The putative role of lutein and zeaxanthin as protective agents against age-related macular degeneration: promise of molecular genetics for guiding mechanistic and translational research in the field[1–4]

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ABSTRACT

Age-related macular degeneration (AMD) is the primary cause of vision loss in elderly people of western European ancestry. Genetic, dietary, and environmental factors affect tissue concentrations of macular xanthophylls (MXs) within retinal cell types manifesting AMD pathology. In this article we review the history and state of science on the putative role of the MXs (lutein, zeaxanthin, and *meso*-zeaxanthin) in AMD and report findings on AMD-associated genes encoding enzymes, transporters, ligands, and receptors affecting or affected by MXs. We then use this context to discuss emerging research opportunities that offer promise for meaningful investigation and inference in the field. *Am J Clin Nutr* 2012;96 (suppl):1223S–33S.

INTRODUCTION

The dietary carotenoids lutein [(3R,3′R,6′R)-β,ε-carotene-3,3′-diol] and zeaxanthin [(3R,3′R)-β,β-carotene-3,3′-diol] are primary constituents of macular pigment (1, 2) and have been examined for their effect on health and disease of the retina for >200 y (3–5). These 2 nutrients and *meso*-zeaxanthin [(3R,3′S)-β, β-carotene-3,3′-diol], a metabolite of lutein (6), are known collectively as macular xanthophylls (MXs)[5]. Biological plausibility of MX-retinal disease relations exists because these compounds exhibit the following characteristics: *1*) intake-dependent and -modifiable accretion to the retina, *2*) preferential concentration and localization in retinal cells manifesting retinal pathology, and *3*) biophysical and biochemical capacity to affect processes implicated in pathogenesis and progression of retinal diseases.

A number of large-scale human studies on age-related macular degeneration (AMD), the primary cause of irreversible retina-based visual impairment in elderly people of western European ancestry (7–9), showed AMD-nutrient relations with MX status and intake (10–17). Bird (18) and Ambati et al (19) provided details on the pathogenesis and pathophysiology of AMD. Atrophic and confluent degeneration of retinal pigmented epithelium (RPE) cells and adjacent photoreceptors is known as geographic atrophy (or "dry" AMD). An estimated 1.7 million (1.5%) US residents are living with advanced AMD (AAMD, ie, geographic atrophy and/or neovascular AMD). Choroidal neovascularization is the hallmark of "wet" or neovascular AAMD, a condition characterized by florid sub- and trans-RPE proliferation of new vessels from the choriocapillaris. A series of photographs (http://www.nei.nih.gov/photo/) and dynamic renderings of AMD progression (www.nei.nih.gov/photo/eyedis/VA04.mov) are available from the National Eye Institute.

In the primate retina, high concentrations of MXs exist within areas manifesting susceptibility to light damage and metabolic challenge. The laminar concentration and topographic distribution of MXs may explain how the human retina is typically capable of handling extreme physical and biochemical exposures without appreciable loss of function for ≥6 decades; even in people with AAMD, remarkable resilience of the MX-rich areas is sometimes seen. Bone et al (20) stated that "in the advanced form of AMD known as geographic atrophy, the foveal center, which contains the highest concentrations of lutein and zeaxanthin, tends to be spared until late in the course of the disease." The phenomenon of foveal sparing in MX-dense regions has also been observed in a number of inherited macular diseases (21).

MODERN HISTORY OF RESEARCH ON THE ROLE OF MXs IN HEALTH AND DISEASE OF THE RETINA

At least 200 y of clinical observation and research on macular pigment preceded the 1980 report of Malinow et al (22) that described an absence of retinal MXs and associated RPE defects in macaque monkeys fed a MX-free diet across their life span. We refer the interested reader to an extensive scholarly review of the field published in 1981 by Nussbaum et al (3) and to works including early historical references (4, 5). A chronology of key findings from reports that applied biochemical analyses of retinal

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[5] Abbreviations used: AAMD, advanced age-related macular degeneration; AMD, age-related macular degeneration; MPOD, macular pigment optical density; MX, macular xanthophyll; RPE, retinal pigmented epithelium.

