matters

A potential mouse model for the erosive vitreoretinopathy of Wagner disease

Barbara Kloeckener-Gruissem, Maria T Dours-Zimmermann, Sergej Skosyrski, Sandra Brunner, Corey H Mjaatvedt, Dieter R Zimmermann, Klaus Rüther, Wolfgang Berger

Institute of Medical Molecular Genetics, University of Zurich; Institute of Surgical Pathology, University Hospital of Zurich; Department of Ophthalmology, Charité Universitätsmedizin; Department of Regenerative Medicine and Cell Biology, Medical University of South Carolina

Correspondence
kloeckener@medgen.uzh.ch

Disciplines
Genetics
Cell Biology
Medical Sciences
Molecular Biology
Physiology

Keywords
Eye Disease
Retina
Mouse Model
Versican
Vitreous

Type of Observation
Standalone

Type of Link
Orphan Data

Submitted Mar 9, 2016
Published Sep 16, 2016

Full Open Access
Supported by the Velux Foundation, the University of Zurich, and the EPFL School of Life Sciences.

Abstract

Patients with the very rare eye pathology Wagner disease (OMIM #143200) present with an abnormal (empty) vitreous, retinal detachment and altered electroretinogram (ERG). The disease is progressive and can eventually lead to blindness. No therapy can be offered to date. The genetic basis is the presence of mutations in the VCAN gene, encoding the large extracellular matrix molecule versican, which is a component of the vitreous. All identified mutations map to the canonical splice sites flanking exon 8, resulting in low number of aberrant splice products and a severe increase in two (V2, V3) of the four naturally occurring splice variants. The pathomechanism of Wagner's disease is poorly understood and a mouse model may afford further insight. The $hdf^{-/-}$ mice, named for their initial phenotype of heart defects, carry a null allele for Vcan that leads to embryonic lethality when homozygous, but heterozygote animals are viable. Here we investigated a possible eye phenotype in the heterozygous animals. While the overall morphology of retina and ciliary body appears to be normal, older (17 months) mutant animals show a decrease in ERG signalling profiles affecting the a-, b- and c-waves. This aspect of altered ERG profile demonstrates similarities to the human disease manifestation and underlines the suitability of heterozygous $hdf^{+/-}$ mice as a model for Wagner disease.

