Review

Animal Models of Choroidal Neovascularization: A Systematic Review

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Purpose. Animal models of choroidal neovascularization (CNV) are extensively used to characterize the pathophysiology of chorioretinal diseases with CNV formation and to evaluate novel treatment strategies. This systematic review aims to give a detailed overview of contemporary animal models of CNV.

Methods. A systematic search was performed in PubMed and EMBASE from November 20, 2015, to November 20, 2020, for mammalian animal models of CNV. Following inclusion by two investigators, data from the articles were extracted according to a predefined protocol.

Results. A total of 380 full articles, representing 409 independent animal models, were included. Mice were by far the most utilized animal (76%) followed by rats and non-human primates. The median age of rodents was 8 weeks but with a wide range. Male animals were used in 44% of the studies, but 32% did not report the sex. CNV was laser induced in 89% of the studies, but only 44% of these reported sufficiently on standard laser parameters. Surprisingly, 28% of the studies did not report a sample size for quantitative CNV evaluation. Less than half of the studies performed quantitative in vivo evaluation, and 73% evaluated CNV quantitatively ex vivo. Both in vivo and ex vivo evaluations were conducted primarily at day 7 and/or day 14.

Conclusions. The laser-induced mouse model is the predominant model for experimental CNV. The widespread use of young, healthy male animals may complicate clinical translation, and inadequate reporting challenges reproducibility. Definition and implementation of standardized methodologic and reporting guidelines are attractive.

Keywords: animal model, choroidal neovascularization, neovascular age-related macular degeneration

Chorioretinal diseases with formation of choroidal neovascularizations (CNVs) represent an extensive problem for patients and for healthcare systems. As the most prominent example, age-related macular degeneration (AMD) is the leading cause of irreversible visual impairment and blindness among the aged population in the Western world, and neovascular AMD (nAMD) constitutes a particular burden.[1,2] The hallmark of nAMD is CNV with the risk of rapid, irreversible destruction of retinal integrity if untreated.[3,4] Therefore, preclinical animal research modeling CNV is extensively used in an attempt to characterize the pathophysiological mechanisms behind its development and progression and to investigate and advance current treatment options or provide novel treatment strategies.[5–10]

In recent years, several reviews in other fields such as cancer biology and resuscitation research have focused on the translational potential of animal models, albeit with discouraging results.[11–14] Furthermore, the susceptibility of animal models to bias and lack of adequate reporting is well studied. This complicates reproducibility and, ultimately, reduces the possibility of translation into a clinical setting.[15]

The pertinent question is how animal models reflect CNV formation in human chorioretinal diseases such as nAMD. Animal modeling of a progressive degenerative retinal disease that develops in the aged population sets some natural limitations for the reflection of "true" nAMD. Nevertheless, the design of such a study should arguably reflect the clinical population and scenario to the highest degree possible to increase the chance of successful translation.[16] In this review, we systematically survey the current field of mammalian animal models of CNV to provide a detailed overview of contemporary models.

Methods

Definitions and Inclusion/Exclusion Criteria

This systematic review was conducted in accordance with the PRISMA guidelines (PRISMA checklist is provided as

Supplementary Material S1).[17] The review was not registered. Search strategy, inclusion/exclusion criteria, and a (broad) definition of an animal model of CNV were established prior to the search. The data extraction protocol was created prior to data extraction. We included all original studies within the past 5 years that used animal models of CNV regardless of induction method. All studies using humans or non-mammalian species, as well as all in vitro and ex vivo models, were excluded. All studies modeling what corresponds to polypoidal choroidal vasculopathy or retinal angiomatous proliferation (i.e., studies without clear modeling of classic/type 2 or occult/type1 CNV) were excluded. We restricted the search to articles written in English. Letters, commentaries, editorials, case reports, reviews, and abstract only were excluded.

Search Strategy

Using an elaborate search strategy, we searched PubMed and EMBASE from November 20, 2015, to November 20, 2020, and a search was performed November 23, 2020, using Google Chrome Version 87.0.4280.67 (x86_64). The search strategy was adapted from Hooijmans et al.,[18] with inspiration from two earlier systematic reviews by Vognsen et al.[13] and Fabian-Jessing et al.[19] The search string was built from several blocks. The first part consisted of synonyms of CNV and the second part types of mammals used in animal model research as described by Hooijmans et al.[18] The rest of the search string consisted of the above-mentioned limitations. The entire search strategy including inclusion/exclusion criteria is provided as Supplementary Material S2. Articles with an Epub date prior to the search period but with a publication date, as assigned to the reference, within the search period were included.

Articles were imported to EndNote X8.2 (Bld 13302), and duplicates were removed using the "Find Duplicates" feature in EndNote, subsequently manually by title, and detected by Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia) during import. Articles were imported into Covidence and independently screened (titles/abstracts) by two investigators, who subsequently screened full-text articles. If included, data from the articles were extracted according to a predefined extraction protocol which is provided as Supplementary Material S3. If the study involved more than one group, and if not specified under the definition of the included variables, data were extracted from the control group. Data were only extracted from the articles; methods referred to in previous papers were not included, in which case the data were generally scored as "Not reported."

