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The influence of L-opsin gene polymorphisms and neural ageing on spatio-chromatic contrast sensitivity in 20–71 year olds

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ABSTRACT

Chromatic contrast sensitivity may be a more sensitive measure of an individual's visual function than achromatic contrast sensitivity. Here, the first aim was to quantify individual- and age-related variations in chromatic contrast sensitivity to a range of spatial frequencies for stimuli along two complementary directions in color space. The second aim was to examine whether polymorphisms at specific amino acid residues of the L- and M-opsin genes (OPN1LW and OPN1MW) known to affect spectral tuning of the photoreceptors could influence spatio-chromatic contrast sensitivity. Chromatic contrast sensitivity functions were measured in 50 healthy individuals (20–71 years) employing a novel pseudo-isochromatic grating stimulus. The spatio-chromatic contrast sensitivity functions were found to be low pass for all subjects, independent of age and color vision. The results revealed a senescent decline in spatio-chromatic contrast sensitivity. There were considerable between-individual differences in sensitivity within each age decade for individuals 49 years old or younger, and age did not predict sensitivity for these age decades alone. Forty-six subjects (including a color deficient male and eight female carriers) were genotyped for L- and M-opsin genes. The Ser180Ala polymorphisms on the L-opsin gene were found to influence the subject's color discrimination and their sensitivity to spatio-chromatic patterns. The results expose the significant role of neural and genetic factors in the deterioration of visual function with increasing age.

1. Introduction

Differences in contrast are the primary signals for vision. Measurements of sensitivity to contrast are informative with regards to an individual's ability to perceive slight changes in luminance and chromaticity and gives important insight to their overall visual function. Some psychophysical studies have reported a decline in spectral sensitivity with age (Fiorentini, Porciatti, Morrone, & Burr, 1996; Knoblauch, Vital-Durand, & Barbur, 2001; Werner & Steele, 1988), with older subjects being less sensitive to spatio-chromatic contrast than younger subjects (Fiorentini et al., 1996; Knoblauch et al., 2001, for a review see reference Werner, Delahunt, and Hardy (2004). The decline in contrast sensitivity for both luminance and chromatic contrast appears to begin somewhere between 30 and 70 years of age (Fiorentini et al., 1996). The reasons for these observed senescent declines are debatable, but are commonly attributed to optical factors (Owsley, Sekuler, & Siemsen, 1983; Steen, Whitaker, Elliott, & Wild, 1994; Weale, 1988; Werner, 1982). An alternate explanation, as supported by the current work, is that the decline in sensitivity can be ascribed to neural factors (Elliott, 1987; Hardy, Delahunt, Okajima, & Werner, 2005; Higgins, Jaffe, Caruso, & deMonasterio, 1988; Sloane, Owsley, & Alvarez, 1988; Sloane, Owsley, & Jackson, 1988; Werner, Schwarz, & Paulus, 1995). An individual's chromatic sensitivity at a given age might therefore be related to the degree of loss in cone photoreceptor sensitivity.

Sensitivity, arising from a combination of signals from cone photoreceptors, is likely also to be dictated by the amino acid sequences of the (L) and (M) opsin genes (OPN1LW and OPN1MW). This is because there are considerable variations in the amino acid sequences of the L- and M-opsin genes even among subjects with normal trichromatic color vision (Neitz, Neitz, & Grishok, 1995; Winderickx, Battisti, Hibiya, Motulsky, & Deeb, 1993). Variations in the amino acid sequences of these cone opsins are responsible for spectral tuning of the photoreceptors (Neitz, Neitz, & Jacobs, 1991), and inherited red-green color vision deficiencies are due to alterations in these cone opsin genes

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(Nathans, Piantanida, Eddy, Shows, & Hogness, 1986; Vollrath, Nathans, & Davis, 1988). Polymorphisms in the L- and M-opsin genes give rise to amino acid differences that influence the spectral sensitivity of the encoded pigments. The peak sensitivity of the L- and M-pigments range from 549 to 559 nm and 530 to 533 nm respectively (Carroll, McMahon, Neitz, & Neitz, 2000). The presence of either serine (Ser) or alanine (Ala) at position 180 of the L-pigment gene has been found to be one of the most prevalent polymorphisms that results in variation of chromatic discrimination in males as measured with Rayleigh anomaloscopy (Carroll, Neitz, & Neitz, 2002; Neitz et al., 1995; Sharpe et al., 1998; Winderickx et al., 1992). Ser180 results in a green-shift in Rayleigh match-midpoints (RMMP) and a shift in peak sensitivity (~3.5 nm) of the L-cone towards longer wavelengths compared with Ala180 (Carroll et al., 2002).

Considerable variation in color vision behavior is observed among female subjects, both normal females and those that are heterozygous carriers of red-green color vision deficiencies (e.g. Crone (1959), Dees and Baraas (2014), Feig and Ropers (1978), Hill (1980), Jordan and Mollon (1993), Waaler (1927)). Females have two X-chromosomes, and the one expressed in a given cone cell is determined by X-chromosome inactivation (Lyon, 1972). Hence, both normal females and carriers may have different forms of L- and M-opsin genes on each X-chromosome (Hunt et al., 1998), and this can result in retinas that express up to five different cone pigments (two different L pigments, two different M pigments, and the S pigment; Bosten, Robinson, Jordan, & Mollon, 2005; Jordan & Mollon, 1993, 1997; Jørgensen et al., 1992). It is not unlikely that the number of expressed cone pigments and the spectral separation between these could influence sensitivity to spatio-chromatic patterns, as this would affect the photon catch by the individual's cone mosaic (Sekiguchi, Williams, & Brainard, 1993) and post-receptoral signals. Expression of more than one L- and one M-cone pigment will result in a narrower spectral separation between the expressed cones and therefore increase their spectral overlap. Alternative suggestions to whether this would affect the level of post-receptoral noise, and an increase or decrease in chromatic sensitivity, has been made following reports of differences between males and females (Murray, Parry, McKeefry, & Panorgias, 2012; Rodríguez-Carmona, Sharpe, Harlow, & Barbur, 2008).

The conundrum of chromatic sensitivity with regards to senescence and male–female differences needs to be resolved if we are to understand what causes the large variation in age-related changes in vision (Spear, 1993). Here we sought to study this from two angles combining genetics and measures of sensitivity with a novel spatio-chromatic stimulus. First, we investigated age-related variations in spatio-chromatic contrast sensitivity for stimuli along two directions in color space across a range of spatial frequencies. Second, we explored whether the deduced spectral separation between the underlying L- and M-cone photopigments influenced between-individual variation in spatio-chromatic contrast sensitivity.

2. Methods

2.1. Subjects, ocular health and color vision testing

Forty-one normal subjects (24 females and 17 males, aged 20–71 years, *Mean* = 41.0, *SD* = 17.5, Table 1), one minimally deuteranomalous male (aged 36 years), two female carriers of protan deficiencies (aged 21 and 36 years) and six female carriers of deutan deficiencies (*Mean* = 32.8, *SD* = 14.0) were included in the study. Genetic analyses of X-chromosome visual pigment genes (see Section 2.3) were carried out for 46 subjects: 16 males: *Mean* = 44.31 years, *SD* = 17.2, 30 females: *Mean* = 34.4 years, *SD* = 14.1), including the color deficient male and the eight female carriers (see also Table 2). Four normal subjects were not available to give a biological sample.

The subjects were healthy with no known ocular abnormalities. Fundus photos of the central 45° (Topcon TRC-NW6S), and spectral domain optical coherence tomography with 30° scan-width with 2 and 49 B-scans (100 and 20 frames, respectively), 512 A-scans/B-scan (SD-OCT, Spectralis™ SD-OCT system, Heidelberg Engineering, Heidelberg, Germany) were performed on each subject and found to be normal and free of eye disease. The density and opacities of the eye lens was evaluated with a slit lamp microscope and graded with The Lens Opacities Classification System III (LOCS III), and were required to be no greater than grade 2 (Chylack et al., 1993). None of the subjects had undergone cataract operation.