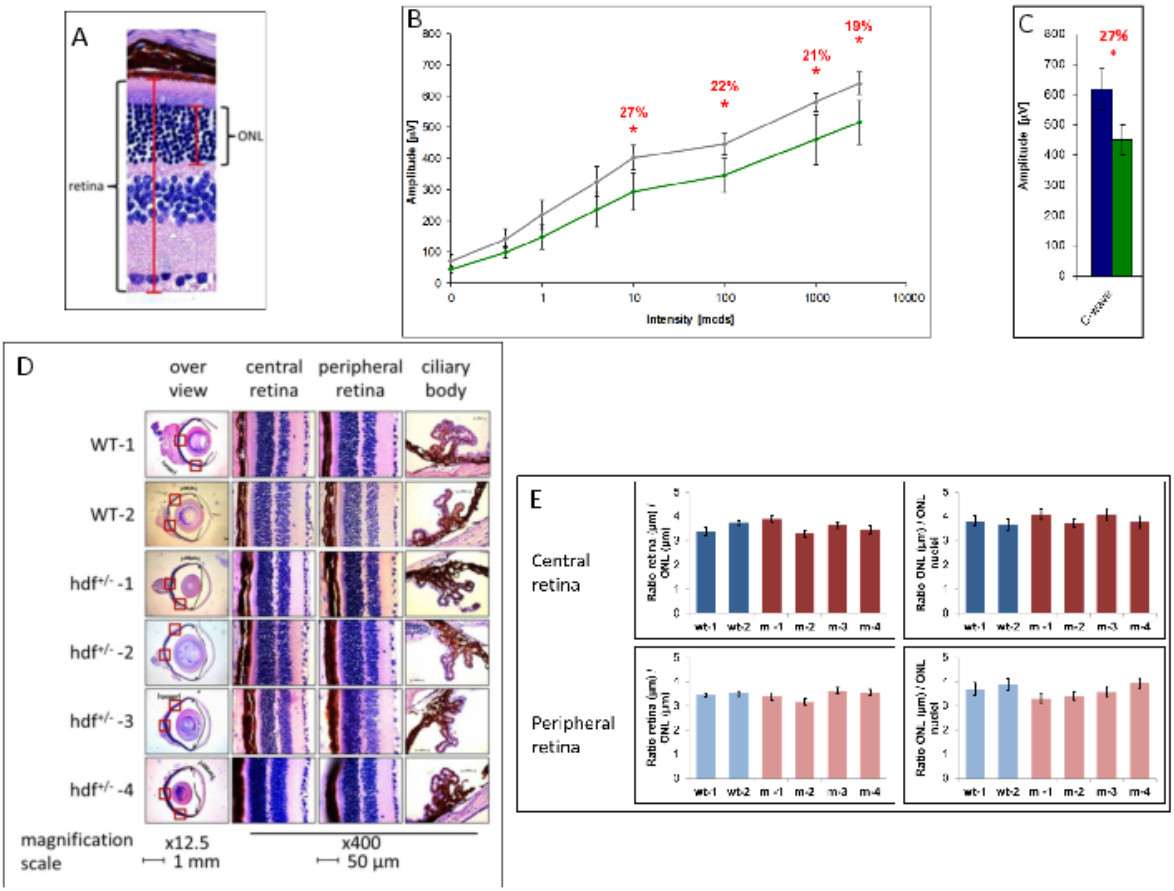
Introduction

Wagner disease (OMIM# 143200) is a rare autosomal dominant progressive disorder belonging to the group of hereditary vitreoretinal degenerations [1] (http://www.ncbi.nlm.nih.gov/books/NBK3821/). Vision impairment causes a major handicap for the affected. Clinically, the disease presents typically with the hallmark features of an empty vitreous cavity with fibrillary condensations, avascular strands and veils. Additionally, chorio-retinal atrophy, which may be reflected in altered ERG, and peripheral tractional retinal detachment are frequently observed [2] [3]. The syndrome shows full penetrance with manifestation at early adolescence. The disease is furthermore characterised by more heterogeneous clinical aspects, that is, cataracts and myopia [1]. As VCAN has been shown to be mutated in the majority of patients with Wagner syndrome, the potential pathomechanism likely involves the large core protein isoforms Vo and/or V1 of the extracellular matrix chondroitin sulphate proteoglycan versican with a length of 3396 and 2409 amino acids, respectively [4]. Furthermore, versican has been found as a component of the vitreous [5] [6]. To date all identified mutations in patients with Wagner disease map to the canonical splice sites bordering exon 8 [7] [8] [9] [10] [11] [12] [13]. Under normal conditions, 4 alternative splice isoforms are produced, which differ in the presence of exons 7 and 8 (variant Vo contains exon 7 and 8, V1 lacks exon 7, V2 lacks exon 8 and V3 lacks exon 7 and 8). It has been suggested that the splice site mutations lead to aberrant splice products and most strikingly to abnormal quantity of the normal transcript isoforms, in particular a dramatic increase in V2 [14] [4] [11]. The protein domains encoded by the large exons 7 and 8 carry 23 potential glucosaminoglycan attachment sites [15], suggesting that the larger isoforms contribute to a gel-maintenance function within the vitreous. In order to shed more light on the disease mechanism, we explored the phenotype of the mouse mutant model hdf which carries a non-functional Vcan gene [16]. Mouse and human versican share a similar genomic organisation of the gene with 77% identity at the DNA sequence level and 64% at the amino acid level, with variations spread throughout the protein rather than being clustered (http://www.ncbi.nlm.nih.gov/gene/, Jan 2016).

Furthermore, alternative splicing of the two largest exons is conserved in both species, yielding the 4 isoforms [17]. These facts led us to investigate the suitability of Vcan knockout mice as a model for Wagner disease. Homozygous hdf mice (Vcan null) have previously been described; they are embryonic lethal due to severe heart and limb developmental defects [17] [16]. In contrast, heterozygous animals are viable and appear at first sight normal, but eye phenotypes have not been investigated. Since the Wagner disease phenotype is dominant and mutations are heterozygous, the occurrence of an eye phenotype in the heterozygous hdf$^{+/-}$ mouse mutant seemed plausible.

Objective

In humans heterozygous mutations in the VCAN gene cause the dominantly inherited erosive vitreoretinopathy of Wagner disease. To understand the underlying pathophysiology we investigated the effects of the heterozygous vcan null mutation in hdf$^{+/-}$ mice on eyes.



a

Figure Legend

Figure 1. Retinal function and morphology of hdf$^{+/-}$ mice and their wild type siblings.

