Analysis of candidate genes for macular telangiectasia type 2

Nancy L. Parmalee,1,2 Carl Schubert,1 Joanna E. Merriam,1 Kaija Allikmets,1 Alan C. Bird,3 Mark C. Gillies,4 Tunde Peto,3 Maria Figueroa,5 Martin Friedlander,4 Marcus Fruttiger,7 John Greenwood,7 Stephen E. Moss,7 Lois E.H. Smith,8 Carmel Toones,9 Chris F. Inglehearn,9 Rando Allikmets3,10

1Department of Ophthalmology, Columbia University, New York, NY; 2Department of Genetics and Development, Columbia University, New York, NY; 3Moorfields Eye Hospital, London, UK; 4Save Sight Institute, Department of Clinical Ophthalmology and Eye Health, The University of Sydney, Sydney, Australia; 5The EMMES Corporation, Rockville, MD; 6Department of Cell Biology, The Scripps Research Institute, La Jolla, CA; 7Department of Cell Biology, University College London Institute of Ophthalmology, London, UK; 8Department of Ophthalmology, Harvard Medical School, Children’s Hospital Boston, Boston, MA; 9Section of Ophthalmology and Neuroscience, Leeds Institute of Molecular Medicine, St James’s University Hospital, Leeds, UK; 10Department of Pathology and Cell Biology, Columbia University, New York, NY

Purpose: To find the gene(s) responsible for macular telangiectasia type 2 (MacTel) by a candidate-gene screening approach.

Methods: Candidate genes were selected based on the following criteria: those known to cause or be associated with diseases with phenotypes similar to MacTel, genes with known function in the retinal vasculature or macular pigment transport, genes that emerged from expression microarray data from mouse models designed to mimic MacTel phenotype characteristics, and genes expressed in the retina that are also related to diabetes or hypertension, which have increased prevalence in MacTel patients. Probands from eight families with at least two affected individuals were screened by direct sequencing of 27 candidate genes. Identified nonsynonymous variants were analyzed to determine whether they cosegregate with the disease in families. Allele frequencies were determined by TaqMan analysis of the large MacTel and control cohorts.

Results: We identified 23 nonsynonymous variants in 27 candidate genes in at least one proband. Of these, eight were known single nucleotide polymorphisms (SNPs) with allele frequencies of >0.05; these variants were excluded from further analyses. Three previously unidentified missense variants, three missense variants with reported disease association, and five rare variants were analyzed for segregation and/or allele frequencies. No variant fulfilled the criteria of being causal for MacTel. A missense mutation, p.Pro33Ser in frizzled homolog (Drosophila) 4 (FZD4), previously suggested as a disease-causing variant in familial exudative vitreoretinopathy, was determined to be a rare benign polymorphism.

Conclusions: We have ruled out the exons and flanking intronic regions in 27 candidate genes as harboring causal mutations for MacTel.

Macular telangiectasia type 2 (MacTel) is a rare, adult onset retinal disease that results in tortuous and dilated retinal vessels, macular pigment changes, macular edema, and in some cases macular holes. It is also referred to in the literature as idiopathic juxtafoveal, or juxtafoveolar, telangiectasia. A classification system was introduced by Gass and Blodi [1] and updated in 2006 by Yannuzzi [2], distinguishing the features of three types of macular telangiectasias. MacTel, or type 2, is a bilateral disease that affects both genders, as opposed to type 1, which is often unilateral, with aneurism and exudates, and generally presents only in men. Type 3, characterized by occlusive telangiectasia, is very rare. The three forms of idiopathic macular telangiectasia are described together to distinguish and classify phenotypically similar pathologies; however, the findings and progression of the three are distinct, and it is believed that each arises from a distinct etiology.

In 2005, The MacTel Project—an international consortium of clinicians and basic science researchers—was established to study the cause, natural history, progression, and epidemiology of the disease, and to explore potential treatments. Publications from collaborators in the MacTel Project have further described the clinical findings, diagnostic methods, and epidemiology related to the disease [3-11]. In advanced cases, neovascularization may be present, arising from the intraretinal vascular plexus. While most reported cases are sporadic, affected sib pairs and affected pairs of monozygotic twins were described in the literature [12,13], leading to the hypothesis that MacTel had a genetic cause in a significant proportion of cases. Ophthalmic examination of relatives of MacTel patients revealed that many families had family members who experienced no vision loss, but did exhibit subtle signs of the disease [14]. Since vertical transmission is observed in several families, an autosomal
dominant model of Mendelian inheritance with reduced penetrance is assumed.

In conjunction with the MacTel Project, we have assembled a cohort of MacTel patients and their relatives for a genetic study. One mode of investigation in our studies has been to identify the disease-causing gene(s) by the direct sequencing of candidate genes in family probands, followed by segregation analysis of families. The current hypothesis is that a dominant causal mutation would be a rare heterozygous variant in all affected individuals. Allele frequencies were determined by screening cases and controls for unknown variants, and for known variants where population frequency data was unavailable.