The subjects were corrected to best logMAR visual acuity, used optimal spectacle or contact lens correction for the distance tested and viewed the stimulus monocularly using their preferred eye with natural pupils. Their statuses of either normal or carrier were confirmed by family history and genetic analysis of the genes encoding the L- and M-cone pigments (for more details see Section 2.2). Color vision was assessed with several standard color vision tests, including Ishihara (24 pl. ed., Kanehara trading INC, Tokyo, Japan), Hardy–Rand–Rittler fourth edition (HRR, Richmond Products, Albuquerque, NM; Bailey, Neitz, Tait, & Neitz, 2004; Cole, Lian, & Lakkis, 2006), Rayleigh anomaloscopy (HMC Oculus Anomaloscope MR, Typ 47700, Oculus Optikgeräte GmbH, Germany) and Medmont C-100 (Medmont Pty Ltd, Vermont, Australia). Each test was administered and performed according to its accompanying guidelines. The HRR and Ishihara plates were administered in an otherwise darkened room with the 'True Daylight Illuminator with Easel' (color temperature 6280 K, model number 1339R, Richmond Products, Albuquerque, NM). The level of illumination was measured at the surface of the test plates with a digital lux meter (Hagner Model EC1, Hagner AB, Solna, Sweden), and averaged about 900 lux. Written test procedures were strictly followed. A single operator (author EWD, a qualified optometrist) collected all the data.

Informed consent was obtained from all subjects after the nature of the study and possible complications were explained both verbally and in writing. The research was approved by the Regional Committee for Medical Research Ethics for the Southern Norway Regional Health Authority and was conducted in accordance with the principles embodied in the Declaration of Helsinki (Code of Ethics of the World Medical Association).

2.2. Spatio-chromatic contrast sensitivity

Spatio-chromatic contrast sensitivity was measured in all subjects, employing a novel pseudo-isochromatic grating stimulus along two complementary directions in color space. The stimulus (Fig. 1) was a chromatic sinusoid grating with a pseudo-isochromatic design that consisted of an array of spatially discrete round spots of varying size and luminance. The principle of the design is similar to that used in pseudo-isochromatic plates (Regan, Reffin, & Mollon, 1994; Stilling, 1918).

The spots that made up the background had a single chromaticity. The spots that made up the grating had a chromaticity determined by the mean color of the region of the grating covered by each spot.

A grating is produced by the addition of two sine waves (L_1, L_2) where luminance and contrast of both of them may be changed in an interrelated manner. L_0 is the mean luminance of the resultant grating. Spatial luminance distribution is described as a function of phase φ, and luminance and contrast ratios r_1 and r_2 respectively: $L_1(r_1, r_2) = r_1 \times L_0 \times (1 \pm r_2) \times m \times \cos\varphi$ and $L_2(r_1, r_2) = (1 - r_1) \times L_0 \times (1 \pm r_2) \times m \times \cos\varphi$.

Table 1
Age (range and mean), sample size, gender, eye tested, distance correction and best-corrected visual acuity (BCVA) for the eye tested of the 41 normal trichromatic subjects included in the study.

Age decades	Age years		Sample size	Gender *n* (% females)	Eye *n* (% OD)	Sph. Eq. [*D*] mean (SD)	BCVA [logMAR] range
	Range	mean (SD)					
20–29	20–27	22.2 (2.11)	15	10 (66.67)	10 (66.67)	−2.75 (3.22)	0.06–(−0.14)
30–39	32–39	35.00 (2.87)	5	4 (80.00)	4 (80)	−1.75 (2.01)	0–(−0.1)
40–49	41–47	42.67 (2.50)	6	5 (83.33)	6 (100)	−1.60 (2.30)	0–(−0.22)
50–59	52–59	55.43 (3.05)	7	2 (28.57)	7 (100)	−0.61 (2.56)	0.14–(−0.01)
60–69	62–67	64.5 (2.17)	6	2 (33.33)	4 (66.67)	0.35 (2.88)	0.02–(−0.08)
70+	71–71	71 (0)	2	1 (50)	1 (50)	−1.94 (3.09)	0.14–0.04

Luminance ratio of the resultant grating is expressed as $r_1 = \frac{L_1}{L_1+L_2}$; values ranging from 0 to 1 with the two component sinusoids being of equal luminance at a ratio of 0.5. Contrast ratio of the resultant grating is expressed as $r_2 = \frac{C_1}{C_1+C_2}$; values ranging from 0 to 1, with the two component sinusoids being of equal modulation at a ratio of 0.5. Here, the cone contrast of the two components was varied simultaneously by adjusting the modulation factor, keeping both the contrast ratio and luminance ratio at 0.5, to find the threshold at each spatial frequency, akin to heterochromatic modulation photometry (Pokorny, Smith, & Lutze, 1989).

The spectral components of the grating varied along either the protan or the deutan dichromatic confusion axes in the CIE 1931 (*x*, *y*, *Y*) chromaticity diagram through a mean chromaticity set to CIE illuminant D65 (0.313, 0.329; appears grey for the luminance level used). The background was the same as the mean chromaticity. The end-point CIE (*x*, *y*)-coordinates of the two axes were (0.393, 0.315) and (0.225, 0.345) for protan, and (0.372, 0.289) and (0.234, 0.381) for deutan. These two stimuli are referred to as the protan and the deutan stimuli, but neither isolates responses for a single cone-type, as they are photometrical isoluminant. Cone excitations were calculated from the CIE (1931) (*x*, *y*, *Y*) coordinates using Smith–Pokorny cone fundamentals (Smith & Pokorny, 1975) based on transformations from Judd color matching functions (Judd, 1951). Relative (Weber) cone contrasts between the grating and the background were calculated for each cone type (C_L, C_M, C_S; Cole & Hine, 1992) and pooled cone contrast as the square root of ($CL^2 + CM^2 + CS^2$; Chaparro, Stromeyer, Huang, Kronauer, & Eskew, 1993). Pooled cone contrast sensitivity is also referred to as chromatic contrast sensitivity.

The space between spots was filled with a mean-luminance-chromaticity and no part of the underlying grating was visible in the stimulus, except that reconstructed by the chromatic pathway by grouping spots of similar hue. The luminance of each spot was chosen at random from a uniform distribution with range 30.0–46.0 cd/m^2, centered on the average luminance of the grating and background of 38.0 cd/m^2. This is equivalent to about 480 photopic Trolands assuming a pupil size of 4 mm. Each spot's luminance was randomized on every trial. The spots covered 65% of the monitor's display area, and the radius of each spot was chosen from a normal distribution with mean 0.06° and standard deviation 0.02°. These parameters were chosen to be above the Nyquist sampling limit such that the mean spot size did not effectively under-sample the grating. Fourier analysis of the stimulus showed that the grating sinusoid was the most dominant frequency present, with the magnitude of the grating frequency being more than 50 decibels stronger than the frequencies introduced by the spots (see Supplementary data). A new distribution of spot sizes and positions was generated at the start of every experiment. There was no observable effect of spot position/size on subjects' contrast sensitivity.

The stimuli were displayed on a calibrated 22-in. CRT monitor (ViewSonic P227f, Walnut CA, USA) via an nVidia Quadro FX2000 graphics card (Santa Clara, CA, USA) at resolutions of: 30 bits per pixel spectral; 1600 × 1200 pixels spatial; 100 Hz temporal. To ensure the stability of the monitor, colorimetric values and the spectral radiance output of each phosphor was measured daily with a spectrophotometer (SpectraScan PR650, Photo Research) before any experiments were carried out. Errors in the displayed CIE (*x*, *y*, *Y*) coordinates of test patches were <0.005 in (*x*, *y*) and <5% in *Y* (within the range of light levels of the stimuli). For the CRT monitor used here, modulation along the protan and deutan axis resulted in maximum L- and M-cone contrast of 7.8% and 14.1% respectively.

Chromatic contrast sensitivity was measured for nine or 12 different spatial frequencies from 0.3 to 3.0 c/deg in all subjects. The subjects viewed the stimulus at a distance of 114.6 cm. The size of the stimulus was 14° in diameter and at least four cycles of the grating were displayed for all spatial frequencies to prevent a possible reduction in sensitivity at lower spatial frequencies (Campbell & Robson, 1968; Findlay, 1969; Savoy & McCann, 1975). The experiment was carried out in an otherwise darkened room.

Prior to each experiment, the subjects were dark adapted for five minutes, then light adapted for 30 s by viewing a neutral grey screen with the same color and luminance as the background of the stimuli. A two-alternative forced-choice procedure was implemented and the contrast of the stimuli were altered using a staircase employing a three-down and one-up rule (Wetherill & Levitt, 1965). The staircase step size was *N*/24, where *N* was the range of tested contrasts, except the first 5 steps which used step sizes of 6*N*/24, 3*N*/24, 2*N*/24, 1.5*N*/24 and 1.2*N*/24 respectively. This strategy quickly localized the subject's threshold and still retained a sufficiently small step size for accurate testing around that threshold. The subject's task was to maintain fixation on a black cross that appeared at the center of the display and indicate whether the top of the grating pointed 30° towards the left (from vertical) or to 30° towards the right. The stimulus presentation time was 500 ms with an inter-trial interval of at least 1000 ms (the exact time depending on how fast the subject responded after viewing the stimuli). During the inter-trial interval, the display showed a uniform field, using the same neutral grey as the background, to avoid pattern adaptation.