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MXs is shown in **Table 1**, starting with the 1984 works by Snodderly et al (23, 24) on MX spatial distribution and absorption properties in the primate retina and subsequent work by Bone et al (1), which identified lutein and zeaxanthin as the major chromatographically separable components of macular pigment. The first evidence of MX-AMD relations in large-scale, well-phenotyped multicenter human cohorts emerged in the early 1990s from observational studies by Seddon et al (11), Mares-Perlman et al (36), and the Eye Disease Case-Control Study Research Group (37). Around this time, Bone et al (38) applied mass spectroscopic methods to determine the stereochemistry of macular pigment, thus providing information with which to model the biophysical properties of MXs in membranes. Conceptual frameworks for MX-AMD research have evolved from international congresses convening over the past 20 y (39–41) and from progressive reviews (4, 23, 24, 28, 42–50). Results from randomized, double-masked, placebo-controlled trials on MXs and visual function emerged in the mid-2000s (51, 52). In the largest of these, Richer et al (51) reported significant improvements in visual acuity among participants randomly assigned to receive 10 mg lutein/d for 12 mo. The Age-Related Eye Disease Study 2 (www.areds2.org) and the Carotenoids and Co-Oxidants in Age-Related Maculopathy Study (53) are large-scale trials designed to examine structural and functional retinal response to MXs in people with AMD.

CHEMICAL STRUCTURE OF MXs

MXs are dipolar dihydroxy carotenoids, existing as structural isomers and characterized by an internally symmetrical form with a conjugated polyene chain and 2 terminally hydroxylated ionone rings. Details on structures of MXs ($C_{40}H_{56}O_2$) and their metabolites appear in Khachik et al (28) and Bernstein et al (54). Two- and three-dimensional renderings may be accessed at http://pubchem.ncbi.nlm.nih.gov/ (lutein, CID: 6433159; zeaxanthin, CID: 5280899).

CORE CONCEPTS TO GUIDE TRANSLATIONAL RESEARCH ON MX-AMD RELATIONS

Primates cannot synthesize lutein and zeaxanthin de novo (6, 22) and have adapted with a capacity for efficient MX uptake (26, 55), transport (56, 57), retention (20, 46, 58–60), and protection (49) in the retina; the efficient operation of these processes may be testimony to the physiologic significance of MXs in retinal health and disease. Biological plausibility for protective actions of MXs in AMD is supported by the following: *1*) MX structure and natural biophysical properties (43), *2*) specific accretion of MXs to the retina from a pool of ~40 dietary (61) and ~15 circulating (62, 63) carotenoids, and *3*) specific laminar and topographic distribution (23, 24, 31, 32, 64) and unique membrane disposition of MXs (65). A number of unifying concepts, which are helpful in guiding the effort to determine how regulatory mechanisms and metabolic fate of MXs may affect MX-AMD associations, have emerged over the past 2.5 decades. These are as follows:

1) MX concentration is increased 1000- to 10,000-fold from the circulation to the retina (43, 54) via active transport mechanisms (43) involving specific binding proteins (19, 21, 22, 60, 61).

2) MXs are selectively concentrated (48) and specifically distributed in the retina with optical detection limits at linear distances out to 1.2–1.5 mm from the center of the fovea and biochemical signals quantifiable out to ~4.0–5.8 mm from this same area (4). Noninvasive in vivo imaging technologies have been used to measure macular pigment optical density (MPOD; a quantitative estimate of the capacity of MXs to attenuate energy in the range of visual blue light), topographic distribution, and membrane disposition of MXs (reviewed in references 54 and 66). MX concentration is ~1 mmol/L in the human fovea (44) and retinal concentrations can be 2 to 3 orders of magnitude higher than those in other tissues (43, 67). The average mass of lutein + zeaxanthin per unit retinal area is 1.33

TABLE 1
Reports on biochemical analysis of MXs in primate retina[1]