(A) Cross-section of mouse retina to demonstrate morphological evaluations by measuring the thickness of the retina (left long red bar) and the outer nuclear layer (ONL) (right short red bar).

(B) Quantitative evaluation of ERGs showing the average amplitude of the b-wave measured in 6 wild types (grey line) and 6 Vcan heterozygous mutants (hdf$^{+/-}$) (green line) at the age of 17 months. At a light intensity of 10 mcds/m^2 a significant reduction in the mutant mice was observed, which remained significant with increasing light intensity.

Error bars display confidence intervals at 95%.
(C) ERG evaluation of the c-wave amplitude obtained from the same mice as shown in (B). Mutant mice show a statistically significant reduction of 27% in amplitude.
(D) Haematoxilin staining of retina and ciliary body from 4 mutant ($hdf^{+/-}$ 1–4) and 2 wild type (WT1-2) animals at the age of 17 months. Representative images were chosen for each animal. The red boxes in the overview panel indicate the central and peripheral regions displayed in more detail (enlarged 400x). Typical arrangement of retinal layers at central and peripheral positions is shown from left to right: RPE (redish-brownish colour), followed by blue staining nuclei of the outer ONL and the inner nuclear layer (INL) and the ganglion cell layer (blue nuclei). The right column shows images of ciliary bodies. Scale for overview is 1 mm, for enlarged areas 50 µm.
(E) Quantitative assessment of retinal thickness in same animals as (E). Top graphs show ratios measured at central retina (retina thickness (µm)/ONL thickness (µm)) and ONL thickness (µm)/number of nuclei in ONL. Bottom graphs show same measurements at the peripheral retinal position. For each animal 15 sections were measured. Displayed are the average values; error bars show confidence intervals. Details for measurement are given in (A). P = <0.05.

Animals

The $hdf^{+/-}$ mouse strain was generated at the Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, USA [13]. The animals were bred to yield a segregating, viable population of heterozygous and wild type siblings. Mice were kept at the Institute of Pathology, University of Zurich.

ERG

At age 5.5 months, animals were transferred to the Department of Ophthalmology, Charité Universitätsmedizin, Berlin, Germany, where Ganzfeld ERG (scotopic and photopic electroretinogram) measurements were performed as described [17] at age 5.5 months, 12 months and 17 months. After the last time point, 12 animals (6 wild type and 6 $hdf^{+/-}$ mice) were sacrificed and eyes were removed for morphological analysis at the Institute of Medical and Molecular Genetics, University of Zurich.

Histology

Eyes were fixed and embedded in paraffin, and 2–3 µm sections were cut and were stained with haematoxylin-eosin as described [18]. Analysis was performed using Zeiss Axioplan2 imaging light microscopy. Sections were evaluated at the ciliary body and also at the central and peripheral retina. Thickness (µm) of the entire retina and ONL (outer nuclear layer) as well as the nuclei in the ONL was measured (Fig. 1A). To correct for normal variation in eye sizes, statistical analysis was done after calculating the ratio of retinal thickness and OLN thickness and the ratio of ONL thickness and cell number in the ONL. For each of the 6 animals, (2 wild types and 4 $hdf^{+/-}$ mutants) 3 slides were evaluated to yield a total of 15 measurements per animal. For statistical evaluation GraphPad v5 was used, first to assess normal distribution of the data using D'Agostino & Pearson test and second to assess significance using one-way ANOVA with Bartlett's test.

Results & Discussion

Electrophysiological response

At age 5.5 months and 12 months, the scotopic and photopic ERGs did not differ between mutant and wild type siblings (data not shown). Furthermore, ophthalmoscopic analysis of wild type and mutant mice at 5.5 months did not reveal a difference (data not shown). At age 17 months, in the mutant animal eyes, the b-wave amplitude of the scotopic ERG was significantly reduced by 22% on average (Fig. 1B; 10 [mcds]: t(10) = 3.9, P = 0.00296; 100 [mcds]: t(10) = 3.97, P = 0.00264; 1000 [mcds]: t(10) = 3.64, P = 0.00454; 3000 [mcds]: t(10) = 4.06, P = 0.002287). The a-wave amplitude showed, for some light intensities, statistically significant differences (10 [mcds]: t(10)=0.91, P = 0.3824; 100 [mcds]: t(10)=2.99, P = 0.0136; 1000 [mcds]: t(10) = 3.07, P = 0.0118; 3000 [mcds]: t(10) = 2.18, P = 0.0544; 30000 [mcds]: t(10) = 2.69, P = 0.0228; 60000 [mcds]: t(10) = 2.02, P = 0.0710; 90000 [mcds]: t(10) = 2.3, P = 0.0439; 300000 [mcds]: t(10) = 1.95, P = 0.0797). The c-wave was also decreased (Figure 1c, t(10) = 4.99, P = 0.00054), indicating that