Three or four different spatial frequencies were tested in each block and each block was repeated three times, with each block taking about 15–20 min to complete. Subjects were tested for 1-h each time, taking approximately 10–13 h for each subject to complete the experiment (including color vision testing).

2.3. Genetic analysis

Blood (whole blood) or saliva samples (Oragene-DNA, OG-500, DNA Self-Collection Kit, DNA Genotek Inc., Ottawa, ON, Canada) were obtained from the subjects to analyze the genes encoding for the L- and M-cone pigments. Genomic DNA was extracted from peripheral leukocytes using ArchivePure DNA Purification

Table 2
Chromatic contrast sensitivity and molecular genetic data. Area under curve (AUC), estimated limiting sensitivity (ELS: *Y*-intercept, protan curve), predicted peak sensitivity of L- and M-cone pigments, L- and M-haplotype (exon 2, 3 and 4) and probable gene array presented for normal females and males, one deuteranomalous male (DA) and protan- (PC) and deutan carriers (DC). Subject 4072 is genotypically mild deuteranomalous with probable gene array LLMM and a 7 nm-spectral separation of L- and M-cone pigment. 4053 and 4047 are carriers of deuteranopia with probably gene array L + LM.

		AUC	ELS (*Y*-intercept)	Predicted peak sensitivity (nm)[a]		Haplotype (exon 2, 3 and 4)[b,c,d]						Probable array[e]
	ID	Protan axis		L	M	L-pigment gene			M-pigment gene			
Normal females	4066	3.7	4.3	559 + 555.5	530	T I S	L/M V A/V I S/A	IAM	I V Y	M V A I/V A	T S V	LM + LMM
	4060	4.7	3.9	555.5	530	T I S	L/M V A I A	IAM	I V Y	M V A I A	T S V	LM + LMM
	4055	6.1	5.0	559	530	T I S	L/M V A/V I S	IAM	I V Y	M V A I/V A	T S V	LMM + LMM
	4036	8.1	4.6	559 + 555.5	530	T I S	L V A I S/A	IAM	I V Y	M V A I A	T S V	LM + LMM
	4052	8.4	5.5	559 + 555.5	530	T I S	L/M V A I S/A	IAM	I V Y	M V A I A	T S V	LM + LMM
	4051	8.6	5.1	555.5	530	T I S	L V A I A	IAM	I V Y	L/M V A I A	T S V	LM + LMM
	4004	9.3	7.9	559 + 555.5	533 + 530	T I S	L/M V A/V I/V S/A	IAM	I V Y	L/M V A/V I/V S/A	T S V	LMM + LMMM
	4077	9.5	4.3	559 + 555.5	533 + 530	T I S	L I/V A I S/A	IAM	I V Y	M V V I/V S/A	T S V	LM + LMM
	4028	9.6	6.1	559 + 555.5	533 + 530	T I S	L V A I S/A	IAM	I V Y	L/M V A I S/A	T S V	LM + LMM
	4070	9.6	6.6	559	530	T I S	L I/V A I S	IAM	I V Y	M V A/V I/V A	T S V	LM + LMM
	4025	9.8	7.3	555.5	530	T I S	L/M V A I A	IAM	I V Y	M V A I A	T S V	LMM + LMM
	4005	9.9	5.3	555.5	530	T I S	L/M V A I A	IAM	I V Y	L/M V A I A	T S V	LM + LMM
	4003	10.1	9.2	559 + 555.5	530	T I S	L/M V A I S/A	IAM	I V Y	M V A I A	T S V	LM + LMM
	4067	11.8	5.9	559	530	T I S	L V A I S	IAM	IVY	M V A I A	T S V	LM + LMM
	4054	12.0	8.0	555.5	530	T I S	L/M V A I A	IAM	I V Y	L/M V A I A	T S V	LMM + LMM
	4078	12.3	7.6	559	530	T I S	L I A I S	IAM	I V Y	M V V V A	T S V	LM + LMM
	4038	12.6	7.9	559	533 + 530	T I S	L/M V A/V I S	IAM/V	I V Y	L/M V A/V I/V S/A	T S V	LM + LM
	4001	12.8	6.6	559	530	T I S	L/M I/V A I S	IAM	I V Y	L/M V A I A	T S V	LMM + LMM
	4080[f]	13.6	7.1	559 + 555.5	530	T I S	L/M V A I S/A	IAM/V	I V Y	L/M V A/V I/V A	T S V	LM + LMM
	4050[f]	14.0	6.2	559 + 555.5	533 + 530	T I S	L V A I S/A	IAM	T/I V Y	M V A I S/A	T S V	LM + LMM
	4010[f]	16.2	8.3	559 + 555.5	530	T I S	M V A I S/A	IAM	I V Y	M V A I A	T S V	LMM + LMM
	4027[f]	16.9	7.5	559 + 555.5	530	T I S	M V A I S/A	IAM/V	I V Y	M V A/V I/V A	T S V	LM + LMM
Normal males	4083	4.0	2.6	555.5	530	T I S	M V A I A	IAM	I V Y	M V A I A	T S V	LMM
	4082	4.4	3.3	555.5	530	T I S	M V A I A	IAM	I V Y	M V A I A	T S V	LM
	4059	4.4	3.3	559	530	T I S	L V A I S	IAM	I V Y	M V A I A	T S V	LM
	4040	5.1	5.8	559	530	T I S	L V A I S	IAM	I V Y	M V V V A	T S V	LM
	4049	6.5	4.1	559	530	T I S	L V A I S	IAM	I V Y	M V V V A	T S V	LM
	4048	6.8	4.3	559	530	T I S	L V A I S	IAM	I V Y	M V A I A	T S V	LMM
	4079	8.0	4.3	559	533 + 530	T I S	L I A I S	IAM	I V Y	L/M V A I S/A	T S V	LM
	4073	8.0	5.0	559	530	T I S	M V A I S	IAM	I V Y	M V A I A	T S V	LMM
	4017	11.2	7.0	559	530	T I S	L V A I S	IAM	I V Y	M V A I A	T S V	LMMM
	4064	11.4	7.3	559	533	T I S	M V V I S	IAV	I V Y	M V V V S	T S V	LM
	4016	12.0	8.0	559	530	T I S	L V A I S	IAM	I V Y	L/M V A I A	T S V	LMM
	4075	12.0	7.5	559	530	T I S	L I A I S	IAM	I V Y	M V V V A	T S V	LM
	4043	12.7	9.7	559	530	T I S	L V A I S	IAM	I V Y	M V A I A	T S V	LMMM
	4015	13.2	8.6	556.5	533 + 530	I V Y	L V A I S	IAM	I V Y	L/M V A I S/A	T S V	LMM
	4071	14.0	8.1	559	530	T I S	M I A I S	IAM	I V Y	M V V V A	T S V	LMM
DA	4072	7.3	7.3	–	530	T/I I/V S/Y	L/M VAIA	I/T A/S M/V	IVY	MVAIA	TSV	LLMM
PC	4011	10.2	6.4	555.5	530	T I S	M V A I A	IAM	T/I I/V S/A	L/M V A I A	T S V	LMM + MMM
	4002	15.1	6.6	–	530	T/I I/V S/Y	M V A I S/A	I/T A/S M/V	T/I I/V S/A	L/M V A I A	T S V	LM + LMM[g]
DC	4047	4.6	4.2	559	536	T I S	L V A/V I S	IAM	I V Y	L V A I S	T A M	L + LM
	4012	6.6	5.5	555.5 + 553	530	T/I I/V S/Y	L/M V A I A	IAM	I V Y	M V A I A	T S V	LLM + LMM
	4041	8.0	6.6	555.5 + 553	530	T/I I/V S/Y	L/M V A I A	IAM/V	I V Y	M V A I A	T S V	LLM + LMM
	4063	9.7	8.2	–	533 + 530	T/I I/V S/Y	L/M V A/V I S/A	IAM/V	I V Y	M V A I S/A	T S V	LLL + LMM
	4053	10.0	5.1	555.5	530	T I S	L V A I A	IAM	I V Y	L/M V A/V I/V A	T S V	L + LM
	4037	11.9	7.7	–	530	T/I I/V S/Y	L/M I/V A/V I/V S/A	I/T A/S M/V	I V Y	M V A I A	T S V	LL + LM

-Peak sensitivity not estimated.
[a] See reference Carroll et al. (2002) for estimate of peak sensitivities.
[b] Amino acid positions: 65, 111, 116 on exon 2; 153, 171, 174, 178, 180 on exon 3; 230, 233, 236 on exon 4.
[c] Single letter amino acid code is as follows: T = threonine, I = isoleucine, S = serine, L = Leucine, V = Valine, M = Methionine, A = alanine, Y = tyrosine.
[d] Two amino acids separated by a slash, for example S/A means that the person had multiple L-opsin genes and at least one had Ser180 and one had Ala180.
[e] Number of long- (L) and medium-opsin genes (M) per X-chromosome array.
[f] The four individuals with highest cone contrast sensitivity.
[g] 4002 is a known carrier of a protan deficiency (inferred from her protan deficient son), and her probable gene array given by the mass array analysis is LM + LMM. This analysis does only look at the total number and type of L- and M-genes on the X-chromosome, but it does not tell the exact order of the genes. Her probable array will therefore be LM + MML due to her son's color vision status.

kit (5 prime GmbH, Thermo Fisher Scientific Company, LLC, USA) and from saliva samples using repIT®•L2P kit (DNA Genotek Inc., Ottawa, ON, Canada).