We selected candidate genes that were known to be causative in diseases with phenotypic similarity to MacTel (e.g., familial exudative vitreoretinopathy [FEVR] and Norrie disease [15-19]), genes with a known molecular genetic role in retinal vascularization or macular pigment transport, and genes of functional interest that lay in regions of interest based on familial linkage studies. Twenty-seven genes were identified and screened based on these criteria.

**METHODS**

**Study population:** Participants were enrolled at 23 centers in seven countries (Australia, Germany, France, the UK, Israel, Switzerland, and the United States). Each center received approval from their governing human subjects review board. Informed consent was obtained in accordance with the Declaration of Helsinki. Records of informed consent and human subject approvals for all participating centers were centrally managed by the EMMES Corporation. Study subjects were enrolled based on a diagnosis of MacTel by the principal investigator at each center. Further criteria for enrollment were that patients be at least 18 years of age, be of European ancestry, and be free of diabetic retinopathy or other retinal disease [20]. Relatives of patients diagnosed with MacTel were actively recruited. Age- and ethnicity-matched controls were concurrently recruited—often a spouse or other unrelated individual present at the clinic appointment with the proband. At the time screening was performed, the MacTel cohort consisted of DNA from 200 unrelated probands diagnosed with MacTel. Since March 2010, study enrollment has been ongoing; a total of 735 samples have been sent to Columbia University’s Center for Human Genetics. Of those samples, 360 are unrelated cases, and the rest are mostly unaffected relatives. The youngest MacTel proband in the study was 25 years old at the time of enrollment, and the oldest was 85. The majority of probands were between 50 and 70 years of age when they were enrolled.

The families of the probands sequenced consisted of five affected sibling pairs (ASPs), two affected sibling trios, one affected parent and child duo, and additional relatives. Specifically, family 8 (Figure 1) consisted of 11 individuals, including an ASP, two additional unaffected siblings, one affected and one unaffected parent, two uncles, and three cousins. Family 9 consisted of an ASP and three unaffected and one possibly affected offspring of the siblings. Families 22, 30, and 81 were ASPs. Family 29 was an affected sibling trio plus six unaffected adult offspring of the siblings. Family 42 was a three-generation family consisting of an affected trio, an affected uncle, three unaffected individuals in the second generation, and three unaffected adult offspring in the third generation. Family 101 was a discordant sibling pair with one affected and one unaffected parent.

The Columbia University control cohort consisted of DNA samples from individuals recruited as controls for studies of age-related macular degeneration (AMD). Participants were matched by age and ethnicity to the AMD cohort, and were found free of macular disease at the time of recruitment, as previously described [21,22]. Briefly, controls underwent ophthalmic examination to screen for retinal disease. Stereo fundus photographs were evaluated using standard classification systems. Individuals accepted as controls had no family history of AMD, and were determined to be free of retinal disease. Three hundred and sixty-eight controls from the Columbia cohort were screened for this study. In addition, a cohort of 639 AMD patients was screened for selected variants. Data obtained from screening the AMD cohort was beneficial in that the patients in this cohort were of advanced age and had undergone thorough retinal examination, thereby ruling out MacTel and increasing the number of individuals classified as controls.

**Diagnosis of MacTel:** Participants were given a standardized ophthalmic examination, including best corrected visual acuity, fundus examination with photography, fluorescein angiography, optical coherence tomography, and blue light reflectance. Images were taken and sent to Moorfields Eye Hospital’s Reading Centre, in London, England, for evaluation. The criteria for diagnosis are described by Clemons et al. [20]. Diagnostic features of MacTel are based on the Gass and Blodi criteria [1], and include loss of transparency in the perifoveal region, dilated and telangiectatic blood vessels, especially in the temporal retina, and crystalline deposits. In cases where the adjudication made at the reading center was not in accordance with the diagnosis made at the recruiting center, the reading center diagnosis was used to code the sample for genetic studies. Each sample was assigned to one of four diagnostic categories: affected, possibly affected, probably not affected, or unaffected. Patients were reevaluated at regular intervals over the course of the study.