Statistics

Descriptive statistics were performed to characterize the included studies. Kappa statistic analysis was performed to assess interrater agreement between the two reviewers performing title/abstract screening and full-text review. Statistical analyses were performed in Stata 17 (StataCorp, College Station, TX, USA). Figures depicting descriptive statistics were created using Prism 9.3.1 for Mac OS X (GraphPad Software, San Diego, CA, USA).

Results

We retrieved 462 articles from PubMed and 490 articles from EMBASE; 406 duplicates were removed. The two reviewers assessed 546 titles/abstracts in Covidence; 146 articles were found irrelevant based on title and abstract screening, and 20 conflicts between reviewers were resolved by consensus. A kappa of 0.92 for agreement between reviewers was calculated. Based on full-text review of 400 articles, 20 articles were excluded (kappa = 0.90). In total, 380 articles published in 142 different journals were included (Fig. 1). A list of these articles is provided as Supplementary Material S4. Twenty-seven articles used more than one model (ranging from two to three models). Thus, 380 full articles represented 409 independent animal models of CNV that were included for data extraction. Throughout the present study, independent animal models will be termed "studies." The top three journals with the highest number of published CNV studies were *Investigative Ophthalmology & Visual Science* (39 studies, 10%), *Experimental Eye Research* (32 studies, 8%), and *Scientific Reports* (19 studies, 5%). Of the studies, 380 (93%) reported approval by local authorities or institutional committees, and 290 (71%) reported that the studies were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Also, 246 studies (60%) referred to other studies for complete methodology (see Supplementary Material S5).

Animal characteristics are presented in Table 1. The mouse was by far the most used animal model for experimental CNV (76%). Due to inaccurate reporting and varying nomenclature, it was difficult to extract data on the type of substrain uniformly. However, our data suggest that approximately one-third of all mouse studies at least once in the Methods section reported strain/substrain as a variation of the annotation "C57BL/6" without further specification of substrain. Rats (especially Brown Norway) were also widely used (17%), whereas non-human primates (5%) and rabbits (2%) were used to some extent. Only one pig model and one dog model were used within the search period.

Young mice and rats with a median age of 8 weeks were primarily used, although the age ranged from 3 to 54 weeks and 5 to 14 weeks for mice and rats, respectively. The median ages for all species are provided in Table 1. Age was unreported in 100 studies (25%). Male animals were predominantly used (44%), whereas 12% of studies used female animals and 12% used animals of both sexes. Sex was not reported in 131 studies (32%). Most studies used normal animals (94% for the control group and 83% for the experimental group). The median number of animals used in the smallest control group (or the smallest experimental group in case of no control group) reported for quantitative CNV evaluation was six (range, 2–23) for mouse studies. The smallest group for quantitative CNV evaluation was defined as the number of animals (not eyes or lesions unless the number of animals could be univocally determined on the basis of either) in the smallest group quantitatively evaluating CNV by any method. In 116 studies (28%), a sample size for quantitative CNV evaluation could not be identified. Also, 196 studies (48%) used animals from a commercial supplier; however, the supplier was not reported in 28% of the studies.

CNV Induction

The characteristics of CNV induction are presented in Table 2 for all studies and stratified by species. The administration route for anesthesia associated with surgical/laser induction models was most commonly intraperitoneal (39%) followed by intramuscular (8%). In a minor fraction, inhalation, intravenous, subcutaneous, or combinatory administration was