in addition to photoreceptors and inner layer of retina, the retinal pigment epithelium (RPE) is also involved [18].

Histological analysis

Eyes from the individual mutants were analysed for ERG beforehand and were subjected to histological analysis of the retina. All retinas displayed a proper morphological distribution of the various cell layers positioned at the central or peripheral retina or at the ciliary body when comparing heterozygous mutant and wild type siblings (Fig. 1D). Furthermore, quantitative analysis of the thickness of the retina or the outer nuclear layer also did not reveal a difference between mutant and wild type siblings (Fig. 1E, central and peripheral retina: ratio retina [µm] /ONL [µm] nuclei ($t(4) = 0.03285$, $P = 0.9754$ and $t(4) = 0.3736$, $P = 0.7277$, respectively) and for ratio ONL [µm] /ONL nuclei ($t(4) = 1.303$, $P = 0.2624$ and $t(4) = 2.724$, $P = 0.0528$, respectively).

Taking the morphological and ERG studies together, a functional defect is likely due to reduced signalling from the retinal cells. Of note, a collagen (Col2a1) mouse model for Stickler Syndrome [19], a connective tissue disease with syndromic features affecting limbs and an eye phenotype very similar to that observed in Wagner disease [1] [20], had previously been shown to display morphological alterations in the ciliary body only in older mice (18 months). Therefore, we also examined potential changes in the ciliary body in the hdf mouse strain. Based on the image observation, no obvious difference between wild type and mutant animals could be detected (Fig. 1D).

The dominantly inherited Wagner disease is associated with heterozygous mutations in the VCAN gene, leading to abnormal splicing. While the hallmark clinical phenotype is affecting the vitreous, retinal complications are common, also reflected by abnormal ERG [1] [3]. The hdf mouse model is characterised by a null mutation in the Vcan gene. We sought to explore an eye phenotype in heterozygous $hdf^{+/-}$ mice, possibly phenocopying the human disease. In younger mice, no difference was seen between the wild type and mutant animals but the ERG showed abnormalities in older animals. A slight reduction of a-wave suggested that the photoreceptor cells are involved. A reduction of the b-wave implicated synaptic or post-synaptic effects on the bipolar cells. Generally, the b-wave may also be affected when the blood flow through the central retinal artery is blocked or the membrane potential of Müller cells is changed as result of an altered extracellular potassium concentration [21]. Which of these conditions apply here needs to be further investigated. In addition to the b-wave, the c-wave has also been reduced in the older $hdf^{+/-}$ mice, suggesting an involvement of the RPE or the RPE-photoreceptor complex. However, as the a-wave is normal a potential damage of the photoreceptors is at least not detectable. The c-wave is not a typical feature of the human ERG, but is found in several animals, including mice. Interestingly, the age-related progressive nature of the phenotype in Wagner patients is mimicked in the $hdf^{+/-}$ mouse model, showing an altered retinal function at increased age. Whether the patho-mechanism is also comparable remains to be investigated because the mouse strain carries an expression null allele [16], while human patients still may express the two smallest splice variants V2 and V3 from the affected allele. It has previously been suggested that the splice defect might result in an imbalanced ratio of transcript isoforms that may lead to an imbalance of versican protein isoforms [9] [4] [11]. The observation that retinal morphology was not altered in $hdf^{+/-}$ mice suggests that the abnormal ERG response may be caused by cellular signalling defects rather than cell death. Furthermore, the intact ciliary body suggests that the heterozygous null allele of Vcan does not exert a major structural function with respect to the ciliary body, unlike the Col2a1 null gene [19].

Conclusions

Taken together, a functional similarity of the $hdf^{+/-}$ mouse to the human Wagner disease has been demonstrated. However, further experiments are needed to establish the hdf-mouse strain as a valid animal model for this hereditary vitreoretinal degeneration.

