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Aanvraagformulier 2017/2018

DEADLINE: Your application (section A, B and C) has to be received by email only by the secretariat of UitZicht on November 1st 2017 (11.59 PM/23.59 uur CET).

Part A: Scientific Quality – in English (mandatory), except for part A2

Explanation: Section B contains information about (patient's) relevance an Section C is for the Administration.

A1. General

Name applicant: Susanne Roosing, PhD

Research team: Blindness Genetics, Department of Human Genetics, Radboudumc

Project title: A long-read whole genome sequencing approach to unravel the genetic cause in inherited retinal dystrophies.

☐ Grant application (max € 250.000) OR ■ Pilot-project application (max € 75.000)
Is your project a Randomized Clinical Trial (RCT)? ☐ YES ■ NO

Planned start: 9/2018

Planned duration: 1 year

Keywords (max. 6): retina, long-read sequencing, structural variations, autosomal dominant retinitis pigmentosa

A2. Summary of contents and aim of the research project for layman in Dutch (max 750 words).

Beschrijf hieronder in maximaal 750 woorden de inhoud van uw onderzoek op VWO-niveau. Ga in op de volgende onderwerpen:
- Achtergrond van het onderzoek
- Doelstelling
- Onderzoeksopzet
- Primaire uitkomstmaten
- Belasting en risico (indien van toepassing) voor deelnemers
- Tijdspad
- Kennisoverdracht en implementatie *(Toelichting Kennisoverdra cht en implementatie: Hoe worden deelnemers en relevante groepen geïnformeerd over de resultaten van het onderzoek? Hoe en in welke fasen betrekt u patiëntenorganisaties bij uw onderzoek?)*

Achtergrond

In de afgelopen decennia zijn er 261 genen beschreven die betrokken zijn bij erfelijke netvliesziekten (inherited retinal diseases-IRDs). Analyse van deze genen levert in ~60% een genetische verklaring voor. Nieuwe technologieën zijn nodig om de overige 40% te verklaren. Whole exome sequencing (WES) analyseert alleen de eiwitcoderende onderdelen van genen (1% van het totale genoom) en herkent grote veranderingen in chromosomen heel matig. Analyse van het gehele genoom via whole genome sequencing (WGS) maakt momenteel zijn intrede in het onderzoeksveld waardoor niet-eiwitcoderende onderdelen van genen wel bestudeerd kunnen worden. De methode werkt echter via het sequencen van korte DNA fragmenten (25-100 baseparen) waardoor het onmogelijk blijft om complexe DNA gebieden (o.a. gebieden die DNA elementen bevatten die vaak meerdere malen in het genoom voorkomen; een hoog of laag GC-basepaar gehalte) goed in kaart te kunnen

brengen. Momenteel is er nog weinig bekend over de betrokkenheid van complexe regio's bij de genetische oorsprong van IRDs. Bij deze vraagstelling kan lange-fragment sequentie analyse (10.000-20.000 baseparen) uitkomst bieden, omdat de DNA moleculen die worden gesequenced langer zijn dan de genoemde repeterende elementen en de gebruikte methode even goed presteert voor DNA fragmenten met een hoog of laag GC gehalte.

Doelstelling

Het doel van deze pilotstudie is om te testen of lange-fragment sequencing van het hele genoom een verbetering kan opleveren voor het genetisch ophelderen van grote aantallen IRD-patiënten waarbij geen genetische oorzaak kon worden gevonden middels de huidige onderzoekstechnieken.

Onderzoeksopzet

Omdat de beschreven technologie zeer innovatief is en nog beperkt kan worden toegepast i.v.m. de aanzienlijke kosten, hebben wij ervoor gekozen om drie autosomaal dominante RD-families (twee personen per familie) te bestuderen waarbij eerdere analyses van whole exome sequencing (WES) en korte-fragment genoom brede analyse (WGS) geen verklaring voor de aandoening had opgeleverd. Voor deze families (Figuur 1) zijn bij eerder onderzoek chromosomale gebieden aangewezen waarin de genetische defecten zich bevinden. De Nederlandse familie W97-079 en de Canadese familie W04-179 koppelen op een 5.1 Mb gebied op chromosoom 17. De Nederlandse familie met Dominante Cystoïde Macula Degeneratie (DCMD/CYMD) toont koppeling op een 2.1 Mb gebied op chromosoom 7. Hierdoor kunnen wij zeer gericht de genetische data bestuderen.

Primaire uitkomstmaten

Doelstelling 1

Het vinden van de genetische oorzaak bij drie autosomaal dominante RD families.

Doelstelling 2

Het bewijs leveren dat lange-fragment sequencing potentie heeft om bij veel genetisch niet-opgeloste IRD-patiënten de oorzaken te vinden.

Belasting en risico voor deelnemers

Er is geen sprake van risico doordat enkel bloedsamples benodigd zijn voor isolatie van DNA. Deze zijn al verkregen voor eerdere onderzoeken in het verleden. Lange-fragment sequencing draagt het risico van nevenbevindingen met zich mee, echter, deze zijn niet groter dan voor de eerder uitgevoerde WES. In het geval van een nevenbevinding met mogelijk invloed op de gezondheid van de persoon zal een onafhankelijke commissie (klinisch geneticus, klinisch-moleculair geneticus, geneticus, lid van de medisch ethische commissie), advies geven of de patiënt hiervan op de hoogte wordt gesteld.

Tijdspad

9/2018 –	10/2018:	Sample voorbereiding voor lange-fragment sequencing
10/2018 –	11/2018:	Lange-fragment sequencing uitvoering en bioinformatische analyse
12/2018 –	5/2019:	Data analyse; indien parallele RNA studie goede resultaten oplevert tevens vergelijking DNA en RNA variaties
5/2019 –	7/2019:	Publicatie van resultaten; nieuwsbrieven en posters voor betrokken families maken

Kennisoverdracht en implementatie

De hoofdaanvrager zal bevindingen adresseren aan de betrokken familieleden. De drie families tonen zich betrokken voor onderzoek naar de genetische oorzaak van de autosomaal dominante RD binnen hun familie. (http://steunfondsrp17.nl/, https://dcmd.info/info/dcmd). Wanneer een (mogelijk) oorzakelijk defect wordt gevonden zal dit tevens met behandelend oogartsen gedeeld worden en met de families besproken worden. De Canadese familie zal wordt geïnformeerd door prof. dr. Robert Koenekoop (Montreal, Canada) en via een podcast.

De bevindingen en navolgende kansen voor patiënten waarbij middels de huidige routine analyses geen genetische oorzaak is gevonden, zullen worden besproken bij algemene patiënten besprekingen en gecommuniceerd worden via een podcast online (www.RD5000.nl; www.oogvereniging.nl). Ook zal een poster worden gemaakt voor de betreffende families.

