Effect of an Intravitreal Antisense Oligonucleotide on Vision in Leber Congenital Amaurosis due to a Photoreceptor Cilium Defect

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Photoreceptor ciliopathies constitute the most common molecular mechanism of the childhood blindness Leber congenital amaurosis (LCA). Ten LCA patients carrying the c.2991+1655A>G allele in the ciliopathy gene *CEP290* (Centrosomal protein 290) were treated (NCT03140969) with intravitreal injections of an antisense oligonucleotide to restore correct splicing. There were no serious adverse events and vision improved at 3 months. The visual acuity of one exceptional responder improved from light perception to 20/400.

Leber congenital amaurosis (LCA) is a childhood blindness with severe vision loss and progressive degeneration of rod and cone photoreceptors. The first breakthrough in therapy for LCA was in the form caused by bi-allelic *RPE65* mutations[1] where the primary defect is in retinal pigment epithelium (RPE) cells[2]. The more common molecular mechanisms of LCA involve a primary ciliopathy of rod and cone photoreceptors. A deep intronic allele (c.2991+1655A>G) is a frequent cause of LCA ciliopathies due to *CEP290* (Centrosomal protein 290) mutations[3]. This allele results in a classic splicing defect, creating a premature truncation codon p.(Cys998*), likely subjecting the transcript to nonsense-mediated decay. Most patients lose all rod photoreceptors[4] but retain a central island of poorly functioning cone photoreceptors (Fig.1a) over many decades[5], thereby creating an opportunity for therapy.

An antisense oligonucleotide (AON) was designed to restore correct splicing in the retina[6] (Fig.1b), and we are assessing its safety and tolerability in a clinical trial involving intravitreal injections (ClinicalTrials.gov number: NCT03140969).

Substantial improvement in vision in one patient prompted the decision to perform interim analyses of all data. Ten subjects were injected at least once and up to four times (Extended Data Table 1, Supplementary Table 1); eight subjects had at least 3 months and four subjects had at least 6 months of follow up after the first injection. There were no severe adverse events and no events met stopping criteria. There was no intraocular inflammation: ocular treatment emergent adverse events were mild to none (Supplementary Information and Supplementary Tables 2 and 3). There were no retinal changes apparent during the three months after the first injection on cross-sectional (Extended Data Fig.1) or en face imaging (Extended Data Fig. 2).

Visual acuity is a standard method to evaluate efficacy. Baseline visual acuities ranged from 1.1 $\log_{10}$ MAR to light perception (LP) in study eyes, and 0.7 $\log_{10}$ MAR to LP in untreated contralateral eyes (Extended Data Table 1). After one month, there were no changes in visual acuity; at three months, one patient had a large (2.7 $\log_{10}$ MAR) improvement and four other patients had smaller improvements from baseline that were equal or greater than the 0.3 $\log_{10}$ MAR commonly considered as clinically meaningful (Fig.1d). Interocular comparison at baseline showed treated eyes to be 0.12 $\log_{10}$ MAR (6 letters) worse than untreated eyes; by three months after intervention, however, interocular asymmetry reversed and treated eyes were 0.54 $\log_{10}$ MAR (26 letters) better than untreated eyes (Extended Data Fig.3c). Statistical analysis showed a significant effect at three months after treatment. At three months, subjects received a second injection (Extended Data Table 1). Six subjects had data to months 4 and 5 and four subjects to month 6. Improvements over baseline were retained at six months (Extended Data Fig.3).

LCA patients tend to show oculomotor instability ranging from fine nystagmus to large amplitude 'wandering' eye movements[5]. Consistent with some clinical observations, imaging of the eyes at three months showed a tendency towards improved ocular stability of treated eyes when presented with a fixation light but not when in a darkened room without fixation (Extended Data Fig.4). The average improvement was 0.13 log mm at three months and grew to 0.27 log mm at six months. The six month time point showed a significant effect.

To better quantify changes in photoreceptor function due to intervention, the intensity of dimmest lights detected in the dark were evaluated with full-field stimulus testing (FST). Before intervention, 7 of 8 patients demonstrated light thresholds ranging from -2.2 to 3.3 $\log_{10}$ cd.m^{-2} for red, and -2.5 to 2.3 $\log_{10}$ cd.m^{-2} for blue flashes (Extended Data Fig.5). Chromatic differences were consistent with detection by cone photoreceptors at baseline in all patients but P7 who had function mediated by rod photoreceptors. By two and three months, both red and blue thresholds showed improvements in many treated eyes (Fig.1e,f). At baseline, there was symmetry between the eyes with interocular differences averaging less than 0.02 $\log_{10}$. After the injections, an interocular asymmetry developed by three months favoring better thresholds in treated eyes (-0.37±0.72 $\log_{10}$ for red, -0.82±0.83 $\log_{10}$ for blue, respectively; Extended Data Fig.5e,f). Statistical analysis showed a significant effect at months 1, 2, 3 and 6 (Extended Data Fig.5).

CEP290 ciliopathy is well known to affect the anatomy of photoreceptors[4,5]. Changes to photoreceptor cilial anatomy were studied with cross-sectional images from a subset of patients with analyzable data at the fovea (Extended Data Fig.6). P2 had foveal atrophy and evidence on microperimetry for fixation located in the

temporal parafovea of the treated eye. P4 and P7 showed an apparent increase in the reflection originating near the junction between the inner and outer segments (Extended Data Fig.6c) and P7 showed lengthening of inner and outer segments (Extended Data Fig.6e). Such findings were not seen in the untreated eyes (Extended Data Fig.6b,d,f).

Functional vision was assayed with a multi-luminance mobility course. Mobility scores showed a tendency for improvement at two and three months but changes were mostly symmetric between the eyes and there was no significant effect (Extended Data Fig.7).

Patient P2 was an exceptional responder who first reported substantial visual improvements 6 weeks after treatment. These findings led to a series of additional research studies. One year previously, P2 had visual acuities of LP in both eyes. At baseline, vision in both eyes remained LP (Fig.2a). The standard ETDRS letter acuity chart at 1m, and all the Berkeley Rudimentary Vision Test cards at 1 and 0.25 m were not seen by either eye. At 1 month after the 160 µg dose, visual acuities remained at LP (Fig.2a). At 6 weeks, the patient self-reported that, for the first time in decades, lights were seen with increasing clarity and brightness, but only in the treated eye.

At month 2, the patient could read the first three lines of the standard ETDRS chart at 1 m with the treated eye (corresponding to a visual acuity of 1.46 $\log_{10}$ MAR or Snellen equivalent of 20/580) but could not distinguish any letters with the untreated eye which remained LP (Fig.2a). Over the next 4 months, including an intervening maintenance dose of 80 µg after the 3-month visit, there was incremental increase in acuity to 1.28 $\log_{10}$ MAR (Fig.2a). To better localize the retinal origin of

129 the improved acuity, we used a modified microperimeter and stimulated the macula
130 directly. With the treated eye at the 2-month visit, the patient was able to distinguish
131 orientation of achromatic gratings at 1.37 $\log_{10}$ MAR similar to the standard ETDRS
132 results supporting a macular origin for improved acuity (Fig.2a). Chromatic gratings
133 were used to distinguish between photoreceptor types mediating acuity. With red
134 stimuli, the patient was able to distinguish orientation of gratings at 1.50 $\log_{10}$ MAR,
135 whereas with blue stimuli he could only see gratings at 1.97 $\log_{10}$ MAR (Fig.2b).
136 Between 3 and 6 months, chromatic acuities improved further (Fig.2b).
137 We used FST to evaluate detection of chromatic stimuli pre- and post-
138 treatment in each eye under dark- and light-adapted conditions (Fig.2c-f). Results
139 indicated mediation by cone photoreceptors under both conditions at all visits, and in
140 both eyes. Thresholds remained without change from baseline in the untreated eye,
141 for all visits, all stimuli and under different adaptation conditions. In the treated eye,
142 however, there was an improvement in thresholds post-treatment compared to
143 baseline. Threshold changes at 6 months were 0.71 and 0.55 $\log_{10}$ units for red
144 FSTs under dark- and light-adapted conditions, respectively, and 1.21 and 0.77 $\log_{10}$
145 units for blue FSTs under dark- and light-adapted conditions, respectively. Statistical
146 analysis showed a significant effect at all post-treatment visits for dark-adapted
147 conditions (Fig.2c,d) and all visits except for month 1 for light-adapted conditions
148 (Fig.2e,f). Importantly, the large improvements of P2 were not the sole driver of the
149 significance of the clinical trial cohort. Removing P2 from the analyses did not
150 change the main statistical conclusion supporting significant improvements of visual
151 acuity and FST at 3 months (Extended Data Table 2).