Additional Information

Methods

Animals

The hdf$^{+/-}$ mouse strain was generated at the Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, USA [13]. The animals were bred to yield a segregating, viable population of heterozygous and wild type siblings. Mice were kept at the Institute of Pathology, University of Zurich.

ERG

At age 5.5 months, animals were transferred to the Department of Ophthalmology, Charité Universitätsmedizin, Berlin, Germany, where Ganzfeld ERG (scotopic and photopic electroretinogram) measurements were performed as described [17] at age 5.5 months, 12 months and 17 months. After the last time point, 12 animals (6 wild type and 6 hdf$^{+/-}$ mice) were sacrificed and eyes were removed for morphological analysis at the Institute of Medical and Molecular Genetics, University of Zurich.

Histology

Eyes were fixed and embedded in paraffin, and 2–3 µm sections were cut and were stained with haematoxylin-eosin as described [18]. Analysis was performed using Zeiss Axioplan2 imaging light microscopy. Sections were evaluated at the ciliary body and also at the central and peripheral retina. Thickness (µm) of the entire retina and ONL (outer nuclear layer) as well as the nuclei in the ONL was measured (Fig. 1A). To correct for normal variation in eye sizes, statistical analysis was done after calculating the ratio of retinal thickness and OLN thickness and the ratio of ONL thickness and cell number in the ONL. For each of the 6 animals, (2 wild types and 4 hdf$^{+/-}$ mutants) 3 slides were evaluated to yield a total of 15 measurements per animal. For statistical evaluation GraphPad v5 was used, first to assess normal distribution of the data using D'Agostino & Pearson test and second to assess significance using one-way ANOVA with Bartlett's test.

Supplementary Material

Please see https://sciencematters.io/articles/201605000004.

Funding Statement

ProRetina Deutschland e.V. to KR.

Acknowledgements

We would like to thank Susanne Hubman for excellent technical work on retinal histology.

Ethics Statement

Animals were kept in compliance with the National Institute of Health guidelines as approved by the Swiss Cantonal Veterinary Office.

Citations

[1] Kloeckener-Gruissem B and Amstutz C. "VCAN-Related Vitreoretinopathy". In: *GeneReviews* (2009).

[2] Roman A. Graemiger et al. "Wagner Vitreoretinal Degeneration". In: *Ophthalmology* 102.12 (Dec. 1995), pp. 1830–1839. DOI: 10.1016/s0161-6420(95)30787-7. URL: http://dx.doi.org/10.1016/S0161-6420(95)30787-7.

[3] Wagner and H. "Ein bisher unbekanntes Erbleiden des Auges (degeneratiohyaloideo-retinalis hereditaria), beobachtet im kanton Zürich." In: *Klinische Monatsblätter Augenheilkunde* 100 (1938), pp. 840–857.

[4] Tatsuro Miyamoto et al. "Identification of a Novel Splice Site Mutation of the CSPG2 Gene in a Japanese Family with Wagner Syndrome". In: *Investigative Opthalmology and Visual Science* 46.8 (Aug. 2005), p. 2726. DOI: 10.1167/iovs.05-0057. URL: http://dx.doi.org/10.1167/iovs.05-0057.

[5] Anthony Reardon et al. "The large chondroitin sulphate proteoglycan versican in mammalian vitreous". In: *Matrix Biology* 17.5 (Oct. 1998), pp. 325–333. DOI: 10.1016/s0945-053x(98)90085-3. URL: http://dx.doi.org/10.1016/S0945-053X(98)90085-3.

[6] Achilleas D Theocharis et al. "Occurrence and structural characterization of versican-like proteoglycan in human vitreous". In: *Biochimie* 84.12 (Dec. 2002), pp. 1235–1241. DOI: 10.1016/s0300-9084(02)00015-9. URL: http://dx.doi.org/10.1016/S0300-9084(02)00015-9.

[7] Brézin et al. “A new VCAN/versican splice acceptor site mutation in a French Wagner family associated with vascular and inflammatory ocular features”. In: *Molecular Vision* 17 (2011), pp. 1669–1678.

[8] Xuejuan Chen et al. “Targeted Sequencing of 179 Genes Associated with Hereditary Retinal Dystrophies and 10 Candidate Genes Identifies Novel and Known Mutations in Patients with Various Retinal Diseases”. In: *Investigative Opthalmology and Visual Science* 54.3 (Mar. 2013), p. 2186. DOI: 10.1167/iovs.12-10967. URL: http://dx.doi.org/10.1167/iovs.12-10967.

