Counterphase modulation flicker photometry: phenotypic and genotypic associations

A. J. Lawrance-Owen,[1] J. M. Bosten,[1] R. E. Hogg,[1,2] G. Bargary,[1,3] P. T. Goodbourn,[1,4] and J. D. Mollon[1,*]

[1]*Department of Experimental Psychology, University of Cambridge, Downing Street, Cambridge CB2 3EB, UK*
[2]*Centre for Vision and Vascular Science, Queen's University Belfast, Institute of Clinical Science—Block A, Royal Victoria Hospital, Grosvenor Road, Belfast BT12 6BA, UK*
[3]*Division of Optometry and Visual Science, City University, School of Health Sciences, Tait Building, Northampton Square, London EC1V 0HB, UK*
[4]*School of Psychology, University of Sydney, Brennan MacCallum Building (A18), Sydney, NSW 2006, Australia*
Corresponding author: jm123@cam.ac.uk

The OSCAR test, a clinical device that uses counterphase flicker photometry, is believed to be sensitive to the relative numbers of long-wavelength and middle-wavelength cones in the retina, as well as to individual variations in the spectral positions of the photopigments. As part of a population study of individual variations in perception, we obtained OSCAR settings from 1058 participants. We report the distribution characteristics for this cohort. A randomly selected subset of participants was tested twice at an interval of at least one week: the test–retest reliability (Spearman's rho) was 0.80. In a whole-genome association analysis we found a provisional association with a single nucleotide polymorphism (rs16844995). This marker is close to the gene *RXRG*, which encodes a nuclear receptor, retinoid X receptor γ. This nuclear receptor is already known to have a role in the differentiation of cones during the development of the eye, and we suggest that polymorphisms in or close to *RXRG* influence the relative probability with which long-wave and middle-wave opsin genes are expressed in human cones.   © 2014 Optical Society of America

1. INTRODUCTION

When red and green lights are equated by flicker photometry, large variations are found between normal observers in the ratio of red to green radiances that they select [1–5], and these variations have traditionally been attributed to individual differences in the relative numbers of long-wavelength (L) and middle-wavelength (M) cones present in the retina. This L:M ratio has been estimated by finding the additive combination of cone fundamentals that best fits the photopic luminosity function derived by flicker photometry [4,6,7]. Rushton and Baker showed that observers who were more sensitive to red light in their flicker-photometric settings did indeed have relatively higher densities of long-wavelength photopigment, as measured by reflection densitometry [3]. De Vries proposed that the L:M ratio was heritable [1], and Lutze and colleagues reported pedigree data in support of this hypothesis [7].

However, it is known that both the L and the M photopigments differ in their exact spectral positions (i.e., in their wavelengths of maximal sensitivity) [8,9], owing to variations in the amino-acid sequence of the opsins [10,11]. This second source of variation should theoretically contribute additional variance to flicker-photometric settings [12]. Empirical evidence for this expectation is that flicker-photometric settings for color-normal observers do show a moderate correlation with Rayleigh matches [3,13].

The "OSCAR" test is a test of the flicker-photometric type that was introduced in 1983 as a screening device for color deficiency [14]. A similar instrument was later marketed as the Medmont C-100 [15,16]. The OSCAR test uses a variant form of flicker photometry that has been called counterphase modulation flicker photometry. The observer views the end of a solid plexiglass rod, within which is mixed light from two broad-band LEDs, one a red with dominant wavelength 635 nm and the other a green with dominant wavelength 558 nm. The two component lights are modulated in counterphase at ~16 Hz. A single control knob allows the observer to increase the depth of modulation of one component while concurrently decreasing the depth of modulation of the second component. The task is to eliminate or to minimize the visible flicker. The advantage of counterphase modulation photometry over conventional flicker photometry is that the time-averaged luminance and the time-averaged chromaticity remain constant as the observer makes adjustments.

