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Urine Nuclear Magnetic Resonance (NMR) Metabolomics in Age-Related Macular Degeneration

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Figure S1

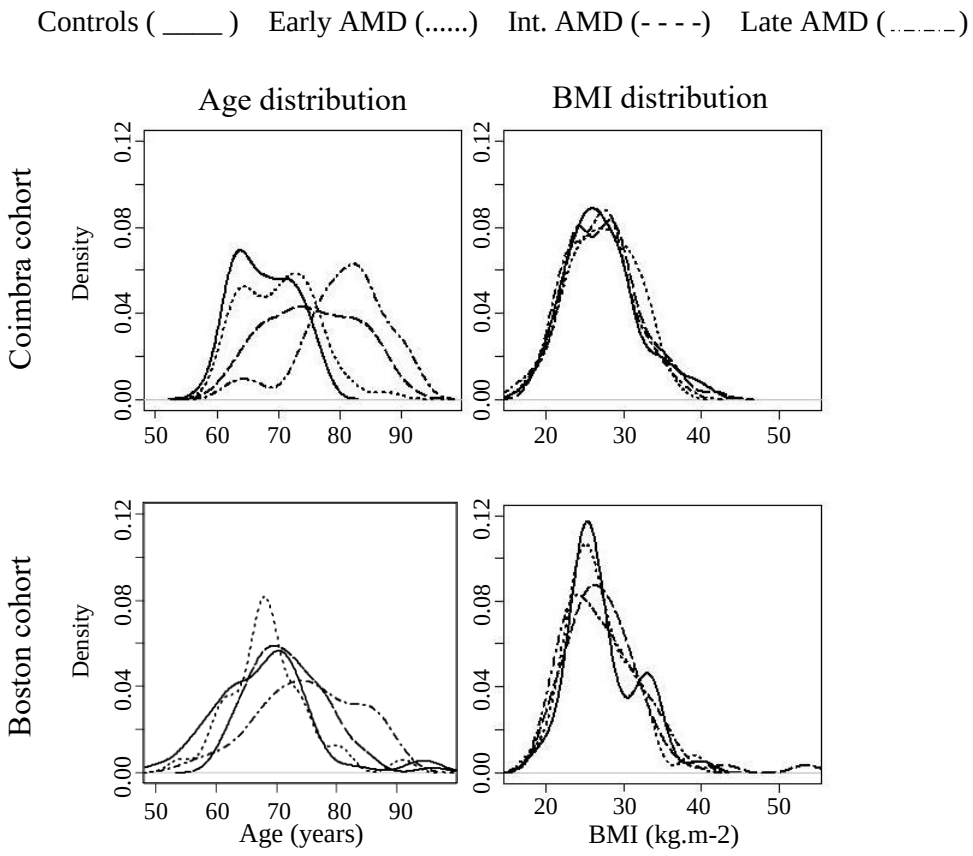


Figure S1. Histograms of age and BMI distributions for controls and AMD patients for Coimbra and Boston cohorts: controls