First author (reference)	Year	Sample	Key contributions
Snodderly (23)	1984	Monkey	MP absorbance spectra and localization in primate
Snodderly (24)	1984	Monkey	MP spatial distribution in primate retina
Bone (1)	1985	Human	L and Z identified as major constituents of MP
Bone (25)	1988	Human	L- and Z-specific distributions within the retina
Handelman (2)	1988	Human	MX quantities accurately estimated in retina
Handelman (26)	1991	Monkey	MX from fixed retina used for densitometry
Bone (27)	1997	Human	L:Z ratio first plotted with retinal eccentricity
Khachik (28)	1997	Primate	3 major xanthophylls and 11 metabolites in retina
Bernstein (29)	1998	Human	MX in vivo imaging method validated on biochemistry
Sommerberg (30)	1999	Human	25% of retinal carotenoids exist in rod outer segments
Rapp (31)	2000	Human	MXs exist in rod outer segments in perifoveal region
Bernstein (32)	2001	Human	Expanded analysis of MX concentration outside of retina
Bone (33)	2001	Human	Retinal MXs lower in people with AMD (vs AMD-free)
Johnson (6)	2005	Monkey	L is a dietary source of *M*-Z
Bhosale (34)	2007	Human	*M*-Z distribution varies with age
Bhosale (35)	2007	Human	MX concentrations higher in persons taking MX supplements

[1] AMD, age-related macular degeneration; L, lutein; MP, macular pigment; MX, macular xanthophyll (lutein, zeaxanthin, *meso*-zeaxanthin); *M*-Z, meso-zeaxanthin; Z, zeaxanthin.

ng/mm^2 at the foveal center and 0.81 ng/mm^2 at an eccentricity of 1.6–2.5 mm (25). The highest concentrations of MXs extend from the center of the fovea to ~0.10 mm and decline exponentially thereafter. At 2.0 mm eccentricity from the foveal center, biochemical analyses show a 300-fold decrease in MX concentration; here, the in vivo optical detection signal for MXs is negligible. Peak MX concentration in all retinal layers exists at the foveola. The Henle fiber layer (a region in the outer plexiform layer of the central retina containing photoreceptor axons) is the most densely MX-concentrated area per unit area in the eye. In the parafovea, the next most concentrated area is the inner plexiform layer (a region occupied by a neuropil of cellular processes connecting interneurons and retinal ganglion cells). MXs are detectable in appreciable quantities in the outer retina within photoreceptor outer segments (30, 31), albeit in relatively lower concentrations than in the inner retina laminae. MX concentrations are relatively lower in the O_2-rich region of the outer retina (photoreceptors and RPE) than they are in the low partial pressure of O_2 environment of the inner retina (layer of Henle and inner plexiform layer); however, highly specific lutein-binding proteins are localized to the metabolically active photoreceptor inner segment [reviewed in Bernstein et al (54)].

The distribution of total MXs within retinal laminae varies with retinal eccentricity (25, 27, 44). With increasing distance from the fovea, pigment concentrations decrease most rapidly in inner retina layers. At ~0.4 mm from the foveal center, the distribution of MXs shows a relatively balanced distribution, with approximately the same density of MXs in the nerve fiber layer as in most other layers. The distribution of specific MXs varies with retinal eccentricity; zeaxanthin and *meso*-zeaxanthin dominate in the fovea with concentrations declining more rapidly than those of lutein as the distance from the fovea increases (25, 27). The lutein:zeaxanthin ratio at 0–5° is ~1.0:1.5; at 5–19°, ~1.5:1.0; and at 19–38°, ~2.0:1.0 (a 1° angular subtense in the retina represents ~0.29 mm of retinal extent]. *Meso*-zeaxanthin is virtually absent in the human food supply and plasma; it is present at similar concentrations to zeaxanthin in the foveola and has negligible signal outside of the fovea (55). The relatively lower concentration of lutein within the central retina has led to speculation that *meso*-zeaxanthin may be metabolized from oxidized lutein via a cone-photoreceptor–specific enzyme (25, 27, 28, 44). In 2005 Johnson et al (6) identified lutein as a dietary precursor of *meso*-zeaxanthin.

There is substantial interindividual variation in global (4) and local (68–70) topographic macular pigment density (59, 71–73). Sharifzedah et al classified 5 major patterns in the distribution of macular pigment in elderly people with resonance Raman (74) and 2 wavelength autofluorescence (75) imaging techniques: very low foveal MPOD existed in 10% of those studied; 1 in 5 persons showed a slightly enhanced foveal MPOD with extension of MXs to eccentric regions; 1 in 3 persons expressed a "sole, sharp, central distribution" of MPOD; 20% of persons showed a dense foveal MPOD with a ring of pigment surrounding this area; and 1 in 10 persons expressed a "uniform, laterally extended distribution" of MPOD.