**Sequencing and genotyping:** DNA was isolated from whole blood by column purification (DNA Blood Maxi, 51194; Qiagen, Valencia, CA). Eight probands of families with more than one affected individual were screened by direct Sanger sequencing for mutations in 27 candidate genes. For each
gene, primers were designed to amplify each exon and flanking intronic sequences. Primer sequences are listed in Appendix 1. PCR reactions were performed with 2 ng of genomic DNA in a total volume of 25 μL, using 25 pmol each of forward and reverse primer, 200 μM dATP, dCTP, dGTP, dTTP, 2.5 mM MgCl$_2$, 1.5 U Taq Polymerase (Hot Fire DNA Polymerase, Solis Biodyne, Tartu, Estonia, or AmpliTaq Gold, Applied Biosystems, Carlsbad, CA), and 10× buffer supplied by the manufacturer. Thermocycling was performed using either the Stepdown protocol, or the Touchdown (68–55 °C) protocol. Stepdown: an initial 12 min denaturation step at 95 °C was followed by 12 cycles of 95 °C for 12 s, 65 °C for 20 s (with a 0.5 °C reduction in temperature for each cycle), and 72 °C for 55 s. This was followed by 30 cycles of 95 °C for 12 s, 50 °C for 20 s, and 72 °C for 55 s, with a final 7 min extension at 72 °C. Touchdown (68–55 °C): an initial 12 min denaturation step at 95 °C was followed by 26 cycles of 95 °C for 15 s, 68 °C for 20 s (with a 0.5 °C reduction in temperature for each cycle), and 72 °C for 45 s. This was followed by 15 cycles of 95 °C for 15 s, 55 °C for 20 s, and 72 °C for 45 s, with a final 7 min extension at 72 °C. Sequencing was performed by Genewiz (South Plainfield, NJ). One hundred and twelve familial samples were genotyped on the Illumina (Illumina, Inc., San Diego, CA) 1M chip for ongoing linkage studies (data not shown). These data were used to evaluate the segregation of variants detected by sequencing when the variant was a known single nucleotide polymorphism (SNP) that was genotyped on the chip.

Sequences were analyzed for known or unknown variants that differed from the reference sequence. Nonsynonymous coding variants were evaluated using the population frequencies published in the Single Nucleotide Polymorphism database (dbSNP). Unknown, infrequent, or disease-associated variants were evaluated to determine whether they co-segregated with the disease within the families in which they were discovered.

For known variants that were present in the Illumina 1M chipset, genotypes of relatives in the family were inspected to determine whether the variant co-segregated with the disease. For unknown variants, and for rare variants not included on the 1M chip, tests for co-segregation were performed by sequencing the family members of the proband in whom the variant was detected. For variants detected in a family with vertical transmission, where both parents were available, segregation was assessed based on whether the allele was inherited from the affected or the unaffected parent. For variants detected in affected sib pairs whose parents were not available, if the variant was present in both siblings, follow-up analysis was performed by TaqMan assay (Applied Biosystems, Foster City, CA) in the cohort of MacTel probands and in controls, to determine allele frequencies in these cohorts. Variants that were determined to be common polymorphisms based on population frequencies from dbSNP were not pursued further. In some cases, population frequencies were not available in dbSNP, yet the variant was highly polymorphic in the genotyped cohort and was present in both affected and unaffected individuals. Such variants were classified as frequent variants and not pursued further. Variants that merited further analysis either because co-segregation of the variant with the disease could not be ruled out, or because the allele detected was rare or had been associated with disease, were screened by TaqMan assay in...
the entire MacTel cohort, and in a cohort of controls. For selected variants, we also screened an available cohort of AMD patients to determine allele frequencies more precisely. Analyzing the large AMD cohort, in addition to the MacTel and control cohorts, provided additional allele frequency data for previously unknown variants and known variants where population frequency data was unavailable. Allele and genotype frequencies were compared between cases and controls with standard statistical tests, such as a 2x2 contingency table and Fisher’s exact text.

RESULTS
A summary of the screened genes grouped by selection criteria is shown in Table 1. Descriptions of the genes screened and the rationale for selecting candidate genes were as follows.

Genes involved in angiogenesis: One of the defining phenotypes of MacTel is the presence of telangiectatic blood vessels in the retina and, in advanced disease, intraretinal neovascularization. This form of neovascularization is prevalent in diabetic retinopathy and in retinopathy of prematurity (ROP), and represents about 10%–15% of neovascular changes in AMD [23-27]; the remaining 85%–90% of neovascularization in AMD involves aberrant vessels arising from the choroidal vasculature (CNV). The vasculature of the retina is highly specialized; thus, the specific location of the aberration is likely a result of highly tissue-specific molecular genetic interactions. We selected 11 genes related to angiogenesis (Table 1), including the Wnt receptor frizzled-4, its ligand, norrin (NDP), and the coreceptor, low-density lipoprotein receptor-related protein 5 (LRP5). Mutations in these genes have been implicated in FEVR, Norrie disease, and ROP [28-30]. Mouse knockout models of frizzled homolog 4 (Drosophila); (FZD4) [15] and NDP [31] show a lack of intraretinal vessel formation. Other genes involved in angiogenesis or vessel regulation that were screened are angiogenic factor with G patch and FHA domains 1 (AGGF1) [32], angiopoietin 1 (ANG1) [33], dickkopf homolog 1 (Xenopus laevis); (DKK1) [34], hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor); (HIF1A) [35], serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1 (PEDF) [36], thrombospondin 1 (THBS1) [37], tyrosine kinase, endothelial (TIE2) [38], and von Hippel-Lindau tumor suppressor (VHL) [39].

Genes involved in macular pigment transport: MacTel is also characterized by macular pigmentary changes, with advanced cases often lacking macular pigment altogether. Little is known about the molecular genetics of macular pigment transport in the retina. In the healthy retina, the two macular pigments, lutein and zeaxanthin, filter damaging blue light [40,41]. The proteins responsible for lutein transport are unknown. The macular pigment genes screened were glutathione S-transferase pi 1 (GSTPI), a binding protein for zeaxanthin [42], and scavenger receptor class B, member 1 (SCARBI), which has been proposed as a lipid transporter in the retina [43].