Advancing from an era of identifying causative genes in LCA, we are now using this information to design molecular-based therapies for these otherwise incurable forms of blindness. We now report that a primary photoreceptor ciliopathy can show improvement in vision using an AON therapy targeting pre-mRNA splicing. The improvement is noticeable to the patients and quantifiable with a number of outcome measures. Many questions remain as to the longevity of the efficacy and the value and safety of further dosage, but this evidence of positive visual change is a large translational step to the clinic for a childhood blindness with a wide window of therapeutic opportunity[5].

Methods

Methods, including statements of data availability and any associated accession codes and references, are available online.

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Author contributions

A.V.C. and S.G.J. contributed to the clinical study design and protocol development, performed clinical investigation of patients, reviewed, analyzed and interpreted the data and wrote the draft manuscript and its revisions; M.T., M.R.S., P.B., W.dW., P.A, D.M.R., G.P., and M.D.T. developed the clinical study protocol, reviewed the data and contributed to all drafts of the manuscript; A.V.D., B.P.L., and S.R.R. performed the clinical investigation of patients and contributed to the clinical study design and protocol development, and contributed to all drafts of the manuscript; A.C.H., F.N., and S.R.R. performed the injections; J.C., A.V.G., A.J.R., A.S., I.C.H., M.D.H., W.P., E.H.S., I.B., and C.V.C. supported clinical investigation of the patients;

A.J.R. performed the statistical analyses; P.B., P.A. and M.E.C. performed in vitro experiments determining clinical dosing strategy.

Competing interests

M.T., M.R.S., P.B., W.dW., P.A., D.M.R., G.P., and M.D.T. are employees and stock holders of ProQR Therapeutics. M.E.C. was a consultant for ProQR Therapeutics.

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Figure Legends

Fig. 1 | Photoreceptor ciliopathy caused by c.2991+1655A>G allele in the *CEP290* gene and its treatment with antisense oligonucleotide QR-110 injected intravitreally. **a**, Boundaries of the retained central elliptical islands in 20 patients with this allele. **b,** Schematic for the mechanism of action of QR-110. Without treatment (left), the mutation creates a strong splice donor site, and aberrant splicing results in the insertion of a cryptic Exon X in many *CEP290* mRNA transcripts. Exon X contains a premature stop codon, predicted to result in an inactive, truncated CEP290 protein, and/or to target the mutant mRNA transcript for nonsense mediated decay, significantly lowering the levels of wild-type CEP290 protein. With treatment (right) QR-110 binds to the pre-mRNA and blocks aberrant splicing, thereby skipping Exon X in mRNA, resulting in increased levels of wild-type transcripts and CEP290 protein. **c**, Injection into the vitreous humor. **d-f**, Change in $\log_{10}$ units from baseline of visual acuity and full-field stimulus testing (FST) using red and blue flashes presented in the dark. All three measures of visual function show significant improvements in treated eyes at 3 months. Larger symbols are averages from 10 patients at BL and M1, and 8 patients at M2 and M3. Smaller symbols are individual data points. Error bars=±1 sd, BL=average of two pre-treatment baselines, M1-M3=post-treatment evaluations at months 1-3 after the administration of an intravitreal dose of 160 or 320 µg after BL. Linear mixed-effects models were used for the statistical analysis.

Fig. 2 | Six month evaluation of patient P2 who had an exceptional improvement in visual function. **a**, Visual acuity (in $\log_{10}$ MAR, minimum angle of resolution) showing large and sustained improvement in the treated eye starting at month 2 (M2) and continuing at least to month 6. Testing performed with achromatic letters and gratings under free-viewing conditions (filled symbols) or achromatic gratings projected onto the macula (open symbols). LP=light perception; NS=not seen. **b**, Acuity with chromatic (Red or Blue) gratings projected onto the macula starting at M2 and continuing to M6. The results suggest long- and middle-wavelength sensitive cone photoreceptors are the dominant contributors to acuity. **c-f**, FST threshold change from baseline using red and blue flashes presented under dark-adapted (DA) and light adapted (LA, 10 $cd.m^{-2}$ white) conditions. Symbols are averages from repeated measures obtained at each visit (for most visits n=12, except for BL DA where n=30-34, M3 DA where n=18, and some visits where n=10-16), error bars=±1 sd, BL=average of two pre-treatment baselines, M1-M6=post-treatment evaluations at months 1 through 6. Linear mixed-effects models were used for the statistical analysis. Patient was administered a single intravitreal dose of 160 µg after the BL visit, and a further maintenance dose of 80 µg after the M3 visit.

Methods

Study medication and trial design. QR-110 is a 17-mer RNA antisense oligonucleotide (AON) consisting of the following 5' to 3' sequence GGUGGAUCACGAGUUCA prepared as the sodium salt ($C_{180}H_{219}N_{67}Na_{16}O_{101}P_{16}S_{16}$ and molecular mass of 6313.51 Daltons). QR-110 drug substance was manufactured with all RNA bases 2'-O-methylribose modified with all inter-nucleotide linkages comprising phosphorothioate (BioSpring GmbH, Frankfurt, Germany). QR-110 was synthesized as GMP grade by solid state synthesis in the direction 3' to 5' and further purified by ion-exchange chromatography. Drug product was prepared by dissolving QR-110 drug substance (10mg/ml) in formulated phosphate buffered saline in depyrogenated vials and sterilized stoppers, and filter sterilized using 0.22 µm double filtration (Pyramid Laboratories, Inc., Costa Mesa, CA, USA).

QR-110 was designed to bind to a sequence within the exonic splicing enhancer sequence at intron 26 of the *CEP290* pre-mRNA[6]. The hybridization of the QR-110 is thought to modulate the RNA splicing process, blocking access to the active cryptic splicing site, and restoring preference for the wildtype splicing sites. A resulting increase of wildtype mRNA transcript leads to an increase of functional CEP290 protein[6].

An open-label, multiple arm, multiple dose, dose escalation study was designed to evaluate the safety and tolerability of QR-110 administered via unilateral intravitreal (IVT) injection (to the worse eye) every three months for up to 1 year (ClinicalTrials.gov no. NCT03140969). The study is being conducted according to the Declaration of Helsinki as well as according to the principles of Good Clinical Practice. There are three sites (Iowa City, US; Philadelphia, US; and, Ghent,

Belgium) and Institutional Review Boards of the University of Pennsylvania, Wills Eye Hospital, University of Iowa and Ghent University approved the studies whick complied with all relevant ethical regulations. Eligible subjects include males and females 6 years of age or older at screening with a clinical diagnosis of LCA and a molecular diagnosis of homozygosity or compound heterozygosity for the CEP290 p.(Cys998*) mutation. Of note, inclusion criteria for best-corrected visual acuity (BCVA) are better than or equal to light perception (LP) in both eyes, and equal to or worse than +0.6 $\log_{10}$ of Minimum Angle of Resolution (MAR) (20/80 Snellen equivalent) in the worse eye and equal to or worse than +0.4 $\log_{10}$ MAR (20/50 Snellen equivalent) in the contralateral eye. Eligible, enrolled subjects receive up to 4 administrations of QR-110 at 3-month intervals over the course of one year.

