Eye Research*, v. 63, p. 69–91, 2018.

10. CLINVAR. *ClinVar* [Internet]. NIH.gov, 2019. Available from: https://www.ncbi.nlm.nih.gov/clinvar/. Accessed 2022 Jul 2.

11. ZAFAR, S.; AHMAD, K.; ALI, A.; BAIG, R. Retinitis pigmentosa genes implicated in South Asian populations: a systematic review. *Journal of the Pakistan Medical Association*, v. 67, n. 11, p. 1734–1739, 2017.

12. GUL, H.; SHAH, A.H.; HARRIPAUL, R.; et al. Homozygosity mapping coupled with whole-exome sequencing and protein modelling identified a novel

missense mutation in GUCY2D in a consanguineous Pakistani family with Leber congenital amaurosis. *Journal of Genetics*, v. 100, n. 2, 2021.

13. HAHN, L.C.; GEORGIOU, M.; ALMUSHATTAT, H.; et al. The natural history of Leber congenital amaurosis and cone–rod dystrophy associated with variants in the GUCY2D gene. *Ophthalmology Retina*, p. 1–12, 2022.

14. JACOBSON, S.G.; CIDECIYAN, A.V.; PEShenko, I.V.; et al. Determining consequences of retinal membrane guanylyl cyclase (RetGC1) deficiency in human Leber congenital amaurosis en route to therapy: residual cone-photoreceptor vision correlates with biochemical properties of the mutants. *Human Molecular Genetics*, v. 22, n. 1, p. 168–183, 2013.

15. BOUZIA, Z.; GEORGIOU, M.; HULL, S.; et al. GUCY2D-associated Leber congenital amaurosis: a retrospective natural history study in preparation for trials of novel therapies. *American Journal of Ophthalmology*, v. 210, p. 59–70, 2020.

16. JACOBSON, S.G.; CIDECIYAN, A.V.; SUMAROKA, A.; et al. Leber congenital amaurosis due to GUCY2D mutations: longitudinal analysis of retinal structure and visual function. *International Journal of Molecular Sciences*, v. 22, n. 4, p. 1–12, 2021.

17. JACOBSON, S.G.; CIDECIYAN, A.V.; SUMAROKA, A.; et al. Outcome measures for clinical trials of Leber congenital amaurosis caused by the intronic mutation in the CEP290 gene. *Investigative Ophthalmology & Visual Science*, v. 58, n. 5, p. 2609–2622, 2017.

18. YI, Z.; SUN, W.; XIAO, X.; et al. Novel variants in GUCY2D causing retinopathy and the genotype-phenotype correlation. *Experimental Eye Research,* v. 208, p. 108637, 2021.

19. ANNEAR, M.J.; MOWAT, F.M.; OCCELLI, L.M.; et al. A comprehensive study of the retinal phenotype of Rpe65-deficient dogs. *Cells*, v. 10, n. 1, p. 1–16, 2021.

20. GRAHAM, K.L.; MCCOWAN, C.I.; CARUSO, K.; BILLSON, F.M.; WHITTAKER, C.J.G.; WHITE, A. Optical coherence tomography of the retina, nerve fiber layer, and optic nerve head in dogs with glaucoma. *Veterinary Ophthalmology*, v. 23, n. 1, p. 97–112, 2019.

21. DUFOUR, V.L.; YU, Y.; PAN, W.; et al. In-vivo longitudinal changes in thickness of the canine postnatal retina. *Experimental Eye Research*, v. 107926, jan. 2020.

22. SOMMA, A.T.; MORENO, J.C.D.; SATO, M.T.; et al. Characterization of a novel form of progressive retinal atrophy in Whippet dogs: a clinical, electroretinographic, and breeding study. *Veterinary Ophthalmology*, v. 20, n. 5, p. 450–459, 2017.

23. VAN DIJK, E.H.C.; BOON, C.J.F. Serous business: delineating the broad spectrum of diseases with subretinal fluid in the macula. *Progress in Retinal and Eye Research*, v. 84, p. 100955, mar. 2021.

24. KELAWALA, D.N.; PATIL, D.B.; PARIKH, P.V.; SHETH, M.J.; JOSHI, C.G.; REDDY, B. Clinical studies on progressive retinal atrophy in 31 dogs. *Iranian Journal of Veterinary Research*, v. 18, n. 2, p. 119–123, 2017.

25. URKASEMSIN, G.; PONGPANICH, M.; SARIYA, L.; et al. Whole genome sequencing identifies a homozygous nonsense mutation in the JPH2 gene in Shih Tzu dogs with progressive retinal atrophy. *Animal Genetics*, v. 52, n. 5, p. 714–719, 2021.

26. Boye Shannon E., Hauswirth William W., Boye Sanford L., inventors; Hospira, Inc., assignee. *Raav-guanylate cyclase compositions and methods for treating*