3) Membrane orientation and localization of MXs affects cytoarchitectural stability, light filtering, and the capacity to modulate oxidative stress in the retina (43, 65).

4) MXs show a capacity to affect processes implicated in AMD pathogenesis, because they have been shown to

- interact with membrane-bound proteins (65, 76) and lipids (65, 77) of the retina
- absorb-attenuate energy in the range of damaging ambient blue light (43, 78, 79)
- modulate oxidative stress and redox balance (80–87) by scavenging oxidizing agents and re-reducing oxidized macromolecules (88), quenching triplet excited states of photosensitizers (44), and neutralizing singlet oxygen, peroxyl radicals, and nitric dioxide
- interact with key molecules in signal transduction cascades (76, 89–91) inhibiting cell growth and stimulating differentiation (92–94), transactivating nuclear receptors (92), antagonizing nuclear receptor activation (95), influencing expression of connexin genes (96, 97) acting in adhesion complexes to maintain cellular homeostasis (98), and binding immunomodulatory lipocalin proteins (76, 99).

5) Biochemical analyses (1, 2, 25, 27, 35) and in vivo imaging studies (54, 66) indicate that genetic (100, 101), dietary (54, 102, 103), and environmental (102) factors can affect MX tissue concentrations within retinal layers and cell types manifesting pathology in AMD (4, 30, 31, 49, 54, 64). Points germane to these issues are as follows:

- Serum and plasma MX concentrations vary directly with dietary MX intake (104–112) and supplement use (113, 114), according to most reports.
- Neuringer et al (103) and Johnson et al (6) used biochemical and in vivo imaging methods to analyze retinal tissue in rhesus monkeys receiving an MX intervention to provide direct evidence that *1*) MX supplementation late in life is capable of strongly increasing retinal MX concentrations, even after "nutritional deprivation", and *2*) lutein is a dietary source of *meso*-zeaxanthin. This in vivo primate model also was used to examine the effect of MX intake on the retinal vulnerability to acute photochemical damage induced by small-spot exposures of coherent light at 476 nm (115). Rhesus monkeys were fed a lifelong MX-free diet, and at the start of the study had no MXs detected biochemically in serum or adipose tissue or by reflectometry in the retina. The absence of retinal MX was confirmed biochemically in post mortem samples (6). Dietary supplementation with either pure lutein or zeaxanthin for 6–24 mo brought retinal MXs to concentrations similar to or higher than those of animals receiving dietary MXs across their life spans (6). After ~6 mo of MX supplementation, the animals showed reduced amounts of ophthalmoscopically determined damage within the fovea, compared with measurements made before supplementation (115).

TABLE 2
Studies that examined relations of genes with MX status and AMD[1]