The X-chromosome visual pigment genes (OPN1LW and OPN1MW) were selectively amplified using long distance polymerase chain reaction (PCR), with reverse primers corresponded to sequences within exon 5 that encode amino acid differences responsible for the spectral differences between L- and M-pigments. Forward primers corresponded to sequences within exon 2 that are common to both L- and M-genes. The L-opsin gene was amplified with forward primer 5' GTCTCTGGCTTGAGGGACAG and the reverse primer was 5′GCAGTACGCAAAGATCATCACC; the M-opsin gene was amplified with forward primer 5′CCTTCGAAGGCCCGAATTA and reverse primer 5′CAGAAGCAGAATGCCAGGAC.

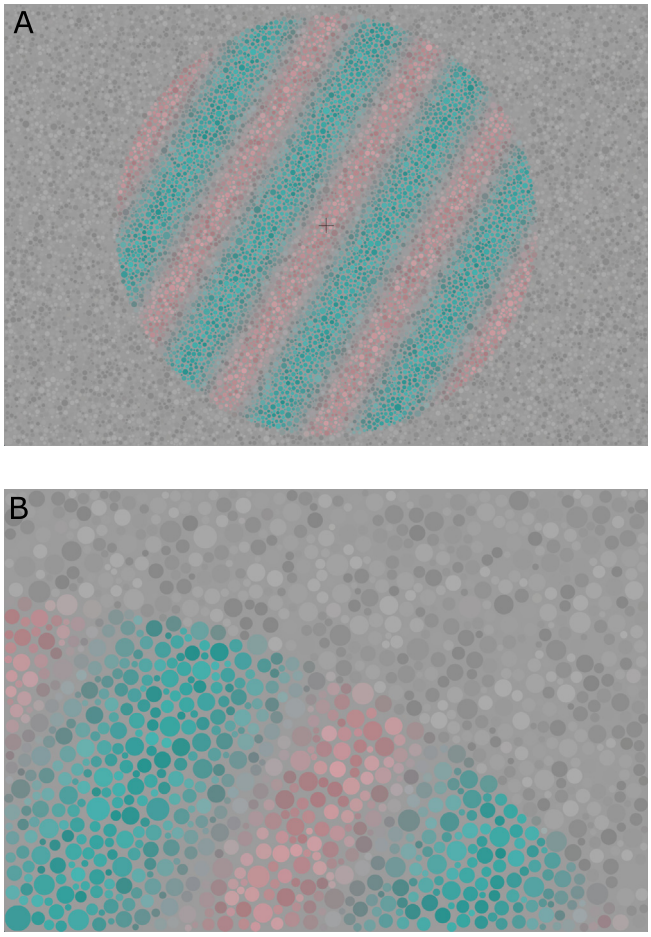

Fig. 1. Image of a typical right-facing protan pseudo-isochromatic grating (A), and a magnified view demonstrating that the grating can only be perceived by grouping spots of similar hue (B).

The amplifications were carried out using Platinium® Taq DNA Polymerase High Fidelity kit (Invitrogen™, Life Technologies, Grand Island, NY, USA) with a hot start. The thermal cycler used was the 96-well 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA), and the thermal cycler parameters were 94 °C for 2 min for one cycle, followed by 35 cycles of 94 °C for 30 s, 62 °C for 30 s and 68 °C for 10 min. The PCR product was further used to amplify the specific regions exon 2, 3 and 4 for the L- and M-opsin genes as described elsewhere (Neitz et al., 2004). The nucleotide sequence of each exon was determined by direct fluorescent sequencing and the sequencing analysis was done with 3500 Genetic analyzer (Applied Biosystems, Foster City, CA, USA). Results from the long PCR are based on polymorphisms on a single opsin gene.

Single nucleotide polymorphism (SNP) genotyping was also carried out with the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer-based instrument Sequenom MassARRAY® (Sequenom Inc., San Diego, CA, USA). The instrument uses specially designed PCR primers where a short length of DNA surrounding the SNP is amplified, followed by single-nucleotide extensions of a primer annealing adjacent to the SNP. An estimate of the relative number of L- and M-genes and probable architecture of the gene array in terms of the relative number of L- and M-genes was measured. Results from MassARRAY are based on polymorphisms on both L- and M-opsin genes combined.

The genetic analysis also revealed if some of the females with normal color vision were obligatory carriers of a red-green color vision deficiency. Protan or deutan carriers were genetically identified by the gene in the first position on one of the X-chromosomes opsin gene arrays. A carrier having M-opsin gene in the first position on one array and L-opsin gene in first position on the other array is a protan carrier (Kainz, Neitz, & Neitz, 1998). Some deutan carriers were identified by virtue of having one array with L-opsin genes in both the first and second positions on one array.

2.4. Analysis and statistics

For each spatial frequency, raw data from 3×80 trials were combined. The contrast thresholds were estimated from the 75% correct point of the psychometric function and 95% confidence intervals on this point were calculated with a bootstrap procedure, based on 2000 data sets simulated from the number of experimental trials at each level tested (Foster & Bischof, 1997; Fründ, Haenel, & Wichmann, 2011).

A parametric model was fitted to each subject's threshold data, and three scalar values were extracted from the fitted chromatic contrast sensitivity functions for further analysis: estimated limiting spatial frequency, estimated limiting sensitivity, and area under the sensitivity curve. To make these values robust to subject variability, the bootstrap sensitivity estimates are fitted first by a model-free non-linear regression (LOESS) effectively removing outliers, and then further fitted by an exponential curve, which is a numerically robust approximation permitting easy extrapolation to the two axes. The exponential curve fit was chosen as a parsimonious model of the low-pass filter behavior observed both for chromatic neural cells (for a review see reference Lee (2011)) and for human chromatic contrast sensitivity functions (Mullen, 1985).

Statistical analysis was done with IBM SPSS statistics Version 20 (International Business Machines Corp., USA). Correlation and linear regression analyses were computed to see whether age, peak sensitivity of the L-cone and RMMP was a predictor for the sensitivity results. Kruskal–Wallis H test was carried out to explore differences between the groups. If the test revealed significant differences, Mann–Whitney U test was carried out for pairwise comparisons. Bonferroni correction for multiple comparisons was applied when appropriate with differences deemed significant when $p \leqslant 0.017$. A one-way between-groups analysis of covariance (ANCOVA) were performed for determining if there was a differences in sensitivity for observers with Ser, Ser/Ala or Ala at position 180 of OPN1LW, while controlling for age. Differences were considered significant when $p \leqslant 0.05$.

3. Results

3.1. Color vision testing

Performance on standard color vision tests are reported based on the following fail criteria: three or more misreadings on the Ishihara, and two or more misreadings on the screening plates on the HRR on the second sitting. None of the carriers made any misreadings on the Ishihara. One male (4072), who was classified as mild deuteranomalous based on genetics, misread 73 as 78 (plate 13) on the Ishihara, but made no errors on the HRR, and was within normal limits on all other tests.