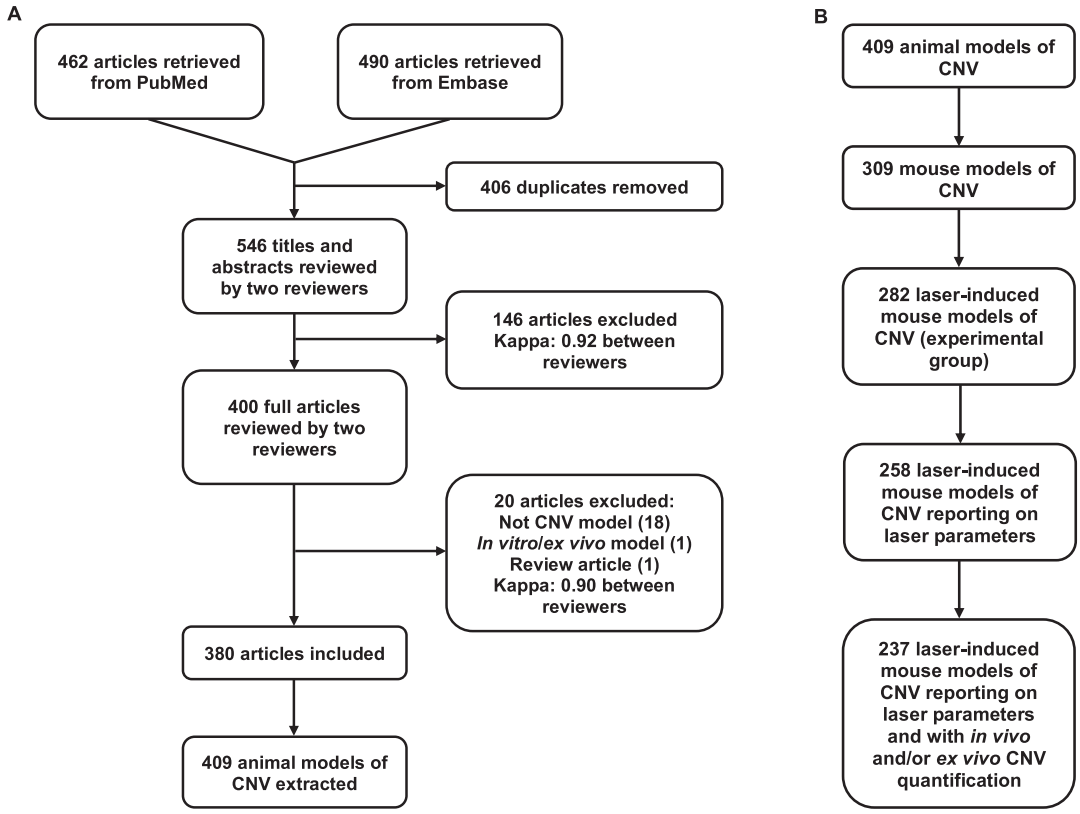

Figure 1. (**A**) Flow diagram depicting the selection process of articles and animal models. (**B**) Flow diagram depicting the selection process of laser-induced mouse models of CNV reporting on laser parameters and with CNV quantification.

used. The administration route was not reported in 194 studies (47%). Two-thirds of studies (66%) reported on specific anesthetics, whereas almost one-third (31%) did not. The most utilized induction method was laser/photocoagulation, which was used in 363 studies (89%) for the control group and 372 studies (91%) for the experimental group. For the control group, 27% of the studies included a group with "no induction," which, typically, was used as a negative control in expression studies and not for parts of the studies quantitatively evaluating the CNV. A minor fraction of mouse studies used transgenic animals for CNV induction (i.e., spontaneous CNV on a genetic background). The laser system was specified in 308 studies (75%), but laser parameters were insufficiently described in more than half of the studies (56%). In 30 studies (7%) utilizing laser for CNV induction, no laser parameters were reported.

Laser Parameters

Specific laser parameters were extracted for laser-induced mouse models of CNV and are presented in Table 3. We confined this extraction to the experimental group, because control groups sometimes displayed no induction. Furthermore, we limited extraction to studies with in vivo and/or ex vivo CNV quantification ($n = 237$) (Fig. 1B). We excluded one single parameter for one single study, which was likely a typographical error that could not be interpreted in a clear and meaningful way. In cases of minor errors (e.g., spot size expressed in millimeters instead of micrometers) we interpreted and extracted data to the best of our knowledge.

The median wavelength of lasers used was 532 nm (range, 514–831). Lasers with a specific wavelength of 532 nm were used in 83% of the studies reporting on wavelength. Lasers with a wavelength of 810 nm were used in 8% of the studies. Other wavelengths were reported in a very limited number of studies. Of the above-mentioned 237 models, 27% did not report on wavelength. The median power/intensity of lasers used was 160mW with a wide range (50–1000mW). The most frequently used intensities were 120mW (17%), 100mW (15%), 200mW (14%), 250mW (9%), and 150mW (8%); 4% did not report on the intensity of the laser. The median duration of laser application was 100 ms. Most studies used a duration of either 100 ms (58%) or 50 ms (23%); 5% did not report on the duration of laser application. The median laser spot size was 75 µm but with an approximately equal distribution among 50 µm (29%), 75 µm (26%), and 100 µm (25%); 14% did not report on laser spot size. The most frequently reported spot location was varying descriptions of placement around the optic disc (41%). "O'clock"/meridians (23%) and disc diameters from the optic disc (18%) were also frequently used; 26% did not report on spot location. In the studies reporting spot location as disc diameters from the optic disc the median number of disc diameters was 2 but with an anatomically relatively wide range (1–4.5). Only five studies reported spot location as distance from the optic disc. The median distance was 0.75 mm (range, 0.4–1.5). The median number of laser spots applied in one eye was 4 (range, 1–8); 14% of studies did not report the number of laser spots.

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Table 1. Animal Characteristics

Variable	All Models ($N = 409$)
Species	
Mouse, *n* (%)	309 (75.6)
Rat, *n* (%)	70 (17.1)
Brown Norway, *n*	53
Long Evans, *n*	10
Dark Agouti, *n*	3
Lister Hooded, *n*	1
Other, *n*	1
Not reported, *n*	2
Non-human primate, *n* (%)	19 (4.7)
Cynomolgus (*Macaca fascicularis*), *n*	9
Rhesus (*Macaca mulata*), *n*	6
Marmoset, *n*	3
Other, *n*	1
Rabbit, *n* (%)	9 (2.2)
New Zealand white, *n*	4
Chinchilla rabbit, *n*	2
Dutch-Belted, *n*	2
Other, *n*	1
Pig, *n* (%)	1 (0.2)
Dog, *n* (%)	1 (0.2)
Age (wk), median (range)	
Mouse	8 (3–54.4)
Rat	8 (5–14.3)
Non-human primate	189.2 (78.3–1800)
Rabbit	15.2 (14–21.7)
Pig	Not reported
Dog	52.2 (52.2)
Not reported, *n* (%)	100 (24.5)
Sex, *n* (%)	
Male	178 (43.5)
Female	50 (12.2)
Male and female	47 (11.5)
Unclear	3 (0.7)
Not reported	131 (32)
Weight (g), median (range)	
Mouse	22.5 (12.5–87.5)
Rat	200 (120–300)
Non-human primate	4000 (300–5000)
Rabbit	2625 (1750–3000)
Pig	Not reported
Dog	Not reported
Not reported, *n* (%)	320 (78.2)
Morbidity, control group, *n* (%)	
Normal animals	384 (93.9)
Aged	6 (1.5)
Genetically modified	56 (13.7)
High-fat diet	0 (0)
Combination	2 (0.5)
Other	2 (0.5)
Morbidity, experimental group, *n* (%)	
Normal animals	340 (83.1)
Aged	6 (1.5)
Genetically modified	90 (22)
High-fat diet	3 (0.7)
Combination	5 (1.2)
Other	2 (0.5)
Number of animals reported for quantitative CNV evaluation, median (range)	
Mouse	6 (2–23)
Rat	5 (2–18)
Non-human primate	3.5 (2–6)
Rabbit	3 (3–5)
Pig	6 (6)
Dog	Not relevant*
Not relevant, *n* (%)	48 (11.7)
Not reported, *n* (%)	116 (28.4)
Supplier of animals, *n* (%)	
Commercial supplier	196 (47.9)
Own institution	67 (16.4)
Both	32 (7.8)
Not reported	114 (27.9)