Demographics and baseline characteristics of patients treated. The date for study start (first visit of first patient) was October 16, 2017. The current report represents an interim analysis of the ongoing study as of the cutoff date of August 15, 2018. Included are all ten subjects who have received one or more injections of QR-110 (Extended Data Table 1). Written informed consent (or informed assent and parental consent for pediatric subjects) was provided by each subject before the initiation of study activities. Ophthalmic and systemic safety aspects of the study were and continue to be monitored by an independent Data Monitoring Committee. There were 6 adult patients between the ages of 19 to 44 years, and 4 pediatric patients between 8 and 16 years. There were 5 males and 5 females. All subjects were compound heterozygotes for the c.2991+1655A>G p.(Cys998*) allele and an additional mutant allele in the *CEP290* gene. Two patients were siblings, and two

patients carried the same mutations but were not known to be related. Subjects were assigned to receive one of two dose levels of QR-110. Three adult and two pediatric patients were assigned to a loading dose of 160 µg and a maintenance dose of 80 µg QR-110. Three adult patients and two pediatric patients were assigned to a dose consisting of 320/160 µg QR-110 (Extended Data Table 1, Supplementary Table 1).

Safety evaluations. Ocular safety was assessed with standard eye examinations, including gradings of the anterior and posterior segment according to the Standardization of Uveitis Nomenclature[7], and of the lens according to the Age-Related Eye Diseases Study Clinical Lens Grading System[8]. Near-infrared excited autofluorescence imaging, when possible, was used to document any changes in RPE pigmentation[9]. Systemic safety was evaluated with physical examinations at baseline and postoperative visits. Routine hematology; testing of serum chemistry, prothrombin time (with international normalized ratio), and partial thromboplastin time; and urinalysis were performed at baseline and postoperatively.

Visual acuity. Visual acuity (VA) was measured using Early Treatment Diabetic Retinopathy Study (ETDRS) methodology[10] at two baseline visits and post-injection visits starting at month 1. Best-corrected VA was scored as the number of letters correctly read after adjusting for distance (4m or 1m) and expressed as $\log_{10}$ MAR to measure the range of acuities from 20/10 to 20/800 (or from -0.30 to +1.6 $\log_{10}$ MAR). For patients not able to correctly read ETDRS letters at 1m, Berkeley Rudimentary Vision Test battery was performed[11] at distances of 1 m and 0.25 m to measure the range of acuities from 20/500 to 20/16,000 (or from +1.4 to +2.9 $\log_{10}$

316 MAR). Hand-motions (HM) acuity was assigned +3.0 $\log_{10}$ MAR and light-perception
317 (LP) was assigned +4.0 $\log_{10}$ MAR.
318
319 **Imaging.** Spectral-domain Optical Coherence Tomography (OCT) was used to
320 obtain cross-sectional imaging of the retina[4,5] (RTVue-100; Optovue, Fremont, CA, or
321 Spectralis, Heidelberg Engineering, Heidelberg, Germany). All OCT images were
322 aligned by straightening the major RPE reflection. In a subset of 4 patients, with
323 reliable foveal scans available in both eyes at BL, month 1 and month 3 time points,
324 quantitative analyses of the photoreceptor cilial anatomy was performed using
325 longitudinal reflectively profiles. Inner segment (IS) length was estimated between
326 the outer limiting membrane peak and the peak originating near the junction of inner
327 and outer segments (IS/OS). The outer segment length was estimated between the
328 IS/OS peak and peak originating near the interface of OS tips and apical RPE
329 processes. En face imaging with near-infrared illumination was performed with
330 autofluorescence mode or reflectance mode using a confocal scanning laser
331 ophthalmoscope[4,5,9] (HRA2 or Spectralis, Heidelberg Engineering, Heidelberg,
332 Germany).
333
334 **Oculomotor Control and Instability (OCI).** To account for the wide spectrum of
335 oculomotor abnormalities encountered in *CEP290*-LCA patients, an infrared video
336 oculography method was used[5]. Two recordings were performed in a darkened
337 room. One with a bright fixation light available along the primary gaze, and another
338 without a fixation light.
339

340 **Full-field Stimulus Testing (FST).** Sensitivity to chromatic light flashes presented in
341 the dark in dark-adapted eyes was measured with full-field stimulus testing (FST)
342 developed specifically for patients with severe vision loss and oculomotor
343 instability[12]. FST was tested with a commercial software[13,14]. For each color, eye,
344 and visit, approximately 12 independent thresholds were obtained thus providing an
345 estimate of intra-session variability. Large variability in FST usually indicates
346 unreliable performance within a session. Exploratory analyses showed that 11
347 sessions (out of 197) were associated with unusually large intra-session variances
348 compared to previously published estimates of variability[12]. These sessions had
349 intra-session standard deviation of >1.01 $\log_{10}$ and were also classified as suspected
350 outliers by Tukey's criteria (1.5xIQR above the third quartile). Statistical analyses
351 were performed twice: one with the full data set and a second time excluding the 11
352 sessions. Both analyses, with and without exclusions, supported the same statistical
353 conclusions regarding a significant treatment effect from month 1 to 6; however,
354 exclusion allowed presentation of the most conservative, internally consistent and
355 repeatable data. Of additional note, at the three month time point, it was found out
356 that one patient (P7) was mistakenly tested with a 'flash' stimulus (duration <4 ms)
357 instead of the 'pulse' stimulus (duration=200 ms) specified in the protocol. At the
358 three month time point, the patient was tested with both types of stimuli to obtain a
359 comparison.
360
361 **Mobility.** The visual navigation challenge (ORA-VNC™) was used to assess mobility
362 performance of patients at multiple levels of luminance[15]. There were four difficulty
363 levels of the courses, and each course had several lighting conditions defining 20

364 'levels'. For each level there were several combinations of random obstacle
365 placements that were used to avoid learning effects. A level of 0 represented failing
366 to pass successfully any of the courses at any light level; and a level of 19
367 corresponded to passing the most difficult course under dimmest light conditions. For
368 each eye and each visit, the highest level course passed was recorded. Results from
369 P7 were censored because of the unavailability of levels 13-19 at the time of both
370 baseline visits. In addition, one of the baseline visits and the month 1 visit of P5 was
371 censored for the same reason.
372
373 **Additional evaluations in the exceptionally responding patient.** Patient P2 was
374 known to the investigators and previously evaluated with detailed non-invasive
375 assessments of visual function and retinal structure[4,5]. Contemporaneous with the
376 clinical trial, the patient was enrolled in additional research studies that had been
377 approved by the University of Pennsylvania Institutional Review Board. The studies
378 included FST under dark- and light-adapted conditions[12,16]. Specifically, the custom
379 thresholding algorithm was based on a 4 dB/2 dB staircase with two response
380 reversals (as opposed to the binary thresholding algorithm used by the
381 manufacturer). In addition, there was a limited response-acceptance window to
382 minimize the effect of extraneous responses not synchronized with the stimulus
383 presentation. Both of these algorithmic features helped reduce variability especially
384 in patients with severe vision loss. Spatial resolution was measured with achromatic
385 and chromatic gratings[17].
386

Statistical analyses. Linear mixed-effects models were used for the statistical analysis of all efficacy outcomes to account for the correlation structure and repeated measures within each data set. The baseline data were pooled from the two visits obtained before the first injection. Results from the two dose groups were pooled for statistical analyses. The models used an unstructured covariance matrix, restricted maximum likelihood estimation and the Satterhwaite's approximation for denominator degrees of freedom. Computations used the lme4 (ver. 1.1-17)[18] and lmerTest (ver. 3.0-1)[19] packages from R statistical software (ver. 3.4.4, 2018-03-15)[20].