3.2. Spatio-chromatic contrast sensitivity

Fig. 2A and B shows mean log pooled cone contrast sensitivity for the two chromatic stimuli as a function of spatial frequency for the six different age decades included in the study. Fig 2C and D shows the sensitivity for the two protan and five deutan carriers in comparison to normal trichromats of the same age

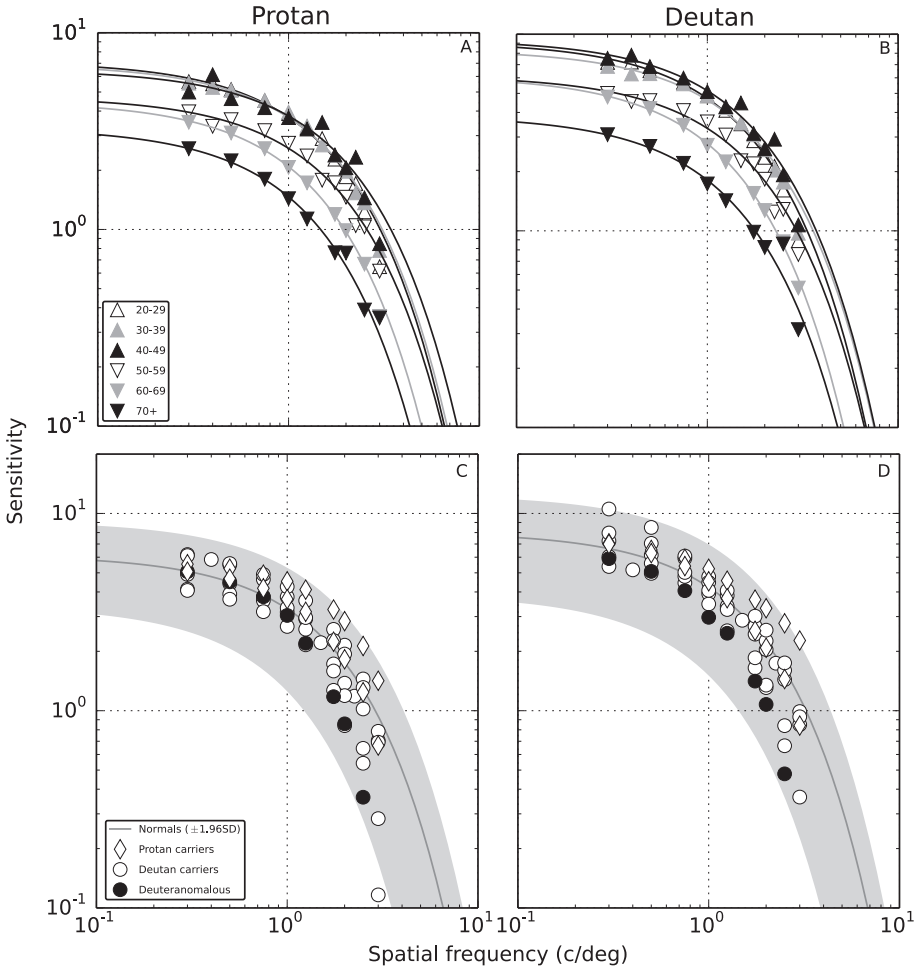

Fig. 2. Mean log pooled cone contrast sensitivity for the protan (A and C) and deutan (B and D) stimuli as a function of spatial frequency. A and B show the means for the six different age decades for all 43 normal subjects included in the study. C and D show the data for the subjects aged 20–49 whom were genotyped (for L- and M-opsin genes) with the mean cone contrast sensitivity function (solid line) and ±1.96 SD (grey area) for the normal subjects and individual data plotted for protan carriers (open diamonds), deutan carriers (open circles) and deuteranomalous male (filled circles).

(20–49). Age-related variations for normal trichromats are presented in Fig. 3A–F. Both area under curve [Fig. 3A and D, for stimuli along protan axis (P): $B = -0.122$, $t(39) = -4.80$, $p \ll 0.001$ and along deutan axis (D): $B = -0.15$, $t(39) = -4.27$, $p \ll 0.001$] and estimated limiting sensitivity were significantly predicted by the age of the subjects [Fig. 3B and E, P: $B = -0.07$, $t(39) = -5.67$, $p \ll 0.001$ and D: $B = -0.079$, $t(39) = -3.57$, $p = 0.001$], and a significant proportion of the variance in area under curve [P: $R^2 = 0.37$, $F(1, 39) = 23.1$, $p \ll 0.001$ and D: $R^2 = 0.32$, $F(1, 39) = 18.3$, $p \ll 0.001$] and estimated limiting sensitivity was explained by age [P: $R^2 = 0.45$, $F(1, 39) = 32.2$, $p \ll 0.001$ and D: $R^2 = 0.25$, $F(1, 39) = 12.7$, $p = 0.001$].

There was a small, but significant decline in estimated limiting spatial frequencies with age [Fig. 3C and F, P: $B = -0.03$, $t(39) = -2.78$, $p = 0.008$ and D: $B = -0.04$, $t(39) = -2.70$, $p = 0.010$], and a significant proportion of the variance in estimated limiting sensitivity was explained by age [P: $R^2 = 0.17$, $F(1, 39) = 7.74$, $p = 0.008$ and D: $R^2 = 0.16$, $F(1, 39) = 7.30$, $p = 0.010$].

It is clear from Fig. 3 that the between-individual variation within each age decade is as large as the age-related decline, particularly for those aged younger than 50 years of age. In fact, the age-related difference between age groups 20–29, 30–39 and 40–49 was not significant (Kruskal–Wallis H test, all $p > 0.16$), and age did not predict any of the results for these age decades alone (linear regression, all $p > 0.10$). Some of this variability may be attributed to response noise. Here, we asked the question whether peak sensitivity of the L-pigment, the deduced spectral separation between the L- and M-cone pigments or the presence of Ser and/or Ala at position 180 of L-opsin gene could explain some of the between-individual variation, both in those aged 20–49 and those aged 50–71. Note that deutan carriers (Fig. 2B, open circles) showed on average reduced sensitivity when compared with the normal subjects (see also Table 3). However, most of the carrier's performance is still within the ±1.96 SD of the normal trichromats.

3.3. Genetics

Molecular genetic data for the 46 subjects included in the genetic analysis are summarized in Table 2. The results show predicted peak sensitivities, haplotypes of the L- and M-opsin genes and probable gene array based on data from long PCR analyses. The quality of the DNA was good enough to determine the identity of the genes in the expressed positions. For all but one subject (4063) all L-genes reside in an expressed position. Subject 4063 has 3 opsin genes on one X chromosome, and we could not determine whether the second gene specified Ser or Ala, therefore this subject was not included in the analysis of associations with Ser180Ala polymorphisms on the L-opsin gene (3.3.1). The measure of the relative number of L- and M-genes was based on data from MassARRAY analyses. Estimated area under curve and limiting sensitivity along the protan axis are also tabulated.

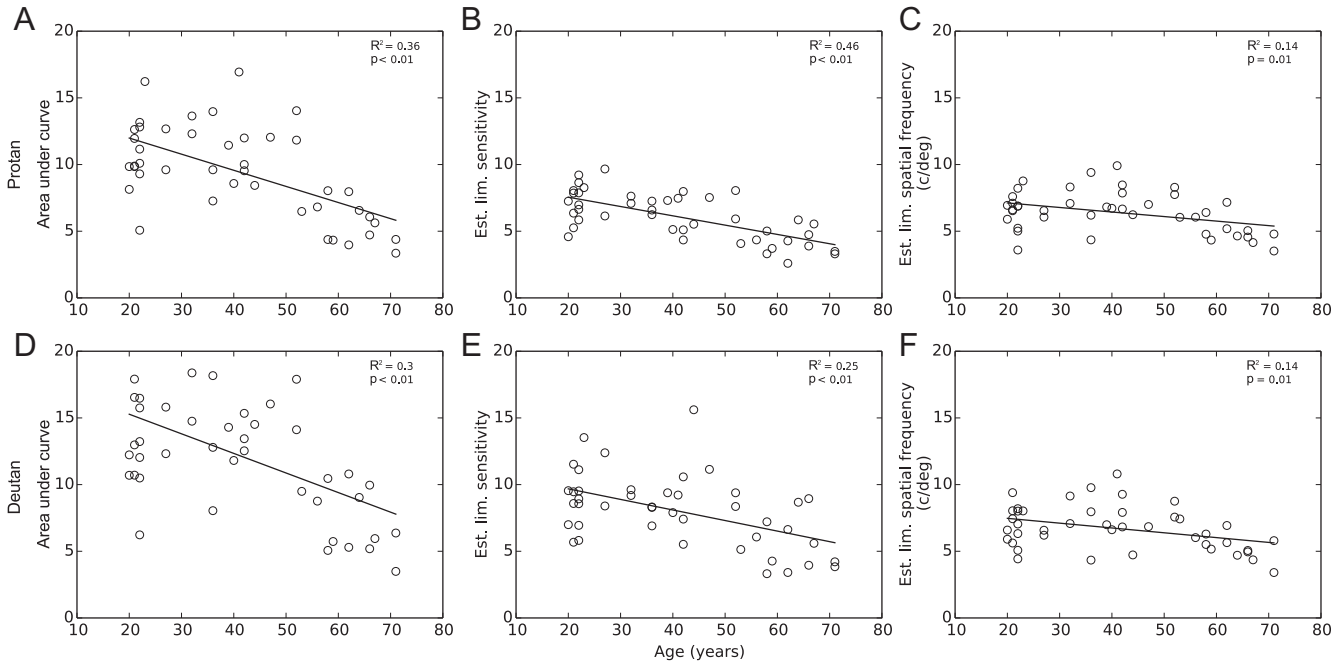

Fig. 3. Three descriptive parameters for performance on the protan (A–C) and deutan stimuli (D–F) are plotted as a function of age for each subject: estimated area under cone contrast curve (integrated between the *X*- and *Y*-axis intercepts, A and D), estimated limiting sensitivity (*Y*-intercept: cone contrast curve cut-off value *Y*-axis for $x = 0.1$, B and E) and estimated limiting spatial frequency (*X*-intercept: cone contrast curve cut-off value *X*-axis for $y = 0.1$, C and F).

