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Adhesion Failures Determine the Pattern of Choroidal Neovascularization in the Eye: A Computer Simulation Study

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Abstract
Choroidal neovascularization (CNV) of the macular area of the retina is the major cause of severe vision loss in adults. In CNV, after choriocapillaries initially penetrate Bruch’s membrane (BrM), invading vessels may regress or expand (CNV initiation). Next, during Early and Late CNV, the expanding vasculature usually spreads in one of three distinct patterns: in a layer between BrM and the retinal pigment epithelium (sub-RPE or Type 1 CNV), in a layer between the RPE and the photoreceptors (sub-retinal or Type 2 CNV) or in both loci simultaneously (combined pattern or Type 3 CNV). While most studies hypothesize that CNV primarily results from growth-factor effects or holes in BrM, our three-dimensional simulations of multi-cell model of the normal and pathological maculae recapitulate the three growth patterns, under the hypothesis that CNV results from combinations of impairment of: 1) RPE-RPE epithelial junctional adhesion, 2) Adhesion of the RPE basement membrane complex to BrM (RPE-BrM adhesion), and 3) Adhesion of the RPE to the photoreceptor outer segments (RPE-POS adhesion). Our key findings are that when an endothelial tip cell penetrates BrM: 1) RPE with normal epithelial junctions, basal attachment to BrM and apical attachment to POS resists CNV. 2) Small holes in BrM do not, by themselves, initiate CNV. 3) RPE with normal epithelial junctions and normal apical RPE-POS adhesion, but weak adhesion to BrM (e.g. due to lipid accumulation in BrM) results in Early sub-RPE CNV. 4) Normal adhesion of RBaM to BrM, but reduced apical RPE-POS or epithelial RPE-RPE adhesion (e.g. due to inflammation) results in Early sub-retinal CNV. 5) Simultaneous reduction in RPE-RPE epithelial binding and RPE-BrM adhesion results in either sub-RPE or sub-retinal CNV which often progresses to combined pattern CNV. These findings suggest that defects in adhesion dominate CNV initiation and progression.

Introduction

Organization

We first review the key components of the retina and the processes commonly hypothesized to underlie CNV. We then discuss our main hypotheses for CNV mechanisms and why we believe adhesion may play an important role in both initiation and progression of CNV. We then use a multi-cell computer simulation of a mechanistic computational model of the choriocapillaris, BrM and photoreceptors to investigate the effects of adhesion variations on CNV initiation and progression. Finally, we focus on how adhesion in the BrM-RPE-POS complex changes due to aging and inflammation both in human retina and in animal models of CNV and discuss the biomedical implications of our results.

Age-Related Macular Degeneration and Pathological Choroidal Neovascularization

Sprouting angiogenesis, growth of new blood vessels from preexisting vessels, occurs in response to chemical and mechanical stimuli and to hypoxia in both adult and embryonic tissues. Sprouting angiogenesis requires activation of normally quiescent endothelial cells in pre-existing blood vessels, breakdown of existing basement membranes, migration of activated cells led by one or more endothelial tip cells or immune cells (which can also function as tip cells) in response to environmental and cell-contact cues and proliferation of a subset of activated endothelial cells (stalk cells) with possible recruitment of support cells (pericytes and smooth muscle cells) during blood-vessel maturation [1].

In pathological angiogenesis, e.g. in vascular tumors, vessels do not mature, resulting in leaky capillary vasculature which causes
severe edema, inefficient blood transport and reduced oxygenation. Maturation failure can result in a pathological feedback loop, where worsening hypoxia leads to higher levels of proangiogenic factors, including vascular endothelial growth factor A (VEGF-A) and platelet-derived growth factor (PDGF), and the greater excess of proangiogenic factors produces even more inefficient capillaries, worsening the hypoxia.

The hallmark of wet or exudative age-related macular degeneration (AMD), which is the leading cause of irreversible blindness in North America, Europe, and Australia [2], is lesioning choroidal neovascularization (CNV), the invasion of the retina by new blood vessels growing from the choriocapillaris (CC). In humans, CNV frequency increases with age, independent of other risk factors or specific insults, though numerous risk factors and insults can greatly increase its probability of occurrence in an individual. CNV in all patients shares the same basic neoangiogenic steps. We distinguish the following three temporal phases: Initiation, when endothelial cells first cross Bruch’s membrane (BrM; Early CNV), when endothelial cells spread and form capillaries in a defined locus, and Late CNV, when additional loci may become involved, often leading to retinal pigment epithelium (RPE) detachment and degeneration, CNV regression/involution and photoreceptor death.

The diverse CNV scenarios are categorized based on histological [3] and clinical observations [4]. Neovascular vessels originating from the choroid can grow in the plane between the RPE and BrM (sub-RPE, occult or Type 1 CNV), between the retina and the RPE (sub-retinal, classic or Type 2 CNV) or in both locations (combined or Type 3 CNV). Type 3 CNV can also form as a late stage of Early Type 1 or Early Type 2 CNV. In wet AMD, severe visual loss results from subretinal hemorrhage from the leaky CNV, which leads to the eventual formation of a disciform scar. While CNV is generally a disease of the elderly, with onset occurring after 70 years, progression after onset may be rapid. According to the Macular Photocoagulation Study Group [5], about 40% of patients with untreated Type 1 CNV loose significant visual acuity within 12 months. 23% of these patients develop Type 3 CNV within 3 months and an additional 23% develop Type 3 CNV within 12 months. Current therapeutic strategies depend on the CNV locus (subfoveal, juxtafoveal or extrafoveal) and include photodynamic therapy, laser photocoagulation and most-commonly, anti-angiogenic drugs (Lucentis, Marcugen or Avastin) [6]. Long-term prognoses are poor; only 20% of patients with Type 1 CNV have stabilized or improved vision 36 months after initial diagnosis and treatment [5].

Developing more effective targeted intervention strategies will depend on understanding CNV mechanisms. However, because of the structural complexity of the normal and diseased retina and the numerous homeostatic and developmental mechanisms operating concurrently, experiments have yet to identify clearly the mechanisms responsible for either CNV initiation or progression. As a novel approach to developing such understanding, this paper applies quantitative models and computer simulations to test hypotheses for the mechanisms leading to CNV initiation and controlling early and late Type 1, 2 and 3 CNV.

Current Hypotheses for CNV Initiation and Progression

Multiple hypotheses compete to explain CNV initiation, growth and patterning (for comprehensive reviews, see [7,8]). These hypotheses form two major groups depending on their primary mechanism of action: 1) VEGF overexpression, and 2) irregularities in BrM (including focal defects and basal deposits). Inflammation affects both mechanisms because it promotes formation of irregularities in BrM and participates in angiogenesis. To better understand CNV, we consider its risk factors and correlated pathological conditions and briefly discuss their relationship to the steps of angiogenesis.

Excessive expression of VEGF, mainly in response to injuries and hypoxia, without balancing expression of angiogenesis inhibitors is a major stimulator of neoangiogenesis in most tissues. Excess VEGF has been considered a primary cause of CNV [9] because anti-VEGF drugs can significantly inhibit CNV progression. This hypothesis seems reasonable, because activation, proteolytic activity and survival of ECs depend on VEGF concentrations, and directional migration of tip cells depends on gradients of VEGF, which in turn depend on the composition of the ECM and the proteolytic activity of ECs [10] (in many cases VEGF-A is bound to the ECM and is only sensed by ECs when released by proteolytic enzymes). In animal models, excess secretion of VEGF by the RPE due to subretinal injections of reactive oxygen species (ROS) [11] or adenovirus [12] can induce CNV. However, other studies in transgenic mice show that increased expression of VEGF-A and/or angiopoietin-2 in RPE is not sufficient to initiate CNV and that overexpression of VEGF can only initiate CNV when combined with subretinal injections [13] which disturb the integrity of the RPE, probably by triggering inflammation, reducing RPE-POS contact adhesion and inducing RPE growth [14–16]. Also in transgenic mice [17], overexpression of VEGF-A in the RPE causes extensive intrachoroidal neovascularization, but does not lead to sub-RPE or sub-retinal CNV, again suggesting that an intact Bruch’s membrane/RPE barrier prevents choroidal neovascularization from penetrating into the subretinal space. Immunohistochemical analyses of human subjects with a history of chorioretinal disease show that compared to age-matched control subjects (mean age about 80 years) their PEDF levels are significantly lower than their VEGF levels in RPE cells, the RPE basal lamina, BrM and the choroidal stroma [9,18]. This imbalance shows that disturbed angiogenic and antiangiogenic factor levels correlate with late-stage chorioretinal disease, but the relative expression levels of these factors would need to be measured experimentally before CNV initiation to establish whether the imbalance is a cause or a result of CNV. However, secreted proteome profiling in human RPE cell cultures derived from donors with AMD shows a 2- to 3-fold increase in their levels of PEDF compared to age-matched healthy donors [19]. Thus, while the experimental evidence shows a clear increase in proangiogenic factors, this increase may be compensated by an increase in antiangiogenic factors. Whether an imbalance develops is thus not definitively established. Another serious objection to the VEGF-hypothesis is that it fails to explain the distinct loci and progression of CNV. VEGF levels are high throughout the retina and cannot provide spatial cues to lead to

Author Summary

This paper tests hypotheses for the mechanisms of choroidal neovascularization (CNV), the pathological growth of capillaries in response to physical defects in a structured tissue, the retina, showing that previously neglected cell-cell, cell-ECM and ECM-ECM adhesion failures suffice to determine the loci and progression of neovascularization. Surprisingly, a simple theory based on classes of adhesion failures, which involve variation of only five parameters, can coherently explain the heterogeneous range of CNV growth patterns and dynamics. Our results are generally applicable to other types of tissues where capillaries are close to an epithelium, e.g., lung and gut.
the localization of CNV to either the RPE-BrM boundary (Type 1) or the RPE-POS boundary (Type 2). Indeed, no experiment has established the presence of VEGF gradients within the BrM-RPE-POS complex.

Both age-related changes of the retina and pathological conditions can increase VEGF expression. Life-long accumulation of lipids in BrM and their gradual oxidation (producing reactive oxygen species and recruiting immune cells) correlate with increased production of VEGF by the RPE and greater likelihood of developing CNV [26]. Hypoxia also temporarily increases the secretion of VEGF in cultured RPE cells by up to 3-fold over 48 hours, followed by a return to baseline [21,22]. Inflammatory cells also secrete VEGF and other proangiogenic and antiangiogenic factors (see Inflammation subsection in supplementary Text S1).

Irregularities of BrM include focal breaks and thinning in BrM, abnormal production of ECM by the RPE, and formation of soft drusen. All of these BrM defects correlate with CNV [23,24]. However, the common hypotheses that BrM presents a physical barrier to the invasion and/or growth of choriocapillaries into the retina and that small gaps in BrM may be responsible for initiation of CNV, contradict several experimental and clinical observations: 1) BrM is never an impenetrable barrier to immune and tip cells and has not been shown to physically block the invasion of activated ECs into the sub-RPE space. BrM is only 2–4 μm thick, with pores up to 0.5 μm diameter [25]. The BrM elastin layer in the macula of healthy young adults (age≤62) can have gaps of up to 2 μm [26]. 2) Activated endothelial cells, which are always present in the normal choriocapillaris, probe their micro-environment by sending out processes (like filopodia) as thin as 0.1 μm and as long as 200 μm even in dense embryonic and adult tissues. Such filopodial processes can easily cross BrM through its pores. Leukocytes can cross BrM rapidly under both normal and inflammatory conditions [27] (taking at most a few hours to cross the BrM-RPE barrier and only a few minutes to cross the endothelium in an in vitro flow model [28]). ECs digest and penetrate an intact BrM in less than a week when RPE is severely damaged due to phototoxicity in a rat model [29]. 3) The rate of CNV in persons younger than 50 years old is negligible (except in cases of excess inflammatory response in the eyes). 4) The CNV initiation probability when BrM is mechanically disrupted in animal models is about 10% [30]. These observations suggest that focal defects and thinning of BrM do not significantly reduce the already minimal efficacy of the physical barrier function of healthy BrM. Instead, other mechanisms may explain the correlation of focal defects in BrM with CNV, e.g., breaks in BrM due to calcification may disrupt both the RBaMs and the basement membranes of CC cells, disrupting the epithelial junctional structure of the RPE [23] and activating CC endothelial cells. This simultaneous activation of ECs and disruption of the RPE may explain the correlation of focal defects in BrM with CNV.

However, while BrM does not form a mechanical barrier to persistent EC penetration of the retina, BrM and the RPE attached to it clearly do form an effective barrier to choroidal penetration, even in the presence of small holes in BrM. The nature of this barrier is not clear. Haptotaxis may play a role. ECs exhibit strong haptotactic preference for their own basement membrane. The basement membrane of the RPE (RBaM) differs in structure and components from the CC basement membrane (CC BaM) (reviewed in [31]). ECs preferentially adhere to their own basal lamina and new blood vessels follow the pattern of any pre-existing EC-manufactured basal lamina after capillary atrophy [32]. Thus, in the absence of factors that induce directed migration of ECs (chemotaxis or haptotaxis), we hypothesize that activated ECs of the choriocapillaris prefer to stay on the outer side of BrM, which has a high level of CC BaM, and not to invade sub-RPE space, which almost entirely lacks CC BaM.

Since overexpression of VEGF and reduction in BrM’s barrier function may not fully explain CNV initiation, multiple types, loci and progression [23], this paper investigates the possible role of additional mechanisms, particularly adhesion defects.

Adhesion Failure and CNV
While not usually considered crucial to CNV, a great deal of experimental evidence suggests that failures of adhesion are essential for the development of CNV (Table 1). The strict spatial separation of the CC from the normal retina and the distinct loci of Type 1 and Type 2 CNV suggest that the physical structure and properties of the BrM-RPE-POS complex may determine both CNV initiation and progression. Experimental evidence suggests that reduced RBaM-BrM adhesion may enable CNV to invade the sub-RPE space. The differences in properties and effects of hard and soft drusen support this hypothesis. Soft drusen are strong risk factors for CNV and are often associated with detachment of the RPE from BrM, suggesting that they substantially reduce RBaM-BrM adhesion [33–35]. However, soft drusen, which contain hyalised material and attach firmly to the inner collagenous layer of BrM (based on EM images) do not greatly increase the likelihood of CNV [24,35]. Softening of hard drusen, which reduces their adhesion to RBaM, correlates with CNV [36,37]. Similarly, the ability of inflammation to induce CNV suggests that impaired lateral adhesion between cells in the RPE promotes Type 2 CNV. Pathological conditions that compromise the integrity of the oBRB by weakening junctional epithelial adhesion in the RPE cause a wide range of neovascular diseases in the retina [38–42]. Recent studies show that subretinal drusenoids, which are drusen-like deposits that accumulate between the RPE and photoreceptors, perturb RPE-POS adhesion and correlate with CNV [43–45]. Finally, the detachment of the POS from the RPE (retinal detachment) reduces the integrity of the oBRB and significantly increases the risk of CNV in animal models of CNV, suggesting that impaired RPE-POS adhesion also promotes CNV.

Since the relative importance, roles and interactions among the different types of adhesion impairment during CNV initiation and progression are unclear, this body of experimental evidence motivated us to study the role of adhesion failures in the BrM-RPE-POS complex in CNV.