For VA, the dependent variable was the minimum angle of resolution expressed in $\log_{10}$ MAR. The model used Treatment-by-Visit interaction as fixed effects. Intercept and Visit were specified as random effects, with Patient as the grouping factor. The Treatment factor had two levels (treated and untreated eyes), and the Visit factor had seven levels (baseline and months 1, 2, 3, 4, 5 and 6). For OCI, the dependent variable was the variation of the radial distance of the center of pupil from the mean normal primary gaze locus over 30 s expressed in $\log_{10}$ mm. The fixed effects and random effects were the same except Visit factor had three levels (baseline, month 3 and 6). Separate analyses were performed for OCI data recorded with and without fixation. For FST, the dependent variable was the visual threshold expressed in $\log_{10}$ phot-cd.m^2. The model used Treatment-by-Condition interactions as fixed effects in addition to Treatment-by-Visit. The Condition factor had two levels (blue and red). The random effects were the same as in the VA model. For mobility the dependent variable was the performance score, an ordinal variable ranging from 0 to 19. The same analysis was used for mobility as for VA by approximating the ordinal variable as a continuous variable. For Fig. 2, separate

analyses were performed for dark-adapted and light-adapted data. Analyses were identical to that of all other FST results except random effects were not specified as there was only a single patient.

Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data availability. All relevant patient-level data are displayed in Figures. All requests for data will be reviewed by ProQR Therapeutics and the Universities involved to verify if the request is subject to any intellectual property or confidentiality obligations. Patient-related data may be subject to confidentiality. Any data that can be shared will be released.

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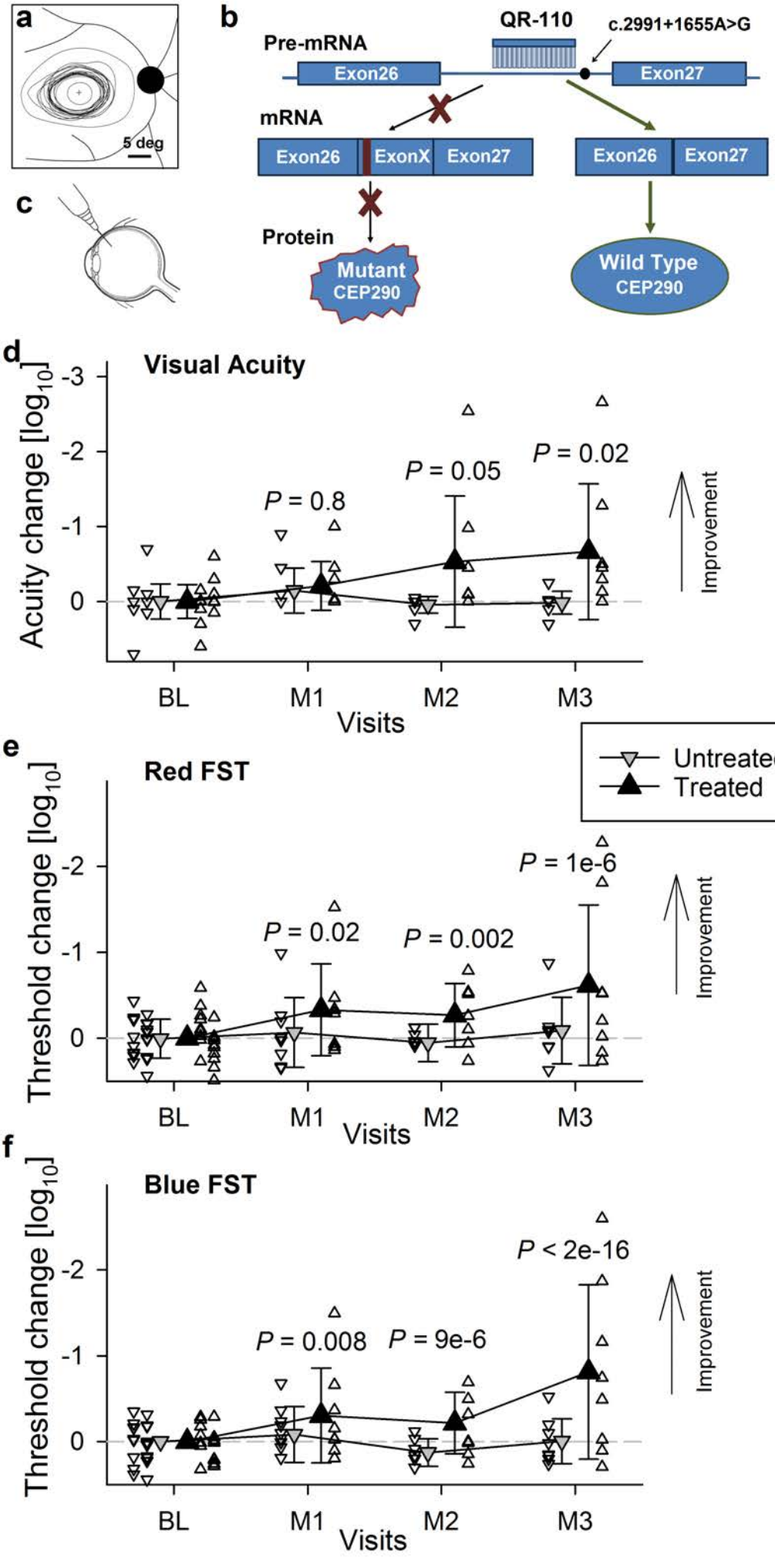
a
5 deg
b
QR-110
c.2991+1655A>G
Pre-mRNA
Exon26
Exon27
mRNA
Exon26
ExonX
Exon27
Exon26
Exon27
Protein
Mutant CEP290
Wild Type CEP290
c
d
Visual Acuity
Acuity change [log10]
-3
-2
-1
0
P = 0.8
P = 0.05
P = 0.02
Improvement
BL
M1
M2
M3
Visits
e
Red FST
Threshold change [log10]
Untreated
Treated
P = 0.02
P = 0.002
P = 1e-6
Improvement
BL
M1
M2
M3
Visits
f
Blue FST
Threshold change [log10]
P = 0.008
P = 9e-6
P < 2e-16
Improvement
BL
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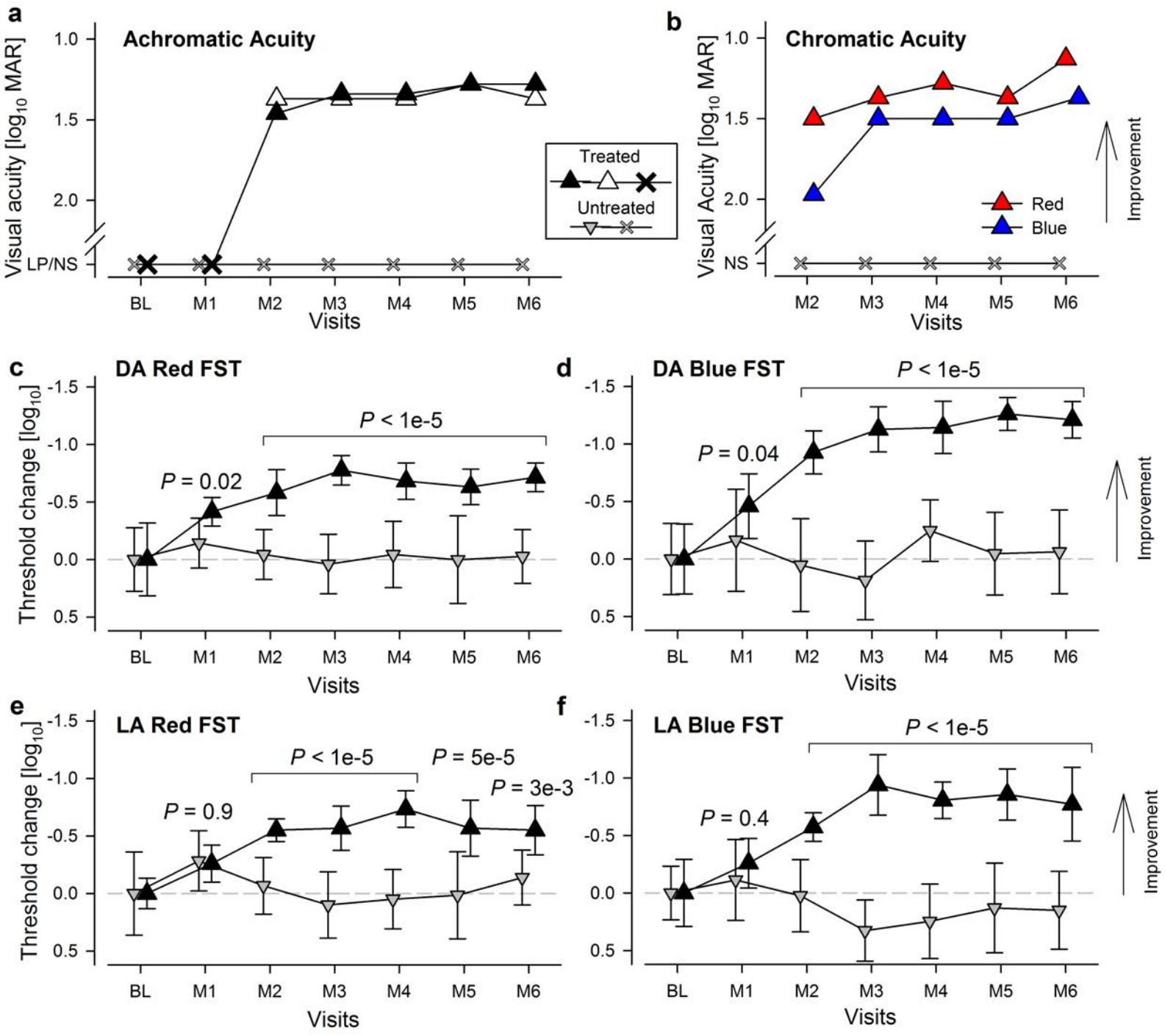
M2
M3
Visits