Table 3
Overview of color vision results and grouped molecular genetic data for subjects aged 20–49. The peak sensitivity of the L-pigment and spectral separation ($\delta\lambda_{max}$) between the L- and M-cone pigments, area under cone contrast sensitivity curve (AUC), estimated limiting sensitivity (*Y*-intercept) and estimated limiting spatial frequency (*X*-intercept) for protan (P) and deutan stimuli (D), Rayleigh matching midpoints (RMMP) and null-point settings on Medmont C-100 presented for normal subjects (NT), protan- (PC) and deutan-carriers (DC), with mean and 95% confidence interval (CI).

	NT		PC		DC	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
Peak L (average)	557.6*	555.5–559.0	555.5†	–	554.7*	554.3–554.3
$\delta\lambda_{max}$	27.3*	25.2–29.0	25.5†	–	24.7*	24.3–24.3
AUC (P)	11.2	6.0–16.9	12.7	10.2–14.9	9.2	6.6–11.5
AUC (D)	14.6	7.5–21.9	15.3	12.4–17.9	12.0	9.6–13.4
Y-intercept (P)	7.0	4.4–9.5	6.5	6.4–6.6	6.6	5.1–8.1
Y-intercept (D)	9.3	5.6–15.0	8.5	8.1–8.9	10.0	6.6–14.5
X-intercept (P)	7.0	4.0–9.8	8.3	6.8–9.6	6.0	4.9–7.7
X-intercept (D)	7.3	4.5–10.5	8.2	6.3–9.8	5.9	4.0–7.8
RMMP	39.8	36.3–43.0	39.1	37.1–39.1	39.5	36.5–42.0
C-100	−1.76	−3.05–(−0.79)	−2.59	−3.25–(−2.59)	−1.49	−2.56–(−1.0)

* Significant difference between normal subjects and deutan carriers.
† Results based on only one subject.

Table 3 shows grouped molecular genetic data; including peak sensitivity of the L-pigment and predicted spectral separation between the L- and M-cone pigments, as well as the results from the Rayleigh anomaloscopy for normal subjects, protan- and deutan-carriers. The deutan carriers' peak sensitivity of L-cone pigment was significantly shifted towards shorter wavelengths ($U = 31.50$, $Z = -2.00$, $p = 0.046$, $r = 0.31$), and, hence, their spectral separation between L- and M-cone pigments ($\delta\lambda_{max}$) was significantly narrower ($U = 4.50$, $Z = -3.13$, $p = 0.002$, r = 0.50) when compared with normal subjects (Tables 2 and 3).

3.3.1. L-opsin gene's influence on spatio-chromatic contrast sensitivity

Fig. 4 shows calculated area under the chromatic contrast sensitivity curve (AUC) for protan stimuli (subjects aged 20–49) and the presence of Ser and/or Ala at position 180 of L-opsin gene (A) and AUC as a function of estimated peak sensitivity of L-cone pigments (B). Peak sensitivity for females with two L-cone pigments was calculated as the average of the two. There is no linear correlation between area under curve and peak sensitivity of the L-cone pigments. The curve is more U-shaped and some of those with peaks between 556 and 558 nm have better sensitivity than those with peaks at shorter or longer wavelengths. Those at either end have typically either Ser (filled black squares) or Ala (open squares) at position 180 with those in-between having both Ser and Ala (filled grey squares). The five subjects with the highest sensitivity (largest AUC) all have both Ser and Ala at this position.

Subjects with only Ser at position 180 of L-opsin gene exhibited significantly higher mean cone contrast sensitivity than subjects with only Ala (Table 4, area under curve for protan and deutan stimuli, calculated for subjects aged 20–49: $U = 17.0$ and 16.0, $Z = -2.47$ and -2.55, $p = 0.014$ and 0.011, $r = 0.55$ and 0.57). The same subjects also exhibited higher limiting sensitivity (cut off *Y*-axis) for protan stimuli ($U = 19.0$, $Z = -2.32$, $p = 0.020$, $r = 0.52$). This was not the case for deutan stimuli ($p = 0.62$). There was no

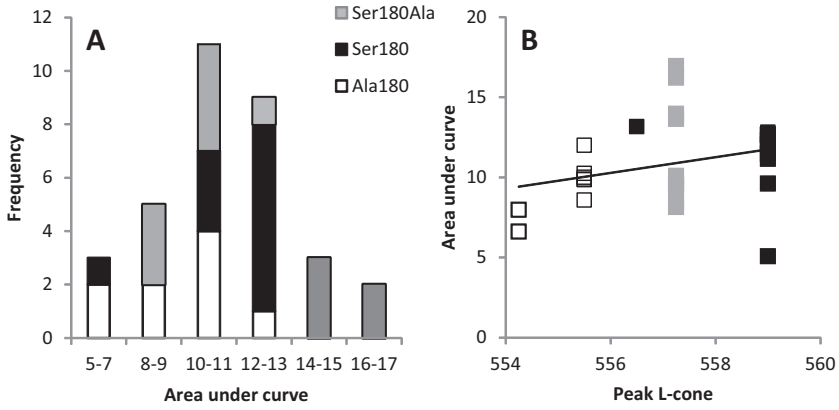

Fig. 4. Spatio-chromatic contrast sensitivity: presence of Ser180Ala and peak sensitivity (L-cone) for the subjects aged 20–49. Frequency distribution of area under curve (AUC) for protan stimuli and the presence of either Ser or Ala at position 180 of the L-opsin gene (A) and area under curve for protan stimuli as a function of peak sensitivity of L-cone pigment (B). The two individuals with the largest AUC are 4027 and 4010. Results are presented for subjects with Ala (white bars/open squares), Ser and Ala (grey bars/filled grey squares) and Ser (black bars/filled black squares) at position 180 of L-opsin gene.

Table 4
Overview of chromatic contrast sensitivity results and polymorphism at position 180 for subjects aged 20–49. Area under curve and *Y*- and *X*-intercepts for protan and deutan stimuli presented for subjects with Ser, Ala or Ser and Ala (Ser/Ala) at position 180 on L-opsin gene (from long PCR analyses), with mean and 95% confidence interval (CI).

Amino acid	Area under curve				*Y*-intercept				*X*-intercept (c/deg)			
	Protan stimuli		Deutan stimuli		Protan stimuli		Deutan stimuli		Protan stimuli		Deutan stimuli	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Serine	11.4*	5.1–13.1	14.5*	6.2–17.6	7.5	5.8–9.5	9.4	5.8–12.2	6.6	3.6–8.0	7.1	4.4–8.2
Alanine	9.2*	6.6–11.1	12.0*	8.0–14.4	6.3	5.1–7.6	9.3	5.7–13.1	6.2	5.1–7.6	6.2	4.0–8.7
Ser/Ala	11.7	8.1–16.7	15.1	10.5–21.9	6.7	4.3–8.8	9.2	5.5–14.8	7.4	5.0–9.9	7.7	4.7–10.5

* Significant difference between subjects with Ser versus Ala.

significant difference in mean area under curve or mean limiting sensitivity between subjects with only Ser versus those with both Ser and Ala ($p \geqslant 0.26$) or between subjects with only Ala versus those with both Ser and Ala ($p \geqslant 0.09$) on the L-opsin gene. The same result was observed for those aged 50–71. Those with only Ser at position 180 of L-opsin gene exhibited significantly higher mean cone contrast sensitivity than subjects with only Ala (Table 4, area under curve for protan and deutan stimuli: $U = 2.0$ and 0.0, $Z = -2.13$ and -2.50, $p = 0.033$ and 0.013, $r = 0.61$ and 0.72. The same subjects also exhibited higher limiting sensitivity (cut off *Y*-axis) for protan and deutan stimuli ($U = 2.0$ and 0.0, $Z = -2.13$ and -2.50, $p = 0.033$ and 0.013, $r = 0.61$ and 0.72).

