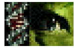
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Differences between rat strains in models of retinopathy of prematurity

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Purpose: Genetic factors appear to modulate the incidence and severity of retinopathy of prematurity (ROP). Different rat strains may be analogous to genetic differences across human ethnic groups. We investigated the incidence and severity of neovascularization (NV) in Brown Norway (BN) and Sprague Dawley (SD) rats in oxygen-induced retinopathy (OIR) and acidosis-induced retinopathy (AIR) models for ROP. We also studied whether there was a difference in retinal vascular endothelial growth factor (VEGF) mRNA levels in OIR animals.

Methods: Newborn SD and BN rats (110 in both groups) were raised in standardized litters of ten (four OIR, twelve AIR, six non-gavaged room air controls). Beginning on day 1 of life, OIR litters were exposed to seven cycles of hyperoxia (80% O_2, 20.5 h) and hypoxia (10% O_2, 0.5 h) with a gradual return to 80% O_2 over 3 h. This was followed by room air recovery for five days. OIR and OIR control rats were sacrificed on day 13 of life. AIR rats were gavaged twice daily with NH_4Cl (10 mmol/kg) from day 2 to day 7 of life, or 15 mmol/kg twice daily on day 2 and then 10 mmol/kg twice daily from day 3 to day 7. AIR and AIR control rats were sacrificed on day 10 or day 13. Retinas from left eyes were dissected, ADPase stained and flatmounted. Presence and severity of NV (clock hours, 0 to 12) was scored by a masked observer. Right retinas from OIR and room air controls were processed for VEGF mRNA analysis.

Results: In OIR rats, the incidence of NV was higher in BN than SD rats (100% compared to 5%, $p<0.0001$). NV was more severe in BN rats (1 to 10 clock hours, median 7 clock hours compared to 0 to 1 clock hours, $p=0.0001$). In contrast, the incidence of NV in AIR rats was similar in BN and SD rats (4% compared to 0%, $p=1.0$) in the 10 mmol/kg study, and 18% compared to 0%, ($p= 0.15$) in the 15 mmol/kg study. Increased levels of retinal VEGF mRNA were found in BN OIR animals when compared to BN room air controls (1.4 fold increase) whereas retinal VEGF mRNA levels were similar between SD OIR and SD room air control animals.

Conclusions: BN rats differ from SD rats in incidence and severity of NV in OIR. The findings in AIR were limited by the low incidence of NV and intolerance to higher multiple doses of NH_4Cl. In OIR, the higher severity of NV was associated with higher retinal VEGF mRNA in BN OIR rats. Studies are warranted to investigate the genetic differences between Brown Norway and Sprague Dawley rats in models of ROP. These genetic studies may yield further clues into the pathogenesis of clinical ROP.

Rat models of retinopathy of prematurity (ROP) include "oxygen-induced retinopathy" (OIR) [1-5] and "acidosis-induced retinopathy" (AIR) [6]. In the OIR model, newborn rats are exposed to cycles of hyperoxia and hypoxia (or normoxia) [1-5], whereas in the AIR model, newborn rats receive systemic agents such as oral gavage of ammonium chloride (NH_4Cl) [6] or intraperitoneal acetazolamide [7]. In both OIR and AIR, neonatal rats develop preretinal neovascularization (NV) that is morphologically similar to ROP [4,6].

Severe ROP occurs with greater frequency and severity in some racial groups than others, for example, Caucasian infants compared to African-American infants [8,9] and Indian infants compared to Caucasian infants [10]. In animal models of ROP, differences between rat strains have been described. Zhang et al. [11] and Gao et al. [12] reported that Brown Norway rats had more severe retinopathy than Sprague Dawley rats in a constant hyperoxia model. We [13] found that even among rats of the same strain, OIR induced by fluctuating hyperoxia-hypoxia was more severe in Sprague Dawley rats from one vendor (Charles River) than from another (Harlan). In contrast to OIR, in AIR we found no difference in the incidence and severity of preretinal NV between Sprague Dawley rats from Charles River and Harlan [13].

Although Zhang et al. [11] and Gao et al. [12] previously studied the difference between Brown Norway and Sprague Dawley strains in a constant oxygen exposure OIR model, few data are available for the more commonly used cyclic hyperoxia-hypoxia model used by many laboratories [1-5]. In the present study, we investigated whether there is a difference in incidence and severity of preretinal NV between Brown Norway and Sprague Dawley rats in both OIR and in AIR.