		Relation					
		Gene-MX status			Gene-AMD		
Action on MXs (reference)	Gene symbol	In vitro	In/ex vivo	Human	In vitro	In/ex vivo	Human
1. Uptake, transport, and accretion of MXs							
MX transport in intestine, choroid, RPE	*CD36* (125)	ARPE-19 (126)	—	(127)	(128)	$CD36^{-/-}$ (128–130)	(131)
MX transport in intestine, choroid, RPE	*SCARB1* (125)	ARPE-19 (126)	Fly (132–134)	(135)	—	$SCARB^{-/-}$ (136)	(137)
MX accretion/transport in OPL (137)	Tubulins (55)	—	Primate (179)	(140)	—	—	(141)
MX transport in circulation	*APOE* (142)	—	$ApoE^{-/-}$ (143)	MPOD (144, 145)	—	—	(146–154)
MX transport /binding on HDL-C	*ABCA1* (125)	—	$WHAM^{-/-}$ (124)	—	—	Human (155)	(156–160)
MX transport /binding on HDL-C	*APOA1* (125)	—	$WHAM^{-/-}$ (124)	—	—	—	(161)
Specific zeaxanthin uptake in retina	*GSTP1* (57)	—	—	Retina (55, 57)	—	—	(162, 163)
Specific lutein uptake in retina	*STARD3* (56)	—	Human (56)	Retina (55, 56)	—	—	—
2. Cleavage of MXs							
15,15′ (symmetric) cleavage	*BCMO1* (164)	—	—	(165), MPOD (127)	—	—	—
9′,10′ (eccentric) cleavage	*BCO2* (264)	(91)[2]	(166–169)	–	—	—	—
Synthesis/degradation of MX-rich HDL-C	*LPL* (169)	—	—	Serum (170)	—	—	(158, 160)
3. Antioxidant potential							
Structural integrity of DNA	*POLB*, *POLL*	(171)	—	—	—	—	—
4. Effect on adaptive cellular response							
Adhesion complex signaling	Connexins	(96)	—	—	(172)	—	—
Proliferation/apoptosis	*CDK2*, *CCND1*	(91)[2]	—	—	–	—	—
5. Interference with growth factors	*IGF* genes	(91)[2]	—	—	(173)	—	(174, 175)[3]
6. Diseases associated with low MX status							
Sjögren-Larsson syndrome	*ALDH3A2*	—	—	Retina (176)	—	—	—
Stargardt disease 1/cone-rod dystrophy	*ABCA4* (177)	—	—	(21)	—	—	(177–184)

[1] Numbers in parentheses correspond to reference numbers. Full names for genes represented by symbols are available from http://www.ncbi.nlm.nih.gov/gene. References in the "Gene symbol" column refer to immunolocalization studies in primate retina. AMD, age-related macular degeneration; ApoE, apolipoprotein E; ARPE-19, human retinal pigment epithelium immortalized cell line; CD36, CD36 molecule (thrombospondin receptor); HDL-C, HDL cholesterol; MPOD, macular pigment optical density; MX, macular xanthophyll; OPL, outer plexiform layer; RPE, retinal pigmented epithelium; SCARB, scavenger receptor class B; WHAM, Wisconsin hypoalpha mutant; $^{-/-}$, double knockout.

[2] Evidence based on tests with lycopene.

[3] Retinopathy endpoint.

The degree of foveal damage in the supplemented animals was similar to that of animals fed since birth on a standard laboratory-based diet containing MXs and significantly less than that of a comparison group maintained on the MX-free diet. Furthermore, in both the standard diet controls and the supplemented animals, the foveal region showed less damage than the parafoveal region located at 1.5 mm ($\sim 6^{\circ}$) eccentricity, outside the area of detectable MPOD, whereas before supplementation no such foveal photoprotection was found. No differences due to MX status or supplementation were seen in the parafoveal region beyond the area of dense macular pigment.

- Biochemical measurement of retinal response to MX intake exists from post mortem human studies. Bhosale et al (35) reported 3-fold higher retinal MX concentrations in $\sim$20% of eye donors from their 131-person cohort aged $\geq$48 y. Retrospective surveys on MX intake for this high-MX-retinal-status group indicated frequent daily use of high-dose MX supplements in 82% of people; the remaining 18% had a history of consuming MX-rich diets. Surveys on a random sample of 20 eye donors with retinal MX concentrations within expected limits indicated that none of these people regularly took MX supplements or consumed foods concentrated in MXs.
- Increased MX intake (11–13, 16, 17) and status (33, 36, 37, 45) are inversely associated with advanced AMD. As with work on MX status-AMD relations, equivocal findings exist for endpoints restricted to early or intermediate AMD.

THE PROMISE OF MOLECULAR GENETICS FOR EXAMINING THE EFFECT OF MXs ON AMD

AMD is a complex disease with a strong hereditary component (116). Aspects of retinal MX absorption, distribution, metabolism, and excretion are genetically determined, as shown by studies in twins (117, 118) and first-degree family members (119–121). Notably, retinal MX status profiles have shown stronger relations among monozygotic than dizygotic twins (101). Findings from a large cohort of married people indicate that interspouse relations exist for dietary intake and serum