*No quantitative CNV evaluation.

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Table 2. CNV Induction

Variable	All Models ($N = 409$)	Mouse ($n = 309$)	Rat ($n = 70$)	Non-Human Primate ($n = 19$)	Rabbit ($n = 9$)	Pig ($n = 1$)	Dog ($n = 1$)
	n (%)						
Type of induction method for control group							
Laser	363 (88.8)	274 (88.7)	65 (92.9)	18 (94.7)	5 (55.6)	0 (0)	1 (100)
Subretinal injection (Matrigel)	2 (0.5)	1 (0.3)	0 (0)	0 (0)	1 (1.1)	0 (0)	0 (0)
Subretinal injection (VEGF gene therapy)	2 (0.5)	1 (0.3)	0 (0)	0 (0)	1 (1.1)	0 (0)	0 (0)
Subretinal injection (other agent)	2 (0.5)	2 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Surgical	1 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
Genetically (transgenic animal)	11 (2.7)	11 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Combination	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other	2 (0.5)	2 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No induction	111 (27.1)	93 (30.1)	14 (20)	1 (5.3)	3 (33.3)	0 (0)	0 (0)
No control group	3 (0.7)	2 (0.7)	1 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)
Type of induction method for experimental group							
Laser	372 (91)	282 (91.3)	66 (94.3)	19 (100)	4 (44.4)	0 (0)	1 (100)
Subretinal injection (Matrigel)	3 (0.7)	1 (0.3)	0 (0)	0 (0)	2 (22.2)	0 (0)	0 (0)
Subretinal injection (VEGF gene therapy)	3 (0.7)	1 (0.3)	1 (1.4)	0 (0)	1 (11.1)	0 (0)	0 (0)
Subretinal injection (other agent)	2 (0.5)	2 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Surgical	1 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)
Genetically (transgenic animal)	13 (3.2)	13 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Combination	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other	3 (0.7)	2 (0.7)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)
No induction	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No experimental group	14 (3.4)	10 (3.2)	3 (4.3)	0 (0)	1 (11.1)	0 (0)	0 (0)
Type of anesthesia for surgical/laser induction method for experimental group							
Intraperitoneal	158 (38.6)	122 (39.5)	36 (51.4)	0 (0)	0 (0)	0 (0)	0 (0)
Intramuscular	33 (8.1)	8 (2.6)	14 (20)	7 (36.8)	3 (33.3)	0 (0)	1 (100)
Subcutaneously	1 (0.2)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)	0 (0)
Intravenously	4 (1)	1 (0.3)	1 (1.4)	1 (5.3)	1 (11.1)	0 (0)	0 (0)
Inhalation	5 (1.2)	5 (1.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Combination	1 (0.2)	0 (0)	0 (0)	1 (5.3)	0 (0)	0 (0)	0 (0)
Not relevant	13 (3.2)	13 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Not reported	194 (47.4)	160 (51.8)	19 (27.1)	10 (52.6)	4 (44.4)	1 (100)	0 (0)
Specific anesthetics for surgical/laser induction reported							
Yes	270 (66)	191 (61.8)	59 (84.3)	13 (68.4)	6 (66.7)	0 (0)	1 (100)
Not relevant	13 (3.2)	13 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Not reported	126 (30.8)	105 (34)	11 (15.7)	6 (31.6)	3 (33.3)	1 (100)	0 (0)
Laser system described							
Yes	308 (75.3)	231 (74.8)	56 (80)	15 (79)	5 (55.6)	0 (0)	1 (100)
Not relevant	23 (5.6)	17 (5.5)	1 (1.4)	0 (0)	4 (44.4)	1 (100)	0 (0)
Not reported	78 (19.1)	61 (19.7)	13 (18.6)	4 (21)	0 (0)	0 (0)	0 (0)
Specification of laser parameters							
Yes, sufficiently	129 (31.5)	96 (31.1)	29 (41.4)	3 (15.8)	1 (11.1)	0 (0)	0 (0)
Yes, but not sufficiently	227 (55.5)	171 (55.3)	36 (51.4)	15 (79)	4 (44.4)	0 (0)	1 (100)
Not relevant	23 (5.6)	17 (5.5)	1 (1.4)	0 (0)	4 (44.1)	1 (100)	0 (0)
Not reported	30 (7.3)	25 (5.7)	4 (5.7)	1 (5.3)	0 (0)	0 (0)	0 (0)

Among the studies, 60% reported (an indication of) confirmation of a break in Bruch's membrane in terms of a so-called bubble formation. Two studies utilized optical coherence tomography (OCT) for confirmation, whereas 39% omitted to report on whether a break could be confirmed. More than two thirds of the studies (71%) did not report exclusion criteria (e.g., hemorrhage after laser application) for excluding single laser spots or burns—or whole eyes—in connection with CNV induction.