Like other flicker-photometric measures, the OSCAR test ought to be sensitive to individual variations in L:M cone ratios. Some empirical evidence that this is so is provided by the protan-like and deutan-like settings of heterozygotes for color deficiency [13,17,18], whose L:M cone ratios must be skewed by random X-chromosome inactivation [19,20]. And settings on the OSCAR test correlate with a psychophysical estimate of cone ratio derived from parafoveal acuity for cone-isolating targets [18].

Little is yet understood of what determines individual differences in the L:M cone ratio. It is thought that a stochastic event—the binding of the upstream locus control region to the promoter region of either the long-wave gene or the

middle-wave gene—determines which opsin gene is expressed in a given cone [21]. However, if there are heritable individual differences in L:M ratio, there must be some factor that alters the probabilities with which L and M opsin genes are favored. It has been suggested that polymorphisms upstream of the opsin genes may alter transcription factor binding sites and thus alter the probability that the L rather than the M gene will be expressed [22–24].

In view of the large individual differences in L:M ratio and their possible heritability, we included the OSCAR test in the PERGENIC project, a genome-wide association study in which 1060 individuals were tested on a 2.5 h battery of optometric, perceptual, and oculomotor tests [25–27]. We here report the distribution and reliability of OSCAR settings for a large population, and we identify a tentative genetic association with individual differences on the test.

2. METHODS

A. Participants
1060 healthy young adults (647 female) were recruited from the Cambridge area by advertisements within the university and online. Participants were of European descent, as established by the nationality of their four grandparents and confirmed by genetic analysis. They were aged from 16 to 40 years (M = 22 years, SD = 4 years). All had normal or corrected-to-normal acuity (less than or equal to 0.00 logMAR). A randomly selected subset of 105 participants returned for a second test after an interval of at least one week; thus we were able to estimate test–retest reliability for the OSCAR test.

The study received approval from the Cambridge Psychology Research Ethics Committee. All participants gave written consent after receiving written information about the study.

B. Tests and Procedures
The OSCAR test used was a commercial version of the instrument, which was formerly manufactured by Medilog (The Netherlands). It is encapsulated in a small rectangular plastic box with the control knob on the upper surface. The 558 and 635 nm lights are mixed within a plexiglass rod, and the end of the rod, slightly roughened to give a diffusing surface, is visible at the front end of the box. At a setting of −10 on the scale, the Michelson contrast of the modulation of the 635 nm light is approximately 0.75 and the modulation of the 558 nm light is zero. At a setting of +10 on the scale, the modulation of the 558 nm light is approximately 0.75 and the 635 nm light is unmodulated. As one modulation is increased, the other is decreased concomitantly in a linear fashion. The red and green component lights are of similar luminance, 5.5 and 6.4 cd $\cdot$ m^{-2}, respectively.

At the viewing distance of 25–30 cm, the stimulus surface subtended approximately 1.4 deg of visual angle. The participant held the instrument in his or her hand and was asked to adjust the control knob to minimize the visible flicker. Three consecutive readings were taken, and the OSCAR test score was defined as the mean of these three readings. Between settings the experimenter randomly offset the control knob. The test was administered in a room lit by daylight fluorescent lamps. OSCAR test data were available for 1058 individuals from the full cohort and for 104 individuals from the subset who were tested twice on different occasions.

We included in the PERGENIC battery five plates from the Ishihara test (10th edition, 1951): Plate 1 (demonstration plate), Plate 4 (transformation plate), Plate 9 (transformation plate), Plate 17 (disappearing plate), and Plate 24 (diagnostic plate). Plates were wall-mounted, were illuminated by daylight fluorescent lamps, and were viewed from 1 m. Ishihara data were available for 1057 individuals from the full cohort.

C. Genetic Analysis
Saliva samples were collected from participants using Oragene OG–500 kits (DNA Genotek, Ottawa, Canada). For 1008 participants, DNA was extracted according to the manufacturer's protocol and was genotyped using the Illumina HumanOmniExpress BeadChip. Genotype calling was by custom clustering, using Illumina GenomeStudio software.

Twenty-one samples were excluded from further genetic analysis: one because phenotypic data were lacking, three because of a genotypic sex anomaly, one because the genotyping call rate was low (<97%), one because the participant was genetically a population outlier, and 15 because they were duplicate or related samples. This left 987 individuals in the genetic analysis (599 female).