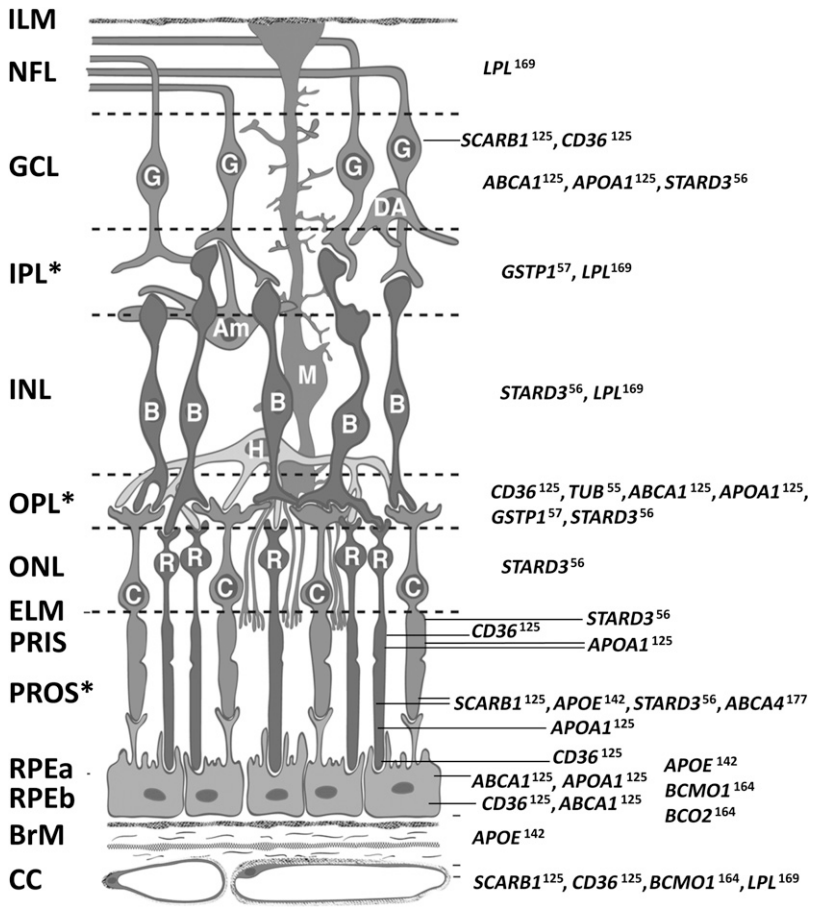

FIGURE 1. Distribution of proteins affecting or affected by macular xanthophylls in primate retina. Full names for genes are available from http://www.ncbi.nlm.nih.gov/gene. Superscripts on gene symbols refer to reference numbers of immunolocalization studies containing micrographs. Am, amacrine cell; B, bipolar cell; BrM, Bruch's membrane; C, cone photoreceptors; CC, retinal choroid layer; DA, displaced amacrine cell; ELM, external limiting membrane; G, ganglion cell; GCL, ganglion cell layer; H, horizontal cell; ILM, inner limiting membrane; INL, inner nuclear layer; IPL, inner plexiform layer (interneurons); M, Müller cell; NFL, nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; PRIS, photoreceptor inner segments; PROS, photoreceptor outer segments; R, rod photoreceptors; RPEa, retinal pigmented epithelium apical area; RPEb, retinal pigmented epithelium basal area. *Retinal layers with macular xanthophyll concentrations. The schematic was created by D Fisher and reproduced with permission from reference 187.

concentrations of MXs (100), but not for MPOD (a proxy for retinal MX status). Work in the field examining the molecular genetics of AMD has focused on both DNA sequence variation in genes encoding constituents of the complement system and those involved in HDL transport and metabolism. The link between MX status, complement genes, and AMD (122) has been examined in a cross-sectional sample of 302 healthy adults (123); carriers of AMD-associated complement factor H and age-related maculopathy susceptibility 2 (ARMS2) variants had lower MPOD values than their peers. An axis of AMD-HDL-MX status relations may also exist, because MXs are carried on HDL (124) and interact with numerous receptors and transporters affecting cholesterol metabolism.