a
Achromatic Acuity
Visual acuity [log10 MAR]
1.0
1.5
2.0
LP/NS
Treated
Untreated
BL
M1
M2
M3
M4
M5
M6
Visits
b
Chromatic Acuity
Visual Acuity [log10 MAR]
1.0
1.5
2.0
NS
Red
Blue
Improvement
M2
M3
M4
M5
M6
Visits
c
DA Red FST
Threshold change [log10]
-1.5
-1.0
-0.5
0.0
0.5
P < 1e-5
P = 0.02
BL
M1
M2
M3
M4
M5
M6
Visits
d
DA Blue FST
P < 1e-5
P = 0.04
Improvement
Visits
e
LA Red FST
P < 1e-5
P = 5e-5
P = 3e-3
P = 0.9
Visits
f
LA Blue FST
P < 1e-5
P = 0.4
Improvement
Visits

Extended Data Table 1: Baseline Participant Characteristics

Code	Sex	2nd *CEP290* Allele #	Age/Grp ~	Baseline VA [log MAR] +	Treated Eye @	Dose [ug] &	Num. of Inj. ^	Length of f/u [mon]$
P1	M	c.2506_2507delGA	19 / A	LP / LP	RE	160 / 80	4	9.0
P2	M	c.4723A>T	41 / A	LP / LP	RE	160 / 80	3	7.0
P3	M	c.5668G>T	44 / A	2.3 / 2.4	LE	160 / 80	2	3.0
P4	F	c.4438-3delC	16 / P	2.5 / 2.5	RE	160 / 80	3	6.0
P5	M	c.6277delG	8 / P	1.9 / 2.1	LE	160 / 80	2	5.0
P6	F	c.3167_3168insA	21 / A	LP / LP	RE	320 / 160	3	6.5
P7	F	c.4723A>T	27 / A	1.1 / 0.7	RE	320 / 160	2	5.0
P9	F	c.4393C>T	24 / A	LP / LP	RE	320 / 160	1	1.0
P8	M	c.6277delG	10 / P	1.9 / 1.4	RE	320 / 160	2	3.0
P10	F	c.547_550delTACC	15 / P	LP / LP	RE	320 / 160	1	1.0

all patients had c.2991+1655A>G/p.(Cys998*) allele in common; nucleotide change and predicted effect of the additional allele shown

~ Age in years at the time of enrollment; A=adult; P=pediatric

+ Visual acuity in right / left eyes in logarithm of minimum angle of resolution (MAR); 0 log MAR corresponds to Snellen acuity of 20/20, 2 log MAR corresponds to 20/2000; LP=Light perception

@ RE=right eye, LE=left eye

& Loading / maintenance dose of QR110 injected intravitreally in a 50 uL volume

^ Intravitreal injections every 3 months

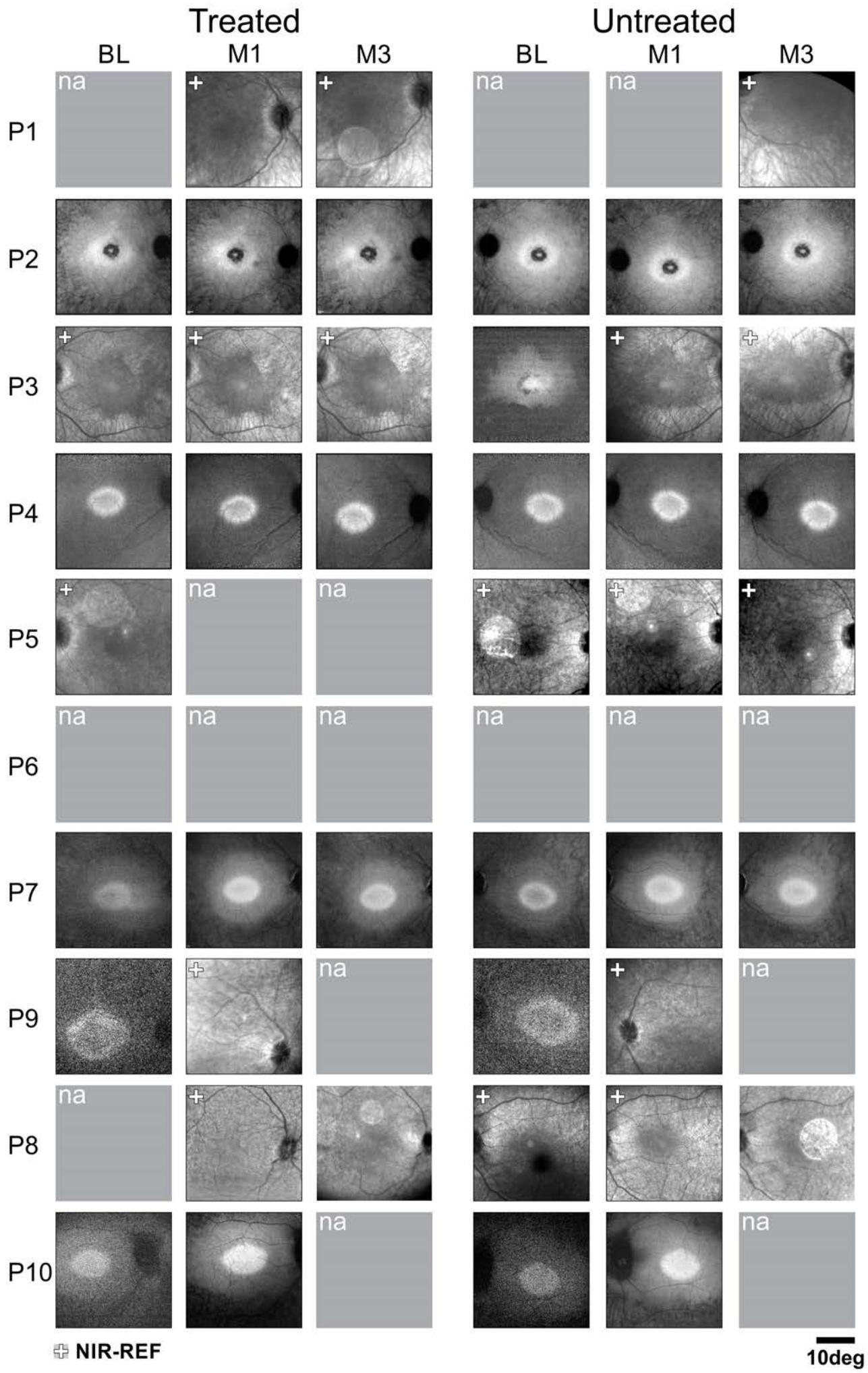
$ Length of followup in months after the first injection