Dr. Roosing zal de resultaten van de pilotstudie bespreking binnen het European Retinal Disease Consortium, bestaande uit 17 research groepen uit 12 landen. In de tweejaarlijkse bijeenkomsten worden de partners geïnformeerd, waardoor zij hun huidig onopgeloste samples gericht kunnen bekijken. In het geval van vergelijkbare bevindingen worden de krachten gebundeld.

De wetenschappelijke gemeenschap zal worden geïnformeerd door een publicatie in een 'open access' tijdschrift. Wanneer deze studie succesvol is, zal een vervolg studie worden geïnitieerd. Gezien de sterke ontwikkeling van de lange-fragment sequencing technologie wordt een enorme daling in de kosten voorzien. Dit biedt mogelijkheden om de technologie aan genetische onopgeloste patiëntengroepen aan te bieden.

Number of words for A2 (max. 750): 749

A3. Scientific summary of contents and aim of the project (in English, max 400 words).

Number of words for A3 (max. 400): 377

Currently, 261 genes have been associated with inherited retinal diseases (IRDs) (RetNet; https://sph.uth.edu/retnet/). Whole exome sequencing (WES) provides a molecular diagnosis in only ~60% of patients with IRDs (Haer-Wigman et al. 2017). WES misses mutations due to incomplete coverage of coding regions and is inadequate to identify all types of structural variations. Furthermore, erroneous variant filtering protocols may contribute to the missing heritability. The pressing reason for missing mutations likely is the location of genetic variants in the 99% of the human genome that is not covered with WES. Whole genome sequencing (WGS) has become a cost-efficient technology, in particular to detect non-coding variants and assess structural variations, however the interpretation of identified single nucleotide variants remains very challenging without proper functional read-outs. Moreover, the presence of large rearrangements cannot be excluded with WGS through short-read sequencing.

New approaches as long-read sequencing may provide proof for causality of large rearrangements in the biology of IRDs. We hypothesize that the missing 40% of causal variants of genetically unexplained IRDs can partially be detected through long-read WGS. To provide evidence for this hypothesis we propose to study three autosomal dominant RD families in whom no causal defects could be identified using WES or short-read WGS. For these families the critical regions have been determined through linkage analysis in the past. For the autosomal dominant cystoid macular dystrophy (CYMD), a locus of 2.1 Mb on chromosome 7 was determined to harbor the elusive defect (Kremer et al. 1994; Roosing et al. unpublished data). For the two autosomal dominant retinitis pigmentosa (adRP) families the original locus was 34.5 Mb (Den Hollander et al. 1999) which has recently been decreased to 5.1 Mb through linkage and haplotype analyses in the fourth generation of one of the two adRP families (Roosing et al. ARVO-meeting 2017). A Canadian adRP family also is linked to the same region on chromosome 17, but we did not perform detailed haplotype studies. We aim to identify the elusive gene defect in these families though long-read WGS supported by already initiated transcriptomic studies. Investigating these specific families enables a focused and functionally supported approach which can provide evidence for the causality of large rearrangements and will open doors for long-read WGS in currently unexplained dominant and recessive RD families.

A4. Information on the research team (maximum of 750 words)

a. Research Line(s) in which the intended study will be incorporated:

From March 2014 – March 2016, Susanne Roosing worked as a postdoctoral fellow at the University of California, San Diego (UCSD) and the Rockefeller University, New York, in the group of Prof. Gleeson, who specializes in pediatric brain diseases amongst which syndromic ciliopathies. Since June 2016, dr. Roosing works in the Department of Human Genetics in Nijmegen in which she previously performed her PhD studies with Prof. Cremers, Prof. den Hollander, Prof. Klaver and Prof. Hoyng. Currently, Dr. Roosing has identified eight genes associated with IRDs or ciliopathies, among genes associated with cone (rod) dystrophy (*MFSD8*, *POC1B*, *RAB28*), two novel genotype-phenotype correlations (*CEP290*, *TULP1*) and three Joubert syndrome-associated genes (*CEP120*, *KIAA0556, KIAA0586)*. She has three additional first author publications on molecular genetics of IRDs, and has contributed to 17 additional publications. Moreover, she coordinates the European Retinal Disease Consortium, consisting of 17 research groups in 12 countries (www.ERDC.info), which has ascertained ~20.000 unrelated families with IRDs, and actively exchange data and technologies.

As we could not find the underlying variants in ~40% of IRDs using WES, we will focus our attention on non-coding defects through WGS. Short-read WGS (read-length 25-100 bp) provides a deepened understanding of genomes, but does not perform well in 'complex' regions and thus potentially results in a knowledge gap regarding genetic causes of IRDs. To overcome this problem, long-read sequencing may provide an opportunity. Long-read sequencing approach employing the PacBio Sequel machine can overcome non-coverage due to long repeats, high GC-content and large rearrangements such as inversions, which could be an unidentified underlying cause of IRDs. Findings in the three proposed families will be combined with currently ongoing studies investigating induced pluripotent stem cells (iPSCs) obtained from patient skin biopsy generated fibroblasts. iPSCs were generated for four cases and differentiated into PPCs. The CYMD iPSCs were also differentiated into RPE cells as we cannot exclude that the underlying defect is not expressed in neural retinal cells. RNA sequence analysis will be performed in a follow-up study.

b. List current grant(s) that are related to this application: (Explanation: Name of funding agency, title of grant application, the amount (€), the granting period and specify the degree of overlap with the current application.)

Co-PI; Foundation Fighting Blindness Program Project Award "Splice Modulation to Treat Inherited Retinal Diseases" (Project 1: PIs: F. Cremers, **S. Roosing**; USD 542.800, 05/2017-04/2022). *[no overlap]*

Co-PI; Uitzicht 2017: "Shedding light on the dark side of the genome in inherited retinal diseases." (PIs: F. Cremers, **S. Roosing**; The Rotterdamse Stichting Blindenbelangen, Stichting Blindenhulp, Stichting tot Verbetering van het Lot der Blinden; EUR 90.000; 06/2017-12/2018). *[no overlap]*

Co-PI; Foundation Fighting Ireland: "Shedding light on unexplained inherited retinal diseases in Ireland and the Netherlands". (PIs: **S. Roosing**, M. Carrigan, J. Farrar, F. Cremers, EUR 350.000; 01/2018-01/2021). *[no overlap]*

c. Recent relevant international publications of the team: (Explanation: name five in case of a full grant application, name three in case of a Pilot project.)

Roosing S, Cremers FPM, Riemslag FCC, Zonneveld-Vrieling MN, Talsma H, Klessens-Godfroy FJM, den Hollander AI, van den Born LI. A rare form of retinal dystrophy caused by hypomorphic nonsense mutations in *CEP290*, Genes (Basel). 2017 Aug 22;8(8). pii: E208.