The need for models and simulation. While a detailed experimental analysis of adhesion effects in CNV is desirable, it is currently impractical. No animal model exhibits the full range of AMD-related CNV pathologies [46], while in vitro experiments do not reproduce the complex layering and porosity of BrM, the interlocking of the RPE and POS, the accumulation of lipids in the BrM layers or the formation of soft drusen. Independent quantitative control of biological mechanisms is experimentally difficult, especially in vivo. We therefore chose to develop computational models which allow us to titrate the effects of specific mechanisms without confounding crosstalk or quantitative uncertainties and to study the synergistic or antagonistic effects of multiple mechanisms acting simultaneously or sequentially. Computational models also allow us to explore many more combinations of bio-mechanistic hypotheses and parameter choices than we could in experiments. Our computational models include key retinal components, cell-cell, cell-ECM and ECM-ECM adhesion mechanisms and major angiogenesis-related processes, like BrM breakdown by proteases, hypoxic signaling upregulating VEGF production and VEGF and oxygen transport.
### Table 1. Pathological conditions and injuries, their effects on adhesion and their correlations with CNV.

| Context | Subject | Adhesion | Effects | CNV Loci | Simulation Results |
|---------|---------|----------|---------|----------|--------------------|
|         |         | RPE-RPE | RBA-M-BrM | RPE-POS | RPE Viability |          |                      |
| 1       | Normal Aging (No Drusen) | Human | + | + | + | - | No initiation even in presence of small holes in BrM (Table S1) |
| 2       | Hard Drusen [23,35] | Human | - | - | + | - | - | See the Current Hypotheses for CNV Initiation and Progression section |
| 3       | Soft Drusen [23,35–37] | Human | -/+ | -/- | -/+ | -/+ | Sub-RPE | S11, T12, P13 CNV (Tables S2, S5, S6, S7) |
| 4       | Subretinal Drusenoid (reticular pseudodrusen) [44,45,96] | Human | -/- | -/-/+ | -/- | -/+ | Sub-Retinal and/or Sub-RPE | T12, S22, P23 CNV (Tables S6, S8, S9) |
| 5       | BrM Calcification [23,24] | Human | -/+ | -/+ | + | -/+ | * | * |
| 6       | Active Inflammation [80] | Young Human | - | + | -/-/+ | -/+ | Sub-Retinal | S22 CNV (Table S8) |
| 7       | Retinal Detachment [14,40] | Cat | -/- | + | - | + | * | S22 CNV (Table S8) |
| 8       | High Fat Diet+Aging+Blue Light [75] | Mouse | -/+ | -/- | + | +/- | Early Sub-RPE | ET1 CNV (Table S2) |
| 9       | Chemotoxicity [81] | Rabbit | - | +/- | - | - | Sub-Retinal | P23 CNV (Table S9) |
| 10      | Sub-Retinal Injection [83–86] | Rat, Rabbit | - | +/- | - | -/+ | Sub-Retinal | S22, P23 CNV (Tables S8, S9) |
| 11      | Sub-retinal Injection and VEGF Overexpression [13] | Rat | - | -/+ | - | -/+ | Sub-Retinal | S22, P23 CNV (Tables S8, S9) |

**Columns:** **Condition:** type of condition, injury or perturbation in clinical or experimental observations. **Subject:** human or animal. **Adhesion:** strength of adhesion (+ = normal, - = moderately impaired, -/ = severely impaired). **Effects:** RPE viability (+ = most RPE cells remain viable, - = some RPE cells die, - = most RPE cells die). CNV loci ( = no or low probability of initiation and progression, sub-RPE = Type 1, sub-retinal = Type 2, combined pattern = Type 3). * = no data presented/available. Simulation results (boldface words = model objects. **CNV Type definitions:** see Table 4 and Table 5. * = no data presented/available. See the Results and Discussion sections for details of simulation results.

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Our computational model of the retina allows us to investigate the significance of these hypothesized mechanisms in both CNV initiation and progression. In this paper, we focus on the significance of adhesion failures in the BrM-RPE-POS complex to CNV early and late progression, deferring a detailed comparative study of the roles of VEGF overexpression and BrM defects to future publications.

A Quantitative Model of the Retina-RPE-CC Complex

CNV involves the interaction of two complex components, the retina, with its supporting structures, and the choriocapillaris. We briefly review the functional and structural properties of these components in the context of CNV in supplementary Text S1.

To allow unbiased study of CNV mechanisms, our model of the retina-RPE-CC includes objects and processes (Table 2) capable of recapitulating all the major CNV hypotheses (VEGF overexpression, BrM defects, adhesion failures and inflammation). We list our key modeling assumptions in supplementary Text S2. We translate our quantitative model into a computational model in the Methods section in supplementary Text S3 (for a detailed explanation of our modeling terminology see supplementary Text S4). To avoid confusion, we use normal fonts for biological objects and boldface to represent objects and times in the quantitative model, e.g. RPE denotes the model’s representation of biological RPE and one year denotes one simulated biological year. We also use boldface to distinguish the specific simulation interactions of junctional adhesion, labile adhesion and plastic coupling in our model from their biological correlates, but we do not use a separate font to distinguish other modeled and biological processes.

Anatomical components of the model. Since CNV is usually limited to the outer retina, we model the choriocapillaris, BrM, RPE and parts of the outer retina in detail, and represent the inner retina implicitly through appropriate boundary conditions at the outer limiting membrane (see Figure 1, for normal anatomical components). Our model does not explicitly represent the OLM which defines the innermost (towards the inner retina) boundary of the modeled outer retina. The properties of the retinal layers depend on the in-layer distance from the fovea. We could represent these typical anatomical/thickness variations of the biological retina in our model by changing a limited number of geometrical and metabolic parameters, though we do not do so in the present paper. Our model explicitly represents BrM, but neglects its layered structure and assumes that the inner and outer basal laminae and basement membrane of BrM provide equivalent adhesion substrates. Modeled BrM is composed of small blocks of non-diffusible solid material (frozen generalized cells). We assume that cells cannot cross intact BrM. So our modeled BrM blocks cell migration (See Discussion section for more details). Our model does not explicitly represent basal deposits, which play a major role in CNV initiation and progression, but includes them implicitly via their effects on the adhesion properties of the RPE-BrM complex. Since CNV originates from the outgrowth of capillaries in the choriocapillaris, our model represents the capillary network of the choriocapillaris (CC) and the endothelial cells (ECs) explicitly. Modeled CNV capillaries are composed of stalk cells (see the Angiogenesis and BrM Degradation subsection, below). Our model represents extracellular fluid in the tissue by a medium that fills spaces unoccupied by cells or BrM.

Oxygen transport and metabolism in the model retina. The choriocapillaris secretes diffusing oxygen at a constant rate (see the Methods section in supplementary Text S3) and PO2 at the OLM boundary is constant. Numerous experimental and theoretical studies of oxygen tension profiles in the retina show that the oxygen consumption by the RPE is negligible compared to that of the PIS [47]. Our model assumes that RPE oxygen uptake is negligible [47,48]. Our model neglects the effects of blood flow entirely and assumes that PO2 is independent of position along a capillary, or whether a capillary is a sprout or has anastomosed with other vessels. The effects of blood flow on vascular remodeling and tumor growth have been extensively studied by Owen et al. [49], Szczepanek and Szczekely [50], Perfahl et al. [51], Alacron et al., Bartha and Rieger [52], Welter et al. [53,54], McDougall et al. [55], Stephaniou et al. [56,57], Pries et al. [58-61] and Macklin et al. [62]. Our flow-related simplifying assumptions generally have the effects of increasing oxygen availability, reducing the rate and extent of neovascularization. We have performed simulations (data not shown) in which PO2 at the choriocapillaris is set to half of its normal level, representing continuous systemic hypoxia. These simulations show that lower PO2 at the choriocapillaris has little effect on the generic behavior of our model.

Adhesion properties of EC, RPE, POS and PIS cells. Our model has two types of cell-cell and cell-BrM adhesion: 1) labile adhesion and 2) junctional adhesion. Modeled labile adhesion represents cell-cell or cell-ECM surface adhesion in the absence of strong junctional structures (e.g. RPE-POS adhesion). Junctional adhesion combines labile adhesion at cell boundaries with plastic coupling (e.g. between neighboring cells or between BrM and cells). The plastic coupling simulates cytoskeletally-coupled junctional structures as breakable springs (see the Methods section in supplementary Text S3) that mechanically connect neighboring cells and also connects cells to BrM.

Junctional adhesion represents biological epithelial/endothelial junctional adhesion or cell-ECM focal adhesion. Our representation of adhesion gives us the flexibility to represent both mesenchymal cells and cells organized in an epithelium. When plastic coupling between neighboring or ECM-adhering cells is strong relative to other effects including labile adhesion, cells are less likely to break their plastic couplings and change their neighbors, as is typical in epithelial-junction-coupled cells in an epithelium (e.g. a layer of differentiated epithelial cells in vitro at 100% confluency). However, when plastic coupling is weak or absent, cells can relatively easily migrate or change their neighbors, as is typical of mesenchymal cells or epithelial cells in vitro well below 100% confluency. In our model, we use this flexibility to vary the strengths of labile adhesion and plastic coupling independently to represent the differing adhesion properties of cells in healthy and pathological tissues.

Our model represents the in-plane epithelial junctions between healthy RPE cells by junctional adhesion with both strong labile adhesion and strong plastic coupling between RPE cells (Figure 2). Since RPE cells adhere strongly to their basement membranes, we treat the RBaM as a part of each RPE cell and assume that attachment of RPE cells to BrM depends on the adhesion of the RBaM (not of basal lamina) to BrM. During RPE detachment, cleavage occurs between the RBaM and BrM. Since we do not model the RBaM, we represent this RPE-RBaM-BrM adhesion as a single junctional adhesion between RPE and BrM cells (RPE-BrM junctional adhesion) (Figure 2). Since no known junctional structures couple RPE to the POS, we represent RPE-POS adhesion by relatively weak RPE-POS labile adhesion between RPE cells and POS cells. RPE-POS labile adhesion is weak relative to the strength of labile adhesion in RPE-RPE junctional adhesion. Neighboring POS and PIS cell segments adhere via junctional adhesion (Figure 2), limiting traverse photoreceptor displacement under normal conditions.
In an endothelium, ECs mainly adhere to other ECs via vascular endothelial cadherin (VE-Cadherins) and tight junctions. When forming the CC, modeled ECs adhere via junctional adhesion to neighboring ECs and BrM (Figure 2). Stalk cells (representing activated ECs) adhere to all other ECs via junctional adhesion with labile adhesion at the same strength as ECs in the CC, but have relatively weak plastic coupling to ECs and BrM (Figure 2). We assume ECs adhere to the RPE and to photoreceptor segments (PIS and POS generalized cells) weakly via labile adhesion representing nonspecific biological surface adhesion (Figure 2).

Adherent cells suspended in liquid assume a spherical shape, meaning that non-specific cell-liquid adhesion is weak. We represent this weak cell-liquid adhesion by weak labile adhesion

### Table 2. Model objects and processes.

| Object Types          | Processes                                                                 |
|-----------------------|---------------------------------------------------------------------------|
| Generalized Cells     | Adhere via junctional adhesion to ECs and BrM                             |
| Endothelial Cells (EC)| Adhere via labile adhesion to RPE, PIS, POS and Medium                   |
| Vascular Cells (of the CC) | Take up RPE-derived VEGF-A                        |
|                       | Secrete short-diffusing VEGF-A                                            |
|                       | Secrete Oxygen                                                            |
|                       | Die when RPE-derived VEGF-A is less than a threshold                       |
|                       | Have intrinsic random motility                                             |
| Stalk Cells (of the CNV) | Migrate via chemotaxis up gradients of short-diffusing VEGF-A (Contact-inhibited) |
|                       | All Processes of Vascular Cells and:                                      |
|                       | Migrate via chemotaxis up gradients of RPE-derived VEGF-A (Contact-inhibited) |
| Tip Cells             | Grow in response to RPE-derived VEGF-A (Contact-inhibited)               |
|                       | All Processes of Vascular Cells except secretion of Oxygen:              |
| RPE Cells             | Adhere via junctional adhesion to RPE cells and BrM                       |
|                       | Adhere via labile adhesion to ECs, PIS, POS and Medium                   |
|                       | Secrete RPE-derived VEGF-A                                                |
|                       | Die in absence of contact with RPE and BrM                                |
|                       | Have intrinsic random motility                                             |
| Photoreceptor Compartments | Adhere via junctional adhesion to POS and PIS cells                      |
| POS Cell-parts        | Adhere via labile adhesion to ECs, RPE, BrM and Medium                   |
|                       | Have intrinsic random motility                                             |
| PIS Cell-parts        | All Processes of POS Cell-parts and:                                      |
|                       | Consume Oxygen                                                            |
| Extracellular Materials | BrM Non-diffusing solid material (implemented as non-motile generalized cells) |
|                       | Adheres via labile and/or junctional adhesion (see EC, RPE, PIS, POS)    |
|                       | Degraded by MMP                                                           |
| Medium                | Adheres via labile adhesion to cells and BrM                              |
| Fields                | Oxygen Diffuses                                                          |
|                       | Decays                                                                    |
|                       | RPE-derived VEGF-A Diffuses                                              |
|                       | Decays                                                                    |
|                       | Short-diffusing VEGF-A Diffuses                                           |
|                       | Decays                                                                    |
|                       | MMP Diffuses                                                             |
|                       | Decays                                                                    |
|                       | Degrades BrM                                                             |

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Figure 1. Retinal structure, the retinal pigment epithelium, Bruch’s membrane and the choriocapillaris. Left large scale: Structure of the outer retinal layers, the RPE and the CC. Right: Detail of the CC-BrM-POS complex. CC: choriocapillaris, BrM: Bruch’s membrane, RPE: Retinal pigment epithelium, CC BaM: Basement membrane of the CC, OCL: Outer collagenous layer, EL: Elastin layer, ICL: Inner collagenous layer, RPE BaM: Basement membrane of the RPE (we abbreviate RPE BaM as RBaM), POS: Photoreceptor outer segment, PIS: Photoreceptor inner segment, ONL: Outer nuclear layer. Light purple shading indicates the location of the inner retina. Scale bars ~10 μm.

3D Simulations of Choroidal Neovascularization

between cells and medium. Thus cells prefer to adhere to other cells or BrM rather than to be surrounded by medium.

We do not model explicitly the differences in adhesion between the apical, lateral, and basal surfaces of biological RPE cells and photoreceptors. In our model labile adhesion strengths depend only on the types of cells or membranes in contact, not on the apical, basal or lateral identity of the contacting regions. However, effective cell polarization in the model develops from the specific geometry and contacts which occur in the normal and diseased retina, so at most times, cells emergently exhibit correctly polarized adhesivity, even though we do not impose it. We also neglect temporal adhesion changes observed clinically or experimentally during CNV progression, assuming temporally constant adhesivity of RPE, POS, PIS cells in our individual simulations.

Since we can vary independently the strength of the labile adhesion and plastic coupling which contribute to junctional adhesion, we refer to the labile components of RPE-RPE or RPE-BrM junctional adhesion as RPE-RPE or RPE-BrM labile adhesion and to the plastic coupling components of RPE-RPE or RPE-BrM junctional adhesion as RPE-RPE or RPE-BrM plastic coupling.