Quantitative CNV Evaluation

The characteristics of quantitative evaluation of CNV are presented in Table 4 for all studies and stratified by species. In vivo, CNV was predominantly evaluated by fluorescein angiography assessing leakage (35%). OCT was used in 49 studies (12%) and OCT angiography was used in 10 studies (2%). More than half of the studies (58%) did not evaluate CNV in vivo. In comparison, CNV was evaluated ex vivo in 73% of the studies, and retinal pigment epithelium

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Table 3. Laser Parameters of Laser-Induced Mouse Models of CNV

Variable	Models (n = 237)
Wavelength (nm)	
Median (range)	532 (514–831)
Not reported, n (%)	63 (26.6)
Power/intensity (mW)	
Median (range)	160 (50–1000)
Not reported, n (%)	10 (4.2)
Duration (ms)	
Median (range)	100 (20–800)
Not reported, n (%)	11 (4.6)
Spot size (µm)	
Median (range)	75 (50–300)
Not reported, n (%)	34 (14.4)
Spot location	
Disc diameters (DD) from optic disc, n (%)	43 (18.1)
If yes, number of DDs, median (range)	2 (1–4.5)
Distance from optic disc (mm)	7 (3)
If yes, distance in mm, median (range)	0.75 (0.4–1.5)
O'clock	55 (23.2)
Quadrants	5 (2.1)
Around disc	97 (40.9)
Between vessels	26 (11)
Other	14 (5.9)
Not reported	61 (25.7)
Number of spots	
Number of laser spots/burns, median (range)	4 (1–8)
Not reported, n (%)	32 (13.5)
Confirmation of break in Bruch's membrane, n (%)	
Reporting of bubble formation	142 (59.9)
OCT	2 (0.8)
Not reported	93 (39.2)
Exclusion criteria for burns/eyes, n (%)	
Yes	69 (29.1)
Not reported	168 (70.9)

Table 4. Quantitative Evaluation of CNV

	n (%)						
Variable	All Models (N = 409)	Mouse (n = 309)	Rat (n = 70)	Non-Human Primate (n = 19)	Rabbit (n = 9)	Pig (n = 1)	Dog (n = 1)
Software for CNV quantification							
Yes	290 (70.9)	227 (73.5)	52 (74.3)	7 (36.8)	3 (33.3)	1 (100)	0
Not reported	71 (17.4)	45 (14.6)	11 (15.7)	12 (63.2)	3 (33.3)	0	0
Not relevant (no CNV quantification)	48 (11.7)	37 (12)	7 (10)	0	3 (33.3)	0	1 (100)
In vivo evaluation (quantitative)							
Fundoscopy/ophthalmoscopy	2 (0.5)	2 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fundus photography	3 (0.7)	2 (0.7)	1 (1.4)	0 (0)	0 (0)	0 (0)	0 (0)
Fluorescein angiography	144 (35.2)	88 (28.5)	33 (47.1)	18 (94.7)	5 (55.6)	0 (0)	0 (0)
FITC-dextran angiography	0 (0.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
OCT	49 (12)	35 (11.3)	7 (10)	6 (31.6)	1 (11.1)	0 (0)	0 (0)
OCT angiography	10 (2.4)	6 (1.9)	4 (5.7)	0 (0)	0 (0)	0 (0)	0 (0)
Other	20 (4.9)	15 (4.9)	5 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)
No in vivo evaluation	237 (58)	199 (64.4)	32 (45.7)	1 (5.3)	3 (33.3)	1 (100)	1 (100)
Ex vivo evaluation (quantitative)							
RPE/choroidal flatmount	280 (68.5)	229 (74.1)	48 (68.6)	3 (15.8)	0 (0)	0 (0)	0 (0)
Retinal sections	35 (8.6)	21 (6.8)	11 (15.7)	2 (10.5)	0 (0)	1 (0.2)	0 (0)
FITC-dextran	60 (14.7)	42 (13.6)	15 (21.4)	3 (15.8)	0 (0)	0 (0)	0 (0)
Other	12 (2.9)	10 (3.2)	2 (2.9)	0 (0)	0 (0)	0 (0)	0 (0)
No ex vivo evaluation	110 (26.9)	71 (23)	15 (21.4)	14 (73.7)	9 (0.7)	0 (0)	1 (0)