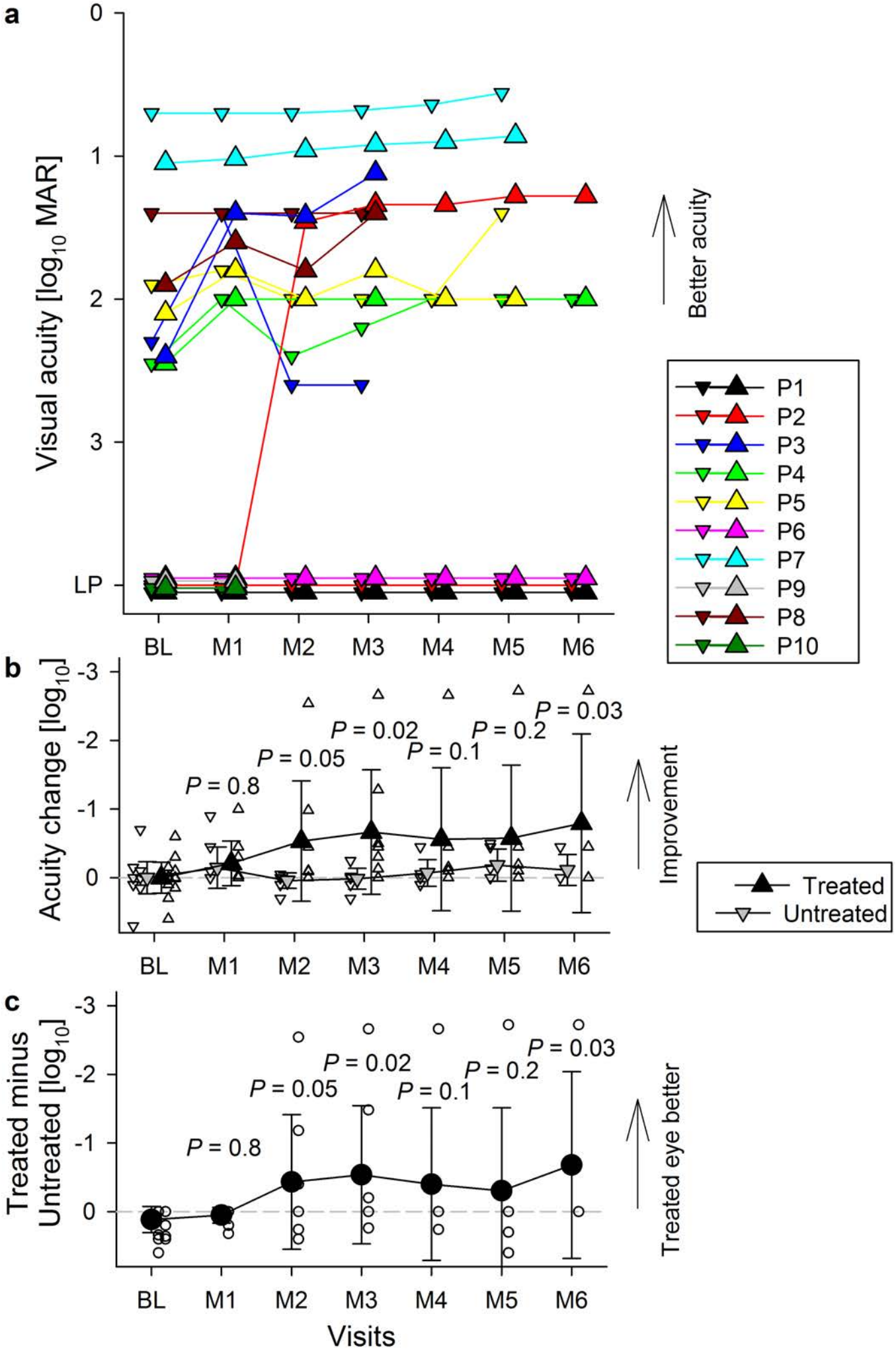
Extended Data Table 2: Treatment effect at 3 months

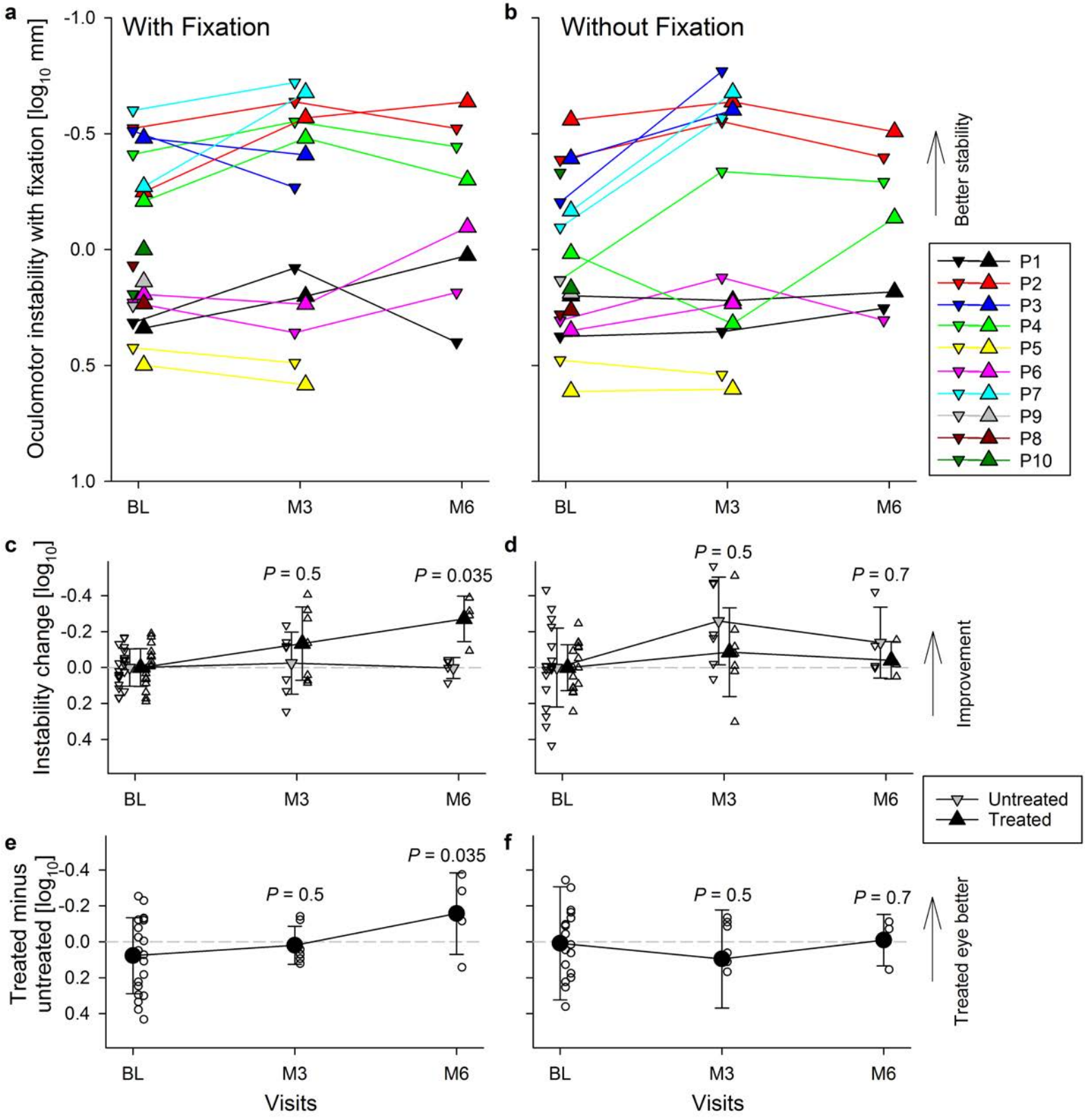
		Mean change from BL* [log_{10}]	P-value +
All patients (n=8)			
VA	Treated eyes	**-0.67**	**0.022**
	Untreated eyes	0.02	
Red FST ~	Treated eyes	**-0.62**	**1E-06**
	Untreated eyes	-0.09	
Blue FST ~	Treated eyes	**-0.81**	**< 2E-16**
	Untreated eyes	-0.01	
Withholding data from P2 (n=7)			
VA	Treated eyes	**-0.38**	**0.018**
	Untreated eyes	0.02	
Red FST ~	Treated eyes	**-0.63**	**3E-04**
	Untreated eyes	-0.16	
Blue FST ~	Treated eyes	**-0.76**	**2E-13**
	Untreated eyes	-0.04	