Angiogenic and antiangiogenic factors. To aggregate the effects of RPE-derived diffusible growth factors on the choriocapillaris and CNV capillaries we include a diffusible growth-factor field, RPE-derived VEGF-A which represents the aggregate proangiogenic effect of all biological long-diffusing proangiogenic and antiangiogenic factors. All types of ECs in our model take up RPE-derived VEGF-A uniformly at a constant rate. We omit growth factors and cytokines from other sources. Since we assume that PEDF affects CNV only as an antiangiogenic factor and that it diffuses at the same rate as RPE-derived VEGF, we can combine the effects of the two RPE-derived VEGF isoforms (120 and 165) and PEDF on CNV growth and regression into an effective cell response to RPE-derived VEGF-A. While numerous other diffusible proangiogenic and antiangiogenic factors [63,64] may play a modulatory role in capillary behavior (see Inflammation subsection in supplementary Text S1), we lack detailed experimental data on their spatial and temporal distribution and function. All modeled ECs (including CC and activated ECs, see below) secrete a short-diffusing VEGF-A. The short-diffusing VEGF-A is not a survival factor for ECs and we ignore its uptake by ECs. Both short-diffusing VEGF-A and RPE-derived VEGF-A decay at constant rates and diffuse uniformly everywhere in our modeled retina. However, the two VEGF-A diffusion lengths differ significantly. Typically, RPE-derived VEGF-A diffuses ~5 times farther than short-diffusing VEGF-A. Assuming the decay rates for both RPE-derived VEGF-A and short-diffusing VEGF-A are the same, a five-fold difference in diffusion length translates into a twenty-five fold larger diffusion constant for RPE-derived VEGF-A compared to short-diffusing VEGF-A.

Angiogenesis and BrM degradation. Computer simulations can help us analyze the role of multiple mechanisms during angiogenesis, both in pathological conditions like tumor-induced angiogenesis [65–68] and corneal angiogenesis [69] and in healthy tissues like muscle [70]. Here, we use a multi-cell 3D angiogenesis model which we have previously described [65] to simulate CNV growth and patterning. Our model includes two types of activated ECs: tip cell and stalk cell types. The tip cell type is a transient cell type that lasts one day, then differentiates into the stalk cell type. Cells of stalk cell type remain stalk cells. Both stalk and tip cells migrate via chemotaxis up gradients of both RPE-derived VEGF-A and short-diffusing VEGF-A at any cell boundaries which are not in contact with other ECs, i.e. they exhibit contact-inhibited chemotaxis. Thus, a stalk cell at the tip of an angiogenic sprout, which has less contact area with
other ECs, migrates more strongly than other stalk cells in response to VEGF-A gradients. Thus stalk cells can function as biological endothelial tip cells, leading other stalk cells in the trunk of an angiogenic sprout, as seen in experiments. Stalk cells self-organize into capillary-like network patterns [71].

Since experiments suggest that both ECs and macrophages can function as tip cells in CNV, we can interpret a tip cell either as an EC tip cell or as an immune cell that invades BrM. Modeled tip cells secrete a single MMP field. Our model is agnostic about the type of MMP, though experiments seem to favor MMP-2 which is activated on contact with tip cells or macrophages expressing MT1-MMP. Our model’s MMP field represents a very short-diffusing molecule that degrades BrM. If we assume that other types of MMP remain bound to the EC, it does not greatly affect our simulations, data not shown. Active tip cells produce MMPs at rates which allow them to invade even dense ECM at speeds of more than a few µm per day (e.g. during intersegmental blood vessel formation in zebrafish embryos). At this rate of degradation, a single tip cell could destroy the entire simulated BrM within a few simulated months. However, the typical hole size in BrM in vivo is less than a few cell diameters [29]. To generate a simulated hole in BrM compatible with these in vivo observations (a roughly one cell-diameter hole), we must switch off MMP secretion by the tip cell after 24 simulated hours. In our simulations we represent this cessation of MMP secretion by the differentiation of the tip cell into a stalk cell at 24 h. We neglect the slow reconstitution of BrM by RPE cells.

Inflammation. We represent the adhesion-reducing effects of inflammation due to inflammatory factors and immune cells implicitly by weakening RPE-RPE, RPE-POS (due to acute inflammation) and RPE-BrM adhesion (due to chronic irregularities in complement cascade). We neglect the role of inflammation on juxtacline Delta/Notch coupling between ECs.

Cell proliferation and death. Typically, adherent cells like RPEs need to adhere to other cells (of the same or different types) or to an appropriate substrate to remain viable. Otherwise they die. Modeled RPE cells require RPE-RPE and RPE-BrM contact to remain viable. In the absence of such contact, RPE cells die. RPE cells do not proliferate or grow. Both CC and ECs require a low concentration of RPE-derived VEGF to remain viable, and die below a threshold level of RPE-derived VEGF-A. Stalk cells grow at a rate depending on the local concentration of RPE-derived VEGF-A unless their growth is inhibited by stalk-EC contact (contact-inhibited growth, see the Methods section in supplementary Text S3 for details).

Results

In this section, we discuss how adhesion in the BrM-RPE-POS complex forms an effective physical barrier to CNV and how adhesion failures increase the risk of CNV initiation. We then relate the different modes of adhesion failure to the resulting CNV loci (CNV types), CNV progression and translocation (changes of CNV locus). We also discuss the CNV dynamics for individual adhesion scenarios and individual simulations representative of those adhesion scenarios.

All simulations begin with either no tip cell or one tip cell. In our simulations, the tip cell degrades BrM via MMP secretion, forming a roughly one cell diameter hole in BrM which allows it to cross BrM into the retina. The tip cell does not divide; 24 hours after the start of the simulation, it differentiates into a stalk cell, ending its degradation of BrM. All other stalk cells descend from this stalk cell. Stalk cells at the tip of angiogenic sprouts behave like biological tip cells. This tip-cell-like behavior allows stalk cells to migrate away from existing stalk-cell clusters, releasing their contact-inhibition. They then grow and divide when they reach a preset doubling-volume. CNV refers to the ensemble of stalk cells in a simulation. We define the time of CNV onset (the CNV initiation time) to be the time at which the total number of stalk cells exceeds three. For implementation parameter values and additional simulation details see the Methods section in supplementary Text S3.

We simulated 108 different adhesion scenarios (sets of adhesion parameters) [ID: 1 to 108] assigning one of three levels: normal; 3, moderately impaired; 2 and severely impaired; 1 to each of the five key adhesion parameters: 1) the RPE-RPE labile adhesion strength (RRl), 2) the RPE-RPE plastic coupling strength (RRp), 3) the RPE-BrM labile adhesion strength (RRl), 4) the RPE-BrM plastic coupling strength (RRp), and 5) the RPE-POS labile adhesion strength (RRl). For each adhesion scenario we
simulated our retina both in the absence and presence of a tip cell for one simulated year. In the presence of a tip cell, CNV may initiate (see below, in the Necessary and Sufficient Conditions for CNV Initiation section). We ran 10 simulation replicas for each adhesion scenario in which we included a tip cell (1000 simulations), and 3 simulation replicas for each adhesion scenario in the absence of a tip cell (324 simulations). Although not all the adhesion scenarios are physiologically likely (for example, Table S7: adhesion scenario ID: 90), a comprehensive exploration of the adhesion-parameter space clarifies the role of each corresponding mechanism in CNV initiation and progression.

The results of our simulations are 3D time-varying structures and fields. Quantitative comparison and classification of patterns and changes in patterns in 3D are often challenging. We have developed a morphometric quantification and classification algorithm (see the Quantification and Classification of Simulations subsection in supplementary Text S3 for details) that is able to classify CNV patterns, i.e. CNV types, and their time-dependent (CNV-type) changes, i.e. CNV progression (Table 3, Table 4, Table 5). Our algorithm calculates a morphometric weight, MW, based on the total contact area between stalk cells and BrM, and between stalk cells and the POS. A MW close to 1 indicates that most stalk cells are confined between the RPE and BrM (sub-RPE) in Type 1 CNV. A MW close to 0 indicates that most stalk cells are confined between the RPE and POS (sub-RPE) in Type 2 CNV. A MW close to 0.5 usually indicates Type 3 CNV (see the Quantification and Classification of Simulations subsection in supplementary Text S3 for additional conditions when MW~0.5). Our computational classification is compatible with current static histological classifications and can be applied to images of appropriately labeled histological sections and 3D microscopy images of the retina.

We use multiple-regression analysis to relate the CNV initiation probability, types, progression and dynamics to the typical adhesion scenarios which cause them (e.g., Figures 3–6). When statistical inference is ambiguous ($R^2<0.7$), we look at individual simulation time series in detail.

### Necessary and Sufficient Conditions for CNV Initiation

CNV initiation in our simulations requires: 1) a tip cell, and 2) adhesion failures. A tip cell is not sufficient to initiate CNV if all adhesions are normal. Even when a tip cell makes a hole in BrM, crosses BrM and differentiates into a stalk cell CNV does not initiate if all adhesions are normal (Table S1, adhesion scenario ID: 1).

The strong adhesion of RPE cells to neighboring RPE cells, POSs and BrM means that the BrM-RPE-POS ensemble behaves as a mechanically stable complex. This complex effectively obstructs CNV by limiting the proliferation and invasion of stalk cells into the sub-RPE and sub-retinal spaces. We discuss, in greater detail below, how different modes of adhesion failure in the complex allow stalk cells to proliferate and invade. Chemotaxis greatly affects how stalk cells invade the sub-RPE and sub-retinal spaces. Stalk cells migrate via chemotaxis up gradients of both short-diffusing and RPE-derived VEGF-A. RPE-derived VEGF-A is especially important because its concentration is maximal in the RPE, encouraging stalk cells to migrate from the CC into the sub-RPE and sub-retinal spaces. Adhesion between components of the BrM-RPE-POS complex opposes such invasion, inhibiting CNV initiation. Our simulations show that finite-strength adhesive interactions among the components of the BrM-RPE-POS complex can prevent invasion by stalk cells if the adhesion forces are greater than the forces which stalk cells exert on the RPE-RPE and RPE-BrM boundaries due to chemotaxis. Because of the complicated interactions among components during angiogenesis, the existence of such adhesion thresholds is not obvious a priori. Our simulations therefore allow us to refine our understanding of these thresholds to show that EC-EC adhesion is also important in vivo. Self-organization of ECs into a capillary network pattern requires: 1) Strong chemotaxis forces that balance EC-EC adhesion (ECs form clusters rather than networks when chemotaxis to both short-diffusing and RPE-derived VEGF-A is weak), 2) the EC-EC adhesion strength must be comparable to the adhesion strengths in the BrM-RPE-POS complex. Thus the strength of adhesion among RPE-RPE, RPE-BrM and RPE-POS required to resist chemotaxing ECs also depends on EC-EC adhesivity.

We performed multiple-regression analysis (see the Methods section in supplementary Text S3 for details) against five adhesion parameters ($RRl$, $RRp$, $RBl$, $RBr$, $RBp$) to relate specific adhesion failures to the probability of CNV initiation. The five adhesion parameters and their (multi)linear combinations account for 88% of the observed variance in the CNV initiation probability (adjusted $R^2 = 0.83$). To visualize the five-dimensional (5D) regression relations, we reduce 5D to 3D by assuming that $RRp = RRl$ and $RBp = RBl$. We call this reduction symmetric since it assumes that impairing RPE-RPE labile adhesion also impairs RPE-RPE plastic coupling. E.g., we would expect changes in

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**Table 3.** Classification of CNV type based on Morphometric Weight.

| Morphometric Weights (MW) | CNV Type |
|---------------------------|----------|
| MW=0.75                   | Type 1   |
| MW=0.25                   | Type 2   |
| 0.25< MW<0.75             | Type 3   |

We define the type of CNV based on the mean morphometric weight during a three month window. A MW=0.75 throughout the window indicates that most stalk cells lie between BrM and the RPE (in the sub-RPE space) and do not contact the POS. We therefore assign the time window to Type 1. A MW=0.25 throughout the window indicates that most stalk cells lie between RPE and the POS (in sub-retinal space) and do not contact BrM. We therefore assign the time window to Type 2 CNV. A MW~0.25 Usually indicates that stalk cells occur in both the sub-RPE and sub-retinal spaces. In a few exceptional cases (Table S5) with a sub-RPE~0.25, most stalk cells lie between neighboring RPE cells rather than in either the sub-RPE or sub-retinal spaces (discussed in the Stable Type 3 CNV subsection in supplementary Text S5).

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**Table 4.** (Temporal) Nomenclature for CNV.

| CNV Classification | Relevant Adhesion Scenarios |
|---------------------|-----------------------------|
| Early Type 1 (ET1)  | Table S2                    |
| Late Type 1 (LT1)   | -                           |
| Early Type 2 (ET2)  | Table S3                    |
| Late Type 2 (LT2)   | -                           |
| Early Type 3 (ET3)  | Table S4                    |
| Late Type 3 (LT3)   | -                           |

To classify CNV progression dynamics during a simulated year, we determine the early and late loci of stalk cells using the mean MWs during the first and last three months of each simulation (we can make this calculation whether or not CNV initiates).

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3D Simulations of Choroidal Neovascularization
cytoskeletal architecture due to inflammation to affect both adhesion mechanisms together. We consider symmetric reductions in adhesion later in this section. Figure 3 shows a 3D volumetric visualization of the CNV initiation probability \( P_{\text{init}} \) in the symmetrically reduced parameter space \( RRp = RRL \) and

**Figure 3.** CNV Initiation probability dependence on key adhesion mechanisms. 3D plot of the regression-inferred CNV initiation probability \( P_{\text{init}} \) vs. three key adhesion strengths using ten simulation replicas for each adhesion scenario in the 3D parameter space obtained by setting \( RRp = RRL \) and \( RBP = RB\). Red corresponds to \( P_{\text{init}} = 1 \) and purple to \( P_{\text{init}} = 0 \). The black region at the top-front corner indicates the locus of normal adhesion. The three isosurfaces of CNV initiation probability correspond to \( P_{\text{init}} = 0.25 \) (front), 0.5 (middle) and 0.75 (back). The five adhesion parameters and their (multi)linear combinations account for 88% of the observed variance in CNV initiation probability (adjusted \( R^2 = 0.83 \)). Regression predicts a minimum CNV initiation probability of 0.08 for normal adhesion, much higher than observed in either our simulations or experiments. For normal RPE-POS labile adhesion, moderate impairment of either RPE-RPE \( (RRp = RRL) \) or RPE-BrM \( (RBP = RB\) junctional adhesion increases the CNV initiation probability to \(-50\%). Severe impairment of RPE-POS increases the CNV initiation probability to \(-50\% even when both RPE-RPE and RPE-BrM are normal.

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**Figure 4.** Sub-RPE CNV dependence on adhesion. 3D plot of the regression-inferred average \( MW \) using 10 simulation replicas for each adhesion scenario in the 3D parameter space obtained by setting \( RRp = RRL \) and \( RBP = RB\). The average \( MW \) shows the stalk cell locus even when CNV fails to initiate, so a region prone to ET1 CNV develops ET1 CNV only if CNV initiates. Red corresponds to \( MW = 1 \) and purple corresponds to \( MW = 0 \). The black region at the top-left corner indicates the locus of normal adhesion. \( MW = 1.0 \) for RPE-RPE junctional adhesion normal, RPE-BrM junctional adhesion severely impaired (weak) and RPE-POS labile adhesion normal. The three isosurfaces correspond to \( MW = 0.25 \) (back), 0.5 (middle) and 0.90 (front). The five adhesion parameters and their (multi)linear combinations account for 93% of the observed variance in average \( MW \) for all 108 adhesion scenarios (adjusted \( R^2 = 0.89 \)). Severe impairment of RPE-POS labile adhesion greatly reduces the MW, so ET1 CNV can only occur when RPE-POS labile adhesion is near normal. Scenarios with severe impairment of RPE-BrM junctional adhesion \( (RBP = RB\) = 1) and normal RPE-POS labile adhesion are prone to ET1 CNV for a wide range of RPE-RPE junctional adhesion impairment \( MW > 0.95 \) for \( RRp = RRL \geq 1.5 \). The red region with \( MW > 0.9 \) has \( P_{\text{init}} \leq 0.8 \). We have rotated the axes from their orientation in Figure 3 to show the regions in the parameter space prone to ET1 CNV. To show the structure of the isosurfaces, we have rotated the axes relative to Figure 3.