[9] Barbara Kloeckener-Gruissem et al. “Novel VCAN mutations and evidence for unbalanced alternative splicing in the pathogenesis of Wagner syndrome”. In: *European Journal of Human Genetics* 21.3 (June 2012), pp. 352–356. DOI: 10.1038/ejhg.2012.137. URL: http://dx.doi.org/10.1038/ejhg.2012.137.

[10] S. P Meredith et al. “Clinical characterisation and molecular analysis of Wagner syndrome”. In: *British Journal of Ophthalmology* 91.5 (Oct. 2006), pp. 655–659. DOI: 10.1136/bjo.2006.104406. URL: http://dx.doi.org/10.1136/bjo.2006.104406.

[11] Arijit Mukhopadhyay et al. “Erosive Vitreoretinopathy and Wagner Disease Are Caused by Intronic Mutations in CSPG2 / Versican That Result in an Imbalance of Splice Variants”. In: *Investigative Opthalmology and Visual Science* 47.8 (Aug. 2006), p. 3565. DOI: 10.1167/iovs.06-0141. URL: http://dx.doi.org/10.1167/iovs.06-0141.

[12] Shawn M. Ronan et al. “Mutational Hot Spot Potential of a Novel Base Pair Mutation of the CSPG2 Gene in a Family With Wagner Syndrome”. In: *Archives of Ophthalmology* 127.11 (Nov. 2009), pp. 1511–1519. DOI: 10.1001/archophthalmol.2009.273. URL: http://dx.doi.org/10.1001/archophthalmol.2009.273.

[13] Pierre-Raphaël Rothschild et al. “De Novo Splice Mutation in the Versican Gene in a Family With Wagner Syndrome”. In: *JAMA Ophthalmology* 131.6 (June 2013), pp. 805–807. DOI: 10.1001/jamaophthalmol.2013.681. URL: http://dx.doi.org/10.1001/jamaophthalmol.2013.681.

[14] Barbara Kloeckener-Gruissem et al. “Identification of the genetic defect in the original Wagner syndrome family”. In: *Molecular Vision* 12 (2006), pp. 350–355.

[15] M T Dours-Zimmermann and D R Zimmermann. “A novel glycosaminoglycan attachment domain identified in two alternative splice variants of human versican.” In: *Journal of Biological Chemistry* 269 (1994), pp. 32992–32998.

[16] H. Yamamura et al. “A heart segmental defect in the anterior-posterior axis of a transgenic mutant mouse”. In: *Developmental Biology* 186.1 (June 1997), pp. 58–72. DOI: 10.1006/dbio.1997.8559. URL: http://dx.doi.org/10.1006/dbio.1997.8559.

[17] C.H. Mjaatvedt et al. “TheCspg2Gene, Disrupted in thehdfMutant, Is Required for Right Cardiac Chamber and Endocardial Cushion Formation”. In: *Developmental Biology* 202.1 (Oct. 1998), pp. 56–66. DOI: 10.1006/dbio.1998.9001. URL: http://dx.doi.org/10.1006/dbio.1998.9001.

[18] Jiang Wu, Neal S. Peachey, and Alan D. Marmorstein. “Light-Evoked Responses of the Mouse Retinal Pigment Epithelium”. In: *Journal of Neurophysiology* 91.3 (Oct. 2004), pp. 1134–1142. DOI: 10.1152/jn.00958.2003. URL: http://dx.doi.org/10.1152/jn.00958.2003.

[19] Kai Kaarniranta et al. “A mouse model for Stickler’s syndrome: Ocular phenotype of mice carrying a targeted heterozygous inactivation of type II (pro)collagen gene (Col2a1)”. In: *Experimental Eye Research* 83.2 (Aug. 2006), pp. 297–303. DOI: 10.1016/j.exer.2005.11.027. URL: http://dx.doi.org/10.1016/j.exer.2005.11.027.

[20] Robin et al. “Stickler Syndrome”. In: *University of Washington and Seattle and Seattle (WA)* (1993).

[21] Perlman and Ido. “The Electroretinogram: ERG”. In: *Webvision: The Organization of the Retina and Visual System* University of Utah Health Sciences Center, Salt Lake City (UT) (1995).