Quality control of individual single nucleotide polymorphisms (SNPs) excluded markers with more than 2% missing genotypes (12,706 SNPs) and markers with a minor allele frequency of less than 1% (77,738 SNPs). This left 642,758 SNPs. Association analysis was performed on the ranked OSCAR settings assuming an additive genetic effect and using the software PLINK (Purcell *et al.*, 2007) with four covariates: sex, and the top three principal components reflecting genetic variation in our cohort (to control for stratification in the population).

In the case of each SNP with a "suggestive" ($p < 10^{-5}$) association, we performed a clumping analysis on the region of interest. We used PLINK's clumping function, with a significance threshold for index SNPs of 0.00001, a significance threshold for clumped SNPs of 0.01, a linkage disequilibrium (LD) threshold for clumping of 0.1, and a physical distance threshold of 1250 kB. Clumping analysis defines a region that is in LD with the significantly associated SNP, and that contains other SNPs also associated with the trait with a specified p value. This region is therefore likely to contain the locus of interest, where lies the polymorphism causing the variation in phenotype.

3. RESULTS AND DISCUSSION

A. Reliability and Distribution of OSCAR Test Settings
Figure 1 shows the relationship between the first and second test scores for the subset of 104 participants who were tested on two independent occasions separated by at least one week. Although we took only three readings on each occasion, the OSCAR test clearly measures a visual characteristic on which observers reliably differ. The test–retest reliability as assessed by a Spearman rank order correlation between the OSCAR test score for session one and session two was $\rho = 0.80$ ($p = 5.0 \times 10^{-24}$). The corresponding Pearson's correlation was $r = 0.81$ ($p = 1.9 \times 10^{-25}$).

From the full cohort, 984 individuals correctly read Ishihara plate numbers 4, 17, and 24. A further 16 made a single mistake on these plates that was not an obviously daltonian
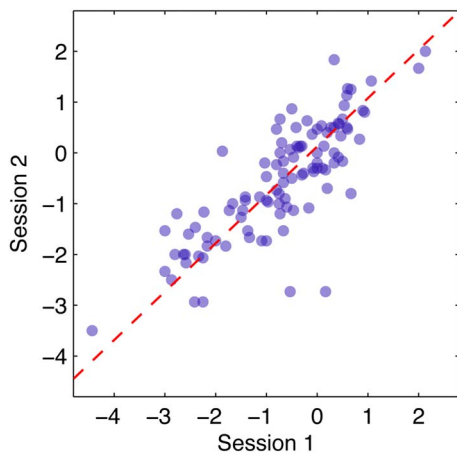
Fig. 1. Relationship between OSCAR settings on Session 1 and Session 2 for the subset of 104 randomly selected participants who were tested on two occasions at least one week apart. For an explanation of the scales, see Section 2.B.

reading but rather a misinterpretation of the digit (e.g., reading a *3* as an *8* or a *7* as a *1*) [28]. In Fig. 2, the solid black line shows the probability density distribution of OSCAR settings for these participants who, very largely, are likely to be color normal. The standard deviation of the distribution is 1.18.

Within this population of presumptive normals, there was no difference in mean OSCAR setting for men and women (M = −0.702, −0.707, respectively; $t(999) = 0.0735$, $p = 0.941$). However, women have a significantly larger variance than men: the standard deviation was 1.072 for men and 1.246 for women; $f(365, 634) = 0.740$, $p = 0.0014$. An explanation for the greater variance in women may be the presence, within the population,

of heterozygotes for color deficiency who have clinically normal color vision but abnormal cone ratios (and thus abnormal spectral luminosity functions), owing to X-chromosome inactivation [13,20,29,30]. Statistically, as many as 15% of our female population might be expected to be heterozygotes for color deficiency. Theoretically, however, opposing the effect of this subgroup will be a central tendency in the remaining women, that is, a tendency to have luminosity functions nearer the average, owing to the presence of two X chromosomes rather than one.