Genes identified from expression arrays: Several genes were screened that were identified as differentially expressed in mouse models intended to mimic some aspects of the MacTel phenotype (data not shown). From the top of this list, five genes were screened that were identified as also having possible functional relevance by playing a role in MacTel: apelin receptor (AGTRL1), apelin (APLN) [44], complement factor B (CFB) [45], leucine-rich alpha-2-glycoprotein 1 (LRG1), and plasmalemna vesicle associated protein (PLVAP) [46].

Genes identified from suggestive linkage regions: Linkage studies were performed using families in which at least one family member in addition to the proband was diagnosed as affected by MacTel. While these results will be reported separately, several genes of possible functional interest were identified in regions of suggestive linkage on chromosomes 1, 7, 10, and 12 during the course of analysis. Cerebral cavernous malformation 2 (CCM2) [47], insulin-like growth factor binding protein 3 (IGFBP3) [48], sarcospan (Kras oncogene-associated gene); (SSPN) [49], and transforming growth factor, beta 2 (TGF2B) [50] were selected and screened as candidates under these criteria. Each of these genes has been proposed to be involved in angiogenesis or regulation of blood vessels; IGFBP3 and TGF2B2 have also been proposed as genes related to diabetes. Linkage studies are ongoing as additional families are recruited.

Genes involved in diseases with related phenotypes: An increased prevalence of hypertension and diabetes are found in MacTel patients [51]. Genes involved in these diseases, which are also expressed in the retina, especially in the vasculature, were considered as candidates. Succinate receptor 1 (SUCNR1) [52], angiotensin II receptor, type 1 (AGTR1) [53], aldehyde dehydrogenase 3 family, member A2 (ALDH3A2) [54,55], very low density lipoprotein receptor (VLDLR) [56], and oxoglutamate (alpha-ketoglutarate) receptor 1 (OXGR1) were screened on this basis, in conjunction with linkage or expression array data, or personal communication with collaborators.

Table 1 summarizes the variants detected by the complete sequencing of all exons and adjacent intronic sequences in 27 candidate genes in eight MacTel probands. In total, we discovered three unknown and 20 known missense changes, and 22 synonymous and 61 intronic variants. Frequent variants with reported minor allele frequencies (MAFs) over 0.10 were not analyzed further unless the variant was reported to be disease-associated (PEDF pThr72Met and GSTP1 p.Ile105Val were screened in cases and controls, though they are frequent variants). Variants with published population frequencies between 5%–10% were assessed for further...
Table 1. Genes sequenced and missense variants detected by Sanger sequencing in 8 macular telangiectasia probands.

| Gene/ category | Variant | rs number | MAF MacTel cases (400 chromosomes) | MAF Controls (736 chromosomes) | MAF dbSNP | Notes |
|----------------|---------|-----------|-------------------------------------|--------------------------------|-----------|-------|
| Vascular/angiogenic | AGGF1 | p.Pro698Thr | rs34400049 | NS | NS | 0.28 | FV, DNS |
| | ANG1 | p.Thr257Arg | - | 0.005 (1/400) | 0 (0/736) | - | DNS |
| | DKK1 | None | | | | | |
| | FZD4 | p.Pro33Ser | rs61735304 | 0.02 (6/400)/0.03 (17/1278) | 0.01 (13/736) | ND | DNS, MT, AMD |
| | | p.Pro168Ser | - | NS | NS | - | |
| | HIF1A | None | | | | | |
| | LRP5 | p.Val667Met | rs4988321 | 0.07 (28/400) | 0.06 (41/736) | 0.03 | MT, DNS |
| | | p.Gln1192Arg | rs3736228 | NS | NS | 0.12 | DNS, FV |
| | FZD4 | None | | | | | |
| | LRP5 | p.Gln1192Arg | rs3736228 | NS | NS | 0.12 | DNS, FV |
| | NDP | None | | | | | |
| | PEDF | p.Thr72Met | rs1136287 | 0.37 (148/400)/0.34 (33/1278) | 0.33 (245/736) | 0.36 | FV, MT, AMD |
| | THBS1 | p.Thr523Ala | rs2292305 | NS | NS | 0.1 | FV, DNS |
| | TIE2 | p.Val486Ile | rs1334811 | NS | NS | 0.03 | DNS |
| | | p.Val600Leu | rs35030851 | NS | NS | 0.04 | DNS |
| | VHL | None | | | | | |
| Expression microarray | AGTR1 | None | | | | | |
| | APLN | None | | | | | |
| | CFB | p.Leu9His | rs4151667 | 0.04 (16/400)/0.02 (23/1278) | 0.04 (29/736) | 0.07 | DNS, MT, AMD |
| | | p.Arg32Gln | rs641153 | 0.10 (40/400)/0.04 (51/1278) | 0.12 (88/736) | 0.1 | DNS, MT, AMD |
| | | p.Gly252Ser | rs4151651 | - | 0.04 (23/1278) | 0.04 (29/736) | 0.04 | DNS, AMD, LD |
| | | p.Lys533Arg | - | NS | NS | 0.35 | DNS, FV |
| | LRG1 | p.Pro133Ser | rs966384 | NS | NS | | |
| | PLVAP | None | | | | | |
| Macular pigment | GSTP1 | p.Ile105Val | rs1695 | 0.33 (132/400)/0.30 (383/1278) | 0.3 | 0.39 | MT, AMD, FV |
| | | p.Ala114Val | rs1138272 | 0.08 (32/400)/0.07 (87/1278) | 0.06 (46/736) | 0.12 | MT, AMD, FV |
| | SCARB1 | p.Val135Ile | rs5891 | 0.03 (12/400) | 0.02 (15/736) | 0.01 | MT DNS |
| | | p.Pro376Leu | rs74830677 | 0.01 (3/400) | 0.01 (5/736) | 0.05 | MT, DNS |
| Genes under suggestive linkage peaks | CCM2 | p.Val120Ile | rs11552377 | NS | NS | 0.17 | FV, DNS |
| | IGFBP3 | None | | | | | |
| | SSPN | None | | | | | |
| | TGFβ2 | None | | | | | |
| Disease related genes | AGTR1 | p.Ala244Ser | rs12721225 | NS | NS | 0.03 | DNS |
| | ALDH3A2 | None | | | | | |
| | OXGR1 | p.Thr205Ala | - | 0.005 (1/400) | 0 (0/736) | - | DNS |
| | SUCNR1 | None | | | | | |
| | VLDLR | None | | | | | |