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METHODS

All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Use Committee at our institution.

Animals and standardization of litter size: Pregnant Sprague Dawley (SD) rats used in this study were obtained from Harlan (Indianapolis, IN). Brown Norway (BN) rats were obtained from our own breeding colony, with the breeding pairs originating from Harlan (BN/SsNHsd, Indianapolis, IN). The BN strain was chosen to represent pigmented rats because BN rats had been studied by other investigators in a different OIR model [11,12]. The SD strain was chosen to represent nonpigmented rats because it has been extensively used by us in previous studies [4,6].

Newborn pups delivered in the same 24 h period were mixed and grouped into litters of ten and raised with a lactating mother for the duration of the study. Light was cycled on a 12 h on, 12 h off schedule, the room temperature was maintained at approximately 21 °C, and mothers were fed *ad libitum*.

Standardization of litter size was performed because litter size influences the development of the retinal vasculature [14], and litter size influences the incidence and severity of NV, in both OIR [1] and AIR models [15]. We hypothesized that BN rats would have a greater incidence and severity of NV in both OIR and AIR. Choosing a model where the incidence and severity of NV was low in SD rats would maximize our ability to detect an increased incidence and severity of NV in BN rats under the same experimental conditions.

OIR model: Twenty newborn SD rats and 20 newborn BN rats were raised in four litters of ten pups each and housed in plastic chambers (46x54x46 cm, PlasLabs, Lansing, MI) where oxygen and carbon dioxide levels were monitored and controlled by an OpMosphere (model A42X03, BioSpherix, Redfield, NY). As in our previous OIR studies [1,4,13], the litters received seven daily cycles of oxygen (a gradual increase from 21% to 80% O_2 over 3 h, 80% O_2 for 20.5 h, and a rapid drop to 10% O_2 that was held for 0.5 h). Inspired CO_2 was maintained at 0.2%, which corresponds to our normocarbic conditions [1,4,16,17]. The rats recovered in room air for five days and then were sacrificed on day 13 of life. This corresponds to the time point of maximal NV in SD rats using this model [17]. OIR rats were weighed at birth and on days 8 and 13. We chose to study OIR in litters of ten because we expected the incidence of NV in the SD rat raised in this litter size to be about 15% based on our previous studies [1]. This would allow us the opportunity to detect an increased incidence of NV in BN rats compared to SD rats. Since the typical natural litter size of BN rats was four or five pups, it was not practical to create larger litter sizes of 25 pups, where we would expect to see a greater incidence of NV [1].

In order to provide age matched controls, 20 additional newborn rats were raised in litters of ten in room air (10 BN and 10 SD) and were sacrificed on day 13.

AIR model: Forty newborn SD rats and 40 newborn BN rats were raised in eight litters of 10 each. To induce AIR, rats received ammonium chloride (NH_4Cl, 10 mmol/kg) by orogastric gavage twice daily from days 2 through 7 of life, as we have previously described [6,15]. Rats were allowed to recover for two days, and then were sacrificed on day 10 of life. Day 10 was chosen because it corresponds to the time point of maximal NV in SD rats using this model [18]. AIR rats were weighed daily. We chose to study AIR in litters of ten because we expected the incidence of NV in SD rats raised in litters of ten to be about 18% based on previous studies of AIR [15]. An additional 40 rats (20 BN and 20 SD) were gavaged with saline. These additional rats were designated AIR control rats and sacrificed on day 10.

A subsequent experiment was conducted to maximize the acidosis by increasing the dose of ammonium chloride. Further test doses were used (15 mmol/kg twice daily, 20 mmol/kg twice daily, and 40 mmol/kg twice daily), but an initial dose to 20 mmol/kg and 40 mmol/kg resulted in unacceptable mortality. A dose of 15 mmol/kg twice daily could only be tolerated for the first day of gavage. Therefore, another set of animals raised in litters of ten (20 BN and 20 SD) were gavaged with 15 mmol/kg twice daily on day 2 (the first day of gavage) and then 10 mmol/kg twice daily on subsequent days until day 7. For this study, the rats were sacrificed on day 13, in parallel to our first report of AIR [6].