Roosing S, van den Born LI, Sangermano R, Banfi S, Koenekoop RK, Zonneveld-Vrieling MN, Klaver CC, van Lith-Verhoeven JJ, Cremers FP, den Hollander AI, Hoyng CB. Mutations in *MFSD8*, encoding a lysosomal membrane protein, are associated with nonsyndromic autosomal recessive macular dystrophy. Ophthalmology. 2015 Jan;122(1):170-9.

*__Roosing S__, *Lamers IJC, *de Vrieze E, *van den Born LI, Lambertus S, Arts HH, Study group, Peters TA, Hoyng CB, Kremer H, Hetterschijt L, Letteboer SJF, #van Wijk E,

#Roepman R, #den Hollander AI & #Cremers FPM. Disruption of the basal body protein *POC1B* results in autosomal recessive cone-rod dystrophy (2014). Am. J. Hum. Genet. 95, 131-142. *Shared first authors; #shared senior authors.

d. In case of collaboration: name the organization(s)/research team(s) within the Netherlands and/or abroad that will cooperate with you on an intense basis within this study:

Radboudumc, Department of Human Genetics
Prof. F. Cremers (Prof. Ophthalmogenetics), Dr. K. Neveling (Advisor PacBio), Dr. M. Nelen (Advisor PacBio), Dr. C. Gilissen (Bioinformatician), Dr. A. Hoischen (Advisor PacBio) and Dr. H. IJntema (Advisor/clinical molecular genetics).

Radboudumc, Department of Opthalmology
C. Hoyng (Advisor/ophthalmologist)

McGill University, Montreal, Canada
R. Koenekoop (Advisor/ophthalmologist)

e. Number of words for A4 (max. 750): 721

A5. Aim and design of the study (in English, maximum of 2.750 words, excluding 'references', including text near figures)

a. Problem definition
A genetic diagnosis for IRDs is essential for a patient's accurate prognosis, genetic counseling and eligibility for therapies. Although WES is routinely used for IRD gene defect identification, ~40% of all cases remain genetically unexplained.(Abu-Safieh et al. 2013; Haer-Wigman et al. 2017) Since the coding regions only represent 1% of the genome it is hypothesized that many unknown pathogenic variants are located in non-coding sequences which can be detected by gene-specific sequence analysis if only one gene is involved in a given IRD or by WGS. Intronic variants can activate protein-truncating pseudo-exons as shown in nine IRD-associated genes (*ABCA4, CEP290, CHM, OA1, OAT, OFD1, PROM1, PRPF31,* and *USH2A* (for references see www.dbass.soton.ac.uk). Non-coding variants can also alter gene transcription or mRNA stability. Recently, *PRDM13* gene duplication and cis-regulatory variants upregulating *PRDM13* transcription were associated with North-Carolina Macula Dystrophy.(Small et al. 2016) To understand the functional consequences of variants in non-coding regions however remains challenging as the predictive value of *in silico* prediction programs is limited. Moreover, little knowledge is available on the contribution of structural variants as a cause of IRDs. Detection of copy number variants through short-read sequencing is possible, however large rearrangements remain uncovered. Detailed identification of large rearrangements may be feasible through long-read sequencing.

b. Relevance
Relevance for patients, families and society
Visual disability has immense consequences in daily life for patients, family and society and is a major health issue worldwide. Participation in society leaves great challenges for those with affected eyesight. In the Netherlands, approximately 320,000 persons are affected by visual impairment and this number is growing steadily. Various classes of retinal dystrophies exist through differences in origin of the primary defect in rods versus cones. Persons with initial rod defects, such as RP, initially show night blindness, progressive peripheral vision loss and tunnel vision. Individuals with cone-dominated defects rather show central vision defects. Age of onset and disease progression also present hallmarks to distinguish various types of retinal dystrophies. RP affects 1:4,000 individuals and often leads to complete blindness around the 3rd of 4th decade of live, whereas Leber congenital amaurosis has a prevalence of approximately 1:50.000 persons leading to total blindness in the first years of life.
A subset of the visual impairments can be restored (partially) through improved visual aids or by surgical intervention. Retinal dystrophies until recently could not be treated. The development of gene and drug therapies for selected IRDs have changed this perspective

completely, and gene or mutation-specific therapies are close to clinical applications (https://clinicaltrials.gov/). While therapeutic approaches are being developed it is essential to broaden the knowledge on the molecular mechanism of disease and identify common denominators involved in IRD.

Short-read WGS (read length 25-100 bp) provides a deepened understanding of genomes, but it only reveals the tip of the iceberg on the genetics complexity. The PacBio long-read sequencer Sequel utilizes a single molecule real-time (SMRT) technology without an amplification step (see Box 1 for details). The advantage of SMRT technology offers i) long read lengths (read length 10-20 kb), ii) high mapping accuracy (>99.999% at 30X coverage depth, without systematic errors), iii) equal coverage across complex regions, and iv) epigenetic characterization of base modifications. The technology has yet resolved hard-to-sequence regions for genomic, transcriptome and epigenetic research purposes (Carneiro et al. 2012, Nanako et al. 2017, Shin et al. 2013). Currently, an additional high quality coverage is obtained for 21 Mb of previously uncovered sequencing per genome. (C. Gillissen and A. Hoischen, personal communication)
A long-read sequencing machine (Sequel) is now available in the Department of Human Genetics, at the Radboudumc in Nijmegen, the Netherlands. This approach will provide proof-of-concept that long-read sequencing can represent the future for (structural) defect identification in IRDs. The plausible identification of causative defects in three selected candidate families provides novel perception into mechanisms underlying disease and improved genetic diagnosis. Addressing the genetic defect in these adRD families may provide direct opportunities in preventing disease reoccurrence for future generations, as pre-implantation diagnostics may be applied for family-planning.

c. Preliminary work
We aim to identify the elusive gene defect in three families though whole genome sequencing supported by already initiated transcriptomic studies (Figure 1). Earlier, we determined for the autosomal dominant cystoid macular dystrophy (CYMD, W91-009), a locus of 2,1 Mb on chromosome 7 is determined to harbor the elusive defect (LOD-score 9.4) (Kremer et al. 1994; Roosing et al. unpublished data) and for the two autosomal dominant RP families linking to chromosome 17, the original locus was 34,5 Mb (Den Hollander et al. 1999) which has recently been decreased to 5,1 Mb (LOD-score is 10.2 for 28 affected persons plus 4.8 for 16 healthy persons) through linkage and haplotype analyses in the fourth generation of W97-079 (Roosing et al. ARVO-meeting 2017). A large Canadian adRP family (W04-179; LOD-score >8.0) also is linked to the same region on chromosome 17, but we did not perform detailed haplotype studies.

Box 1: Technical details Long-read sequencing:
SMRTsequencing captures sequence data as a target DNA molecule is replicated. The template, SMRTbell, is created by ligating hairpin-adapters to both ends of a double-stranded DNA molecule. This is loaded onto a chip (SMRTcell) where it diffuses into separate units called a zero-mode waveguide. Each zero-mode waveguide contains an immobilized single DNA polymerase which can bind to the hairpin adapters and initiates replication. The four differently labeled fluorescent nucleotides are also added. As a base is fixed by the polymerase a distinct light pulse is emitted for each base. These light pulses are recorded and subsequently interpreted to produce the sequence generated in each zero-mode waveguide. The newer chemistries can produce reads up to 60 kb.