The broken line in Fig. 2 shows the probability density distribution for the 57 participants (11 female) who misread at least one plate out of plates 4, 17, and 24. Most of these participants are likely to be either color deficient or carriers of color deficiency, and it is clear that they exhibit two distinct populations on the OSCAR test. Those with positive scores (i.e., average settings in the deutan direction) are more common than those with negative (protan) scores—as would be expected from the known frequencies of protan and deutan deficiencies [31–33]. Twelve participants gave a clearly protan or clearly deutan response on the Ishihara diagnostic plate no. 24: in Fig. 2, we indicate the settings of the three "protans" by **P** and the nine "deutans" by **D**. The "protan" falling in the middle of the OSCAR distribution is a female participant who also misread the remaining plates in a daltonian manner. The "deutan" giving an extreme protan setting may be an example of misdiagnosis by the Ishihara.

B. Note on Ishihara Plate No. 9

In identifying those participants likely to be color deficient (see previous paragraph), we did not use Plate 9, owing to the large number of misreadings of this plate. Plate 9 is of
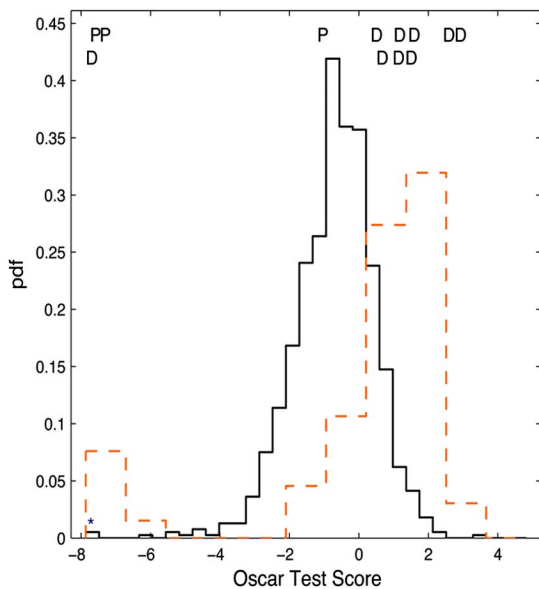

Fig. 2. Distribution of OSCAR settings for the full population of 1058 participants. The solid line shows the probability density function (pdf) for those participants who were estimated to be probably color normal from their responses to the Ishihara plates. The broken line shows the probability density function for participants who gave abnormal readings on the Ishihara plates. A subset of these participants were classified as "protan" or "deutan" by Ishihara plate 24: the settings of these subjects are indicated by "P" or "D."
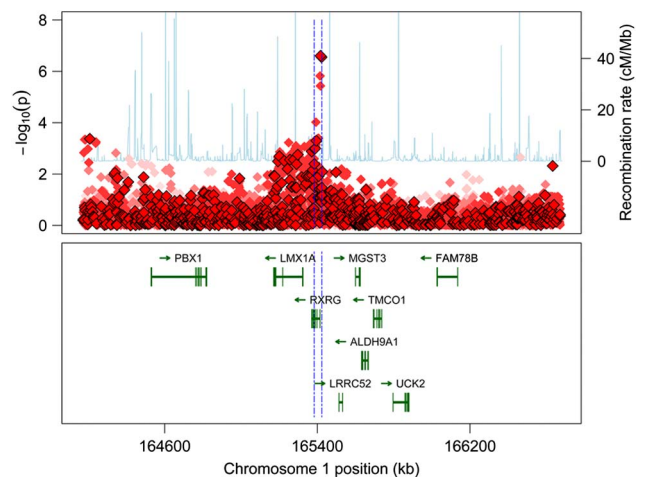

Fig. 3. Manhattan diagram for the region around rs16844995. In the upper panel, $-\log(p)$ values for the associations between rank of OSCAR setting and genotyped SNPs are indicated by the diamonds with black borders. Over a region 1.25 Mbp on either side of rs16844995, we imputed additional common SNPs identified by the 1000 genomes project. Associations with these imputed SNPs are indicated by the diamonds without borders, with saturation corresponding to imputation quality. Recombination rate is plotted with a solid blue line. The lower panel shows the genes present in this region. Vertical rectangles indicate exons. The vertical blue dashed lines in both panels define the region identified by a clumping analysis. This is the region in which the putative causal variant is likely to lie. Additional details of the methods for these analyses have been given by Lawrance-Owen *et al.* [26].