Key references on relations of AMD with variants in genes encoding receptors, targets, transporters, enzymes, and hormones affecting or affected by MXs, their metabolites, and cofactors are shown in **Table 2**; findings are presented in the context of information on MX status relations with these same genes. Findings from immunolocalization studies on constituents of this MX gene set in primate retina are summarized in **Figure 1** (55, 57, 125, 142, 164, 169). Information on gene expression for these genes exists at the Retina Central database (University of Regensburg, http://www.retinacentral.org/) and the NEIBank (http://neibank.nei.nih.gov/index.shtml). We are currently applying the evidence base presented in Table 2 and Figure 1 to guide investigations designed to identify core elements of a "molecular phenotype" (a pattern gene regulation/expression and DNA variation) representing individual capacity to use transporters, receptors, enzymes, and hormones targeting or affected by MXs in ways that may reduce risk of AMD incidence or progression. Although we have not yet identified any single sequence variant explaining a proportion of variance in AMD risk comparable to those of the complement pathway genes, there is now informative work examining putative AMD-associated single-nucleotide polymorphisms present in genes encoding proteins involved in MX transport (121, 131, 137, 146, 148–154, 156–161), binding/capture (141, 162, 163), cleavage (158, 160), and diseases associated with lower MX status (177–184). Projects examining the associations of MX-related genes with retinal pathophysiology in in vivo models have supported inferences on AMD relations with variants in MX transport genes (128, 130, 136, 143).

KNOWLEDGE GAPS AND RESEARCH OPPORTUNITIES

Findings on the biochemical and biophysical actions of MXs in primates may be integrated with bioinformatic data on MX-related genes and proteins to investigate the putative actions of these factors in AMD pathogenesis and pathophysiology. We see promising opportunities for meaningful advances in the field with the following:

1) Ultrastructural localization studies on MX-affected proteins in the retina. Although specific binding proteins for lutein and zeaxanthin exist in the human and monkey retina (19, 21, 22, 25, 67), sites of subcellular MX localization are still unknown (65).

2) Broader analysis of genetic variation and regulation in DNA sequence encoding proteins responsible for MX binding in the retina. Genotyping efforts for *STARD3* and *GSTP1* have not been comprehensive. Exome-focused sequencing and analysis of regulatory elements (microRNA and transcription factor binding sites, histone methylation and acetylation marks) are likely to yield informative results. We provide a list of single-nucleotide polymorphisms capable of producing deleterious peptide shifts in *STARD3* and *GSTP1* (*see* the supplemental material under "Supplemental data" in the online issue).

3) Development of model systems (in vivo animal models and targeted mutagenesis in human retinal cells) based on findings from

- natural models of metabolic MX insufficiency (*ALDH3A2* and *ABCA4* genes)
- findings from efforts discussed in number 2

4) Integrated systems-based approaches to examine metabolic fate of MXs, their precursors, and metabolites. AMD is a polygenic disease, and we must reasonably examine relations of gene variation, regulation, and expression in dynamic biological systems. We give the example for GSPT1 and the protein encoded by the Fanconi anemia complementation group C (FANCC, 9q22.3). The FANCC gene product increases catalytic activity of GSTP1 during apoptosis by preserving the structure of bonds in GSTP1 that normally sustain disruptions in disulfide structure with exposure to oxidizing agents (158). Direct interaction of FANCC and GSTP1 has been shown in a model by using the in vitro coimmunoprecipitation paradigm (186). We have observed a number of coinherited AMD-associated variants in FANCC in a number of our large-scale cohorts (rs356666, $P \leq 0.004$; rs356677, $P \leq 0.001$; rs356669, $P \leq 0.0005$). rs356669 exists in a binding domain for MafK and MafF transcription factors; this relation may provide leads in the study of GSTP1 activation in AMD. Although P values are not in the range of those reported for complement system genes, the biological plausibility of these findings in the context of the evidence base supports the rationale to extend investigations in future studies.

Concepts discussed in this article provide a foundation for planning mechanistic, translational, and applied clinical research projects for investigation of MX-AMD relations. To make meaningful inferences from such efforts, multidisciplinary teams must work to *1*) develop a mechanism- and system-centered evidence base on the molecular genetics of enzymes, transporters, ligands, and receptors affecting or affected by MXs and influencing MX uptake, transport, retention, and protection in the neural and vascular retina, and *2*) apply this evidence base in the context of the science on AMD pathogenesis and pathophysiology.

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