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### Table 5. Nomenclature for CNV dynamics.

| Dynamics Classification | CNV Dynamics | Relevant Adhesion Scenarios |
|-------------------------|--------------|-----------------------------|
| Stable Type 1 \( (S1T) \) | Early Type 1 \( \rightarrow \) Late Type 1 | Table S5 |
| Sub-RPE to Sub-Retinal Translocation \( (T1T) \) | Early Type 1 \( \rightarrow \) Late Type 2 | Table S6 |
| Sub-RPE to Sub-Retinal Progression \( (P1T) \) | Early Type 1 \( \rightarrow \) Late Type 3 | Table S7 |
| Sub-Retinal to Sub-RPE Translocation \( (T2T) \) | Early Type 2 \( \rightarrow \) Late Type 1 | Not Observed |
| Stable Type 2 \( (S2T) \) | Early Type 2 \( \rightarrow \) Late Type 2 | Table S8 |
| Sub-Retinal to Sub-RPE Progression \( (P2T) \) | Early Type 2 \( \rightarrow \) Late Type 3 | Table S9 |
| Type 3 to Sub-RPE Translocation \( (T3T) \) | Early Type 3 \( \rightarrow \) Late Type 1 | Not Observed |
| Type 3 to Sub-Retinal Translocation \( (T3T) \) | Early Type 3 \( \rightarrow \) Late Type 2 | Not Observed |
| Stable Type 3 \( (S3T) \) | Early Type 3 \( \rightarrow \) Late Type 3 | Table S10 |

We classify CNV progression dynamics in each simulation based on its early and late CNV types, allowing for nine CNV-dynamics scenarios. We use the term progression when a simulation replica initially develops either Early Type 1 CNV or Early Type 2 CNV and then develops Late Type 3 CNV and translocation when a replica initially develops either Early Type 1 CNV or Early Type 3 CNV, then Late Type 2 CNV or initially develops either Early Type 2 CNV or Early Type 3 CNV, then Late Type 1 CNV. Three translocation scenarios, \( T21, T31 \) and \( T32 \), did not occur in our simulations.

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for all 108 adhesion scenarios (MW combinations account for 93% of the observed variance in average 0.90 (left). The five adhesion parameters and their (multi)linear
at the top-back corner indicates the locus of normal adhesion. The
ET2 CNV locus even when 

2) When RPE-RPE junctional adhesion is normal, RPE-BrM junctional adhesion is moderately impaired, and RPE-POS labile adhesion is severely impaired (weak). 2) When RPE-RPE junctional adhesion is severely impaired (weak) and RPE-BrM junctional adhesion is normal, independent of RPE-POS labile adhesion. 3) When RPE-RPE adhesion is weak, RPE-BrM adhesion is moderately to severely impaired, and RPE-POS adhesion is severely impaired. The red region does not include all adhesion scenarios in Table S3 leading to
impaired, and RPE-POS labile adhesion

impaired and RPE-POS labile adhesion is severely impaired, CNV initiation probability increases to 100%. Table S1 shows that asymmetrical impairment of either RPE-RPE or RPE-BrM plastic coupling alone, without impairment of the corresponding labile adhesion barely increases the probability of CNV initiation. Thus, the plastic coupling strengths have only a minor effect on the ability of the BrM-RPE-POS complex to oppose CNV.

We believe that our simulated initiation probabilities are higher than those observed in experiments due to two simplifying assumptions of our model: 1) We assumed that all stalk cells can divide indefinitely. Limiting the number of stalk-cell divisions (due to senescence), would lower the probability of CNV initiation. In in vivo experiments, less than 2% of endothelial cells have high proliferation potential [72,73], so angiogenic sprouts often fail to grow, or even regress, if the ECs forming the sprouts have low proliferation potential. 2) We assumed that stalk cells adhere equally to both sides of BrM, while BrM and CC basement membrane are histologically distinct. In experiments, ECs haptotax to the basement membrane of the CC on the outer side of BrM (Figure 1), reducing their probability of crossing BrM compared to our simulations. We expect that increasing adhesion between stalk cells and the CC-side of BrM would reduce the CNV initiation probability, since stalk cells would then prefer to remain on the CC-side of BrM, where contact with the preexisting vasculature would inhibit their growth.
Early and Late CNV and CNV Progression

To classify CNV progression during a simulated year, we determine the early and late loci of stalk cells, using the mean (weighted by the number of stalk cells) MW's during the first and last three months of each simulation whether or not CNV initiates (we can calculate the MW even if CNV fails to spread and thus in the absence of initiation). The mean MW measures the ability of the BrM-RPE-POS complex to confine stalk cells to specific regions. A MW≥0.75 during a given time interval indicates that most stalk cells lie between BrM and the RPE (in the sub-RPE space) and that they do not contact the POS. We therefore assign the time interval to Type 1 CNV. A MW≤0.25 during a given time interval indicates that most stalk cells lie between the RPE and the POS (in the sub-retinal space) and do not contact BrM. We therefore assign the time interval to Type 2 CNV. 0.25<MW<0.75 usually indicates that stalk cells occur in both the sub-RPE and sub-retinal spaces (since few sub-RPE stalk cells touch the POS and few sub-retinal stalk cells touch BrM). We therefore assign the time interval to Type 3 CNV. In a few exceptional cases (Table S10) with 0.25<MW<0.75, most stalk cells lie between neighboring RPE cells rather than in either the sub-RPE or sub-retinal spaces (discussed in the Stable Type 3 CNV section in supplementary Text S5). The type of CNV during the early window which we call the early CNV type (the first three months of a simulation) is especially revealing, because all simulations start from the same initial condition. The early CNV type shows the efficacy of the BrM-RPE-POS complex in blocking stalk cell invasion of the sub-RPE and sub-retinal spaces. Since tissue structure and cell function can change significantly in the BrM-RPE-POS complex during a simulated year, changes in CNV type between the early and late windows can result from either structural or barrier-function changes in the BrM-RPE-POS complex. For example, the adhesion failures which typically lead to Early Type 3 CNV differ from those which typically leading to Late Type 3 CNV.

Because our simulations are stochastic, different replicas of the same adhesion scenario can lead to different combinations of early and late types of CNV. Such variation is common clinically and indicates that a simple population average of MWs over simulation replicas may reveal neither the types, progression dynamics nor degree of heterogeneity of outcomes. To retain this dynamic and population information, we classify CNV dynamics (progression) in each simulation separately based on its early and late CNV type, for a total of 9 CNV dynamics scenarios (Table 5). We use the term progression when a simulation replica initially develops either Early Type 1 CNV or Early Type 2 CNV and then develops Late Type 3 CNV and translocation when a replica initially develops either Early Type 1 CNV or Early Type 2 CNV, then Late Type 3 CNV, then Late Type 3 CNV, then Late Type 2 CNV or Early Type 2 CNV, then Early Type 3 CNV, then Late Type 1 CNV. Three translocation scenarios sub-retinal to sub-RPE CNV (T21 CNV), Type 3 to sub-RPE CNV (T31 CNV) and Type 3 to sub-retinal CNV (T32 CNV) did not occur in our simulations.

Early Type 1 (ET1) CNV and its progression. Stalk cells remain confined to the sub-RPE space in two main classes of adhesion scenarios: 1) When RPE-BrM labile adhesion is moderately to severely impaired, RPE-BrM plastic coupling satisfies RBl+RBp≤4, and both RPE-RPE and RPE-POS labile adhesion are normal (RRl=3 and R0l=3). 2) When both RPE-RP and RPE-BrM labile adhesion are severely impaired (RRl=1 and RBp=1), RPE-BrM plastic coupling is moderately to severely impaired (RBp≤2), and both RPE-RPE plastic coupling and RPE-POS labile adhesion are normal (RRp=3, R0p=3) (Table S2, adhesion scenario ID: 83 and 84). In both classes of adhesion scenarios, CNV initiation leads to Early Type 1 CNV (ET1). Table 6 shows the MW and CNV initiation probabilities for the adhesion scenarios most prone to ET1 CNV (MW>0.9).

Multiple-regression analysis of the five adhesivities accounted for 93% of the observed variance in the average MW for all 108 adhesion scenarios (adjusted 𝐼 kullack− 𝑁ow− 𝐵e 𝑁. Figure 4 shows the stalk cell locus regression-inferred from the average MW as a function of the adhesion parameters obtained by setting RRl=RRi and RRp=RRi. Since Figure 4 shows the stalk cell locus even when CNV fails to initiate, a region prone to ET1 CNV, develops ET1 CNV only if CNV initiates. Severe impairment of RPE-POS labile adhesion greatly reduces the MW, so ET1 CNV can only occur when RPE-POS labile adhesion is near normal.

Scenarios with severe impairment of RPE-BrM junctional adhesion (RRp=RRi=1), and normal RPE-POS labile adhesion are prone to ET1 CNV over a wide range of RPE-POS junctional adhesion impairment (MW>0.95 for RRp=RRi>.15). The red region with MW>0.9 has Pinit>0.8 (Figure 4).

In most adhesion scenarios that develop Early Type 1 CNV with MW>90% the CNV remains in the sub-RPE space during one simulated year (Late Type 1 CNV, MW>75%). I.e., they exhibit Stable T1 CNV (S11 CNV) (see Figure 6, Table 7 and supplementary Text S5 for details). Generally, CNV growth speed differs from replica to replica in adhesion scenarios prone to S11 CNV (compare to S22 CNV dynamics, below). Figure 7 shows typical S11 CNV dynamics for 10 simulation replicas of a single adhesion scenario (RRl=3, RRp=3, RBp=2, RBp=2, ROl=3) (Table S3, adhesion scenario ID: 38). We visualize snapshots of S11 CNV dynamics in one replica in Figure 8 and Video S1.

Adhesion scenarios in which some replicas exhibit T12 CNV can also have replicas which exhibit either S22 or S11 over one simulated year. Figure 9 shows T12 CNV dynamics for 10 simulation replicas of the adhesion scenario (RRl=3, RRp=3, RBp=1, ROl=1) (Table S6, adhesion scenario ID: 93). We visualize snapshots of the T12 CNV dynamics in one replica in Figure 10 and Video S2.

CNV dynamics is very similar across all replicas in adhesion scenarios prone to P13 CNV and much less heterogeneous than for T12 CNV. Figure 11 shows typical P13 CNV dynamics for 10

### Table 6. Adhesion scenario classification based on early CNV type.

| Early CNV Sub-classes | RRl | RRp | RBl | RBp | ROl |
|------------------------|-----|-----|-----|-----|-----|
| Type 1                 | 1   | 3   | *   | 1.2 | 3   |
|                        | 2   | 3   |     | 1.2 | 3   |
| Type 2                 | 1   | 3   | *   | 2.3 | *   |
|                        | 2   | 1   | *   | 2.3 | *   |
|                        | 3   | 1   | RBp+RBp>3 | 1 | RBp+RBp>3 | 1 |
| Type 3                 | 1   | 1   | *   | 1.2 | 3   |

Key: RRl: RPE-BrM labile adhesion strength, RRp: RPE-POS labile adhesion strength, RBl: RPE-BrM plastic coupling strength, RBp: RPE-BrM plastic coupling strength, ROl: RPE-POS labile adhesion strength, S3: 3D Simulations of Choroidal Neovascularization.
simulation replicas of the adhesion scenario \((RR_l = 1, \quad RR_p = 3, \quad RB_l = 1, \quad RB_p = 2, \quad RO_l = 3)\) (Table S7, adhesion scenario ID: 83).

We visualize snapshots of \(P_{13}\) CNV dynamics in one replica in Figure 12 and Video S3.

**Early Type 2 (ET2) CNV and its progression.** Stalk cells initially invade the sub-retinal space (Early Type 2 CNV) in three main classes of adhesion scenarios: 1) When RPE-RPE labile adhesion is normal \((RR_l = 3)\), RPE-BrM labile adhesion is normal or moderately impaired \((RB_l \geq 2)\), and RPE-POS labile adhesion is severely impaired \((RO_l = 1)\). 2) When RPE-RPE labile adhesion is severely impaired \((RR_l = 1)\) and RPE-BrM labile adhesion is either normal or moderately impaired

| CNV Progression Dynamics | Sub-classes | \(RR_l\) | \(RR_p\) | \(RB_l\) | \(RB_p\) | \(RO_l\) |
|--------------------------|-------------|---------|---------|---------|---------|--------|
| S_{11}                   | 1           | 3       | 2, 3    | 1, 2    | 3 \leq RB_l + RB_p \leq 4 | 3      |
| T_{12}                   | 1           | 3       | \(RR_p + RB_p \geq 4\) except \(RB_p = 2\) | 1       | \(RR_p + RB_p \geq 4\) except \(RR_p = RB_p = 2\) | 1      |
| P_{13}                   | 1           | 1       | *       | 1, 2    | 3      |
| S_{22}                   | 1           | 3       | *       | 2, 3    | *      |
|                         | 2           | 1       | *       | 2, 3    | *      |
|                         | 3           | 1       | \(RB_p + RB_p > 3\) | 1       | \(RB_p + RB_p > 3\) | 1      |
| P_{23}                   | 1           | 1       | 1, 2    | 1       | 1      |
| S_{33}                   | 1           | 1       | *       | 2       | \(RB_l + RB_p \leq 4\) | 3      |

To simplify, we list only the adhesion scenarios most prone to each type of CNV progression dynamics. Key: \(RR_l\): RPE-RPE labile adhesion strength, \(RR_p\): RPE-RPE plastic coupling strength, \(RB_l\): RPE-BrM labile adhesion strength, \(RB_p\): RPE-BrM plastic coupling strength, \(RO_l\): RPE-POS labile adhesion strength. Scaled adhesion strengths: 3: normal, 2: moderately impaired, 1: severely impaired (weak), *: all strength levels. See Table 5, for nomenclature for CNV dynamics.

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**Figure 7. Dynamics of stable Type 1 CNV (S_{11} CNV).** A) Total number of stalk cells vs. time. B) Total number of stalk cells confined in the sub-RPE space vs. time. C) Total number of stalk cells in contact with the POS (stalk cells in the sub-retinal space) vs. time. D) Total number of RPE cells vs. time. E) Total contact area between RPE cells and BrM vs. time. F) Total contact area between POS cells and BrM vs. time. The different colors represent the dynamics of 10 simulation replicas of the adhesion scenario \((RR_l = 3, \quad RR_p = 3, \quad RB_l = 2, \quad RB_p = 2, \quad RO_l = 3)\) (Table S5, adhesion scenario ID: 38). (A, B) CNV initiates in 9 out of 10 simulation replicas. All develop Early Type 1 CNV. CNV remains confined in the sub-RPE space during one simulated year (Stable Type 1 CNV). A Fully developed sub-RPE capillary network contains about 45 stalk cells \((\sim 3000\text{ cells/mm}^2)\).

In 5 simulation replicas a few stalk cells die during the simulated year due to lack of RPE-derived VEGF-A. (C) Stalk cells have minimal contact with the POS. (D, E) The RPE remains viable and its total contact area with BrM decreases as stalk cells proliferate. (F) The POS never contacts BrM, indicating that the RPE does not develop any holes.