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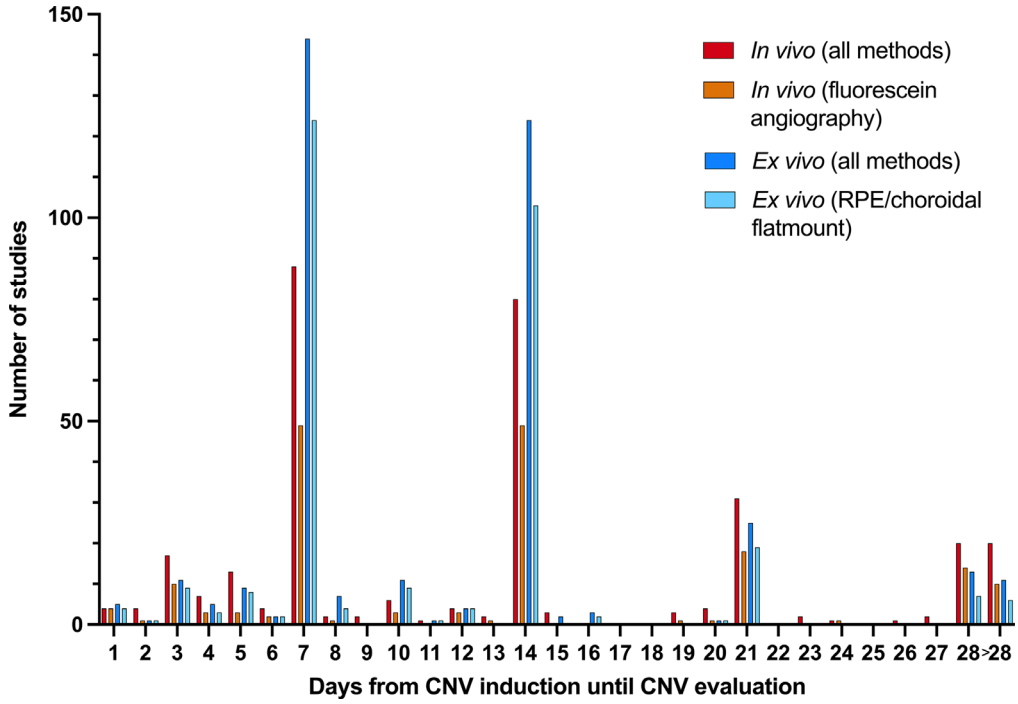

Figure 2. Frequency distribution of days from CNV induction until CNV evaluation, where >28 represents all time points beyond day 28 from CNV induction. *Red columns* represent studies evaluating CNV in vivo by all methods, and *orange columns* represent studies evaluating CNV in vivo only by fluorescein angiography. *Dark blue columns* represent studies evaluating CNV ex vivo by all methods, and *light blue columns* represent studies evaluating CNV ex vivo only by RPE/choroidal flatmount with or without FITC-dextran.

(RPE)/choroidal flatmounts were used in 69% of the studies. The software utilized by investigators for CNV quantification was reported in 71% of the studies. The number of days from CNV induction until quantitative evaluation of CNV in vivo and ex vivo are presented in Figure 2. Overall, the time between induction and evaluation varied among studies. For both in vivo and ex vivo evaluation, most evaluations were performed on day 7 and/or day 14.

Discussion

The purpose of this systematic review was to provide a detailed overview of contemporary mammalian animal models of CNV including a synthesis of animal characteristics and induction and evaluation parameters. Comprehensive narrative reviews with detailed description of the specific models by Pennesi et al.[10] and Grossniklaus et al.[9] should be acknowledged. A strength of the current review is the inclusion of all mammalian species used to model CNV formation, although the laser-induced mouse model dominates the field. Notably, all models across species display large heterogeneity in terms of animal characteristics. This not only questions the reproducibility but also the general translational potential, as the models differ substantially on many parameters that could affect the resemblance with the target diseases.

A concerning issue identified during the screening process was studies with very problematic methodology passing the review process in journals indexed in PubMed and EMBASE. We do not present specific examples, but laser induction of CNV in albino animals (lack of melanin makes photocoagulation very difficult) and sections of intact retinal tissue reportedly showing CNV induction are examples of severe methodological shortcomings found in some published studies.

To outline the best methodological and reporting practices, we created a flowchart/checklist describing mandatory items to be addressed in planning and conducting animal models of CNV (Fig. 3). We suggest that future guidelines should be based on adherence to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and key articles from the literature.

Reporting

The most surprising finding was the insufficient reporting of variables critical for the reliability, comparability, and reproducibility of studies of experimental CNV. Recently, a large survey among U.S. biomedical researchers revealed that 74% reported lack of reproducibility of preclinical animal studies as an important problem.[20] In support of these findings, a *Nature* survey from 2016 revealed that 70% of researchers had failed to reproduce the experiments of others and that more than half had failed to reproduce their own experiments.[15] The ARRIVE guidelines (https://arriveguidelines.org/arrive-guidelines) are the key reporting guidelines for all fields of biomedical research using animals. The guidelines present a minimum of items that should be included in the reporting of animal research to judge the reliability of the study and its findings. Our findings suggest inadequate reporting of variables corresponding to several of the essential items in these guidelines. In ophthalmology, the key guidelines for research using animals are the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Although it is encouraging that 71% refer to these