NS represents not screened, ND represents not determined, FV represents frequent variant, DNS represents Does not segregate with disease, MT represents Screened in MacTel cases and controls, AMD represents Screened in AMD cohort. LD represents the CFB p.Lys533Arg variant is in complete linkage disequilibrium with p.Leu9His. No comparison between MacTel cases and controls reached statistical significance.
screening, based on whether the variant had been reported to be associated with any diseases with phenotypes similar to that of MacTel. Twelve missense variants were screened by TaqMan assay (Table 1) in MacTel cases and unaffected controls; six of these variants were also screened in a large AMD cohort. Of the variants detected, three were unknown (ANG1 p.Thr257Arg, LRP5 p.Gln192Arg, and SCARB1 p.Ile135Val), three had been reported as possibly disease-associated (FZD4 p.Pro33Ser, GSTP1 p.Val105Ile, and PEDF p.Thr73Met). The remainder had low reported MAFs. None of the variants found by sequencing segregated with the disease. Of the variants screened by TaqMan assay, only GSTP1 p.Val105Ile showed a trend toward a statistically significant frequency difference between cases and controls (p=0.09), suggesting it could be a possible modifier, but not a causal gene for MacTel.

The FZD4 variants p.Pro33Ser and p.Pro168Ser were detected in the proband III2 (family 8, Figure 1). We sequenced all members of family 8 and found both the p.Pro33Ser and p.Pro168Ser variants present in two affected daughters, one unaffected daughter, and one unaffected cousin of the proband, indicating that the complex allele containing both mutations did not segregate with the disease (Figure 1). The p.Pro33Ser variant was analyzed by TaqMan assay in 200 MacTel cases, 368 unrelated controls, and 639 AMD cases to determine allele frequencies. This variant was found in one other unrelated MacTel proband (AS). The p.Pro168Ser mutation was also present in each individual carrying p.Pro33Ser. Thirteen controls were heterozygous for p.Pro33Ser (MAF=0.018). In 639 unrelated AMD samples, 16 heterozygotes and one homozygote for p.Pro33Ser were detected (MAF=0.013). In conclusion, there was no statistically significant difference in allele frequencies between cases and controls.

**DISCUSSION**

We have shown that the FZD4 p.Pro33Ser /p.Pro168Ser complex allele, which has been reported as causative in FEVR and ROP [57,58], is present in 2% of unaffected controls, and therefore is not a disease-causing variant in MacTel, FEVR, or ROP. The same allele was detected in one MacTel patient, prompting the hypothesis that FEVR and MacTel may be allelic diseases. Given that FEVR has phenotypic similarities to MacTel, in that the intraretinal vascular plexus is perturbed in both diseases, dysregulation of FZD4 was a plausible hypothesis in the etiology of MacTel. Both FEVR and MacTel also exhibit variable expressivity [59]. In both diseases, affected family members are often unaware that they are affected until a diagnosis is made after thorough examination. Segregation analysis in one MacTel family and case-control association analysis using a large cohort of controls revealed that p.Pro33Ser /p.Pro168Ser, which had been reported as a disease causing mutation, rather, is a benign polymorphism present at a low frequency in the general population. Accordingly, we conclude that it is not causative in either MacTel or FEVR, because our control cohort had no documented retinal disease. This result highlights the importance of segregation analysis in families, and of screening a sufficient number of controls to distinguish between causal mutations and rare benign polymorphisms.