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When RPE-RPE, RPE-BrM, and RPE-POS labile adhesion are severely impaired, the combination of RPE-RPE and RPE-BrM plastic coupling satisfies \( RB_p + RB_p > 3 \). Unless all labile adhesions are severely impaired, impairment of either RPE-RPE or RPE-BrM plastic coupling has little effect on the average MW, though it does increase the CNV initiation probability. For example, adhesion scenarios ID: 22 and 24, which differ only in their RPE-BrM plastic coupling, exhibit the same mean MW; however, \( P_{init} = 0.8 \) for normal RPE-BrM plastic coupling (ID: 22) and \( P_{init} = 1 \) for severely impaired RPE-BrM plastic coupling (ID: 24).

Figure 5 shows \((1 - MW)\), which measures the degree of confinement of stalk cells to the sub-retinal space, based on the regression-inferred average MW (see, the Early Type 1 (ET1) CNV subsection, above) as a function of the five adhesion types.

Figure 8. Snapshots of a simulation replica with stable Type 1 CNV. 3D visualization of a simulation replica exhibiting Stable Type 1 CNV over one simulated year (adhesion scenario ID: 38, simulation ID: 902) \((RB_l = 3, RB_l = RB_l = 2, RO_l = 3)\). Snapshots of the simulation at months 3 (A), 6 (B), 9 (C) and 12 (D). (A) Stalk cells (black arrows) invade the sub-RPE space through a hole (black outline arrow) in BrM (light blue outline arrow) that the tip cell opens during the first 24 hours. (B) Brown outline arrow shows the RPE cells. Red outline arrow shows the CC (B, C) Stalk cells proliferate until they fill the sub-RPE space in month 9, after which proliferation slows down (D) The 45 stalk cells form a connected capillary network in the sub-RPE space. Cell type colors: 1) POS and PIS: light purple, 2) RPE: brown, 3) Stalk cells: green, 4) Vascular cells (CC): red, 5) BrM: light blue. Scale bar \( \approx 50 \mu m \). We have rendered the boundaries of individual cells as semi-transparent membranes. POS, PIS and RPE cells are more transparent to show the underlying structures. See also Video S1.

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The contact area between the RPE-RPE junctional adhesion scenario prone to CNV (S5). Generally, CNV exhibited MW (Table S6) adhesion scenario ID: 93). (A, B) CNV initiates in all replicas. By 3 months, most replicas form a developed sub-RPE capillary network composed of 1–20 to 40 stalk cells (1–1500 to 3000 cells/mm²). 8 replicas develop Early Type 1 (ET1) CNV. Only one replica shows Stable Type 1 (S11) CNV. Some stalk cells in most replicas die due to lack of RPE-derived VEGF-A. (C) Two replicas show Stable Type 2 (S22) CNV (Early ET2) and Late Type 2 (LT2) CNV, black and dark red lines). 7 replicas show LT2 CNV. (D) The RPE remains viable in all replicas. (E) The contact area between the RPE and BrM decreases as either ET1 CNV or S11 CNV develops, and remains constant during ET2 CNV. RPE reattaches to BrM during T12 CNV. (F) The POS contacts BrM once, but the contacts area and duration are both small, so the RPE does not develop any persistent or substantial holes.

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Figure 9. Dynamics of sub-RPE to sub-retinal translocation (T12 Translocation). A) Total number of stalk cells vs. time. B) Total number of stalk cells confined in the sub-RPE space vs. time. C) Total number of stalk cells in contact with the POS (stalk cells in the sub-retinal space) vs. time. D) Total number of RPE cells vs. time. E) Total contact area between RPE cells and BrM vs. time. F) Total contact area between POS cells and BrM vs. time. The different colors represent the results of 10 simulation replicas of the adhesion scenario (RRl = 3, RRP = 3, RRb = 1, RBR = 1, ROI = 1) (Table S6 adhesion scenario ID: 93). (A, B) CNV initiates in all replicas. By 3 months, most replicas form a developed sub-RPE capillary network composed of 1–20 to 40 stalk cells (1–1500 to 3000 cells/mm²). 8 replicas develop Early Type 1 (ET1) CNV. Only one replica shows Stable Type 1 (S11) CNV. Some stalk cells in most replicas die due to lack of RPE-derived VEGF-A. (C) Two replicas show Stable Type 2 (S22) CNV (Early ET2) and Late Type 2 (LT2) CNV, black and dark red lines). 7 replicas show LT2 CNV. (D) The RPE remains viable in all replicas. (E) The contact area between the RPE and BrM decreases as either ET1 CNV or S11 CNV develops, and remains constant during ET2 CNV. RPE reattaches to BrM during T12 CNV. (F) The POS contacts BrM once, but the contacts area and duration are both small, so the RPE does not develop any persistent or substantial holes.

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parameters, reduced to 3D by setting RRP = RRl and RBR = RRb. The red region with (1–MW)>0.9, can be divided into three sub-regions: 1) When RPE-RPE junctional adhesion is normal, RPE-BrM junctional adhesion is moderately impaired, and RPE-POS labile adhesion is severely impaired (weak). 2) When RPE-RPE junctional adhesion is severely impaired (weak) and RPE-BrM junctional adhesion is normal, independent of RPE-POS labile adhesion. 3) When RPE-RPE junctional adhesion is severely impaired (weak) and RPE-BrM junctional adhesion is moderately to severely impaired, and RPE-POS labile adhesion is severely impaired. Figure 5 does not include all the adhesion scenarios in Table S3 leading to Early Type 2 CNV.

In Stable Type 2 CNV (S22 CNV), stalk cells initially invade the sub-retinal space to develop Early Type 2 CNV and remain confined in the sub-retinal space in Late Type 2 CNV. Most adhesion scenarios that develop ET2 CNV in which the MW remains less than 0.15 during the first three months also exhibit S22 CNV (Figure 13, Table 7 and supplementary Text S5). Generally, CNV dynamics is very similar across all replicas of the adhesion scenarios prone to S22 CNV (Table 7 and supplementary Text S5). As for P13 CNV, the variability from replica to replica is smaller than for S11 CNV. Figure 14 shows typical S22 CNV dynamics for 10 simulation replicas of the adhesion scenario (RRl = 1, RRP = 1, RRb = 3, RBR = 3, ROI = 3) (Table S8, adhesion scenario ID: 16). We show snapshots of the S22 CNV dynamics in one replica in Figure 15 and Video S4.

CNV dynamics is very similar across all replicas of the adhesion scenarios prone to P23 CNV (Table 7 and supplementary Text S5): Variability from replica to replica is low and comparable to the variability observed in P13 CNV and S22 CNV. Figure 16 shows typical P23 CNV dynamics for 10 simulation replicas of the adhesion scenario where all adhesions are severely impaired (RRl = 1, RRP = 1, RRb = 1, ROI = 1) (adhesion scenario ID: 108). We visualize snapshots of the P23 CNV dynamics in one replica in Figure 17 and Video S5.

Early Type 3 (ET3) CNV and its progression. In Early Type 3 (ET3) CNV, Stalk cells initially grow both between the RPE and BrM and between the RPE and the POS. Most adhesion scenarios have severely impaired RPE-RPE labile adhesion (RRl = 1), normal RPE-POS labile adhesion (ROI = 3) and either severely or moderately impaired RPE-BrM labile adhesion (RRb = 2) (Table S4). In these adhesion scenarios, RPE-RPE and RPE-BrM plastic coupling have little effect on mean MW and CNV initiation probability. For example, adhesion scenarios ID: 32 (RRp = 3), 85 (RRp = 2) and 88 (RRp = 1) have similar mean MW and CNV initiation probability, despite differing in their RPE-RPE plastic coupling strengths (RRp).

Generally, CNV dynamics is very similar across all replicas of the adhesion scenarios prone to S33 CNV (Table 7 and supplementary Text S5). Variability from replica to replica is comparable to the variability in P13 CNV, S22 CNV and P23 CNV. Figure 18 shows the typical S33 CNV dynamics in 10 simulation replicas of the adhesion scenario (RRl = 1, RRP = 1, RRb = 2, RBR = 2, ROI = 3) (Table S10, adhesion scenario ID: 53). We visualize snapshots of S33 CNV dynamics in one replica in Figure 19 and Video S6.
Discussion

Our simulations show that variations in five key adhesion strengths suffice to explain many of the experimentally and clinically observed dependencies of CNV initiation on drusen, inflammation, retinal detachment and iatrogenic to reproduce the main observed types and progression dynamics of CNV associated with those defects. Since pathological conditions can cause multiple adhesion failures in the BrM-RPE-POS complex, we simulated factorial combinations of graded impairments of the five adhesion types to explore the effects of biologically-coupled adhesion failures. In this section, we discuss the effects of both

Figure 10. Snapshots of a simulation replica showing sub-RPE to sub-retinal translocation (T12 Translocation). 3D visualization of a simulation replica exhibiting T12 CNV translocation during one simulated year (RRl = 3, RRp = 3, RBi = 1, RBp = 1, ROI = 1) (adhesion scenario ID: 93, simulation ID: 849). Snapshots of the simulation at months 3 (A), 5 (B), 9 (C) and 12 (D). (A) Stalk cells (solid black arrow) invade the sub-RPE space through a hole in BrM (black outline arrow) and form a capillary network. All stalk cells remain in the sub-RPE space during the first 3 months. A few vascular cells fill the hole in BrM (black outline arrow) to connect CNV capillaries to the CC (red outline arrow). Brown outline arrow shows an RPE cell. (B) Half of the stalk cells (black outline arrow) have crossed the RPE and transmigrated into the sub-retinal space, forming a new capillary network in the sub-retinal space. The black arrow shows a stalk cell in the sub-RPE space. (C) Most stalk cells have transmigrated into the sub-retinal space and the RPE has completely reattached to BrM (Figure 9E, dark green line). A few vascular cells of the CC have transmigrated into the sub-retinal space (red outline arrow). The sub-retinal capillary network has fewer stalk cells than (C) since stalk cells that migrate into the retina far from the RPE die. (D) The sub-retinal capillary network has fewer stalk cells than (C) since stalk cells that migrate into the retina far from the RPE line. Cell type colors: 1) POS and PIS: light purple, 2) RPE: brown, 3) Stalk cells: green (stalk cells in the sub-retinal space have lighter shading), 4) Vascular cells (CC): red, 5) BrM: light blue. Scale bar = 50 μm. We have rendered the boundaries of individual cells as semi-transparent membranes. POS, PIS and RPE cells are more transparent to show the underlying structures. See also Video S2. doi:10.1371/journal.pcbi.1002440.g010
individual adhesion failures and their combinations on CNV
initiation, type and dynamics and explain their clinical and
experimental relevance.

Table 1 aggregates a spectrum of clinical and experimental
observations for a variety of conditions, with rough estimates of the
degree of impairment of the three main inter-component
adhesions present in the BrM-RPE-POS complex. Table 1 also
lists the most common types of CNV associated with each
condition. In this section, we compare clinical and experimental
observations for these conditions to the results of our simulations
for appropriate adhesion scenarios. Since we assume adhesion is
costant over the duration of the simulation, to understand the
effects of the gradual changes in adhesion which occur in patients
as CNV develops, we must look successively at the results of multiple
simulations for an appropriate series of adhesion
impairments comparable at each time to those of the disease as
it progresses.

Clinical and Experimental Types of CNV and their
Relation to Simulations

CNV due to soft drusen in older humans. As we discussed
earlier (see the section Current Hypotheses for CNV Initiation and
Progression), soft drusen significantly reduce the adhesion of the
basement membrane of the RPE to BrM (RBaM-BrM adhesion).
Sub-RPE CNV often starts by growing between these weakly
adhered layers. Although soft drusen are seen clinically, the early
stages of invasion of ECs into the sub-RPE space have little effect
on visual acuity, so early EC invasions can remain unreported and
unnected. In these patients, initial vision acuity prior to CNV
initiation is not severely impaired compared to typical age-
controlled visual acuity, suggesting that their photoreceptors
remain healthy or at most moderately impaired compared to
age-matched controls. Since the RPE plays multiple roles in
maintaining both photoreceptors (e.g. through the phagocytosis of
spent disks from the photo receptors) and the outer retina (e.g.
through the transport of fluids), near-normal photoreceptors can
only persist in the presence of near-normal RPE cells. Near-
normal vision also requires that the POS remain attached to the
RPE without accumulation of sub-retinal fluid or retinal detach-
ment, indicating that RPE-POS adhesion must remain near-
normal. Based on these observations, we hypothesize that in
patients with soft drusen, both RPE-RPE and RPE-POS adhesion
are near-normal, but BaM-BrM adhesion is impaired with the
level of impairment differing patient-to-patient. This spectrum of
adhesion impairments in the BrM-RPE-POS complex is compara-
able to the first sub-class of adhesions scenarios which are prone
to Early Type 1 CNV (Table 6). Within this sub-class, variation
in the degree of adhesion impairment of RPE-RPE plastic
coupling, RPE-BrM labile adhesion and RPE-BrM plastic
coupling affects many aspects of CNV in simulations, including
the CNV initiation probability, CNV onset time and CNV
dynamics. Additionally, some adhesion scenarios in this class show
significant variability among simulation replicas with identical
adhesivities.

Patients with multiple large soft drusen or confluent drusen (soft
indistinct drusen) have a higher chance of developing CNV [74],
suggesting that CNV initiation probability depends on total area of
drusen and their shape. In standard clinical usage, drusen with a
diameter <63 μm are called small drusen and those with a diameter
>125 μm are called large drusen. Confluent drusen or soft indistinct

3D Simulations of Choroidal Neovascularization

Figure 11. Dynamics of sub-RPE CNV to sub-retinal CNV progression (P13 Progression). A) Total number of stalk cells vs. time. B) Total number of stalk cells confined in the sub-RPE space vs. time. C) Total number of stalk cells in contact with the POS (stalk cells in the sub-retinal space) vs. time. D) Total number of RPE cells vs. time. E) Total contact area between RPE cells and BrM vs. time. F) Total contact area between POS cells and BrM vs. time. The different colors represent the results of 10 simulation replica of the adhesion scenario (RRl = 1, RRp = 3, RBl = 1, Rbp = 2, ROl = 3) (Table S7, adhesion scenario ID: 83). CNV initiates in all replicas and all develop ET1 CNV. A few stalk cells in most replicas die due to lack of RPE-derived VEGF-A. (C) Stalk cells cross the RPE and invade the sub-retinal space once the number of stalk cells in the sub-RPE space reaches ~60 cells, which usually occurs within first two months after initiation. CNV progression to the sub-retinal space is complete around month 5. (D) The RPE remains viable in all replicas. (E) The contact area between the RPE and BrM decreases as ET1 CNV develops, and remains constant afterwards throughout LT3 CNV. (F) The POS contacts BrM a few times, but the contact area and duration are both small, so the RPE does not develop any persistent or substantial holes.

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drusen form when multiple soft drusen touch and merge. Confluent drusen strongly correlate with CNV and serous RPE detachment due to fluid accumulation between BrM and the RPE, indicating severe impairment of RBrM-BrM adhesion due to the larger area of impaired RBrM-BrM adhesion and high lipid levels in the drusen affected regions. These experimental observations suggest that the probability of CNV initiation increases with both the degree and total area of RBrM-BrM adhesion impairment. In our simulations, the total area of impaired adhesion is the same in all replicas, so it does not affect the CNV initiation probability. However, the CNV initiation probability increases with the degree of impairment of RPE-BrM junctional adhesion in those
The three isosurfaces correspond to regions at the top-back corner indicating the locus of normal adhesion. S22 CNV and purple corresponds to a sub-class of ET1 CNV. However, CNV did not progress in this mouse model [75].