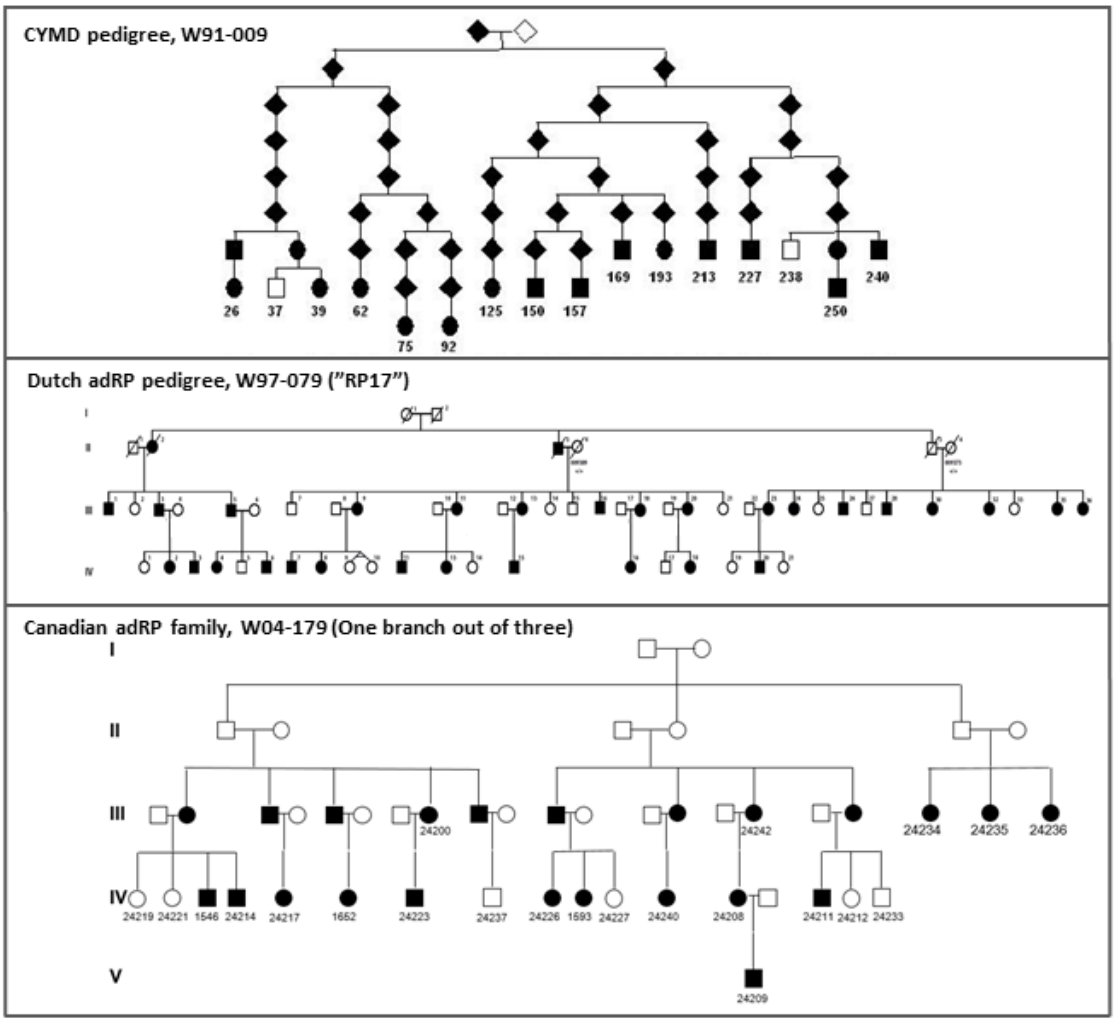

Figure 1: Autosomal dominant RD Pedigrees for long-read sequencing pilot project. A) Simplified CYMD pedigree of family W91-009 that consists of 98 affected and 82 unaffected persons. B) Dutch adRP family W97-079 consisting of 28 affected and 16 unaffected persons. C) One branch out from the Canadian adRP W04-179 family consisting of 49 affected and 85 unaffected persons.

WES was performed in two cases of each of the three adRD families in which no causal (heterozygous) variants were detected thus far. Moreover, short-read WGS in single cases of W91-009 and W97-079 was performed. All heterozygous locus variants in the WGS data were verified using publically available control datasets in view of the autosomal dominant inheritance of the genetic defect and uniqueness of the locus and phenotype, for W97-079 leaving 615 variants of which two are coding variants. Both coding variants are present in a public database (GnomAD) in a frequency too high to cause disease in this family. In the WGS data we identified a 226-kb duplication spanning *GDPD1* and *YPEL1*, as well as the last exons of *SMG8* and *LINC01476* (Figure 2). Although the duplication segregates with the phenotype in the family, the breakpoints and its significance remain unknown. Other structural variations such as inversions, could not be uncovered by employing short-read sequencing nor through chromosome focused karyotyping. WGS for the single case in W91-009 did not reveal novel clues or unique variants in the pathogenicity of CYMD.

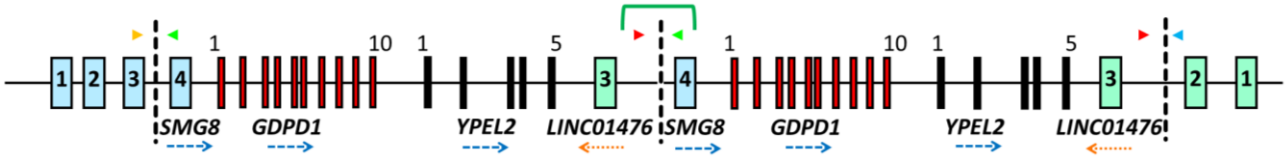

Figure 2: Identified duplication in the Dutch adRP W97-079 family. Partial or complete genes in the duplicated region. Arrowheads indicate primers. Dotted arrows indicate reading direction of genes. Primers ▶ and ◀ generated a unique fragment, indicative of tandem duplication.

d. Objective of the study

Main objective 1:

To perform a focused genome-wide long-read sequencing approach for three adRP families with highly significant loci containing the causative gene defects.

Main objective 2:

Provide a proof-of-concept for the integration of long-read sequencing into the field of IRD research and explore its opportunities. Future studies in cases in which no causative gene defect may be found through WES, may benefit from long-read sequencing WGS over short-read WGS.

e. Study design

For this pilot, we propose to study three adRD families in whom no causal defects could be identified using WES or short-read WGS. For these families the critical regions have been determined through linkage analysis in the past. We will analyze two cases for each of the three families, selecting DNA samples from individuals that –in genetic terms– are most distant and thus share the least amount of genomic segments other than the pathogenic gene defect and the flanking sequences.