Candidate gene screening is a widely used method for detecting disease-associated variants and genes. While often criticized as a “needle in a haystack” approach, it has been successful in determining some disease-associated genes, most notably the major AMD-associated genes, CFH and CFB [21,22]. In this study, 27 possible candidate genes for MacTel were selected based on a combined set of criteria. The exons and flanking intronic regions of all genes were screened by direct sequencing, with follow-up segregation analysis in families and TaqMan genotyping in large cohorts for rare, unknown, or previously disease-associated missense variants. No variants segregated with the disease, and none showed significant association with MacTel, allowing exclusion of the coding regions of these genes as harboring a causal mutation for MacTel. The causal gene(s) for MacTel are currently being searched for by a combination of linkage analyses and whole-genome sequencing.

**ACKNOWLEDGMENTS**

The authors are very thankful to collaborators in the entire project, including the coordinating center, the reading center, clinical enrollment sites, and basic science laboratories. For more information on the MacTel Project, and for a list of collaborators and enrollment centers, please see the MacTel Project website. The authors also wish to express our gratitude to the Lowy Medical Research Foundation for their generous support in making this work possible. We offer our deepest thanks to the macular telangiectasia patients and their families for choosing to participate in this work.

**REFERENCES**

1. Gass JD, Blodi BA. Idiopathic juxtafoveal retinal telangiectasis. Update of classification and follow-up study. Ophthalmology 1993; 100:1536-46. [PMID: 8414413]
2. Yannuzzi LA, Bardal AM, Freund KB, Chen KJ, Eandi CM, Blodi B. Idiopathic macular telangiectasia. Arch Ophthalmol 2006; 124:450-60. [PMID: 16606869]
3. Charbel Issa P, Finger RP, Helb HM, Holz FG, Scholl HP. A new diagnostic approach in patients with type 2 macular telangiectasia: confocal reflectance imaging. Acta Ophthalmol (Copenh) 2008; 86:464-5.
4. Charbel Issa P, Berendschot TT, Staurenghi G, Holz FG, Scholl HP. Confocal blue reflectance imaging in type 2 idiopathic macular telangiectasia. Invest Ophthalmol Vis Sci 2008; 49:1172-7. [PMID: 18326746]
5. Charbel Issa P, van der Veen RL, Stijns A, Holz FG, Scholl HP, Berendschot TT. Quantification of reduced macular pigment optical density in the central retina in macular telangiectasia type 2. Exp Eye Res 2009; 89:25-31. [PMID: 19233170]
6. Aung KZ, Wickremasinghe SS, Makeyeva G, Robman L, Guymer RH. The prevalence estimates of macular
telangiectasia type 2: the Melbourne Collaborative Cohort Study. Retina 2010; 30:473-8. [PMID: 19952995]

7. Chew E, Gillies M, Bird A. Macular telangiectasia: a simplified classification. Arch Ophthalmol 2006; 124:573-4. [PMID: 16606887]

8. Charbel Issa P, Holz FG, Scholl HP. Metamorphopsia in patients with macular telangiectasia type 2. Doc Ophthalmol 2009; 119:133-40. [PMID: 19711108]

9. Finger RP, Charbel Issa P, Fimmers R, Holz FG, Rubin GS, Scholl HP. Reading performance is reduced by parafoveal scotomas in patients with macular telangiectasia type 2. Invest Ophthalmol Vis Sci 2009; 50:1366-70. [PMID: 18997085]

10. Charbel Issa P, Scholl HP, Gaudric A, Massin P, Kreiger AE, Schwartz S, Holz FG. Macular full-thickness and lamellar holes in association with type 2 idiopathic macular telangiectasia. Eye (Lond) 2009; 23:435-41. [PMID: 18259211]

11. Charbel Issa P, Helb HM, Rohrschneider K, Holz FG, Scholl HP. Microperimetric assessment of patients with type 2 idiopathic macular telangiectasia. Invest Ophthalmol Vis Sci 2007; 48:3788-95. [PMID: 17652753]

12. Hannan SR, Madhusudhana KC, Rennie C,洛try AJ. Idiopathic juxtapfoveal retinal telangiectasia in monozygotic twins. Br J Ophthalmol 2007; 91:1729-30. [PMID: 18024833]

13. Menchini U, Virgili G, Bandello F, Malaro C, Rapizzi E, Lanzetta P. Bilateral juxtapfoveal telangiectasia in monozygotic twins. Am J Ophthalmol 2000; 129:401-3. [PMID: 10704569]

14. Gillies MC, Zhu M, Chew E, Barthelmes D, Hughes E, Ali H, Holz FG, Scholl HP, Charbel Issa P. Familial asymptomatic macular telangiectasia type 2. Ophthalmology 2009; 116:2422-9. [PMID: 19815294]