Blood pH measurements were made on additional day-2 pups (sample size of three to eight pups per dose) following gavage with NH_4Cl 10 mmol/kg, 15 mmol/kg, or sodium chloride (control). As previously described [7], arterial blood samples were collected at 3 h following gavage which corresponds to the time of maximal acidosis [6]. All pups underwent urethane anesthesia via an intraperitoneal injection (1.5 g/kg) in the flank, near the lumbar vertebrae and hind limb, while breathing room air. Body temperature was preserved using 39 °C warming pads (Deltaphase isothermal pad, Braintree Scientific, Braintree, MA). The left carotid artery was exposed through a skin incision. The artery was transected and 50 µl of arterial blood was collected in a heparinzed micro-hematocrit capillary tube (Fischer Scientific, Pittsburg, PA) and analyzed immediately using a blood gas analyzer (Synthesis 10, Instrumentation Laboratory, Lexington, MA).

Analysis of retina: All animals were deeply anesthetized with an intramuscular injection of ketamine (80 mg/kg) and xylazine (15 mg/kg). To evaluate retinal vessel morphology and to assess the presence or absence of preretinal NV, left eyes were removed and fixed with 10% neutral buffered formalin for 90 min at 4 °C. The cornea, lens, and vitreous were surgically removed under an operating microscope and the retinas were dissected from the eye cup. Retinas underwent

Table 1. PCR primers used to quantify retinal VEGF mRNA expression

Name	Primer
VEGF 3' UTR 5'	5'-CAGAAACACGACAAACCCATCC-3'
VEGF 3' UTR 3'	5'-TAAGCCACTCACACACACAGCC-3'
36B4 5'	5'-CCGTGTGAGGTCACAGTACC-3'
36B4 3'	5'-GTAGTCAGTCTCCACAGACAAAGC-3'

PCR primers for VEGF and 36B4 were designed for use with the LightCycler (Roche, Indianapolis, IN) as described previously [17] and were used to quantify retinal VEGF mRNA expression.

magnesium activated adenosine phosphatase (ADPase) staining as described by Lutty and McLeod [19]. ADPase stained retinas were flatmounted on microscope slides in Aquamount (Lerner Laboratories, Pittsburgh, PA) with a coverslip.

ADPase stained retinas were graded by a masked examiner under light microscopy using a standard method previously validated in our laboratory [20]. Each retinal quadrant was visually divided into three equal parts, or clock hours, and each clock hour was evaluated for the presence or absence of NV. As in previous studies, NV was defined as clumps, sheets, or tufts of endothelial cells that are distinct from the normal vasculature and arise at the junction of the vascular and avascular retina [1,6,13,20]. Severity was scored based on the number of clock hours containing NV (0 to 12 clock hours) [20]. Cross sectional evaluation of the retinas was not performed in the present study, since we have established that there is a high correlation between the number of clock hours of NV and the number of cells above the inner limiting membrane [20].

Retinal VEGF mRNA: All right retinas from the OIR rats (n=40) and OIR room air control rats (n=20) were processed for mRNA analysis, as previously described [17]. Right eyes were removed from anesthetized rats and retinas were dissected in chilled phosphate buffered saline. Vitreous and ciliary bodies were removed and the retinas were snap frozen in liquid nitrogen. Retinas were homogenized in 0.8 mL TriPure™ Isolation reagent (Roche Molecular Biochemicals, Indianapolis, IN) that included 200 μg of glycogen. Total RNA was treated with DNase I (10 units, Roche Molecular Biochemicals, Indianapolis, IN) for 30 min at 37 °C followed by heat inactivation (65 °C, 15 min). RNA was purified by two acid phenol: chloroform extractions (5:1, pH 4.5, Ambion RNA Diagnostics, Austin, TX) followed by a chloroform:isoamyl alcohol extraction (24:1, Invitrogen, Carlsbad, CA). RNA was precipitated overnight at -20 °C.

Single-retina mRNA expression for VEGF was determined by quantitative real-time reverse transcriptase PCR (qRT-PCR) as described previously [17]. First strand cDNA was synthesized with Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions from 1 μg total RNA primed with oligo dT. PCR reactions were set up using the FastStart master SYBR Green I reagents (Roche Molecular Biochemicals, Indianapolis, IN), 2 μl of cDNA and 10 pmol (VEGF) or 8.5 pmol (36B4) PCR primers. PCR was performed on a LightCyler (Roche Molecular Biochemicals, Indianapolis, IN) and quantified using RelQuant relative quantitation software (version 1.01; Roche Molecular Biochemicals, Indianapolis, IN). Previously described primers [17] were used as listed in Table 1. 36B4 was used as a reference gene and was amplified in a separate reaction at the same time as VEGF. Concentrations of mRNA were reported as a ratio of VEGF to 36B4 and compared relative to an analogous ratio in a day 3 SD control for VEGF included in the same run. This allowed samples to be compared across PCR runs.