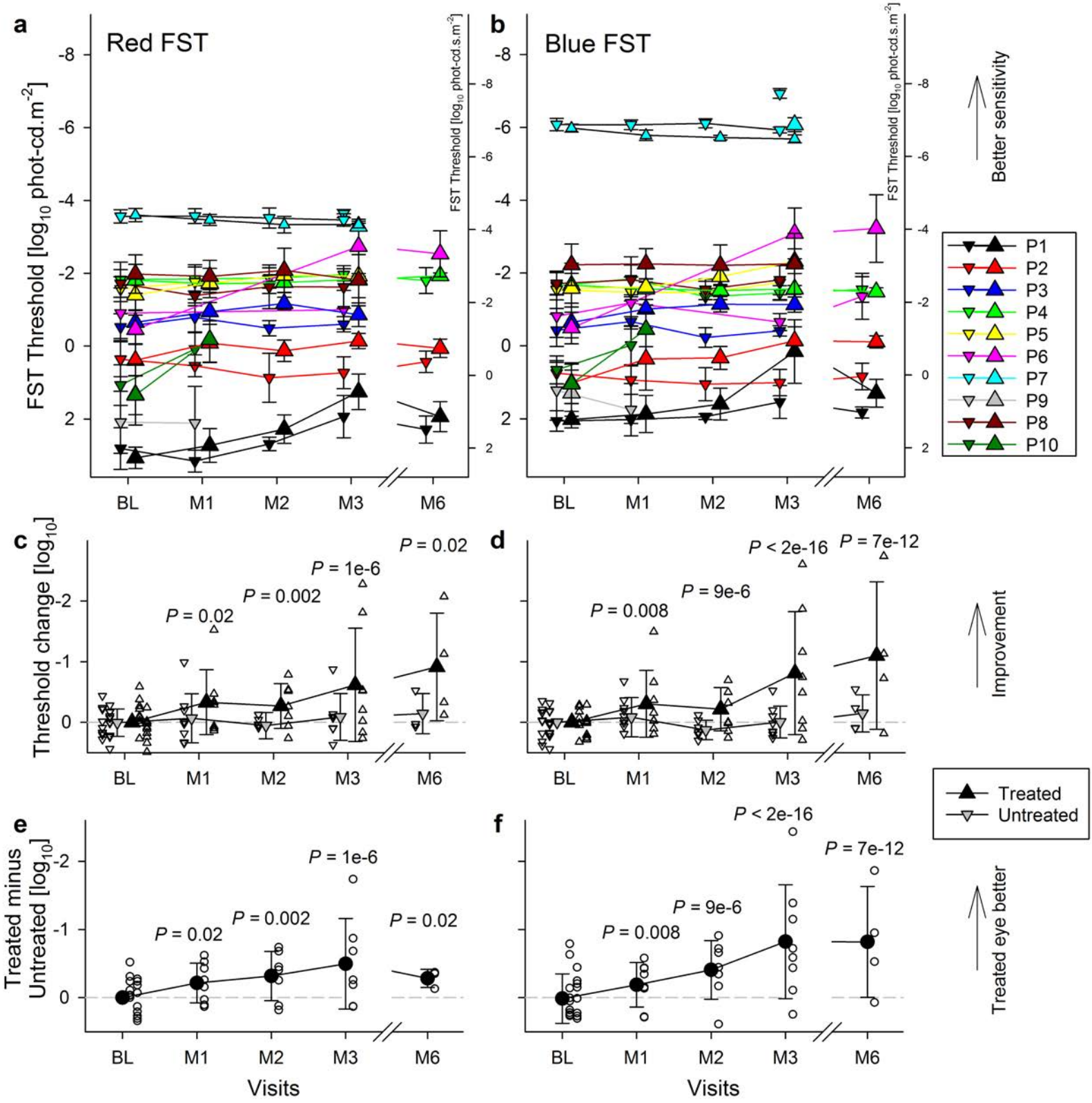
* Negative values correspond to improvement of function compared to baseline (BL).
+ Linear mixed-effects models were used for the statistical analysis of all efficacy outcomes to account for the correlation structure and repeated measures within each data set. P-values for the significance of treatment-by-visit interactions
~ Sessions with an intravisit sd greater than 1.01 have been censored; conclusions are unchanged when censoring is not used