15. Xu Q, Wang Y, Dabdoub A, Massin P, Kreiger AE, Schwartz S, Holz FG. Macular full-thickness and lamellar holes in association with type 2 idiopathic macular telangiectasia. Eye (Lond) 2009; 23:435-41. [PMID: 18259211]

16. Charbel Issa P, Helb HM, Rohrschneider K, Holz FG, Scholl HP. Microperimetric assessment of patients with type 2 idiopathic macular telangiectasia. Invest Ophthalmol Vis Sci 2007; 48:3788-95. [PMID: 17652753]

17. Hannan SR, Madhusudhana KC, Rennie C,洛try AJ. Idiopathic juxtapfoveal retinal telangiectasia in monozygotic twins. Br J Ophthalmol 2007; 91:1729-30. [PMID: 18024833]

18. Menchini U, Virgili G, Bandello F, Malaro C, Rapizzi E, Lanzetta P. Bilateral juxtapfoveal telangiectasia in monozygotic twins. Am J Ophthalmol 2000; 129:401-3. [PMID: 10704569]

19. Gillies MC, Zhu M, Chew E, Barthelmes D, Hughes E, Ali H, Holz FG, Scholl HP, Charbel Issa P. Familial asymptomatic macular telangiectasia type 2. Ophthalmology 2009; 116:2422-9. [PMID: 19815294]

20. Xu Q, Wang Y, Dabdoub A, Smallwood PM, Williams J, Woods C, Kelley MW, Jiang L, Tasman W, Zhang K, Nathans J. Vascular development in the retina and inner ear: control by Norrin and Frizzled-4, a high-affinity ligand-receptor pair. Cell 2004; 116:883-95. [PMID: 15035898]

21. Kondo H, Hayashi H, Oshima K, Tahira T, Hayashi K. Frizzled 4 gene (FZD4) mutations in patients with familial exudative vitreoretinopathy with variable expressivity. Br J Ophthalmol 2003; 87:1291-5. [PMID: 14507768]

22. Warden SM, Andreoli CM, Mukai S. The Wnt signaling pathway in familial exudative vitreoretinopathy and Norrie disease. Semin Ophthalmol 2007; 22:211-7. [PMID: 18097984]

23. Tasman W, Augsburger JJ, Shields JA, Caputo A, Annesley WH Jr. Familial exudative vitreoretinopathy. Trans Am Ophthalmol Soc 1981; 79:211-26. [PMID: 7342402]

24. Chen ZY, Battinelli EM, Fielder A, Bundey S, Sims K, Breaksfield XO, Craig IW. A mutation in the Norrie disease gene (NDP) associated with X-linked familial exudative vitreoretinopathy. Nat Genet 1993; 5:180-3. [PMID: 8252044]

25. Clemens TE, Gillies MC, Chew EY, Bird AC, Peto T, Figueroa MJ, Harrington MW, MacTel Research Group. Baseline characteristics of participants in the natural history study of macular telangiectasia (MacTel) MacTel Project Report No. 2. Ophthalmic Epidemiol 2010; 17:66-73. [PMID: 20100102]

26. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM, Smith RJ, Silvestri G, Russell SR, Klaver CC, Barbazzotto I, Chang S, Yannuzzi LA, Barile GR, Merriam JC, Smith RT, Olsh AK, Bergeron J, Zernant J, Merriam JE, Gold B, Dean M, Allikmets R. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA 2005; 102:7227-32. [PMID: 15870199]

27. Gold B, Merriam JE, Zernant J, Hancox LS, Taiber AJ, Gehrs K, Cramer K, Neel J, Bergeron J, Barile GR, Smith RT, Hageman GS, Dean M, Allikmets R. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. Nat Genet 2006; 38:458-62. [PMID: 16518403]

28. Yannuzzi LA, Negaro S, Iida T, Carvalho C, Rodriguez-Coleman H, Slakter J, Freund KB, Sorenson J, Orlock D, Brodorok N. Retinal angiomatous proliferation in age-related macular degeneration. Retina 2001; 21:416-34. [PMID: 11642370]

29. Rajappa M, Saxena P, Kaur J. Ocular angiogenesis: mechanisms and recent advances in therapy. Adv Clin Chem 2010; 50:103-21. [PMID: 20521443]

30. Heidary G, Vanderveen D, Smith LE. Retinopathy of prematurity: current concepts in molecular pathogenesis. Semin Ophthalmol 2009; 24:77-81. [PMID: 19373690]

31. Bradley J, Ju M, Robinson GS. Combination therapy for the treatment of ocular neovascularization. Angiogenesis 2007; 10:141-8. [PMID: 17372853]

32. Polioa A, Napolitano MC, Bandello F, Chiodini RG. The role of optical coherence tomography (OCT) in the diagnosis and management of retinal angiomatous proliferation (RAP) in patients with age-related macular degeneration. Ann Acad Med Singapore 2006; 35:420-4. [PMID: 16865194]

33. Hendrickx M, Leyns L. Non-conventional Frizzled ligands and Wnt receptors. Dev Growth Differ 2008; 50:229-43. [PMID: 18366384]