Statistical analysis: The incidence of NV was compared for both OIR and AIR using Fisher's exact tests. Severity of NV (clock hours), rat weights, and VEGF to 36B4 ratios were

Table 2. Incidence and severity of neovascularization

Conditions and strain	Animal survival	Incidence of NV	Severity of NV in clock hours: Median	Severity of NV in clock hours: Range
OIR BN	20/20 (100%)	20/20 (100%)	7	1 to 10
OIR SD	20/20 (100%)	1/19 (5%)	0	0 to 1
AIR BN (10 mmol/kg)	33/40 (83%)	1/23 (4%)	0	0 to 1
AIR SD (10 mmol/kg)	36/40 (90%)	0/33 (0%)	NA	NA
AIR BN (15 mmol/kg)	11/20 (55%)	2/11 (18%)	1.5	0 to 2
AIR SD (15 mmol/kg)	17/20 (85%)	0/17 (0%)	0	NA
Controls (OIR + AIR)	60/60 (100%)	1/57 (2%)	0	0 to 1

In oxygen-induced retinopathy (OIR) rats, the incidence of neovascularization (NV) was higher in Brown Norway (BN) than Sprague Dawley (SD) rats. NV was more severe in BN rats. The incidence of NV in acidosis-induced retinopathy (AIR) rats was similar in BN and SD rats. Any difference between animal survival and retinas analyzed was due to a number of retinas deemed ungradable by the masked examiner (e.g., 20 OIR SD rats survived and 19 OIR SD retinas were analyzed). The highest dose tolerated in AIR was 15 mmol/kg twice daily for the first day of gavage and then 10 mmol/kg twice daily thereafter.

compared using Wilcoxon tests. Arterial blood pH values were compared using Student's t-tests. An α level of 0.05 was chosen.

RESULTS

OIR study: All 40 OIR rats and all 20 OIR room air control rats survived (Table 2). Of these 60 rats, only one retina was ungradable (one SD OIR).

In OIR, preretinal NV occurred in 1 of 19 (5%) retinas from the SD rats and in 20 of 20 (100%) retinas from the BN rats (p=0.0001; Figure 1, Table 2). The BN rats had more severe NV than the SD rats (median clock hours were 7 and 0, respectively, p=0.0001, Table 2). Severity of NV ranged from 1 to 10 clock hours for the BN group and from 0 to 1 clock hours for the SD group. None of the 20 age matched OIR room air controls were graded as positive.

There was a difference in rat growth between BN and SD rats. SD room air control rats had accelerated growth compared to BN control rats (day 13 weights were 22.43±1.50 g and 19.30±1.36 g, respectively; p=0.004; Figure 2), but the growth retardation associated with OIR was greater in BN rats than SD rats. The difference between BN OIR rats and BN controls at day 13 was greater than SD OIR rats and SD controls (difference of means was 1.56 g compared to 0.56 g; Figure 2).