The result was confirmed with an ANCOVA across the whole age range (20–71), to compare the difference in cone contrast sensitivity with regards to the Ser180Ala polymorphism, controlling for age. Those with only Ser at position 180 of L-opsin gene exhibited significantly higher mean cone contrast sensitivity than subjects with only Ala after adjusting for differences in age [$F(2, 42) = 3.43$, $p = 0.043$, $\eta_p^2 = 0.14$].

3.4. Rayleigh match

The Ser/Ala difference in overall contrast sensitivity led us to return to the Rayleigh match to examine the Ser/Ala polymorphism's influence on RMMPs and its correlation with spatio-chromatic sensitivity. Age did not predict the RMMPs ($B = -0.03$, $t(46) = -1.51$, $p = 0.14$). Fig. 5 shows (A) the presence of Ser and/or Ala at position 180 of L-opsin gene as a function RMMPs and (B) RMMPs as a function of estimated peak sensitivity of L-cone pigments. Subjects with Ser ($U = 18.50$, $Z = -3.95$, $p \ll 0.000$,

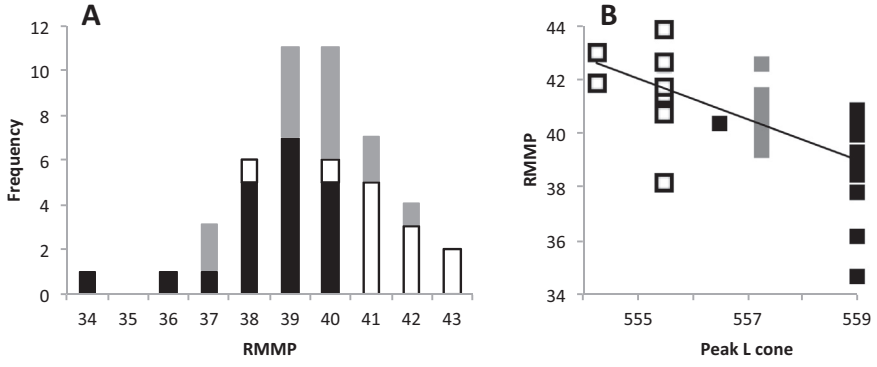

Fig. 5. Rayleigh anomaloscopy: presence of Ser180Ala and peak sensitivity. Frequency distribution of RMMP and the presence of either Ser or Ala at position 180 of the L-opsin gene (A) and RMMP as a function of peak sensitivity of L-cone pigment (B). The individual with RMMP 38 and Ala at position 180 is a deutan carrier 4053 (Table 1) with polymorphisms on M- rather than L-opsin gene as the other deutan carriers. Other details as for Fig. 4.

r = 0.70) or Ser and Ala ($U = 23.00$, $Z = -3.14$, $p = 0.001$, r = 0.62) at position 180 of the L-opsin gene showed a more green-like shift in RMMP compared with only Ala at this position (Fig. 5B). Ser or Ala at position 180 of the M-opsin gene did not influence the RMMP ($p = 0.20$).

As expected, RMMP were significantly predicted by the peak sensitivity of the L-cone pigment, where subjects with peak sensitivity towards longer wavelengths used significantly more green in their RMMP [$B = -0.76$, $t(41) = -5.75$, $p \ll 0.001$]. A significant proportion of the variance in midpoint settings were explained by the peak sensitivity of the L-pigment [$R^2 = 0.45$, $F(1, 40) = 33.1$, $p \ll 0.001$].

There was a small, but significant, correlation between RMMP and sensitivity to spatio-chromatic stimuli along the protan axis (calculated for subjects aged 20–49), where subjects that used more green in their RMMP also showed higher estimated limiting sensitivity and higher AUC [Fig. 6A and B, $B = -0.56$ and -0.232, $t(31) = -2.48$ and -2.04, $p = 0.019$ and 0.051]. A significant proportion of the variance in RMMP was explained by estimated limiting sensitivity and AUC [$R^2 = 0.17$ and 0.12, $F(1, 31) = 6.14$ and 4.18, $p = 0.019$ and 0.051]. There was no correlation between RMMP and the sensitivity results for stimuli along the deutan axis ($p \geqslant 0.17$).

4. Discussion

This is the first study that has investigated spatio-chromatic contrast sensitivity along two directions in color space for a range of spatial frequencies with stimuli where an individual's luminosity function and its variation across the visual field is accounted for. The results reveal a decline in overall sensitivity with age, but the decline is most pronounced at lower spatial frequencies (Figs. 2A, B and 3A, F). The results also reveal that polymorphisms at position 180 of the L-opsin gene may explain some of the between-individual variation in spatio-chromatic contrast sensitivity (Fig. 4) as well as Rayleigh matches (Fig. 5). Firstly, and consistent with previous reports for males, presence of Ser at position 180 significantly predicted the subjects' midpoint settings on Rayleigh anomaloscopy, for both males and females. Secondly, presence of only Ser, as compared with only Ala, at position 180 increased subjects' spatio-chromatic contrast sensitivity, independently of age.

Only a few studies have attempted to determine senescence of chromatic contrast sensitivity at low spatial frequencies [0.31–8.1 c/deg at 1 Hz, protan stimuli (Werner et al., 1995); 1 c/deg at 2 Hz, L–M opponent stimuli (Fiorentini et al., 1996); 0.43 c/deg 1 Hz protan and deutan stimuli (Knoblauch et al., 2001); 0.5, 1, 2, 4 c/deg, L–M opponent stimuli (Hardy et al., 2005)]. Werner et al. (1995) looked at sensitivity along the protan confusion line for different spatial frequencies with an isoluminant sinusoidal grating modulated at 1 Hz. They accounted for individual's luminosity function only in the central part of the test and such stimuli are known to residually stimulate the luminance (achromatic) pathway. They examined 40 subjects aged 18–67 years and reported a decline in sensitivity with age, but only for the highest spatial frequencies. Their result is reminiscent of the age-related decline observed for achromatic gratings, as age has not been found to affect achromatic contrast sensitivity at the lowest spatial frequencies (Elliott, 1987; Owsley et al., 1983). Such a decline in sensitivity has been ascribed to preretinal factors, such as increased density of the lens, light scattering and senile miosis (Owsley et al., 1983; Steen et al., 1994; Weale, 1988; Werner, 1982; Wright & Drasdo, 1985). Here, sensitivity to high spatial frequencies changes little with age (Fig. 3C and F). This may be because all subjects regardless of age had lenses of grade less than 2 on LOCS III and those aged 50 years or older had logMAR letter acuity that differed from that of the young ones by no more than 0.08 (4 letters). Therefore, blur and or preretinal factors are unlikely to have been an important factor in the subjects' performance. It is therefore reasonable to ascribe the significant senescent linear decline in general sensitivity (area under curve, Fig. 3A and D) and decline in sensitivity to low spatial frequencies (Fig. 3B and E) to neural factors. The senescent changes at low spatial frequencies confirms the result reported for a single low spatial frequency by Knoblauch et al. (2001).

The sensitivity to pseudo-isochromatic gratings appears to be stable between 20 and 49 years of age (Fig. 2A and B), but this may be related to the large between-individual differences in sensitivity within the different age decades. The youngest subjects with the lowest sensitivity had approximately the same sensitivity as the oldest subject with the highest sensitivity (Fig. 3). Similar sensitivity patterns have been described previously (Hardy et al., 2005; Spear, 1993). Here we show that some of this variation can be explained by presence of Ser versus Ala at position 180, where individuals with only Ser had significantly higher chromatic contrast sensitivity than those with Ala (Tables 2 and 4, Fig. 4A), independently of age. A similar correlation was observed between RMMP and Ser at position 180, regardless of sex, with presence of Ser180 resulting in a green-shifted RMMP, which is well known for males (Neitz & Jacobs, 1986; Neitz, Neitz, & Jacobs, 1993; Sanocki et al., 1993; Winderickx et al., 1992).