Table 1.   List of Single Nucleotide Polymorphisms that have Suggestive Associations with Settings on the OSCAR Test

SNP	Chr	Position	MAF	p	Clustered Region	Center of Clustered Region	Genes Inside Clustered Region
rs1389623	19	6684197	0.1205	3.549×10^{-6}	18 kbp	6693602	*TNFSF14; C3*
rs17060644	18	74909937	0.1251	4.294×10^{-6}	53 kpb	74901974	*N/A*
rs11768179	7	34208711	0.0876	4.701×10^{-6}	67 kbp	34233270.5	*Downstream of BMPER*
rs4468954	3	10566940	0.0233	9.574×10^{-6}	26 kpb	10557396	*ATP2B2*

the transformation type [34]: the normal reading is "74" and the color-deficient reading is "21." These alternative readings reflect the relative salience of the two chromatic subsystems of the early visual system [35,36]. No fewer than 148 of our participants gave the response "71" while making no error on other plates. It has already been suggested that heterozygotes for color deficiency are prone to give the reading "71" on this plate [18,37]. However, in the present population the proportion (0.139) of women who read "71" was not significantly different from the corresponding proportion (0.163) for otherwise color-normal men ($\chi^2(1, 4) = 1.16$, $p = 0.15$). The frequent misreading of Plate 9 by normal observers has been noted before [38,39]. Clearly a reading of "71" on this plate should not be taken to indicate color deficiency, but the plate may be an indicator of the relative salience of the two subsystems of color vision.

C. Genetic Associations
The strongest genetic association of the ranked OSCAR setting was with the SNP rs16844995 ($p = 2.8 \times 10^{-7}$). This polymorphic marker lies on the q arm of chromosome 1 (Fig. 3), and in the present sample it accounts for 2.6% of the phenotypic variance in OSCAR settings. Homozygotes for the majority allele (T) have a mean OSCAR setting of –0.54. The corresponding value for homozygotes for the minority allele (C) is –1.2, and for heterozygotes it is –0.8. Thus the effect of the minor allele is to shift settings in a protan direction. The minor allele frequency in our sample is 0.212.

The genetic association cannot be explained by the presence of color-deficient participants in the sample: when the association was run including Ishihara status as a covariate, the strength of the association between the rank of the OSCAR score and rs16844995 remained similar ($p = 4.97 \times 10^{-7}$).

In a whole-genome association study the correction needed for multiple testing depends on the number and identity of SNPs that are genotyped, and on the genetic linkage between SNPs in the population tested. We used the Genetic Type 1 Error Calculator [40] to calculate the number of independent tests that we had to correct for in our analysis. The required probability value for significance is $p = 1.35 \times 10^{-7}$. So our association with rs16844995 is at the margin of significance and can only be tentative, but we discuss it in more detail below because the marker lies 7.8 kb upstream of *RXRG*, a gene that independently is a particularly prominent candidate for control of the L∶M cone ratio.

In Table 1 we list other loci that have "suggestive" ($p < 10^{-5}$) associations with the rank of the OSCAR setting. For each suggestive SNP, we list the SNP identifier, the chromosome number, the position of the base, the frequency of the minor allele, the size of the region identified by clustering, the central base position of the clustered region, and any

genes that are found inside the clustered region. Two further SNPs, rs8097978 ($p = 4.294 \times 10^{-6}$) and rs7242877 ($p = 4.432 \times 10^{-6}$) were also suggestively associated with OSCAR setting, at the same genetic locus as rs17060644.