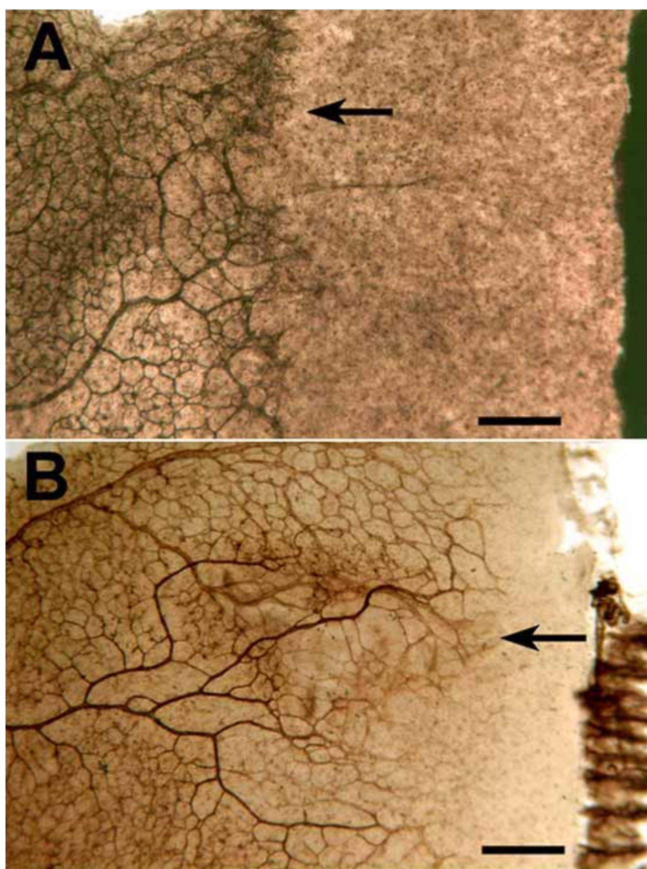

Figure 1. ADPase stained flatmounted retinas from oxygen-induced retinopathy litters. Representative ADPase-stained flatmounted retinas from oxygen-induced retinopathy (OIR) litters. Much more severe NV (arrows) was seen in Brown Norway OIR rats (**A**) than in Sprague Dawley OIR rats (**B**). Scale bars represent 300 μm.

Retinal VEGF mRNA in OIR: Retinal VEGF to 36B4 ratios were similar between SD OIR and SD age matched control animals (medians were 2.76 and 2.68, respectively, p=0.8; all values were normalized to a 3-day SD internal control). In contrast, BN OIR animals had a 1.4 fold increase in retinal VEGF mRNA compared to BN room air controls (median 2.60 and 1.79, respectively; p=0.03; Figure 3).

AIR study: In the first AIR study using 10 mmol/kg twice daily NH_4Cl, 36 of 40 (90%) SD rats, 33 of 40 (83%) BN rats, and all 40 control rats survived. Of the 109 surviving rats, a total of 16 left retinas were ungradable (3 SD AIR, 10 BN AIR, 1 SD control and 2 BN control retinas). Animals that died before the end of the study were not included because autolysis of retinal tissue precludes analysis of NV.

Preretinal NV occurred in 0 of 36 (0%) retinas from the AIR SD rats and in 1 of 23 (4%) retinas from the AIR BN rats (p=0.4; Table 2). Only 1 of the 37 (3%) age matched AIR room air control retinas was graded as positive. The only NV that occurred, in the affected BN AIR rat, was graded as a severity of one clock hour.

In the arterial blood pH study, 3 h after a 10 mmol/kg dose, BN rats had a less severe acidosis than SD rats (pH 7.39±0.03 and 7.29±0.03, respectively; p=0.0009). In fact, the BN rats would not be considered acidotic. We therefore increased the dose of NH_4Cl to a maximally tolerated dose in 5 mmol/kg increments using the same twice daily regime. This maximum dose was found to be 15 mmol/kg twice daily for the first day of gavage and 10 mmol/kg thereafter. Continuing 15 mmol/kg, or higher doses, twice daily resulted in unacceptable mortality. In the arterial blood pH study, with 15 mmol/kg, the BN rats were clearly acidotic (pH 7.26±0.05), though still less acidotic than SD rats (pH 7.17±0.05; p=0.008). Arterial blood pH was similar between BN and SD rats under control conditions of saline gavage (pH 7.42±0.02 compared to 7.41±0.06, respectively; p=0.79).

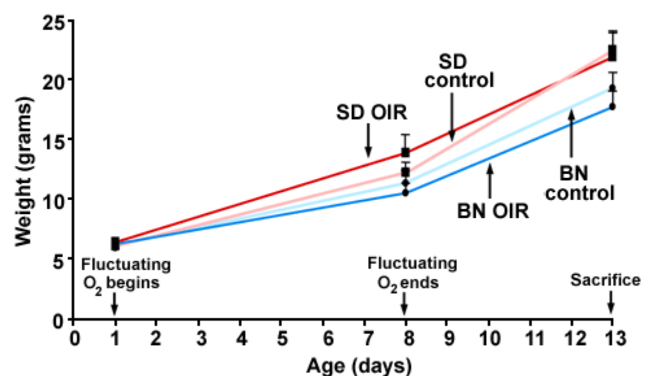

Figure 2. Comparison of rat weights in oxygen-induced retinopathy. Brown Norway (BN) control rats show slower growth than Sprague Dawley (SD) control rats. Under conditions of oxygen-induced retinopathy (OIR), BN rats show greater growth retardation than SD rats.