While experimental and clinical results are incomplete, as we discussed above, they do suggest that patients with stable sub-RPE CNV do not suffer high rates of severe RPE detachment, indicating that their RBaM-BrM adhesion is not severely impaired. Among simulations with ET1 CNV, those in which RPE-BrM junctional adhesion is not too severely impaired (Table 7, $RBl = 1$ or $2$ and $3 \leq RBp + RBp \leq 4$), exhibit Stable Type 1 CNV, agreeing with our interpretation of this clinical observation.

Since we hypothesize that more severe impairment of RBaM-BrM adhesion facilitates CNV spreading and progression, we expect higher variability of outcomes in patients with moderately impaired adhesion than in patients with severely impaired adhesion. In older patients, CNV progression timing and the size and growth rate of the CNV-affected area vary significantly patient-to-patient [5, 76–78]. About one third of untreated sub-RPE CNV cases in older patients remain stable for extended periods of time (~30% remain stable 3 years after diagnosis). A slightly larger population of apparently similar patients progress to more damaging sub-retinal CNV in a short period of time (~40% develop sub-retinal CNV within the 12 months after diagnosis). According to a different study [76], sub-RPE CNV lesion size doubles in 12 months in ~30% of patients and quadruples in ~40% of patients and remained stable in the remainder. Such patient-to-patient variability corresponds closely to the variability we observe in stalk cell proliferation, CNV area growth and onset time in our corresponding simulations. As in clinical observations, simulations of adhesion scenarios with moderately impaired RPE-BrM junctional adhesion (Table 7, $RBl = 1$ or $2$ and $3 \leq RBp + RBp \leq 4$) have greater variability in stalk cell proliferation, CNV growth rate and CNV onset time than simulations with severely impaired RPE-BrM junctional adhesion (Table 6, $RBl = 3$). For example, in the 10 simulation replicas of an adhesion scenario with moderately impaired RPE-BrM junctional adhesion and all other adhesion normal (Table 7, $RBl = 3$, $RBp = 2$, $RBl = 1$, $RBl = 1$, $ROI = 3$), CNV initiates in only 30% of simulations, the initiation onset time is around 10 months (after formation of the initial hole in BrM). For less impaired adhesion, when the plastic component of RPE-BrM junctional adhesion is severely impaired but the labile component is normal (Table 6, $RBl = 3$, $RBp = 3$, $ROI = 3$), CNV initiates in only 30% of simulations, the initiation onset time is around 10 months (after formation of the initial hole in BrM) and the total number of stalk cells remains less than 6 in all replicas during the simulated year.

Overall, the stalk cell growth and proliferation rate among simulations that develop ET1 CNV is slowest in those simulations exhibiting S11 CNV and fastest in those simulations exhibiting P13 CNV. The stalk cell division rate (the frequency of stalk cell division within the tissue) in S11 CNV ranges from one to two cell divisions per year to one cell division every 48 hours. The stalk cell division rate in P13 CNV is less variable, about one cell division every ~12 hours during ET1 CNV (Figure 11A, the first month in most replicas). The long initiation delays and slow sub-RPE CNV development observed in some of our simulation replicas correspond to the slow sub-RPE CNV development and long-term CNV stability observed in a small population of patients (~30% as mentioned in [78]). Fast-progressing ET1 CNV in some of these simulations agrees with the clinically-observed rapid increase in sub-RPE CNV size in a different set of otherwise similar patients. Based on the observed variability in CNV onset time and growth rate in our simulations and its dependence on the degree of adhesion impairment, we believe that the observed clinical patient-to-patient variability may result from both intrinsic stochasticity in certain adhesion regimes and from small patient-to-patient differences in the degree and type of adhesion impairments in the patient’s BrM-RPE-POS complex.

In older patients, sub-RPE CNV may later also invade the sub-retinal space (sub-RPE CNV to CNV sub-retinal progression is a common CNV progression scenario). The factors involved in this transition are not well understood. Gradual degradation of the RPE due to sub-RPE hemorrhaging, formation of sub-RPE fibrosis and inflammation triggered by initial sub-RPE CNV are associated with this transition. The death of RPE cells during this degradation indicates that RPE-RPE adhesion is impaired. The rapid vision loss associated with the transition from sub-RPE CNV...
to sub-retinal CNV, indicates impaired RPE-POS adhesion, though, clinically, we do not know whether RPE-POS adhesion impairment is a cause or result of the transition. Thus these conditions imply impairment of RPE-RPE junctional adhesion and/or RPE-POS labile adhesion in addition to preexisting impairment of RPE-BrM junctional adhesion (due to lipid accumulation). Three classes of adhesion scenarios are relevant: 1) When both RPE-POS labile adhesion and RPE-BrM junctional adhesion are impaired, 2) when both RPE-RPE and RPE-BrM junctional adhesion are impaired, and 3) when RPE-RPE junctional adhesion, RPE-POS labile adhesion, RPE-BrM junctional adhesion are impaired. These three classes include all the sub-classes of adhesion scenarios leading to T12 CNV, P13 CNV, S22 CNV, S23 CNV and S33 CNV. These three classes never lead to S11 CNV (for definitions of the sub-classes of adhesion scenarios see Table 7), suggesting that impairment of RPE-RPE and/or RPE-POS adhesion in addition to preexisting impairment of RBaM-BrM in patients is the primary mechanism leading to the sub-RPE CNV to sub-retinal CNV transition.

Clinically, adhesion strengths may change as CNV progresses. However our simulations show that primary P13 CNV progression can occur in patients even for time-independent adhesion. We can thus use our simulations to develop a prognosis for patients with sub-RPE CNV and in whom RPE-RPE and/or RPE-POS adhesion are impaired in addition to preexisting impairment of RBaM-BrM. Simulations that exhibit ET1 CNV and later develop the sub-retinal space in P13 CNV (Table 7, $RRI = 1$, independent of $RRp$, $RBl = 1$, $RBrp = 1$ or 2, $ROI = 3$) correspond to the most common clinically observed progression of AMD-induced CNV, which begins as sub-RPE CNV and later progresses to involve the sub-retinal space. Simulations with T12 CNV (Table 7, $RRI = 3$, $RBl = 1$, $ROI = 1$, $RRp+RBp\geq4$ except $RRp = RBp = 2$) do not appear to correspond to any standard clinical CNV progression dynamics, perhaps, because the transient nature of the sub-RPE to sub-retinal translocation makes its clinical detection difficult; we hypothesize that T12 CNV may be occurring but is not being diagnosed. Clinically, depending on the time of observation, T12 CNV could be diagnosed as sub-RPE CNV, Type 3 or sub-retinal CNV. Only frequent prospective eye examinations and long-term follow up can determine whether our prediction of clinical sub-RPE to sub-retinal translocations is correct.

Clinically, ET2 and ET3 CNV are not common in drusen-induced CNV in patients. However, the adhesion scenarios that exhibit ET2 and ET3 in our simulations might correspond to secondary CNV which develops later to a primary site of CNV in patients with pre-existing ET1 CNV. To explore the relevance of these scenarios, we could conduct simulations beginning with pre-existing ET1 CNV instead of a single tip cell in the CC. For example, we would expect the pre-existing ET1 CNV to translocate to the sub-retinal space when simulated in adhesion scenarios that exhibit primary S22 CNV. Such CNV dynamics would look like T12 CNV (discussed above). Clinically, this result implies that if we were to increase RBaM-BrM adhesion therapeutically (e.g. by extraction of lipids from BrM or by removing/reducing fluids between RBaM and BrM) in the presence of pre-existing sub-RPE CNV, stalk cells could translocate to the sub-retinal space in a transition to sub-retinal CNV. Since sub-retinal CNV is much more damaging to vision than sub-RPE CNV, this translocation would be a serious iatrogenic side effect. We can make similar analogies for the significance of simulated S23 CNV and S33 CNV.

Vascular RPE detachment caused by growth of CNV under the RPE is a common complication of sub-RPE CNV in AMD. We...
observe a corresponding pathology when RPE-BrM junctional adhesion is severely impaired (Table 6, Early Type 1 sub-class 1, \( RBl = RBp = 1 \)). In this case, Early Type 1 CNV results in later RPE detachment, leading to either T12 CNV translocation or P13 CNV progression in conjunction with the formation of holes in the RPE (we will discuss late-stage CNV complications in detail in future papers).

Inflammation-induced CNV. Sub-retinal CNV without prior diagnosed sub-RPE CNV occurs, but is not as common in older patients as drusen-induced CNV [79]. It also occurs in young patients in conjunction with acute inflammatory conditions, particularly in cases of serpiginous choroidopathy, multifocal choroiditis and panuveitis [80]. The mechanisms leading to this type of CNV are not well understood and we lack clinical insight.

Figure 15. Snapshots of a simulation replica showing stable Type CNV (S22 CNV). 3D visualization of a simulation replica showing S22 CNV in one simulated year \( (RRl = 1, RBp = 1, RBl = 3, RBp = 3, ROl = 3) \) (adhesion scenario ID: 16, simulation ID: 556). Snapshots of the simulation at months 1 (A), 2 (B), 6 (C) and 12 (D). (A) Stalk cells (solid black arrow) invade the sub-retinal space through a hole in BrM (black outline arrow) and form a partially developed capillary network (B). CNV finishes sub-retinal invasion around month 5 and remains in the sub-retinal space throughout LT2 CNV (C–D). A few vascular cells (A, black outline arrow) fill the hole in BrM to connect the CNV capillaries to the CC (red outline arrow). Brown outline arrow shows an RPE cell. Cell type colors: 1) POS and PIS: light purple, 2) RPE: brown (stalk cells in the sub-retinal space have lighter shading), 3) Stalk cells: green, 4) Vascular cells (CC): red, 5) BrM: light blue. Scale bar \( \sim 50 \mu m \). We have rendered the boundaries of individual cells as semi-transparent membranes. POS, PIS and RPE cells are more transparent to show the underlying structures. See also Video S4. doi:10.1371/journal.pcbi.1002440.g015
Figure 16. Dynamics of sub-retinal CNV to sub-RPE CNV progression (P23 CNV Progression). A) Total number of stalk cells vs. time. B) Total number of stalk cells in the sub-RPE space vs. time. C) Total number of stalk cells in contact with the POS (stalk cells in the sub-retinal space) vs. time. D) Total number of RPE cells vs. time. E) Total contact area between RPE cells and BrM vs. time. F) Total contact area between POS cells and BrM vs. time. The different colors represent the results of 10 simulation replicas of the adhesion scenario (ROI = 1, RBP = 1, RBl = 1, RBp = 1, ROI = 1) (Table 59, adhesion scenario ID: 108). CNV initiates in all replicas and all develop ET2 CNV. A few stalk cells in most replicas die due to lack of RPE-derived VEGF-A. (B) Stalk cells cross the RPE and invade the sub-RPE space once the number of stalk cells in the sub-RPE space reaches ~50 cells, which usually occurs during the first month after initiation. Stalk cells gradually invade the sub-RPE space during one simulated year. (D) Up to 30 RPE cells (30% of the total) die. The number of RPE cell deaths increases with the number of sub-RPE stalk cells. (E) The contact area between the RPE and BrM decreases as P23 CNV develops. (F) In all replicas the POS contacts BrM persistently and extensively, as the RPE develops substantial holes (see Figure 17).

doI:10.1371/journal.pcbi.1002440.g016

into how specific risk factors affect the probability of CNV initiation. Younger patients usually lack both drusen [80] and significant dispersed build up of lipids in BrM. As we discussed earlier active inflammation reduces RPE-RPE epithelial adhesion. Alteration of RPE-RPE epithelial adhesion combined with edema due to active inflammation may also reduce RPE-POS adhesion. We do not know whether inflammation always impairs RPE-RPE and RPE-POS adhesion at the same time, or to the same degree. Since CNV initiation occurs promptly during acute inflammation, we do not expect acute inflammation to decrease RBaM-BrM adhesion significantly. Thus, in young patients inflammation both RPE-RPE epithelial adhesion and RPE-POS adhesion are impaired, but not RBaM-BrM adhesion. This adhesion impairment corresponds to impairments of RPE-RPE junctional adhesion and RPE-POS labile adhesion, while RPE-BrM junctional adhesion is near-normal (Table 6, Early Type 2 CNV sub-class 1 and 2). Simulations of these adhesion scenarios consistently exhibit Early Type 2 CNV (ET2 CNV), in which stalk cells initially invade the sub-retinal space without prior invasion of the sub-RPE space, in agreement with clinically-observed sub-retinal CNV in these patients.

In our simulations, when RPE-RPE labile adhesion is severely impaired and all other adhesions are normal (ROI = 1, RBP = RBl = RBp = 1) (Table 58, adhesion parameter set ID: 11) CNV always initiates, followed by Early Type 2 CNV (ET2 CNV). When RPE-POS labile adhesion is severely impaired and all other adhesions are normal (ROI = 1, RBP = RBl = RBp = 3, ROI = 1) (Table 58, adhesion parameter set ID: 19), the CNV initiation probability is 50% and initiation always leads to ET2 CNV. When both RPE-RPE junctional adhesion and RPE-POS labile adhesion are impaired CNV, always initiates and leads to ET2 CNV (see also Figure 5, for more information on how sub-retinal CNV depends on adhesion). Our results make two predictions that can be tested against clinical and experimental observations: 1) Disruption of RPE-RPE epithelial junctions, due to inflammation, by itself should lead to sub-retinal CNV in patients with a relatively intact retina, independent of any defects in RPE-POS adhesion 2) Disruption of RPE-POS contact, by itself, should increase the probability of developing sub-retinal CNV.

Our understanding of CNV dynamics in young patients is incomplete. Neither sub-retinal CNV to sub-RPE CNV progression (P23 CNV) nor sub-retinal CNV to sub-RPE CNV translocation (T21 CNV) has been observed clinically or histologically (since CNV is not fatal, histological data for young patients with CNV is rare). We currently do not know whether this absence of observation is due to major retinal damage due to sub-retinal CNV, which precludes the later transition (and is not included in our model), or whether Late Type 1 CNV is simply overlooked clinically because sub-retinal CNV causes much more severe vision loss. Our simulations make three predictions relevant to inflammation-induced CNV in young patients: 1) If RPE-BrM adhesion junctional is near normal (ROI = 2 or 3, independent of RBp) any combination of severely impaired RPE-RPE junctional adhesion (ROI = 1, independent of RBp) and RPE-POS labile adhesion (ROI = 1) will exhibit Stable Type 2 CNV. 2) When all adhesions are severely impaired (ROI = 1, RBP = RBl = RBp = 1) (Table S8, adhesion parameter set ID: 20) sub-RPE CNV to sub-RPE CNV progression (P23 CNV) will occur. 3) Sub-retinal CNV to sub-RPE CNV translocation (T21 CNV) is
unlikely. These predictions mean that once CNV invades the sub-retinal space, it will not leave this space, so CNV lesions will expand primarily in the sub-retinal space as long as RBaM-BrM is not severely impaired.