D. Retinoid X Receptor γ
Our strongest association was with rs16844995, which lies upstream of the gene *RXRG*. From Fig. 3 it can be seen that several exons of *RXRG* lie within the region that is likely to contain the causal variant. This gene encodes the retinoid X receptor γ (RXRγ), a member of the family of nuclear receptors that regulate gene expression [41]. It is activated by 9-*cis* retinoic acid. The molecule has a ligand-binding domain and a central DNA-binding domain: it binds to specific six-base-pair sequences of DNA in the promoter regions of genes.

What makes RXRγ a provocative candidate in the present context is that it is already is known to have a role in the differentiation of cones [42,43]. It can form a heterodimer with another nuclear receptor, the thyroid hormone receptor ß2 (TRß2), which controls the differentiation of S and L/M cones [44,45]. Deeb and Liu [46] have reported that retinoic acid will increase by 12-fold the level of expression of L and M pigment genes in a human retinoblastoma cell line. In chicks, mice, and man, RXRγ is known to be expressed in developing cones [47–49]. In a study of developing mouse and human retinas, Roberts and colleagues have shown that the receptor is transiently but substantially downregulated at the time of onset of S-opsin [48]. At this stage, nearly all mouse cones express either RXRγ or S-opsin, but very rarely both—a result consistent with the hypothesis that downregulation of RXRγ promotes the onset of S-opsin. In mice that were knockouts for *RXRG*, the normal dorsal-ventral gradient of S-opsin expression was abolished and S-opsin was expressed in all cones. However, the knockout mice had a normal pattern of expression of M-opsin.

Mice have cones only of short-wave and middle-wave types, and nothing is so far known of a possible role of RXRγ in controlling the differentiation of human L and M cones, but the association provisionally reported here suggests that RXRγ is a candidate factor in determining the phenotypic proportion of L and M cones.