In the second AIR study, using this maximally tolerated dose of 15 mmol/kg twice daily for the first day of gavage (day 2) and 10 mmol/kg twice daily for subsequent days (days 3 to 7), 11 of 20 (55%) BN rats and 17 of 20 (85%) SD rats survived. All retinas were gradable, and NV occurred in 2 of 11 (18%) left retinas from BN rats, but none of the 17 (0%) SD rats (Table 2). This difference was not statistically different (p=0.15). The two positive BN retinas had a severity of one clock hour and 2 clock hours, respectively.

DISCUSSION

In our study of oxygen-induced retinopathy using a commonly used fluctuating oxygen protocol, we found that retinopathy was more frequent and more severe in BN rats than SD rats. The increased incidence of preretinal NV in BN rats was also associated with a greater increase in retinal VEGF mRNA compared to SD rats.

Our finding of differences in incidence and severity of OIR in different rat strains is analogous to our previous findings [13] of differences in incidence and severity of OIR between rats of the same strain but from different vendors. Such differences suggest that genetic factors influence the development and severity of preretinal NV in immature retinas. Parallel genetic differences may contribute to the finding of racial differences in incidence and severity of ROP in human infants [8,10]. Nevertheless, increased pigment *per se* does not appear to account for the differences in severity of NV across species, since Indian Asian infants had a lower incidence and severity of ROP than Caucasian infants [10]. In pigmented rats, such as the BN rats used in our study, the incidence and severity of NV was greater than in unpigmented rats. In human premature infants, the influence of pigment appears more complex. Although any stage of ROP was similarly distributed among races, severe ROP characterized by preretinal NV does have a racial predilection [8,10]. African American infants have a lower incidence of stage 3 threshold ROP than Caucasian infants [8], but premature infants from the Indian subcontinent have a higher incidence of severe stage 3 or 4 ROP than Caucasian infants [10].

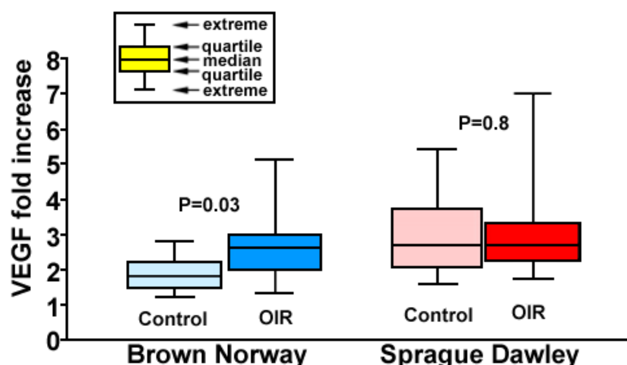

Figure 3. Retinal vascular endothelial growth factor mRNA levels in oxygen-induced retinopathy. Box and whisker plots of VEGF to 36B4 ratios in oxygen-induced retinopathy (OIR) measured by qRT-PCR. Retinal VEGF mRNA levels were increased in OIR conditions, when compared to age-matched room air controls, in Brown Norway rats but not Sprague Dawley (SD) rats. All samples, including age-matched controls, were normalized to a 3 day SD internal control.

Our findings of increased VEGF mRNA in strains of rats that exhibit more severe retinopathy are analogous to those of Gao et al. [12], who reported a greater increase in retinal VEGF protein in BN rats, which suffered more severe retinopathy in a constant hyperoxia model. Gao et al. [12] also reported less severe retinopathy in SD rats associated with a less dramatic rise of retinal VEGF protein levels. In addition, Gao et al. [12] reported a decrease in retinal PEDF, an anti-angiogenic growth factor, prior to maximal NV, which was more marked in the BN rats, resulting in a VEGF to PEDF ratio that was particularly elevated in BN rats. We did not investigate PEDF in our study. Nevertheless, in our OIR study, it appears that differences in NV between strains are mediated in part by differences in responsiveness of retinal VEGF to the fluctuating oxygen insult. These differences in VEGF expression may be analogous to the recent report of genetic differences in VEGF expression in infants with severe ROP [21].

In OIR, other investigators have reported differences between pigmented and nonpigmented rats. In a hyperoxia model, the pigmented Long Evans rat strain is more severely affected by OIR than the nonpigmented SD rat [22]. In other models of angiogenesis, genetic differences in neovascular response have been found by various assays between different inbred mouse strains, such as the corneal micropocket assay and the aortic ring assay [23]. These data, together with data from our present study, provide strong support for the suggestion that genetic factors are critical in determining angiogenic responses.