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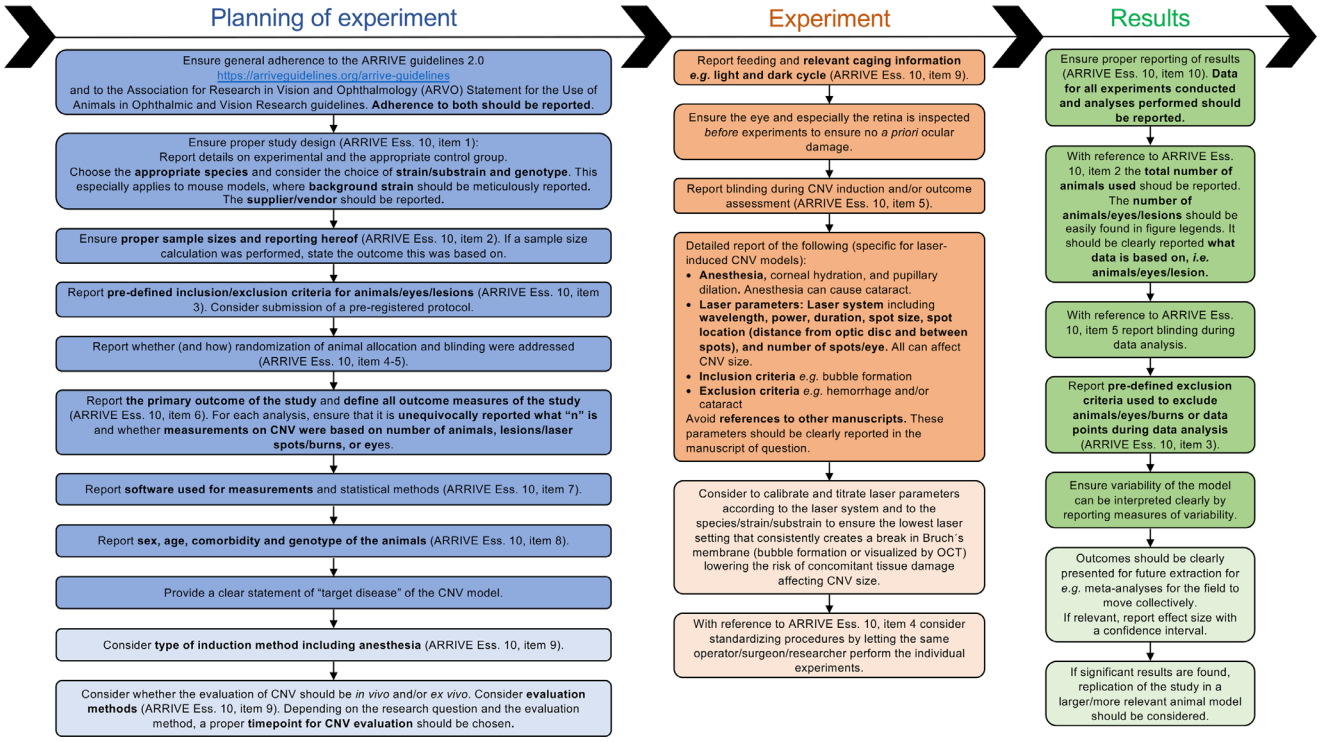

Figure 3. Outline of a CNV animal model flowchart/checklist. Items were chosen according to the ARRIVE guidelines and the main findings of the present review (in *bold*). *Dark colors* represent mandatory items that should be a requisite for conducting and reporting on research on animal models of CNV, and *bright colors* represent optional items that should be considered.

guidelines, the guidelines primarily address the humane use of research animals and do not contain reporting standards. In other fields of biomedical research, specific guidelines for reporting preclinical research on animal models have been established, and it may be time to develop consensus-based methodological *and* reporting guidelines for animal models of CNV.

Biologic Validity and Variation: Lost in Attempted Translation?

Historically, one has to acknowledge the impact of animal models of CNV as the foundation for clinical trials supporting, for example, current standard anti-vascular endothelial growth factor (VEGF) treatment in nAMD.[21–23] However, as in many other fields of medicine, translation of preclinical results has also been discouraging,[11,24] and, despite the reporting of safety and tolerability, several studies have failed to reproduce the same promising preclinical efficacy in a clinical setting.[25–27]

An essential question is the extent to which models of CNV recapitulate CNV formation in the context of human chorioretinal disease such as nAMD. As emphasized by Ryan in 1979,[28] the laser-induced animal model of CNV in rhesus monkeys resembles an acute, traumatic-inflammatory process with substantial neuroretinal damage rather than a chronic, progressive, age-related degeneration.[29,30] Such biological differences between the model and modeled disease warrant cautious extrapolation of, for example, evidence of inflammatory processes.