Currently, ~30 SMRTcells are required to span a single genome at 30X sequencing depth. This coverage is considered sufficient in long-read sequencing to overcome short-read sequencing's issues, such as repeats, or high GC-content. Moreover, 30X coverage enables genome assembly to unravel heterozygosity, built phased haplotypes and accurately pinpoint structural variations, such as large insertions, duplications, inversions or translocations (Figure 3). Additionally, as long-read sequencing takes place in real-time, kinetic variant interpretation from the recorded light-pulse movie can be analyzed to identify base modifications, such as methylation, which provides an additional layer of information. Having two datasets per family enables to generate an overlap to vastly narrow down the data shared by affected relatives and focus on the overlapping structural variants as well as the single nucleotide variants. The availability of DNA samples for almost all affected and unaffected from the three families provides a unique opportunity for large scale segregation analysis for plausible disease causing findings.

The initial focus employing long-read WGS data will be on coding variants which may have been missed in WES or short-read WGS family due to incomplete coverage. All variants will be assessed based on their frequencies in population databases (ExAC, EVS and 1,000 genome project). Missense variants will undergo *in silico* predictions (PhyloP, Grantham, CADD-score) to score their severity. Next, large rearrangements such as duplications, deletions or inversions will be evaluated. These will be assessed for causality by evaluating in-house frequency data from microarrays routinely performed in non-ocular genetic disorders. Lastly, by applying *in silico* predictions, other species' nucleotide conservation scores and genome-wide population frequency scores (GoNL, Kaviar, GnomAD), we intend to reduce the number of variants in non-coding regions. For datasets corresponding to affected individuals from the same family, an overlap file will be created based and focused on their disease specific locus and overlapping variants can be assessed.

Validation of the findings of high interest to the disease phenotype may be assessed through segregation analysis in DNA samples or the corresponding family. Effects of the causative defect may be studied using transcriptome data which is being generated in parallel for selected cases from the two Dutch families. Currently, induced pluripotent stem cells were generated for four cases from W97-079 and W91-009 and differentiated into iPSCs. The iPSCs obtained from CYMD cases were also differentiated into retinal pigment epithelium cells as the underlying defect could be exclusively expressed in RPE cells. RNA sequence analysis will be performed in a parallel study.

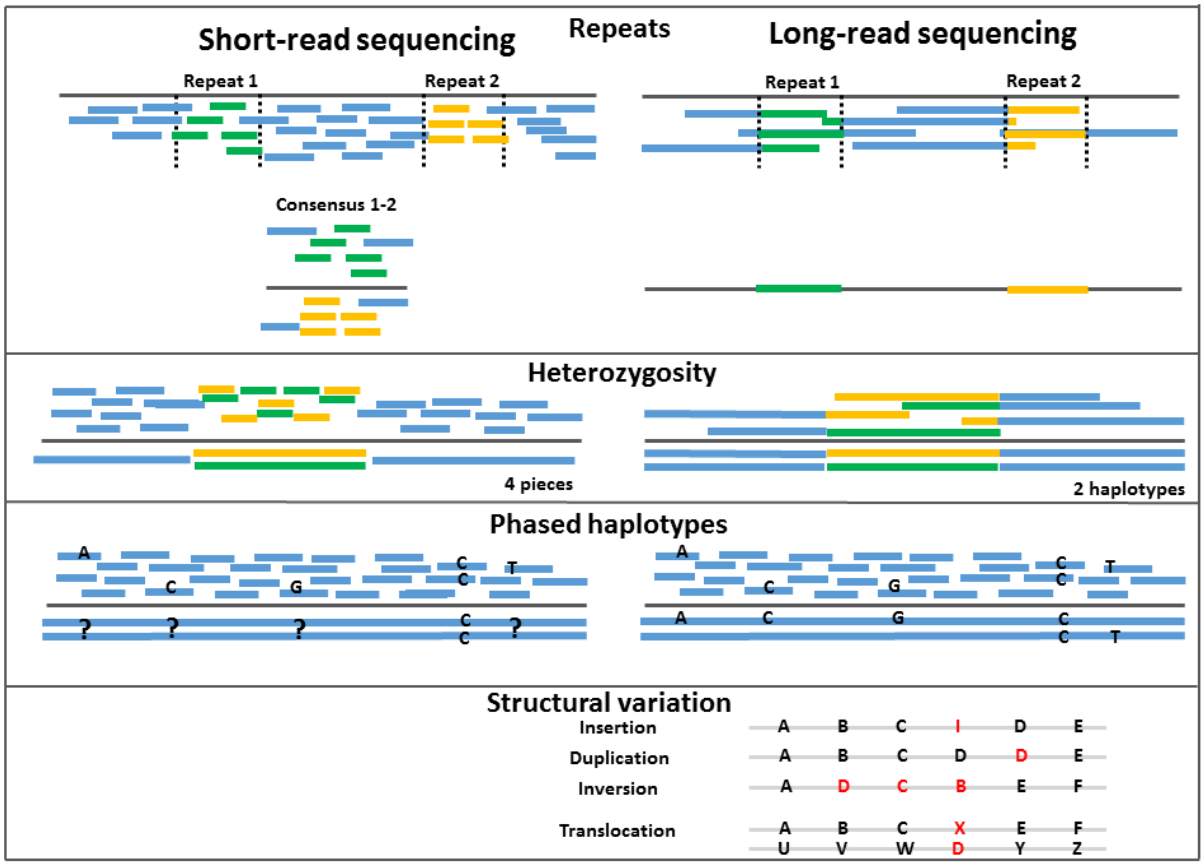

Figure 3: A comparison of short-read vs long-read sequencing for different purposes. Short-read sequencing is unable to differentiate reads of repeats or to cover high GC regions, cannot distinguish or phase haplotypes and proficiently detect structural variants. Long-read sequencing is able to assemble through repeats or GC-rich regions, assess alleles and phase haplotypes, as well as large structural variations.

f. Study population (Include a power calculation if applicable)
Two persons from the Dutch autosomal dominant RP family (W97-079), two persons from the Canadian adRP family (W04-179) linking to the same locus on chromosome 17, two persons from the autosomal dominant cystoid macular dystrophy family (W91-009).