REFERENCES

1. Hl. de Vries, "The heredity of the relative numbers of red and green receptors in the human eye," Genetica **24**, 199–212 (1948).
2. Hl. de Vries, "An extension of Helmholtz's theory of colorvision," in *Réunions d'Opticiens tenue à Paris en Octobre 1946*, P. Fleury, A. Maréchal, and C. Anglade, eds. (Éditions de la Revue D'Optique, 1950), pp. 361–370.
3. W. A. H. Rushton and H. D. Baker, "Red-green sensitivity in normal vision," Vis. Res. **4**, 75–85 (1964).
4. Hl. de Vries, "Luminosity curve of trichromats," Nature **157**, 736–737 (1946).
5. J. Kremers, H. P. N. Scholl, H. Knau, T. T. J. M. Berendschot, T. Usui, and L. T. Sharpe, "L/M cone ratios in human trichromats assessed by psychophysics, electroretinography, and retinal densitometry," J. Opt. Soc. Am. A **17**, 517–526 (2000).
6. R. M. Boynton, *Human Color Vision* (Holt, Rinehart & Winston, 1979).
7. M. Lutze, N. J. Cox, V. C. Smith, and J. Pokorny, "Genetic-studies of variation in Rayleigh and photometric matches in normal trichromats," Vis. Res. **30**, 149–162 (1990).
8. M. Alpern and J. Moeller, "The red and green cone visual pigments of deuteranomalous trichromacy," J. Physiol. **266**, 647–675 (1977).
9. H. J. A. Dartnall, J. K. Bowmaker, and J. D. Mollon, "Human visual pigments: microspectrophotometric results from the eyes of seven persons," Proc. R. Soc. B **220**, 115–130 (1983).
10. J. Winderickx, D. T. Lindsay, E. Sanocki, D. Y. Teller, A. G. Motulsky, and S. S. Deeb, "Polymorphism in red photopigment underlies variation in colour matching," Nature **356**, 431–433 (1992).
11. S. L. Merbs and J. Nathans, "Absorption spectra of human cone pigments," Nature **356**, 433–435 (1992).
12. M. L. Bieber, J. M. Kraft, and J. S. Werner, "Effects of known variations in photopigments on L/M cone ratios estimated from luminous efficiency functions," Vis. Res. **38**, 1961–1966 (1998).
13. G. Jordan and J. D. Mollon, "Sons and mothers: classification of colour-deficient and heterozygous subjects by counterphase modulation photometry," in *Colour Vision Deficiencies XIII* (Springer, 1997), pp. 385–392.
14. O. Estévez, H. Spekreijse, J. T. W. van Dalen, and H. F. E. Verduyn Lunel, "The Oscar color vision test: theory and evaluation (objective screening of color anomalies and reductions)," Am. J. Optom. Physiolog. Opt. **60**, 892–901 (1983).
15. H. Le Sueur, J. D. Mollon, J. Granzier, and G. Jordan, "Counterphase modulation photometry: comparison of two instruments," J. Opt. Soc. Am. A **31**, A34–A37 (2014).
16. A. Metha and A. J. Vingrys, "The C-100: a new dichotomiser of colour vision defectives," Clin. Exp. Optom. **75**, 114–123 (1992).
17. J. D. Mollon, "On the origins of polymorphisms," in *Frontiers of Visual Science: Proceedings of the 1985 Symposium* (National Academy, 1987), pp. 160–168.
18. M. V. Danilova, C. H. Chan, and J. D. Mollon, "Can spatial resolution reveal individual differences in the L : M cone ratio?" Vis. Res. **78**, 26–38 (2013).
19. M. F. Lyon, "Gene action in the X-chromosome of mouse (*Mus Musculus* L)," Nature **190**, 372–373 (1961).
20. S. M. Hood, J. D. Mollon, L. Purves, and G. Jordan, "Color discrimination in carriers of color deficiency," Vis. Res. **46**, 2894–2900 (2006).
21. Y. Wang, P. M. Smallwood, M. Cowan, D. Blesh, A. Lawler, and J. Nathans, "Mutually exclusive expression of human red and green visual pigment-reporter transgenes occurs at high frequency in murine cone photoreceptors," Proc. Natl. Acad. Sci. USA **96**, 5251–5256 (1999).
22. S. S. Deeb, M. Dorschner, A. Shafer, T. Kutyavin, and J. Stamatoyannopoulous, "Novel regulatory regions of the human L/M photopigment gene locus," Investig. Ophthalmol. Vis. Sci. **45**, E-Abstract 654 (2004).
23. S. S. Deeb, "Genetics of variation in human color vision and the retinal cone mosaic," Curr. Opin. Genet. Dev. **16**, 301–307 (2006).
24. K. L. Gunther, J. Neitz, and M. Neitz, "Nucleotide polymorphisms upstream of the X-chromosome opsin gene array tune L : M cone ratio," Vis. Neurosci. **25**, 265–271 (2008).
25. P. T. Goodbourn, J. M. Bosten, R. E. Hogg, G. Bargary, A. J. Lawrance-Owen, and J. D. Mollon, "Do different "magnocellular tasks" probe the same neural substrate?" Proc. R. Soc. B **279**, 4263–4271 (2012).