One potential explanation for the difference between OIR in BN and SD rats is the previously described difference between these strains' pulmonary physiology [24-26]. Further studies could address whether there are specific differences in arterial PaO_2, $PaCO_2$ and pH during the periods of hyperoxia, hypoxia and recovery in our fluctuating oxygen model.

Other mechanisms may involve differences in electrophysiology. BN and SD retinas demonstrated differences in amplitude and implicit time of the electroretinogram (ERG) b-wave [27]. The contribution of the photoreceptors to the pathogenesis of preretinal NV in immature retinas remains controversial [28] and further studies are needed to resolve the issues of cause and effect. It is not clear whether changes at the photoreceptor level drive the changes in the accompanying developing vasculature or vice versa [28].

The important role of growth retardation in the pathogenesis of preretinal NV in immature retinas has been described by us previously [1,15]. In the present study, we observed slower postnatal growth in BN rats than in SD rats, particularly under OIR conditions. It is possible that the yet to be elucidated factors that link growth retardation to preretinal NV may differ between rat strains and in part explain the difference in incidence and severity of retinopathy. Further work is needed in this area.

Our finding of a low incidence of NV (5%) in OIR SD rats raised in litters of ten is worthy of comment since we had

previously reported an incidence of 15% in this smaller litter OIR model [1]. This apparent difference in incidence rates is not statistically significant (p=0.6, Fishers exact test). Therefore, the incidence of NV in SD rats raised in litters of ten is not markedly different between the two studies.

Our findings in AIR are limited by the low incidence of NV in this small litter model and limited by our inability to increase the dose of NH_4Cl gavage without unacceptable mortality. Nevertheless we found no large difference between BN and SD rats in AIR. This finding is consistent with our previous report of similar incidence and severity of AIR in SD rats from different vendors [13].

The finding of similar very low incidence of NV in BN AIR rats and SD AIR rats might be explained by being below the stimulus threshold for AIR. Indeed, our blood pH study confirmed negligible systemic acidosis in BN rats when given a 10 mmol/kg dose. Nevertheless, we conducted additional pilot studies to maximize the acidotic insult to these animals raised in litters of ten. After this maximum 15 mmol/kg dose, both the BN rats and SD rats were clearly acidotic. Even in these conditions of maximally tolerated acidosis, the incidence of NV was only 18% in BN rats. Given our sample size, we do not have sufficient statistical power to conclude that any of our AIR groups (in litters of ten [15]) are statistically different at such low levels of NV. Our previous report of 18% NV in AIR SD rats raised in litters of ten [6,15] (6 of 34 pups) is not markedly different from our current report of 0 of 36, despite the fact that the difference reaches statistical significance (p=0.01, Fishers exact test). This difference does not detract from the primary purpose of the study, which was a direct comparison of SD and BN rats. We had sufficient statistical power in our current AIR experiments to detect large differences in incidence between strains, but these large differences do not appear to be present in AIR, in contrast to OIR. Given the very small natural litter size in BN rats, it is not feasible to raise BN rats in foster litters of 25, where the incidence of NV would be expected to be greater [6,15] with increased ability to detect moderate differences between strains, if they in fact existed. In summary, our reported lack of difference in AIR between BN and SD strains should be considered limited by study design, but it is difficult to conceive of additional feasible studies using NH_4Cl gavage in SD and BN strains that would further address the issue, short of studying hundreds of animals to achieve the statistical power needed to potentially distinguish between low levels of NV.

Our arterial blood pH data suggest the possibility that BN rats and SD rats handle systemic acidosis differently. BN rats had a less severe systemic acidosis with the same dose of NH_4Cl. This is worthy of further study and may provide clues as to potential mechanisms of differential susceptibility to preretinal NV. Genetic differences may manifest themselves as different responses to systemic triggers of NV or differences in local molecular determinants of NV.

In conclusion, we found a difference in the incidence and severity of OIR in Brown Norway compared to Sprague Dawley rats. These findings are important for those working with models of ROP, since models with more severe preretinal NV are desirable for studies of pathogenesis and for testing potential anti-angiogenic agents. More importantly, these strain differences may yield clues as to the pathways that are critical to abnormal angiogenesis and may lead to novel therapeutic approaches. Elucidating these genetic factors may also help explain the racial differences in the incidence and severity of human ROP. Further study of angiogenesis differences among animal strains and human racial groups is warranted.

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