Power calculation is not applicable as the location of the defect based on linkage studies is clear.

g. Primary study parameters/outcome of the study
Robust long-read WGS data, with fragments up to 50 kb spanning the disease loci in six samples from three adRD families.

h. Secondary study parameters/outcome of the study (if applicable)
Identification of novel DNA abnormalities in persons with defects unrelated to the disease locus with unknown significance.

i. Nature and extent of the burden and risks associated with participation (if applicable)
There are no physical risks as merely blood samples are required, which has been done in the previous studies for these families. WGS carries a risk for unsolicited findings, but these risks are not higher than those for the previously performed WES studies in these three families. Patients therefore will be well-informed on these risks. Upon unsolicited findings which are deemed to potentially affect the healthcare of the person, an independent committee consisting of a clinical geneticist, a clinical molecular geneticist, a geneticist, and a member of the medical ethical committee, will discuss these in great detail and decide whether the caretaker of the patient will be informed.

j. Knowledge transfer, Implementation, Consolidation

The main applicant will address the findings in newsletters to the two Dutch families and the Canadian family. The currently involved adRD families are highly collaborative to enable the identification of the causative gene defect in their family (http://steunfondsrp17.nl/, https://dcmd.info/info/dcmd). When the pathogenic defect is elucidated this will be communicated with their treating ophthalmologists, family meetings will be organized and the Canadian family will be informed in collaboration with prof. Koenekoop and through a podcast.

The findings and subsequent opportunities for currently genetically unexplained WES cases will be presented in general patient meetings and communicated though a podcast online (www.RD5000.nl; www.oogvereniging.nl). A laymen's poster will be posted online to update the community, affected individuals and their relatives with an IRD.

Dr. Roosing will discuss the results of the pilot project in the European Retinal Disease Consortium, which comprises of 17 research groups from 12 countries. In the biannual meetings all partners can be informed and they can analyze their currently unexplained adRD cases. In that case, these teams will join their forces to confirm the cause of the adRD in these patients.

The scientific community will be informed of our findings by publication of the study results in open access journals. When the genetic defects have been identified, follow-up studies will be performed to investigate the mechanisms of the CYMD and adRP in more detail, which is a prerequisite to design new treatment strategies. As the price for the long-read sequencing technology is expected to decrease enormously, this will provide opportunities for a large group of genetically unexplained IRD cases.

k. Study time schedule (Explanation: In case of a Pilot-project, the study period can be up to 2 years.)

9/2018 – 10/2018: Sample preparation for long-read sequencing
10/2018 – 11/2018: Long-read sequencing and bioinformatic analyses
12/2018 – 5/2019: Data analyses; if parallel RNA studies yield promising results also comparisons DNA and RNA variations
5/2019 – 7/2019: Publication of results; prepare newsletters and laymen's poster

l. In case your study is a Pilot-project: please explain why you chose for a Pilot-project instead of a regular study. Please mention the Go/No Go criteria for a possible follow-up study.

A long-read WGS approach has not been done yet in IRD cases. Currently, to provide a 30X coverage for a long-read genome, ~30 SMRTcells are required with a corresponding price of €33.154. However, due to fast developments in long-read sequencing in increased SMRTcell sequencing capacity, we anticipate a 4-times reduction in SMRTcells needed leading to a price per single genome of ~€8.273 at the start of this study. This study is considered successful if new clues are found for the elusive defects underlying CYMD at chromosome 7 and adRP at chromosome 17. If long-read sequencing technology will continue to become more cost-effective it can be offered in more unexplained IRD cases with or without prior knowledge on the location of the genetic defect.

m. Number of words for A3 (max. 2.750): 2439

n. References (not to be included in the word count): (Explanation: A max. of 10 key publications in international literature - References should include titles of papers, and use standard abbreviations of journals.)

Abu-Safieh L, Alrashed M, Anazi S, Alkuraya H, Khan AO, Al-Owain M, Al-Zahrani J, Al-Abdi L, Hashem M, Al-Tarimi S, Sebai MA, Shamia A, Ray-Zack MD, Nassan M, Al-Hassnan ZN, Rahbeeni Z, Waheeb S, Alkharashi A, Abboud E, Al-Hazzaa SA, Alkuraya FS. Autozygome-guided exome sequencing in retinal dystrophy patients reveals pathogenetic mutations and novel candidate disease genes. Genome Res. 2013 Feb;23(2):236-47.

Carneiro MO1, Russ C, Ross MG, Gabriel SB, Nusbaum C, DePristo MA. BMC Genomics. Pacific biosciences sequencing technology for genotyping and variation discovery in human data. 2012 Aug 5;13:375.

den Hollander AI, van der Velde-Visser SD, Pinckers AJ, Hoyng CB, Brunner HG, Cremers FPM. Refined mapping of the gene for autosomal dominant retinitis pigmentosa (RP17) on chromosome 17q22. Hum Genet. 1999 Jan;104(1):73-6.

Haer-Wigman L, van Zelst-Stams WA, Pfundt R, van den Born LI, Klaver CC, Verheij JB, Hoyng CB, Breuning MH, Boon CJ, Kievit AJ, Verhoeven VJ, Pott JW, Sallevelt SC, van Hagen JM, Plomp AS, Kroes HY, Lelieveld SH, Hehir-Kwa JY, Castelein S, Nelen M, Scheffer H, Lugtenberg D, Cremers FPM, Hoefsloot L, Yntema HG. Diagnostic exome sequencing in 266 Dutch patients with visual impairment. Eur J Hum Genet. 2017 May;25(5):591-599.

Kremer H, Pinckers A, van den Helm B, Deutman AF, Ropers HH, Mariman EC. Localization of the gene for dominant cystoid macular dystrophy on chromosome 7p. Hum Mol Genet. 1994 Feb;3(2):299-302.

Nakano K, Shiroma A, Shimoji M, Tamotsu H, Ashimine N, Ohki S, Shinzato M, Minami M, Nakanishi T, Teruya K, Satou K, Hirano T. Advantages of genome sequencing by long-read sequencer using SMRT technology in medical area.Hum Cell. 2017 Jul;30(3):149-161.

Roosing S, Khan MI, Micheal S, Zonneveld-Vrieling MN, Royer-Bertrand B, Rivolta C, den Hollander AI, Hoyng CB, Cremers FPM, Genomics approaches to identify an elusive defect at chromosome 17q22 in an autosomal dominant retinitis pigmentosa family, the Association for Research in Vision and Ophthalomology (ARVO) conference 2017, Baltimore, USA

Shin SC, Ahn DH, Kim SJ, Lee H, Oh TJ, Lee JE, Park H. Advantages of Single-Molecule Real-Time sequencing in high-GC content genomes. PLoS One. 2013 Jul 23;8(7):e68824.

Small KW, DeLuca AP, Whitmore SS, Rosenberg T, Silva-Garcia R, Udar N, Puech B, Garcia CA, Rice TA, Fishman GA, Héon E, Folk JC, Streb LM, Haas CM, Wiley LA, Scheetz TE, Fingert JH, Mullins RF, Tucker BA, Stone EM. North Carolina Macular Dystrophy is caused by dysregulation of the retinal transcription factor PRDM13. Ophthalmology. 2016 Jan;123(1):9-18.

A6. Financial details

Budget, to be approved by the local management
Approved by: Peter van Woensel (managing director) and Dennis Vissers (financial controller), Department of Human Genetics, Radboudumc, Nijmegen, the Netherlands.