The group of subjects with L-cone peak sensitivity at 559 nm was those that on average showed the highest spatio-chromatic contrast sensitivity (Fig. 4B). All of these 10 subjects have only Ser at position 180 of the L-opsin gene. Six out of seven normal males are in fact in this last group compared with four out of 18 normal females (Table 2). Fig. 6 shows a small correlation between green shifted RMMP and chromatic contrast sensitivity (AUC and limiting sensitivity). The results imply that only 12–17% of the variance was shared by the two measures. It is evident when comparing the RMMP and AUC versus frequency of Ser180Ala plots (Figs. 4A versus 5A) with peak L plots (Figs. 4B versus 5B) that RMMPs for those with only Ser is associated with larger spectral separation between the L- and M-cone pigments, whereas the individuals with the highest spatio-chromatic sensitivity have both Ser and Ala, and therefore estimated intermediate spectral separation (but this will also depend on degree of X-inactivation). Ten out of 18 normal females have both Ser and Ala at position 180. Others have reported differences in color discrimination between males and females, with males outperforming females (Birch, Young, & David, 1991; Rodríguez-Carmona et al., 2008). This is often attributed to the Ser and Ala polymorphism at position 180 on

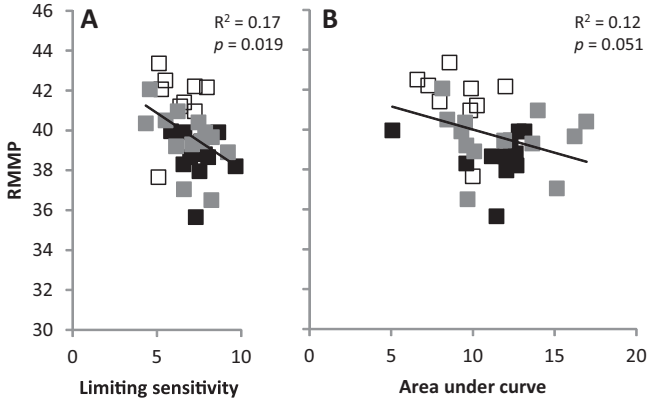

Fig. 6. Color vision performance predicted by sensitivity to spatio-chromatic patterns. RMMP as a function of (A) estimated limiting sensitivity and (B) estimated area under curve for protan stimuli for subjects aged 20–49. Other details as for Fig. 4.

the L-cone pigment on the assumption that females with two L-cone pigments may perform worse, because the added pigment and larger spectral overlap will lead to increased post-receptoral noise (Rodríguez-Carmona et al., 2008). It looks like this might not be the case. On average, females perform worse than males if we exclude the five females with highest sensitivity (four normal and one protan carrier), but not if they are included. One of the four normal females also has two different M-cone pigments, and it can be deduced from her array that it is possible she could have five cone pigments expressed (as two genes from each L/M array may be expressed: Sjoberg, Neitz, Balding, & Neitz, 1998; Winderickx, Battisti, Motulsky, & Deeb, 1992a; Yamaguchi, Motulsky, & Deeb, 1997). Other studies have reported differences in color discrimination with females outperforming males followed by suggestions that expression of more than two L- and M-cones could be the reason why some females outperfrom males (Murray et al., 2012). Jordan, Deeb, Bosten, and Mollon (2010) suggested that photopigment optical density should be considered as a possible factor in color discrimination in general, reflecting upon the possibility that substitution of Met for Leu at position 153 of the L-opsin gene might reduce the effective photopigment optical density. Lower photopigment optical density narrows the pigments spectral sensitivity curves (Brindley, 1953) and is known to improve color vision in animals (Vorobyev, 2004) and color deficient males (e.g. He and Shevell (1995), Neitz, Neitz, He, and Shevell (1999), Thomas, Formankiewicz, and Mollon (2011)). The two normal females with the highest spatio-chromatic sensitivities both have Met at 153 (4010 and 4027, see Table 2), as does both protan carriers. Overall, reported difference between females and males is multifactorial and must partly be related to cone opsin genotype of the individuals included in a particular study, and partly related to variations in expression resulting from X-chromosome inactivation (Bosten et al., 2005; Jordan & Mollon, 1993; Jordan & Mollon, 1997; Jørgensen et al., 1992; Lyon, 1972).

The cone fundamentals used in the stimuli in present study were those of Smith–Pokorny, with pigment extinction spectra of 554 nm for protan and 530 nm for deutan stimuli (Smith & Pokorny, 1975). The choice of cone fundamentals may favor one group of subjects. This could also affect sensitivity to the protan- more than the deutan-stimuli, since the variation in L- is greater than for M-cone peak sensitivity (Bieber, Kraft, & Werner, 1998; Carroll et al., 2000). But, the subjects with predicted L-cone peak sensitivity closest to 554 nm (555.5 or 553 + 555.5 nm) were not the ones with the highest or the lowest chromatic contrast sensitivity (Fig. 4B). The individuals who show highest spatio-chromatic contrast sensitivity are four normal females with two L-cone pigments, with predicted peak sensitivity of 555.5 and 559 nm and with both Ser and Ala at position 180. Six other females had the same two L-cone pigments and amino acid sequence at position 180, but these females show performance that is poorer than the average of the groups with just Ser or just Ala at position 180. It is possible that the increased chromatic contrast sensitivity observed among the four females could be related to the mismatch between the cone fundamental used and the predicted peak sensitivity of the individual cones, creating achromatic intrusion. This, however, can neither explain why just four of those ten females show improved performance, nor why these six have higher sensitivity than the individuals with predicted peak at 559 nm. If there were any achromatic intrusion related to mismatch between cone fundamentals and predicted peak sensitivity for an individual, then the individuals with predicted peak at 559 nm would be expected to have had the highest sensitivity. This was not the case (Fig. 4B).

The number of carriers included in the study is small, but it is noteworthy that deutan carriers perform worse with lower mean spatio-chromatic contrast sensitivity than other subjects (Fig. 2B), whereas protan carriers are as good as the best half of the normal females. The arguments for why deutan carriers tend to show reduced discrimination on conventional (e.g. Baraas (2008), Crone (1959), Dees and Baraas (2014), Feig and Ropers (1978), Hill (1980), Jordan and Mollon (1993), Schmidt (1934), Waaler (1927)) and experimental color vision tests (Gunther & Dobkins, 2002; Hood, Mollon, Purves, & Jordan, 2006; Miyahara, Pokorny, Smith, Baron, & Baron, 1998) has mainly been related to either presence of Ser or Ala at position 180 and differences in L:M-cone ratios. Carriers have been found to exhibit a shift in Nagel matching midpoints (Hill, 1980; Jordan & Mollon, 1993; Krill & Schneiderman, 1964; Waaler, 1927; Waaler, 1967), and this observed shift has been attributed to Ser and Ala polymorphisms at position 180 of L-opsin gene (Jordan & Mollon, 1993). The subjects' RMMP was predicted by the Ser and Ala polymorphism at position 180 of the L-opsin gene (Table 3 and Fig. 5A), but the RMMP could not be used to distinguish between protan and deutan carriers, as carriers of either type had either Ser and/or Ala at postion 180. Furthermore, it has been suggested that the Medmont C-100 test can be used to predict an individual's L:M-cone ratio (Le Sueur, Mollon, Granzier, & Jordan, 2014). We had the opportunity to correlate Medmont C-100 null-point settings (last row, Table 3) with other measures, but found no correlation with the presence of Ser at position 180 ($p = 0.24$) or with spatio-chromatic contrast sensitivity (all $p > 0.17$).

The results reveal a senescent decline in spatio-chromatic contrast sensitivity that we ascribed to neural factors. The observed between-individual differences in sensitivity may partly be explained by the Ser180Ala polymorphisms on the L-opsin gene. Williams, Sekiguchi, and Brainard (1993, 1990) reported that both spectral position of cone pigments and retinal cone density would be expected to influence the vertical position of the chromatic contrast sensitivity curve. Parts of the observed between-individual variation within each age group as well as the age-related decline in spatio-chromatic sensitivity might also be related to variation in cone density. Cone photoreceptor density has been reported to decline with age (Panda-Jonas, Jonas, & Jakobczyk-Zmija, 1995; Song, Chui, Zhong, Elsner, & Burns, 2011), with accompanying reports of large between-individual variation in the fovea, para- and perifovea (Dees, Dubra, & Baraas, 2011; Lombardo, Serrao, Ducoli, & Lombardo, 2013; Song, Chui, Zhong, Elsner, & Burns, 2011). The redundancy of cones in the fovea might offer some protection as measures of achromatic foveal visual function is quite often preserved even though a reduction in cone photoreceptor density is evident (Carroll et al., 2009; Ratnam, Carroll, Porco, Duncan, & Roorda, 2013; Talcott et al., 2011). In comparison, L- and M-cones can detect chromatic signals that are many times smaller than achromatic signals under the stimulus conditions used here (Mullen, 1985). Thus, measures of spatio-chromatic contrast sensitivity appear to be more promising in detecting loss of visual function caused by neural cell loss from the retina and beyond.

The combination of psychophysics and genetics of L- and M-opsin genes utilized in this study exposes the importance of understanding variation in individual trajectories of age-related changes in vision. It highlights the possibility that both cone opsin pigments and cone density are decisive factors related to whether senescent changes will lead to increased vulnerability to degenerative eye disease.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.visres.2015.08.015.

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