The widespread use of young, healthy, male mice optimizes standardization and decreases biologic variation; however, this must be assumed to limit the generalizability of the results. Indeed, it has been proposed that samples of experimental animals should be more heterogeneous to incorporate biologic variation into the model.[31] The strategy also raises some questions related to the natural history of CNV formation in a clinical context. AMD, as the major target disease, is first and foremost associated with age, and the risk of neovascular AMD is higher in women than in men. Furthermore, extraocular comorbidities are more prevalent among patients with nAMD than patients without AMD.[32,33] Interestingly, it has been shown that sex (e.g., estrogen levels) and age account for some of the variability in severity of CNV in animal models.[34] Espinosa-Heidmann et al.[35] showed that circulating estrogen levels play a significant role in CNV formation by interacting with proangiogenic factors. Zhu et al.[36] found that female mice developed more severe CNVs compared with male mice. Zhu et al.[36] also found that CNV was more distinct in mice 17 to 20 weeks of age compared with 5 to 8 weeks of age. Similarly, Gong et al.[37] found that mice 12 to 16 weeks of age developed more pronounced CNV than those 6 to 8 weeks of age, and the sex difference was only found for 12- to 16-week-old mice. This notion was supported by a recent study showing that sex was not a significant contributor to CNV formation and healing in young animals (8 weeks).[38] Hence, as the present study shows that the median age of mice used in CNV studies was 8 weeks, a substantial number of studies might not be prone to identifying a sex difference regarding CNV formation and development. Finally, in a paper by Schnabolk et al.,[39] sex-related differences were only observed in C57BL/6J mice subjected to collagen-induced arthritis (CIA) prior to laser-induced CNV, implying that increased comorbidity (in this case, CIA) may result in a sex-specific response. In another study, Dot et al.[40] found a significant difference in retinochoroidal healing/regression of CNV between young and old mice;

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1-year old mice showed more extensive CNV formation and slower regression compared with 4-, 6-, and 10-12-week old mice. Poor et al.[41] reported that not only does genetic background influence the response to laser induction, but also wild-type mice from different commercial suppliers may exhibit different sizes of CNV after laser induction. These findings provide an impetus to model CNV on a broader biological background and, importantly, to report these parameters.

Another essential feature is the specific parameters used for laser induction. Zhu et al.[36] emphasized the importance of the localization of the laser burn. Laser burns 1 papillary diameter from the optic disc produced larger CNVs than more distant laser burns. The authors also found a significant increase in CNV size 14 days after laser induction, indicating that day 14 may be the optimal time point to evaluate CNV area/volume. Also, CNV is highly affected by the induction procedure, the laser operator, and the settings. In this review, we found that laser parameters were insufficiently reported or not reported at all in 63% of the studies. This makes it impossible to reproduce a near-identical break in Bruch's membrane with the same effect on CNV formation. Extraction of specific laser parameters (for laser-induced mouse models of CNV models with CNV quantification) (Table 3) showed that parameters such as spot location that also impact CNV formation[37] were often vaguely reported. Reporting on indication/confirmation of a break in Bruch's membrane as a result of laser application was not reported in 39% of these models. The consideration of these aspects is important, as the artificially induced CNV is somewhat wound-like with spontaneous regression, and it might be difficult to differentiate between the true effects of an intervention and spontaneous regression if these aspects are not standardized, especially if the sample size is small.

Comparative Anatomy

The primary use of rodents as animal models may result in a knowledge gap. Mice and rats do not possess a macula. From an ethical perspective, rodents may be more favorable as experimental animals than higher mammals, and this laser model has proved itself fast, reliable (producing CNVs in up to 100% of burns), and inexpensive compared with larger animals such as non-human primates, pigs, and dogs.[42] Larger animals seem more translationally relevant, and confirmation of significant findings in rodent models could be supported by confirming the results in a larger animal model. It may be appropriate to use an animal with retinal specialization such as the monkey, which has a macula with fovea centralis similar to humans, or the dog or the pig with an area centralis to model a disease like AMD largely confined to the macula. Studies on the practices of translational science show that the choice of rodents as model animals is primarily based on practical constraints and can somewhat be seen as the "default choice of vertebrate animal model."[20] It has been proposed that the collective group of study animals should be more heterogeneous to incorporate biologic variation into the model. The selection of an appropriate animal model in combination with a calculated minimum sample size (based on pilot studies and power calculations), well-established induction/evaluation methods, and careful reporting of all relevant information would provide more reliable, reproducible, and comparable results. Furthermore, this might reduce the total number of experiments.

Limitations

Lack of reporting may have been overestimated, as 60% of studies referred to other articles regarding the methodology used. We did not explore information in the articles to which the studies referred. We included all mammalian CNV models according to the above-mentioned inclusion/exclusion criteria. We did not systemically assess authors' considerations and/or statements on whether the models reflected a specific target disease. However, it is our clear impression that most articles refer to AMD when describing the modeling of CNV. To successfully interpret and compare future studies, we suggest that "the target disease(s)" of the CNV model should be reported. This is included as an item in Figure 3.

Our study was limited to CNV originating from the choroid; that is, we excluded studies with neovascularization originating from the retina although anastomosing with choroid vessels. In the nAMD nomenclature, we limited the studies to classic (type 2) or occult (type 1) neovascular membranes, excluding everything that resembled or was described as retinal angiomatous proliferations and polypoidal choroidal vasculopathy. Furthermore, we did not perform a bias assessment. We found that sample sizes on which quantitative CNV evaluations are based vary and are often small, but we did not address power calculation specifically.

Conclusions

Contemporary animal models of CNV represent a wide range of animal characteristics, induction methods, and evaluation parameters. The laser-induced mouse model is by far the most widely used. Notably, most studies have used young, healthy, male animals, which may not reflect the clinical scenario. This review clearly suggests that a substantial number of studies display inadequate reporting and methodological shortcomings that limit reproducibility and make comparison among studies difficult. This emphasizes the need for definition and implementation of methodologic and reporting guidelines.

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