Chemotoxicity (10% solution of naphthalene force-fed by gavage for 5 weeks) in a rabbit model [81] causes degradation of photoreceptors and proliferation of RPE leading to sub-retinal CNV. In this rabbit model, the RPE cells proliferating phagocyt...
Iatrogenic CNV. Iatrogenic sub-retinal CNV may develop after laser photocoagulation treatment of diabetic macular edema, central serous retinopathy, proliferative diabetic retinopathy, choroidal vascular and neoplastic lesions, vascular occlusive disease and degenerative retinal-pigment-epithelium disorders (reviewed in [82]). Some believed that the primary mechanism for such iatrogenic induction of sub-retinal CNV to be the creation of breaks in Bruch’s membrane, with inflammatory cells, angiogenic factors and choroidal ischemia contributing to the development of CNV in some cases [82]. However, we believe that RPE phototoxicity due to excess (local) laser exposure is more likely primary cause. Phototoxicity stresses RPE cells which can decrease RPE-RPE epithelial adhesion and RPE-POS adhesion and also promote excess expression of VEGF-A by RPE cells. This condition of pathologies is similar to those caused by inflammation, so we would expect these classes of iatrogenic CNV to resemble inflammation-induced CNV, as is indeed observed.

CNV due to sub-retinal injections in animal models. Subretinal injections in most animal models (Table 1) lead to sub-retinal CNV adjacent to the site of injection within weeks, but not to sub-RPE CNV [83–85]. CNV initiation probability depends on the type and amount of material injected (Matrigel, polystyrene beads suspended in liquid, vitreous humor, …) (see [46], for a detailed review). Secondary sub-RPE CNV may follow sub-retinal CNV in some animal models, eg, in a rabbit model [86], a sub-retinal injection of a cocktail containing endotoxins and growth-factors, incorporated in heparin-sepharose leads rapidly to primary sub-retinal CNV and secondary sub-RPE CNV forms farther from site of injection between 2 weeks and 8 months later [86]. The mechanisms leading to this secondary sub-RPE CNV are not well understood. Ni et al. [86] believed that the major factors were changes in RPE cell function due to diffusion of soluble mediators originating from the area of primary CNV, eg, from atrophied primary RPE cells or newly-formed activated secondary RPE cells or changes in function of endothelial cells, treated damaged photoreceptors during the first three weeks. Subretinal CNV follows about 3 months after the beginning of treatment. Because RPE cells with normal epithelial adhesion do not proliferate, we hypothesize that chemotoxicity in this animal model reduces RPE-RPE epithelial adhesion, allowing RPE cells to proliferate. Since the photoreceptors are damaged, we infer that RPE-POS adhesion is severely disrupted. BrM remains intact for the first 3 weeks of treatment. Initial signs of BrM invasion by RPE cells and ECs appear about 3 months after the beginning of treatment. Due to newly synthesized extracellular matrix at the location of the CNV, the RPE basement membrane and BrM become irregular after 6 months, with bundles of extracellular microfilaments connecting RPE basement membrane to BrM. These modifications of the RPE basement membrane and BrM, suggest that RBaM-BrM adhesion is initially normal and gradually decreases to moderately impaired over a period of six months. These adhesion impairments resemble adhesion impairments in younger patients with inflammation. For these adhesion scenarios (Table 6, Early Type 2 CNV sub-class 1 and 2) our simulations always predict early sub-retinal CNV, in agreement with experiments.

Figure 18. Dynamics of stable Type 3 CNV (S33 CNV). A) Total number of stalk cells vs. time. B) Total number of stalk cells confined in the sub-RPE space vs. time. C) Total number of stalk cells in contact with the POS (stalk cells in the sub-retinal space) vs. time. D) Total number of RPE cells vs. time. E) Total contact area between RPE cells and BrM vs. time. F) Total contact area between POS cells and BrM vs. time. The different colors represent the results of 10 simulation replicas of the adhesion scenario (RRl = 1, RBp = 1, RBl = 2, RBp = 2, ROI = 3) (Table 5, adhesion scenario ID: S33). (A, B, C) CNV initiates in all replicas and all replicas develop ET3 CNV. During the first month after initiation, stalk cells gradually invade both the sub-RPE space and the sub-retinal space, with more invading the sub-RPE space. Between months 1 and 2 about 30% of the sub-RPE stalk cells transmigrate into the sub-retinal space. After month 3, the number of sub-RPE stalk cells increases slowly, while the number of sub-retinal stalk cells remains constant. (E) During the first month of the simulation, the contact area between the RPE and BrM rapidly decreases as stalk cells invade the sub-RPE space. Between months 1 and 2, the contact area between the RPE and BrM rapidly increases as sub-RPE stalk cells transmigrate into the sub-retinal space. The contact area between the RPE and BrM slowly decreases after month 3 throughout the simulated year. (D) A few RPE cells die in most replicas. (F) In a few replicas the POS persistently contacts BrM, as the RPE develops small holes.
photoreceptors and Müller cells in regions peripheral to the primary CNV, e.g., increased expression of FGF-1, FGF-2, TGF-alpha and VEGF.

Subretinal injections can cause acute physical retinal detachment, instantaneously destroying RPE-POS contact at the site of injection. However, RPE active pumping and passive flow gradually remove the excess sub-retinal fluid, allowing the retina to reattach within a few days. Sub-retinal injection also almost always induces significant inflammation, which gradually reduces RPE-RPE epithelial adhesion over a period lasting a few days to a week.

Figure 19. Snapshots of a simulation replica exhibiting stable Type 3 CNV (S33 CNV). 3D and 2D visualization of a simulation replica developing S33 CNV in one simulated year (RRl = 1, RRp = 1, RRl = 2, RRp = 2, R0l = 3) (adhesion scenario ID: 53, simulation ID: 917). Snapshots of the simulation at months 1 (A), 2 (B), 6 (C) and 12 (D). (A2-D2) Cross-sections of (A1-D1). All cross-section planes in (A1-D1) panels defined by the two thick black lines in A1. (A) Stalk cells invade the sub-RPE space through a hole in BrM (A1-2, black outline arrows) that the tip cell form during the first 24 hours of the simulation. These stalk cells then form a fully developed sub-RPE capillary network. (A2) Only a few stalk cells (black arrow, A1-2) reach the sub-retinal space during the first month. (B1, C1) The sub-retinal and sub-RPE capillary networks do not grow significantly. (C2) A capillary (black arrows), enveloped by a bilayer of RPE cells, connects the sub-retinal space to the CC via the hole in BrM (D) Stalk cells disrupt the RPE, forming small holes in the RPE (D2, black arrow). The stalk cells at the location of the hole in the RPE (D2, black arrow) contact both the POSs and BrM. The black outline arrow shows sub-retinal stalk cells. Cell type colors: 1) POS and PIS: light purple, 2) RPE: brown, 3) Stalk cells: green, 4) Vascular cells (CC): red, 5) BrM: light blue. Scale bar ~50 μm. We have rendered the boundaries of individual cells in A1-D1 as semi-transparent membranes. POS, PIS and RPE cells are rendered more transparent to show the underlying structures. See also Video S6.

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few weeks. Such condition of transient detachment and followed by long-lasting inflammation induces RPE migration [93] and proliferation [86] (RPE activation). Because RPE cells neither proliferate nor migrate when they are in epithelium and RPE-RPE epithelial adhesion remain normal, the combination of RPE migration and proliferation suggest that RPE-RPE epithelial adhesion may decrease significantly over a period of a few days to a few weeks. Inflammation can also cause the death of photoreceptors [81], suggesting prolonged disruption of RPE-POS contact. Sub-retinal injections can also directly cause focal rupture in all layers of BrM, suggesting that RBaM-BrM adhesion is initially (near) normal at least far from the location of the rupture.

Based on these experimental observations, sub-retinal injections appear mainly to impair RPE-RPE and RPE-POS adhesion comparable to the adhesion scenarios prone to Early Type 2

### Table 8. Geometrical and transport parameters.

| Name          | Description                                           | Values                  |
|---------------|-------------------------------------------------------|-------------------------|
| \( \ell_{\text{POS}} \) | POS layer thickness                                     | \( \sim 30 \mu m \) (compare to [97]) |
| \( \ell_{\text{PIS}} \) | PIS layer thickness                                     | \( \sim 24 \mu m \) (compare to [97]) |
| \( \ell_{\text{OLM}} \) | Location of OLM measured from RPE side of BrM         | \( \sim 67 \mu m \) (compare to [97]) |
| \( \ell_{\text{BrM}} \) | BrM thickness                                          | 6 \( \mu m \) (compare to [24]) |
| \( \ell_{\text{RPE}} \) | RPE thickness                                          | 12 \( \mu m \) (compare to [24]) |

### Oxygen Transport Parameters

| Name          | Description                                           | Values                  |
|---------------|-------------------------------------------------------|-------------------------|
| \( D_{\text{O}_2} \) | Diffusion coefficient of oxygen in retinal tissue      | \( 2 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} \) [98] |
| \( QO_{\text{POS}} \) | Light-adapted (dark-adapted) Oxygen consumption per 100 g of PIS tissue | 13 (26) \( \text{ml O}_2 \text{ min}^{-1} \) [47,48,99] |
| \( P_{\text{O}_2}^{\text{CC}} \) | Oxygen partial pressure in choriocapillaries under normoxia | 80 mmHg [48,99] |
| \( P_{\text{O}_2}^{\text{CC}} \) | Oxygen partial pressure in choriocapillaries under systemic hypoxia | 60 mmHg (extrapolated from rat [100]) |
| \( P_{\text{O}_2}^{\text{OILM}} \) | Oxygen partial pressure at OLM under normoxia and hypoxia | 18 mmHg [47] |
| \( P_{\text{O}_2}^{\text{RPE}} \) | Average oxygen partial pressure in the RPE under normoxia | 65 mmHg during light-adaptation, 61 mmHg during dark-adaptation (see Oxygen Transport Parameters in Text S3) |
| \( P_{\text{O}_2}^{\text{RPE}} \) | Average oxygen partial pressure in the RPE under systemic hypoxia | 49 mmHg during light-adaptation, 45 mmHg during dark-adaptation (see Oxygen Transport Parameters in Text S3) |
| \( S_{\text{O}_2}^{\text{in}} \) | Oxygen flux from 100 g of choriocapillaris tissue to the retina under normoxic condition | 3.42 (CC3D) or 102 ml \( \text{O}_2 \text{ (100 g tissue min)}^{-1} \) during dark-adaptation 2.67 (CC3D) or 80 ml \( \text{O}_2 \text{ (100 g tissue min)}^{-1} \) during light-adaptation |
| \( S_{\text{O}_2}^{\text{in}} \) | Oxygen flux from 100 g of choriocapillaris tissue to the retina under systemic hypoxia | 2.81 (CC3D) or 84 ml \( \text{O}_2 \text{ (100 g tissue min)}^{-1} \) during dark-adaptation 2.05 (CC3D) or 61 ml \( \text{O}_2 \text{ (100 g tissue min)}^{-1} \) during light-adaptation |

### VEGF Transport Parameters

| Name          | Description                                           | Values                  |
|---------------|-------------------------------------------------------|-------------------------|
| \( \lambda_{\text{VEGF}} \) | Decay rate of RPE-derived VEGF-A and short-diffusing VEGF-A | 1 h\(^{-1}\) [101,102] |
| \( \lambda_{\text{VEGF}}^{\text{EC}} \) | Diffusion length of RPE-derived VEGF-A secreted by RPE cells | 13.4 \( \mu m \) \( \sqrt{ \frac{D_{\text{VEGF}}}{\lambda_{\text{VEGF}}} } \) |
| \( D_{\text{VEGF}}^{\text{EC}} \) | Diffusion coefficient of short-diffusing VEGF-A secreted by ECs | \( 0.25 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1} \) |
| \( S_{\text{EGF}}^{\text{EC}} \) | Diffusion length of short-diffusing VEGF-A secreted by ECs | \( 3 \mu m \) \( \sqrt{ \frac{D_{\text{EC}}}{\lambda_{\text{VEGF}}} } \) |
| \( S_{\text{VEGF}}^{\text{EC, tip}} \) | Secretion rate of VEGF by HRPE cells                  | \( \sim 50 \text{ pg (cell h)}^{-1} \) (compare to [103]) (0.1 molecules (voxel MCS\(^{-1}\)) ) |
| \( S_{\text{VEGF}}^{\text{EC, basal}} \) | Basal secretion rate of VEGF by RPE cells              | \( \sim 25 \text{ pg (cell h)}^{-1} \) (compare to [103]) (0.05 molecules (voxel MCS\(^{-1}\)) ) |
| \( S_{\text{VEGF}}^{\text{EC}} \) | Basal secretion rate of short-diffusing VEGF by ECs     | \( \sim 25 \text{ pg (cell h)}^{-1} \) (0.2 (voxel MCS\(^{-1}\)) ) |
| \( Q_{\text{EC}}^{\text{tip}} \) | Basal uptake rate of RPE-derived VEGF-A by vascular cells | \( \sim 300 \text{ ligated molecules per EC} \times 2.8 \times 10^{-4} \) (internalization rate) [104] \( \sim 0.084 \text{ molecule (cell sec}^{-1} \) \( \times 0.28 \text{ molecule (voxel MCS)}^{-1} \) |

### MMP Transport and BrM Degradation Parameters

| Name          | Description                                           | Values                  |
|---------------|-------------------------------------------------------|-------------------------|
| \( S_{\text{MMP}}^{\text{tip}} \) | Secretion rate of MMP by the tip cell                 | 0.148 molecule (cell sec\(^{-1}\)) (1 molecule (voxel MCS\(^{-1}\)) ) |
| \( L_{\text{MMP}} \) | Diffusion length of MMP secreted by the tip cell       | 0.2 \( \mu m \) \( \sqrt{ \frac{D_{\text{MMP}}}{\lambda_{\text{MMP}}} } \) |
| \( G_{\text{BrM}} \) | BrM degradation rate                                   | 0.0094 \( \mu m^3 \text{ (sec molecule)}^{-1} \) (0.075 voxel (MCS molecule\(^{-1}\)) ) |

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CNV (Table 6, Early Type 2 CNV sub-class 1 and 2) and in inflammation-induced CNV in younger patients. Thus our simulations both agree with the cause, initiation and progression of sub-retinal CNV in these animal models.

Our simulations predict that for secondary sub-RPE CNV to develop near a pre-existing sub-retinal CNV RPE-BrM (RBaM-BrM) adhesion must be severely impaired, to develop, independent of the degree of impairment of other types of adhesion in the BrM-RPE-POS complex. To validate our prediction, experiments would need to examine in detail the interface between BrM and the RPE basement membrane in retinal regions far from site of injection and before any initiation of sub-RPE CNV.

In these animal models, sub-retinal injection often causes rapid (less than a month) CNV initiation. We can infer that injection impairs RPE-RPE adhesion because of observed inflammation and RPE proliferation and RPE-POS adhesion because of photoreceptor degradation [81,83,86]. In agreement with these experiments, our simulations show that severe impairment of RPE-RPE labile adhesion, when all other adhesions are normal (RRl=1, RRp=RRp=ROl=3) (Table S6, adhesion parameter set ID: 10), increases the probability of CNV initiation within a month to 100%. In our simulations prolonged impairment of RPE-POS labile adhesion when all other adhesions are normal (RRl=RRp=RRp=ROl=3, ROl=1) (Table S6, adhesion parameter set ID: 19) increases initiation probability to 50%, higher than observed for experimental RPE-POS detachment. This discrepancy may result from the long (up to six month) onset time for CNV in this adhesion scenario. Six months is much longer than the CNV initiation time due RPE-RPE adhesion impairment and the typical time for photoreceptor reattachment and is too long to see in most experiments. Experiments exploring the effects of RPE-POS adhesion impairment on CNV would need to disrupt RPE-POS adhesion for several months without inducing severe inflammation, e.g. by sub-retinal injection of neutral polystyrene microbeads (coated with anti-inflammation compounds).