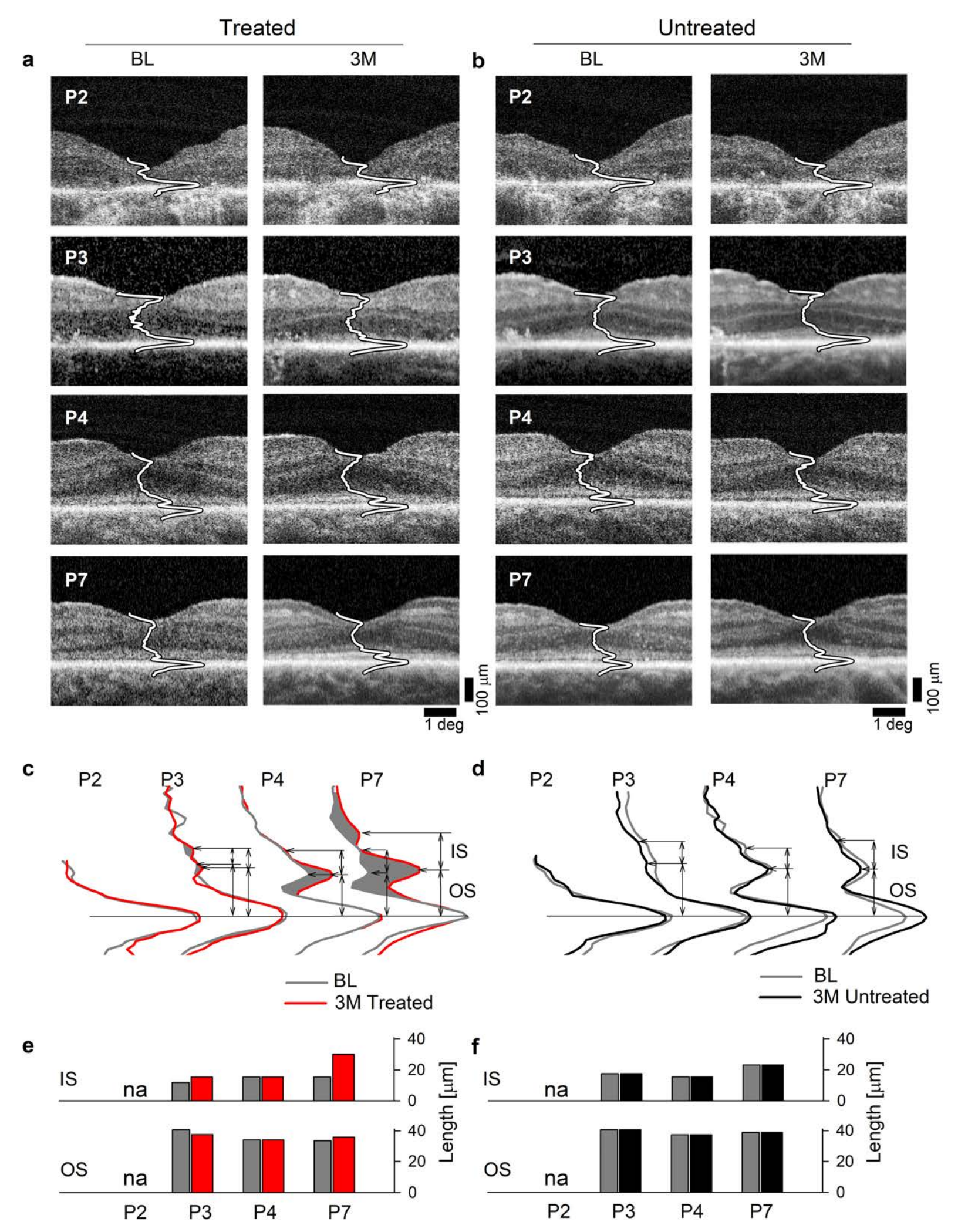

Treated
Untreated
BL
M1
M3
BL
M1
M3
P1
P2
P3
P4
P5
P6
P7
P9
P8
P10
na
na
na
100 um
1 deg

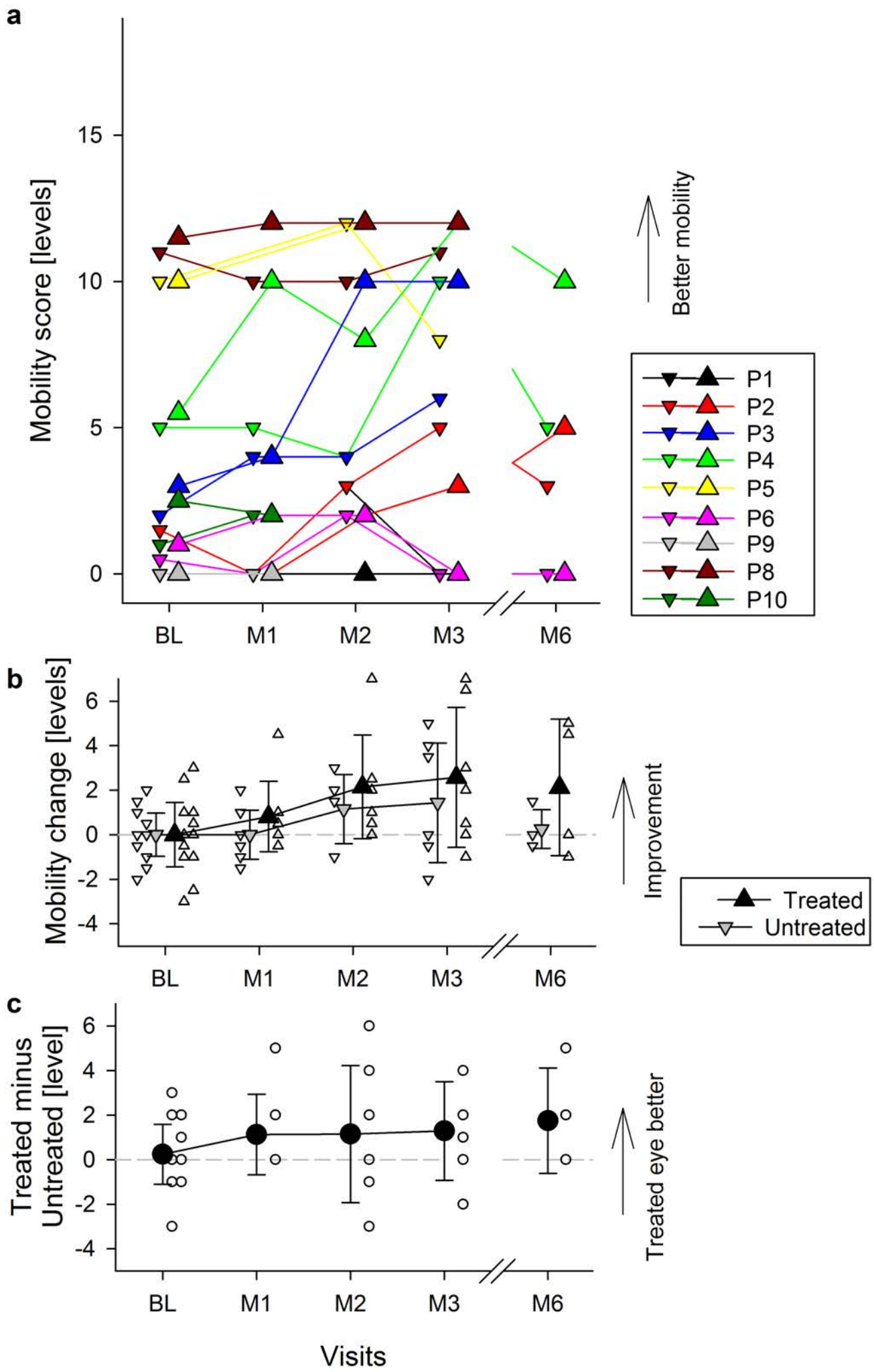

Treated
Untreated
BL
M1
M3
BL
M1
M3
P1
P2
P3
P4
P5
P6
P7
P9
P8
P10
na
NIR-REF
10deg


a
Visual acuity [log10 MAR]
0
1
2
3
LP
BL
M1
M2
M3
M4
M5
M6
Better acuity
P1
P2
P3
P4
P5
P6
P7
P9
P8
P10
b
Acuity change [log10]
-3
-2
-1
0
P = 0.8
P = 0.05
P = 0.02
P = 0.1
P = 0.2
P = 0.03
Improvement
Treated
Untreated
c
Treated minus Untreated [log10]
P = 0.8
P = 0.05
P = 0.02
P = 0.1
P = 0.2
P = 0.03
Treated eye better
Visits


a
With Fixation
Oculomotor instability with fixation [log10 mm]
-1.0
-0.5
0.0
0.5
1.0
BL
M3
M6
b
Without Fixation
Better stability
P1
P2
P3
P4
P5
P6
P7
P9
P8
P10
c
Instability change [log10]
P = 0.5
P = 0.035
-0.4
-0.2
0.0
0.2
0.4
d
P = 0.5
P = 0.7
Improvement
Untreated
Treated
e
Treated minus untreated [log10]
P = 0.035
P = 0.5
Visits
f
P = 0.5
P = 0.7
Treated eye better
Visits


a
Red FST
FST Threshold [log10 phot-cd.m-2]
FST Threshold [log10 phot-cd.s.m-2]
b
Blue FST
Better sensitivity
P1
P2
P3
P4
P5
P6
P7
P9
P8
P10
BL
M1
M2
M3
M6
c
Threshold change [log10]
P = 0.02
P = 1e-6
P = 0.002
P = 0.02
d
P < 2e-16
P = 7e-12
P = 9e-6
P = 0.008
Improvement
Treated
Untreated
e
Treated minus Untreated [log10]
P = 1e-6
P = 0.002
P = 0.02
P = 0.02
f
P < 2e-16
P = 7e-12
P = 9e-6
P = 0.008
Visits
Treated eye better


Treated
Untreated
a
b
BL
3M
P2
P3
P4
P7
100 µm
1 deg
c
d
IS
OS
BL
3M Treated
3M Untreated
e
f
na
40
20
0
Length [µm]


a
Mobility score [levels]
15
10
5
0
BL
M1
M2
M3
M6
Better mobility
P1
P2
P3
P4
P5
P6
P9
P8
P10
b
Mobility change [levels]
6
4
2
0
-2
-4
BL
M1
M2
M3
M6
Improvement
Treated
Untreated
c
Treated minus Untreated [level]
6
4
2
0
-2
-4
BL
M1
M2
M3
M6
Treated eye better
Visits