Personnel (post-doc/**scientific researcher**/technician)* amount of FTE:
*underline
Salary including social charges:

Total year 1 (1fte, 8 months)	€43.172

Total Personnel costs:	€43.172

Other

Supplies and equipment:	€ -
Technology service:	
PacBio sequencing (+consumables)(Sequel; n=6)	€49.482
Publication costs:	€ 2.000
Implementation costs (please explain):	€ -
Reimbursement personnel:	€ -
Travel costs participants:	€ -
Other costs (e.g. insurance, please explain):	€ -

Total other costs:	€51.482
Charges by university (8%)	€ 7.572

TOTAL	**€102.226**

Co financing
Name(s) of the source(s)

Requested from UitZicht	
max € 250.000 (PILOTS max € 75.000)	**€75.000**

Own contributions:

Materials:	€ -
Supervising (0.2 fte, 8 months, S.Roosing)	€ 8.938
Technical support (analist) (0.1fte, 4 months)	€ 1.658
Other (please explain)	

Other Funds being requested (already) and to what amount:
Name(s) of the Fund(s)

DCMD-onderzoek (W91-009 foundation)	€15.000
Stichting Steunfonds RP17 (W97-079 foundation)	€ 7.226
Stichting Blindenhulp	€ 5.000
Total:	€27.226

Other Funds to be requested (in the near future) and to what amount:
Name(s) of the Fund(s) €

*Personal communication Dr. K. Neveling (PacBio advisor, Department of Human genetics, Radboudumc): The costs for a single long-read genome assembly as per November 1st 2017 are estimated at ~€33.154. For September 2018, we anticipate on a 4-times increased capacity per SMRTcell leading to a decrease in costs to a price per genome of ~€8.274.

Deel B: Ten behoeve van het Panel Relevantie – in het Nederlands

Toelichting: Onderdeel A bevat informatie over wetenschappelijke kwaliteit en onderdeel C administratieve informatie.

B1. Onderwerp en opzet van de studie

a. Kruis aan welk onderdeel van het oog onderwerp is van de studie (meerdere antwoorden mogelijk)

☐ Hoornvlies ☐ Lens ☐ Oogbol ■ Netvlies
■ Macula ☐ Oogzenuw ☐ Ander onderdeel, te weten:
☐ Geen onderdeel, toelichting:

b. Wordt er onderzoek gedaan naar een specifieke oogaandoening? ☐ Nee ■ Ja
Zo ja, naar welke oogaandoening wordt onderzoek gedaan?
Toelichting: Als eerste worden autosomaal dominante retinitis pigmentosa (Nederlandse en Canadese familie, beide koppelend op een gebied op chromosoom 17) en dominante cystoïde macula dystrofie (koppelend op chromosoom 7) bestudeerd. Met overtuigende resultaten kan de nieuwe methode geïmplementeerd worden in andere families met autosomaal dominante of autosomaal recessieve families met retina dystrofie.

c. Het Panel Relevantie heeft graag scherp wat het hoofddoel is van het beoogde onderzoek. Kruis aan wat het hoofddoel is (meerdere antwoorden mogelijk)

■ Ontstaan ☐ Epidemiologie ☐ Risico analyse ☐ Behandeling ☐ Preventie
☐ Anders, te weten:

B2. Organisatie van de studie

a. Kruis aan op welk stadium de studie betrekking heeft (meerdere antwoorden mogelijk)

■ Retroperspectief ☐ Prospectief ☐ Niet van toepassing
☐ Anders, te weten:

B3. Omvang van de doelgroep

a. Wat is de prevalentie van de oogaandoening in Nederland?
Antwoord:
De twee hier bestudeerde Nederlandse families omvatten in totaal 126 aangedane personen. Ter vergelijking, de patiënten groepen die tot dusverre de meeste aandacht hebben gekregen vanwege de ontwikkeling van nieuwe therapieën, nl. personen met *RPE65* en *CEP290* defecten, omvatten circa 45 en 35 personen, respectievelijk.

b. Welk percentage hiervan heeft een restvisus van < 0.3 of een kokervisus van < 30 graden? Antwoord:
>60% van de families W91-009 (98 aangedane personen), W97-079 (28 aangedane personen) en W04-179 (49 aangedane personen) (Persoonlijke communicatie C. Hoyng) hebben een visus van 0.3 of lager en kokervisus van minder dan 30 graden.

c. Hoeveel patiënten nemen deel aan het onderzoek? Antwoord:
Bij deze pilot worden zes individuen van drie families geïncludeerd. Deze zijn representatief voor de gehele families (Figuur 1).

d. Hoeveel patiënten hebben (naar schatting) baat bij de resultaten van het onderzoek?
Antwoord:
Momenteel ontvangt ~40% van alle patiënten met een netvliesaandoening die genetisch onderzoek laat uitvoeren nog een negatieve uitslag. Deze pilot studie is erop gericht om dit percentage te verhogen. Korte-fragment WGS zal naar schatting in 10% van de huidige onopgeloste gevallen een genetische diagnose opleveren. Een additionele 10% van de patiënten die momenteel een negatieve uitslag via WES hebben ontvangen zal naar schatting een genetische diagnose worden gegeven middels lange-fragment WGS.

B4. Toetsing METC/DEC en patiëntenorganisaties

Betreft uw aanvraag medisch-wetenschappelijk onderzoek met mensen? ☐ Nee ■ Ja
Heeft u reeds toestemming aan de METC gevraagd?
☐ Nee; let op: een eventuele toezegging van een fonds zal gelden onder de voorwaarde dat de toestemming van de METC verkregen is.
☐ Ja
Zo ja, heeft u reeds toestemming van de METC gekregen? ☐ Nee ☐ Ja
Zo ja, indien toestemming al is verkregen, dan deze bijvoegen bij de stukken.

Betreft uw aanvraag medisch-wetenschappelijk onderzoek met dieren? ■ Nee ☐ Ja

Het Panel Relevantie hecht aan de uitgangspunten van de patiëntenbijsluiter. Deze kunt u downloaden via http://www.uitzicht.nl/home/requests-and-downloads

Hoe betrekt u patiëntenorganisaties bij uw onderzoek? (meerdere kruisjes zijn mogelijk)
☐ Studieopzet ■ Implementatie, resultaten ■ Patiënten media ☐ Algemene media

B5. Wanneer zijn welke resultaten van het onderzoek te verwachten?

■ Onderzoeksresultaat te verwachten binnen 3 jaar.
☐ Onderzoeksresultaat te verwachten tussen 3 en 5 jaar.
☐ Onderzoeksresultaat te verwachten tussen 5 en 10 jaar
☐ Onderzoeksresultaat te verwachten tussen 10 en 20 jaar.
☐ Onderzoeksresultaat te verwachten na meer dan 20 jaar.
Toelichting:

B6. Op welke termijn zijn de resultaten van het onderzoek bruikbaar/toepasbaar voor patiënten, zodat slechtziendheid of blindheid voorkomen wordt?