26. A. J. Lawrance-Owen, G. Bargary, J. M. Bosten, P. T. Goodbourn, R. E. Hogg, and J. D. Mollon, "Genetic association suggests that SMOC1 mediates between prenatal sex hormones and digit ratio," Hum. Genet. **132**, 415–421 (2013).
27. P. T. Goodbourn, J. M. Bosten, G. Bargary, R. E. Hogg, A. Lawrance-Owen, and J. D. Mollon, "Variants in the 1q21 risk region are associated with a visual endophenotype of autism and schizophrenia," Genes Brain Behav., doi: 10.1111/gbb.12096 (in press).
28. J. Birch, *Diagnosis of Defective Colour Vision* (Oxford University, 1993).
29. R. A. Crone, "Spectral sensitivity in color-defective subjects and heterozygous carriers," Am. J. Ophthalmol. **48**, 231–238 (1959).
30. A. Adam, "Foveal red-green ratios of normals, colourblinds and heterozygotes," in *Proceedings of the Tel-Hashomer Hospital* (Tel-Aviv, 1969), pp. 2–6.
31. J. François, G. Verriest, V. Mortier, and R. Vanderdonck, "Over de frekwentie der aangeboren kleurzin-deficienties bij de mannelijke bevolking," Nederlands Tijdschrift voor de Psychologie **12**, 24–37 (1957).
32. O. Vierling, *Die Farbensinnprüfung bei der Deutschen Reichsbahn* (Melsungen, 1935).
33. J. Pokorny, V. C. Smith, G. Verriest, and A. J. L. G. Pinckers, *Congenital and Acquired Color Vision Defects* (Grune & Stratton, 1979).
34. R. Lakowski, "Theory and practice of colour vision testing: a review. Part 2," Brit. J. Indust. Med. **26**, 265–288 (1969).
35. J. D. Mollon, ""Cherries among the leaves": the evolutionary origins of color vision," in *Color Perception: Philosophical, Psychological, Artistic and Computational Perspectives*, S. Davis, ed. (Oxford University, 2000), pp. 10–30.
36. D. M. Dacey, "Colour coding in the primate retina: diverse cell types and cone-specific circuitry," Curr. Opin. Neurobiol. **13**, 421–427 (2003).
37. G. Jordan and J. D. Mollon, "A study of women heterozygous for colour deficiencies," Vis. Res. **33**, 1495–1508 (1993).
38. S. J. Belcher, K. W. Greenshields, and W. D. Wright, "Colour vision survey using the Ishihara, Dvorine, Boström and Kugelberg, and American Optical Hardy-Rand-Rittler test," Brit. J. Ophthalmol. **42**, 355–359 (1958).
39. R. Lakowski, "Colorimetric and photometric data for the 10th edition of the Ishihara plates," Brit. J. Physiolog. Opt. **22**, 195–207 (1965).
40. M. X. Li, J. M. Y. Yeung, S. S. Cherny, and P. C. Sham, "Evaluating the effective numbers of independent tests and significant *p*-value thresholds in commercial genotyping arrays and public imputation reference datasets," Hum. Genet. **131**, 747–756 (2012).
41. M. I. Dawson and Z. Xia, "The retinoid X receptors and their ligands," Biochim. Biophys. Acta **1821**, 21–56 (2012).
42. A. Onishi, G. H. Peng, S. M. Chen, and S. Blackshaw, "Pias3-dependent SUMOylation controls mammalian cone photoreceptor differentiation," Nat. Neurosci. **13**, 1059–1065 (2010).
43. A. Swaroop, D. Kim, and D. Forrest, "Transcriptional regulation of photoreceptor development and homeostasis in the mammalian retina," Nat. Rev. Neurosci. **11**, 563–576 (2010).
44. L. Ng, J. B. Hurley, B. Dierks, M. Srinivas, C. Salto, B. Vennstrom, T. A. Reh, and D. Forrest, "A thyroid hormone receptor that is required for the development of green cone photoreceptors," Nat. Genet. **27**, 94–98 (2001).
45. D. Forrest and A. Swaroop, "Minireview: the role of nuclear receptors in photoreceptor differentiation and disease," Mol. Endocrinol. **26**, 905–915 (2012).
46. S. S. Deeb and Y. Liu, "Thyroid hormone and 9—cis retinoic acid transcriptionally activate the human L/M cone opsin genes," Investig. Ophthalmol. Vis. Sci. **46**, E-Abstract 3074 (2005).

47. F. Hoover, E. A. Seleiro, A. Kielland, P. M. Brickell, and J. C. Glover, "Retinoid X receptor gamma gene transcripts are expressed by a subset of early generated retinal cells and eventually restricted to photoreceptors," J. Comp. Neurol. **391**, 204–213 (1998).

48. M. R. Roberts, A. Hendrickson, C. R. McGuire, and T. A. Reh, "Retinoid X receptor γ is necessary to establish the S-opsin gradient in cone photoreceptors of the developing mouse retina," Investig. Ophthalmol. Vis. Sci. **46**, 2897–2904 (2005).

49. M. Mori, N. B. Ghyselinck, P. Chambon, and M. Mark, "Systematic immunolocalization of retinoid receptors in developing and adult mouse eyes," Investig. Ophthalmol. Vis. Sci. **42**, 1312–1318 (2001).