The role of overexpression of VEGF. We now consider how local cytokines and growth factors that can increase the chemotactic activity of endothelial cells could affect CNV initiation and progression. Our simulations did not explore the role of these factors on the chemotactic activity of ECs directly. However, our simulations using different VEGF-A levels suggest that increased chemotactic activity of stalk cells should increase the CNV initiation probability, as in our simulations in which RPE expresses RPE-derived VEGF-A at twice the normal level. We would also expect that if these factors were short-diffusing and thus formed local gradients, they should promote sub-RPE CNV if their source were localized in the sub-RPE space, or promote sub-retinal CNV if their source were localized in the sub-retinal space. If these factors were long-diffusing, we would expect that the resulting global increase in chemotactic activity of stalk cells would not affect either early or late CNV loci, only the probability of initiation and the rate of progression.

In transgenic mice with inducible overexpression of VEGF in their RPE cells, induction of excess VEGF only induces CNV if combined with sub-retinal injections which disrupt the RPE [13]. In our simulations, when RPE overexpresses RPE-derived VEGF-A at twice the normal level, the probability of CNV initiation increases (data not shown), but the Early CNV types and CNV dynamics do not change. Thus, our simulations show that VEGF overexpression can increase the CNV initiation probability, but does not determine either the early or late CNV loci.

In experiments, ocular hypoxia caused by systemic hypoxia usually promotes retinal angiogenesis, but has no observed effect on the RPE and does not induce choroidal angiogenesis (reviewed in [40]). We do not know what levels of PO2 can trigger hypoxic signaling by RPE cells. Based on experimentally-measured parameters, our simulations show that the PO2 at the RPE decrease from ~63 mmHg to ~49 mmHg when the PO2 at the BrM decreases from 80 mmHg to 60 mmHg during systemic hypoxia in the anatomically normal retina (under light-adapted condition). If we assume that biological RPE cells are hypoxic during systemic hypoxia, then the PO2 below which RPE cells become hypoxic is 49 mmHg. However, PO2~50 mmHg is significantly higher than both PO2~20 mmHg, the typical PO2 in the inner retina and PO2~1 mmHg, the PO2 at which mitochondria work at their maximum metabolic rate. In our simulations, RPE cells become hypoxic (PO2<49 mmHg) only after RPE detachment, a CNV-associated complication which we will discuss in a future paper. Thus, neither the threshold for RPE hypoxia nor RPE hypoxic signaling affects the results we present in this paper.

The nature of the BrM-RPE-POS complex barrier to CNV (activated ECs). As we discussed in the Angiogenic and Antiangiogenic factors section in supplementary Text S1, activated endothelial cells are present in the normal choriocapillaris, so the frequency of ECs crossing BrM is significant even in the normal eye. Clinically, the probability of CNV initiation before age 50 in a normal retina is negligible. Our adhesion-based hypotheses for CNV initiation and progression may resolve this discrepancy. In simulations when all adhesions are normal (referenced to a normal human eyes aged less than 50 years old) activated ECs and small holes in BrM never initiate CNV, suggesting that strong adhesion among BrM-RPE-POS components is the crucial mechanism preventing activated ECs from invading the sub-RPE and sub-retinal space once they have crossed BrM.

Future Directions and Suggestions

Our current model does not include several mechanisms which may also be important to CNV. In future refinements, we will include multiple types of basal deposits and fibrosis (synthesis of new ECM) explicitly to clarify their role in the initiation and progression of CNV. We particularly are interested in how differences in the size and structure of soft and hard drusen affect the initiation and progression of CNV and the frequency of occurrence of RPE detachment and RPE tear formation after therapeutic intervention to treat CNV.

Many hypothesized mechanisms for CNV initiation and progression involve irregularities in transport. We plan more realistic models including capillary maturation, blood flow and in

| Name | Description | Units |
|------|-------------|-------|
| PO(\(x\)) | Oxygen partial pressure at \(x\) | mmHg |
| \(V_a(\(x\))\) | RPE-derived VEGF-A at \(x\) | molecule/voxel |
| \(V_s(\(x\))\) | Short-diffusing VEGF-A secreted by ECs at \(x\) | molecule/voxel |
| \(M(\(x\))\) | MMP secreted by tip cells at \(x\) | molecule/voxel |

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retinal-CC fluid flow to study how oxygen, nutrient and waste transport promote or inhibit CNV.

Since cell adhesion is essential to multicellularity and is important in embryonic development, homeostatic maintenance of adult tissues and diseases like cancer, its importance in CNV is, perhaps, not surprising, given CNV’s many parallels with tumor-induced angiogenesis. However, the role of adhesion in CNV has not been widely appreciated, so neither the relationship between known CNV risk factors and specific adhesion failures, nor the actual adhesivities in the retina and RPE have been quantified experimentally. Quantitative measurements of these adhesion properties and their regulation in the normal and pathological retina would allow more clinically relevant models. Such experiments would greatly reduce uncertainty in our model definition, improve our understanding of CNV initiation and progression. Since measuring adhesivities directly may be difficult, especially in humans, our model also allows us to quantify adhesion failures by looking at how they affect CNV initiation and progression and matching those computational outcomes with experimental observations, then correlating the simulated adhesion changes with experimental risk factors.

Beyond retinal CNV, our results on CNV initiation and epithelial breakdown apply to any tissue in which a basement membrane separates a capillary network from a nearby epithelium, e.g. lung and gut. We expect that the relationships between specific classes of adhesion failures and the loci and dynamics of CNV which we observe in our simulations should carry over to the neovascularization-dependent pathologies of those tissues.

Ultimately, a database of verified simulations for different adhesion scenarios might allow systematic CNV prediction based on clinically-measurable properties of the eye, especially if the adhesion properties can be inferred noninvasively, e.g. by measuring optically, changes in CC or RPE morphology or autofluorescence due to lipid accumulation. In the absence of direct measurements of adhesivity, our simulations allow us to infer adhesion defects from pathologies. For example, if a patient with a functional retina exhibits micro-detachments of the RPE (due to lipid accumulation or small soft drusen), the relationship between the number and degree of detachments and the underlying classes of adhesion failures predicts the probability of CNV initiation and the distribution of CNV onset times, making clinically-useful suggestions for frequency of follow-up examinations and possible prophylactic interventions. To aid diagnosis and treatment we can also develop statistical analyses of the most significant scenarios capable of producing the observed pattern of disruption in each patient (including the probability of initiation, the time of onset of progression and progression speed, ...). Follow-up observations could then narrow the range of admitted hypothetical scenarios to improve the accuracy of the prognosis. More accurate diagnoses may improve both administration of drugs and disease management.

One crucial aspect of a model-based approach to CNV diagnosis, prognosis and treatment is that both the simulation database and the statistical predictors could be continuously refined using feedback from both clinical and histopathological sources, so they would improve with use, providing a platform to integrate clinical and histopathological data for even more accurate diagnosis and prognosis.

### Methods

Our simulations use the Glazier-Graner-Hogeweg model (GGH, also known as the Cellular Potts Model, CPM), a multi-cell, lattice-based, stochastic methodology which describes biological cells and their interactions in terms of Effective Energies and constraints [87]. GGH applications include models of vascular tumors [63], avascular tumor growth [88], biofilms [89], chick limb growth [90], somitogenesis [91], blood flow and thrombus development [92] and angiogenesis [66,71,93,94]. See supplementary Text S3 for details of implementation of objects and processes listed in Table 2. Tables 8–12 summarize the key variables and parameters in our simulations (see also Text S6 for simulation files used to run replicas of adhesion scenario ID: 38).

### Implementation

All our simulations use the open-source CompuCell3D simulation environment [http://www.compcell3d.org/][95]. We ran our simulations on a computer cluster (Quarry, Indiana University) using CompuCell3D v3.4.2. A typical simulation replica takes about 30 hours on a single core of a 2.0 GHz quad-core Intel

| Table 10. Labile adhesion parameters (contact energies). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Cell Types** | **Stalk** | **BrM** | **RPE** | **POS** | **PIS** |
| **Stalk** | −20 | −10 | −10 | −10 | −10 | 3 |
| **BrM** | −12 | −38/−28/−18 | 0 | 0 | −1 |
| **RPE** | −40/−18 | −16/−1 | −16/−1 | 3 |
| **POS** | −16 | −16 | 3 |
| **PIS** | −16 | 3 |

Negative contact energies represent adhesive interactions; positive contact energies represent repulsive interactions. More negative contact energies indicate stronger adhesive interactions. () separates the reference, moderately impaired and severely impaired levels of labile adhesion.

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| Table 11. Labile adhesion strengths (contact energies). |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Cell-Type Pairs** | **Labile Adhesion Strength** | **Name** | **Normal: 3** | **Moderately Impaired: 2** | **Severely Impaired: 1** |
| **RPE-RPE** | RHI | −40 | - | -18 |
| **RPE-BrM** | RHI | −38 | −28 | -18 |
| **RPE-POS** | ROI | −16 | - | -1 |
| **POS-POS** | - | −16 | - | - |
| **PIS-PIS** | - | −16 | - | - |

More negative contact energies indicate stronger adhesive interactions. () denotes labile adhesion strengths not used in our simulations.

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We stored the cell and field-lattice configurations every 6 simulated hours and rendered each snapshot using the integrated post-processing rendering provided by CompuCell3D.

Supporting Information

Table S1 Adhesion Scenarios with Infrequent or No CNV Initiation.

| Cell-Type Pairs | Plastic Coupling Strength |
|-----------------|--------------------------|
| RPE-RPE         | RRp                      |
| RPE-BrM         | RRp                      |
| POS-POS         | -                        |
| PIS-PIS         | -                        |
| PIS-POS         | -                        |
| Vascular-Vascular | -                      |
| Stalk-Vascular  | -                        |
| Tip-Vascular    | -                        |
| Stalk-Stalk     | -                        |
| Stalk-Tip       | -                        |
| Vascular-BrM    | -                        |
| Stalk-BrM       | -                        |
| Tip-BrM         | -                        |

Larger plastic coupling strengths represent stiffer linear springs. (-) denotes values of $\lambda_{plastic}(\tau(\sigma),\tau(\rho))$ not used in our simulations. doi:10.1371/journal.pcbi.1002440.t012

Table S10 Adhesion Scenarios Prone to Stable Type 3 CNV (S33 CNV) (S33 Probability>0.9).

Table S11 Adhesion Scenarios Prone to Stable Type 1 CNV (S11 CNV Probability>0.9).

Video S1 Time-Series of a Simulation Replica with Stable Type 1 CNV. 3D visualization of a simulation replica exhibiting Stable Type 1 CNV over one simulated year (adhesion scenario ID: 38, simulation ID: 902) ($RRl = 3$, $RRp = 3$, $RBi = 2$, $RBP = 2$, $ROl = 3$). Stalk cells invade the sub-RPE space through a hole in BrM that the tip cell opens during the first 24 hours. Stalk cells proliferate until they fill the sub-RPE space in month 9, after which proliferation slows down. The 45 stalk cells form a connected capillary network in the sub-RPE space. Cell type colors: 1) POS and PIS: light purple, 2) RPE: brown, 3) Stalk cells: green, 4) Vascular cells (CC): red, 5) BrM: light blue. We have rendered the boundaries of individual cells as semi-transparent membranes. POS, PIS and RPE cells are more transparent to show the underlying structures. (AVI)

Video S2 Time-Series of a Simulation Replica Showing Sub-RPE to Sub-Retinal Translocation (T12 Translocation). 3D visualization of a simulation replica exhibiting T12 CNV translocation during one simulated year ($RRl = 3$, $RRp = 3$, $RBi = 2$, $RBP = 2$, $ROl = 3$). Stalk cells form a connected capillary network in the sub-RPE space. Cell type colors: 1) POS and PIS: light purple, 2) RPE: brown, 3) Stalk cells: green, 4) Vascular cells (CC): red, 5) BrM: light blue. We have rendered the boundaries of individual cells as semi-transparent membranes. POS, PIS and RPE cells are more transparent to show the underlying structures. (AVI)
$RBl = 1, RBp = 1, ROl = 1$) (adhesion scenario ID: 93, simulation ID: 849). Stalk cells invade the sub-RPE space through a hole in BrM and form a capillary network. All stalk cells remain in the sub-RPE space during the first 3 months. A few vascular cells fill the hole in BrM to connect CNV capillaries to the CC. Half of the stalk cells have crossed the RPE and transmigrated into the sub-retinal space by month 5, forming a new capillary network in the sub-retinal space. Most stalk cells have transmigrated into the sub-retinal space and the RPE has completely reattached to BrM by month 9. A few vascular cells of the CC have transmigrated into the sub-retinal space. The sub-retinal capillary network has fewer stalk cells than the capillary in month 9 since stalk cells that migrate into the retina far from the RPE die. Cell type colors: 1) POS and PIS: light purple, 2) RPE: brown, 3) Stalk cells: green (stalk cells in the sub-retinal space have lighter shading), 4) Vascular cells (CC): red, 5) BrM: light blue. We have rendered the boundaries of individual cells as semi-transparent membranes. POS, PIS and RPE cells are more transparent to show the underlying structures. (AVI)

**Video S3** Time-Series of a Simulation Replica Showing Sub-RPE CNV to Sub-Retinal CNV Progression (P13 Progression). 3D visualizations of a simulation replica exhibiting P13 CNV progression during one simulated year ($RBp = 1, RBp = 3, RBl = 1, RBp = 2, ROl = 3$) (adhesion scenario ID: 93, simulation ID: 513). Stalk cells invade the sub-RPE space through a hole in BrM and form a capillary network. The vascular cells of the CC occupy the hole that the tip cell forms during the first 24 hours of the simulation, connecting the CNV capillaries to the CC. All stalk cells remain in the sub-RPE space during the first month of the simulation. Month 2: A few stalk cells cross the RPE into the sub-retinal space. Month 6: Additional stalk cells migrate into the sub-retinal space and form vascular cords. The stalk cells form a sub-RPE capillary network connected to a sub-retinal capillary network. Cell type colors: 1) POS and PIS: light purple, 2) RPE: brown, 3) Stalk cells: green (stalk cells in the sub-retinal space have lighter shading), 4) Vascular cells (CC): red, 5) BrM: light blue. We have rendered the boundaries of individual cells as semi-transparent membranes. POS, PIS and RPE cells are more transparent to show the underlying structures. (AVI)

**Video S4** Time-Series of a Simulation Replica Showing Stable Type CNV (S22 CNV). 3D visualization of a simulation replica showing S22 CNV in one simulated year ($RBp = 1, RBp = 1, RBp = 3, RBp = 2, ROl = 3$) (adhesion scenario ID: 16, simulation ID: 556). Stalk cells invade the sub-retinal space through a hole in BrM and form a partially developed capillary network by month 2. CNV finishes sub-retinal invasion around month 5 and remains in the sub-retinal space throughout LT2 CNV. Cell type colors: 1) POS and PIS: light purple, 2) RPE: brown (stalk cells in the sub-retinal space have lighter shading), 3) Stalk cells: green, 4) Vascular cells (CC): red, 5) BrM: light blue. We have rendered the boundaries of individual cells as semi-transparent membranes. POS, PIS and RPE cells are more transparent to show the underlying structures. (AVI)

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**Author Contributions**

Conceived and designed the experiments: AS JAG JSG FF YJ HEG. Performed the experiments: AS. Analyzed the data: AS JAG. Contributed reagents/materials/analysis tools: AS MS. Wrote the paper: AS JAG.
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