34. Nikopoulos K, Venselaar H, Collin RW, Rivero-Alvarez R, Boonstra FN, Hooymans JM, Mukhopadhyay A, Shears D, van Bers M, de Wijs J, van Essen AJ, Sjimons RH, Tilanus MA, van Nouhuys CE, Ayuso C, Hoefsloot LH, Cremers FP. Overview of the mutation spectrum in familial exudative vitreoretinopathy and Norrie disease with identification of 21 novel variants in FZD4, LRP5, and NDP. Hum Mutat 2010; 31:656-66. [PMID: 20340138]

35. Els A, Guernsey DL, Wallace K, Zheng B, Vincer M, Allen A, Ingram A, DaSilva O, Siebert L, Sheidow T, Beis J, Robitaille JM, Seeliger MW, Hammes HP, Berger W. Role of the Norrie disease pseudoglioma gene in sprouting angiogenesis during development of the retinal vasculature. Invest Ophthalmol Vis Sci 2005; 46:3372-82. [PMID: 16123442]

36. Brod RD, Shields JA, Shields CL, Oberkircher OR, Sabol LJ. Unusual retinal and renal vascular lesions in the Klippel-Trenaunay-Weber syndrome. Retina 1992; 12:355-8. [PMID: 13366161]
33. Nambu H, Nambu R, Oshima Y, Hackett SF, Okoye G, Wiegand S, Yancopoulos G, Zack DJ, Campochiaro PA. Angiopoietin 1 inhibits ocular neovascularization and breakdown of the blood-retinal barrier. Gene Ther 2004; 11:865-73. [PMID: 15402118]

34. Fedi P, Bafico A, Nieto Soria A, Burgess WH, Miki T, Bottaro DP, Kraus MH, Aaronson SA. Isolation and biochemical characterization of the human Dkk-1 homologue, a novel inhibitor of mammalian Wnt signaling. J Biol Chem 1999; 274:19465-72. [PMID: 10383463]

35. Hughes JM, Groot AJ, van der Groep P, Sersansie R, Vooijs M, van Diest PJ, Van Noorden CJ, Schlingemann RO, Klaassen I. Active HIF-1 in the normal human retina. J Histochem Cytochem 2010; 58:247-54. [PMID: 19901273]

36. Kozulin P, Natoli R, O'Brien KM, Madigan MC, Provis JM. Differential expression of anti-angiogenic factors and guidance genes in the developing macula. Mol Vis 2009; 15:45-59. [PMID: 19145251]

37. Sun J, Hopkins BD, Tsuikawa K, Perruzzi C, Adini I, Swerlick RA, Ko EA, Garewal H, Hageman GS. Angiopoietin 2 expression in the retina: upregulation during physiologic and pathologic neovascularization. J Cell Physiol 2000; 184:275-84. [PMID: 10911358]

38. Hackett SF, Ozaki H, Strauss RW, Wahlin K, Suri C, Maisonneuve P, Yancopoulos G, Campochiaro PA. Angiopoietin 2 expression in the retina: upregulation during physiologic and pathologic neovascularization. J Cell Physiol 2000; 26:1-10. [PMID: 11361022]

39. Loane E, Nolan JM, O'Donovan O, Bhosale P, Bernstein PS, Curletti CR, Hancox LS, Hu J, Ebrigton JN, Malek G, Hauser FA, Rickman CB, Bok D, Hageman GS, Johnson LV. The pivotal role of the complement system in age and age-related macular degeneration: hypothesis re-visited. Prog Retin Eye Res 2010; 29:95-112. [PMID: 19961953]

40. Carson-Walter EB, Hampton J, Shue E, Geynisman DM, Pillai PK, Sathanoori R, Maddren SL, Hamilton RL, Walter KA. Plasmalemmal vesicle associated protein-1 is a novel marker implicated in brain tumor angiogenesis. Clin Cancer Res 2005; 11:743-50. [PMID: 16278383]

41. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. Arch Biochem Biophys 2001; 385:28-40. [PMID: 11788591]

42. Loane E, Nolan JM, O'Donovan O, Bhosale P, Bernstein PS, Curletti CR, Hancox LS, Hu J, Ebrigton JN, Malek G, Hauser FA, Rickman CB, Bok D, Hageman GS, Johnson LV. The pivotal role of the complement system in age and age-related macular degeneration: hypothesis re-visited. Prog Retin Eye Res 2010; 29:95-112. [PMID: 19961953]

43. Carson-Walter EB, Hampton J, Shue E, Geynisman DM, Pillai PK, Sathanoori R, Maddren SL, Hamilton RL, Walter KA. Plasmalemmal vesicle associated protein-1 is a novel marker implicated in brain tumor angiogenesis. Clin Cancer Res 2005; 11:743-50. [PMID: 16278383]

44. Kunduzova O, Alet N, Delesque-Touchard N, Millet L, Castan-
Appendix 1. Primer sequences of candidate genes sequenced (5′−3′).

To access the table, click or select the words “Appendix 1.” This will initiate the download of a pdf archive that contains the table.