■ Praktische bruikbaarheid te verwachten binnen 3 jaar.
☐ Praktische bruikbaarheid te verwachten tussen 3 en 5 jaar.
☐ Praktische bruikbaarheid te verwachten tussen 5 en 10 jaar
☐ Praktische bruikbaarheid te verwachten tussen 10 en 20 jaar.
☐ Praktische bruikbaarheid te verwachten na meer dan 20 jaar.
■ Het onderzoek beantwoordt een wetenschappelijke vraagstelling, zonder directe praktische toepasbaarheid
Toelichting: Indien de pilot de genetische oorzaak in (een van) de onderzochte families aanwijst, kan familieleden met kinderwens in de toekomst pre-implantatie diagnostiek worden aangeboden. Echter voor de huidig aangedane personen binnen deze familie(s) zal het een accurate genetische diagnose zijn en de mogelijkheid bieden tot het ontrafelen van het ziektemechanisme en het ontwikkelen van therapieën.

B7. Kruis aan op welk(e) terrein(en) uit de top 10 van de onderzoeksagenda van de Oogvereniging/Maculavereniging uw onderzoek past? (meerdere antwoorden mogelijk) Zie: https://www.oogvereniging.nl/oogaandoeningen/onderzoeksagenda/

- **Nieuwe & regeneratieve behandelingen**

1 ☐ Stamceltherapie
2 ☐ Gentherapie
3 ☐ Vervanging of herstel van netvlies.

- **Preventie & diagnose**

4 ☐ Invloed van voeding en leefstijl om mijn oogaandoening te voorkomen, of de progressie ervan te vertragen of te stoppen
5 ■ Erfelijkheidsonderzoek voor mijn oogaandoening om nauwkeuriger te voorspellen of (klein)kinderen van mensen met mijn oogaandoening een grotere kans hebben op mijn oogaandoening
6 ☐ Verbetering van oogmetingen en gezichtsveldonderzoek zodat onderzoek minder belastend is voor de cliënt en een betrouwbaarder resultaat geeft

- **Oorzaak en ziekte mechanisme**

7 ☐ Verloop en progressie van oogaandoeningen
8 ■ De invloed die een vroege diagnose kan hebben op het beloop van de oogaandoening verbetering huidige behandelingen
9 ☐ Voorkomen van ernstige complicaties bij oogoperaties met name staar/netvlies) specifiek voor glaucoom
10 ☐ Werking en effectiviteit van oogmedicatie

Toelichting:

B8. Heeft u opmerkingen?

n.v.t.

Deel C: Administratie – in het Nederlands

Hoofdaanvrager

Initialen:	S.
Achternaam:	Roosing
Geslacht:	Mevrouw
Titulatuur:	Dr.
Naam universiteit/instituut:	Radboudumc

Mede-aanvragers(s)

Initialen:
Achternaam:
Geslacht: De heer / mevrouw
Titulatuur: Prof. / Dr. / MSc / anders …..
Naam universiteit/instituut:

Correspondentieadres

Huispost code:	Route 855
Postadres:	Geert groteplein zuid 10
Postcode en woonplaats:	6525 GX Nijmegen
IBAN:	nvt
Telefoon:	024-3655266
Email:	Susanne.Roosing@radboudumc.nl

Kruis aan bij welk fonds u uw aanvraag indient:

Algemene fondsen:

- [x] Algemene Nederlandse Vereniging ter Voorkoming van Blindheid
- [ ] Stichting Blinden-Penning
- [ ] Landelijke Stichting voor Blinden en Slechtzienden (LSBS)
- [ ] Stichting Oogfonds Nederland
- [ ] Vereniging Bartiméus Sonneheerdt
- [ ] Rotterdamse Stichting Blindenbelangen

Specifieke fondsen:

- [ ] Stichting Glaucoomfonds – glaucoom
- [x] Stichting Maculafonds – macula degeneratie
- [ ] Stichting Retina Nederland Fonds – retinale ziekten
- [ ] Dr. F.P. Fischer-Stichting – uitsluitend aanvragen vanuit universiteit/instituut te Utrecht

Kruis aan onder welk Panel Kwaliteit uw aanvraag valt (meerdere opties mogelijk)

Het Panel Kwaliteit bestaat uit enkele subgroepen van elk circa 3 leden. Eén van deze subgroepen gaat voor uw aanvraag de reviewers voorstellen en aanschrijven. Naar aanleiding van de reviews en het wederhoor zal deze subgroep uw aanvraag beoordelen. Deze ranking van de subgroep wordt verstrekt aan de fondsen.

☐ Cornea	☐ Cataract	☐ Diversen	☐ Diabetes	☐ Low Vision
☐ Refractiechirurgie	■ Genetica	☐ Glaucoom	☐ Neuro-ophthalmologie	
☐ Strabologie	☐ Orbita	■ Retina	☐ Oncologie	☐ Uveitis

By signing this form the applicant agrees with the definitions, preconditions and the procedure as being described on the website www.uitzicht.nl and in the file 'Call 2018.'

Place	**Date**	**Signature Applicant**
Nijmegen	***1 November 2017***	***Susanne Roosing***

VERTROUWELIJK

Wetenschappelijke Reviewers
Deze pagina wordt gezonden aan het Sub-Panel waar uw aanvraag onder valt dat besluit welke reviewers worden aangeschreven. Deze pagina wordt niet aan de reviewers gezonden.

Verplicht:
Hieronder dient u namen van 6 mogelijke reviewers (tenminste 3 van buiten Nederland) door te geven. Bij een Pilot-aanvraag namen van 5 mogelijke reviewers (ter keuze uit Nederland of daarbuiten).
- De voorgestelde reviewers mogen niet van hetzelfde instituut zijn als de aanvrager. - - Aanvragers mogen niet in de afgelopen 5 jaren met de voorgestelde reviewers hebben gepubliceerd.

De reviewers dienen zich in een zodanige positie te bevinden ten opzichte van de aanvrager dat zij een volledig onafhankelijke en objectieve beoordeling te kunnen geven.

Namen van reviewers die bij voorkeur WEL benaderd dienen te worden inclusief volledige titulatuur en mailadres:

1. Prof. dr.	Camiel J.F Boon,	C.J.F.Boon@lumc.nl
2. Prof. dr.	Stephen P. Daiger,	Stephen.P.Daiger@uth.tmc.edu
3. Dr.	Suzanne Yzer,	S.Yzer@oogziekenhuis.nl
4. Dr.	Kinga Bujakowska,	Kinga_Bujakowska@meei.harvard.edu
5. Prof. Dr.	Radha Ayyagari,	RAyyagari@ucsd.edu

Namen van reviewers die bij voorkeur NIET benaderd dienen te worden:
N.v.t.