Figure S2

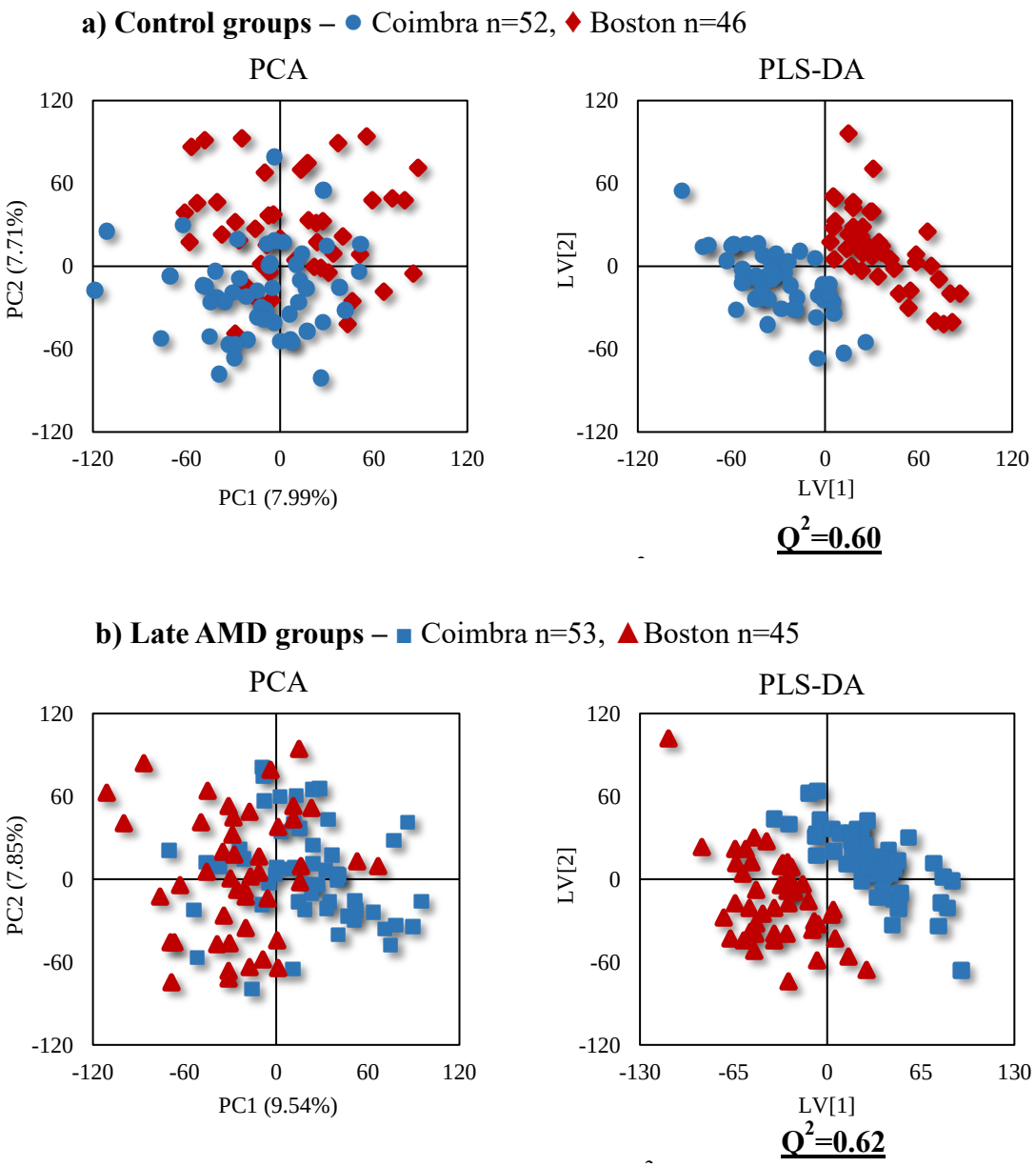


Figure S2. PCA and PLS-DA scores obtained for a) control and b) late AMD groups from each cohort. In the PCA of controls, two outlier samples were removed from the Coimbra cohort; in the PCA of late AMD, two Boston outliers and one Coimbra outlier were removed.

Figure S3

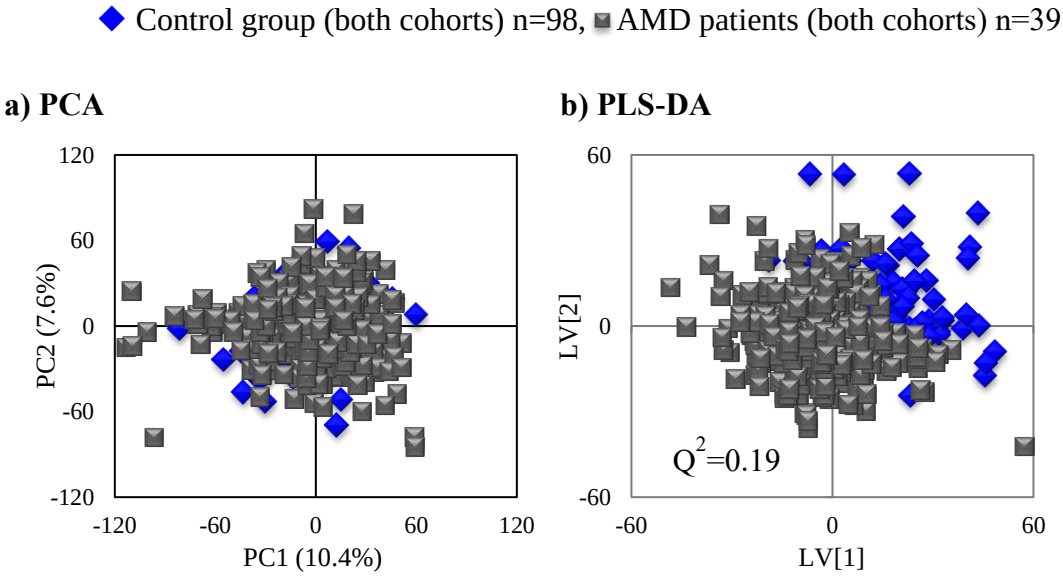
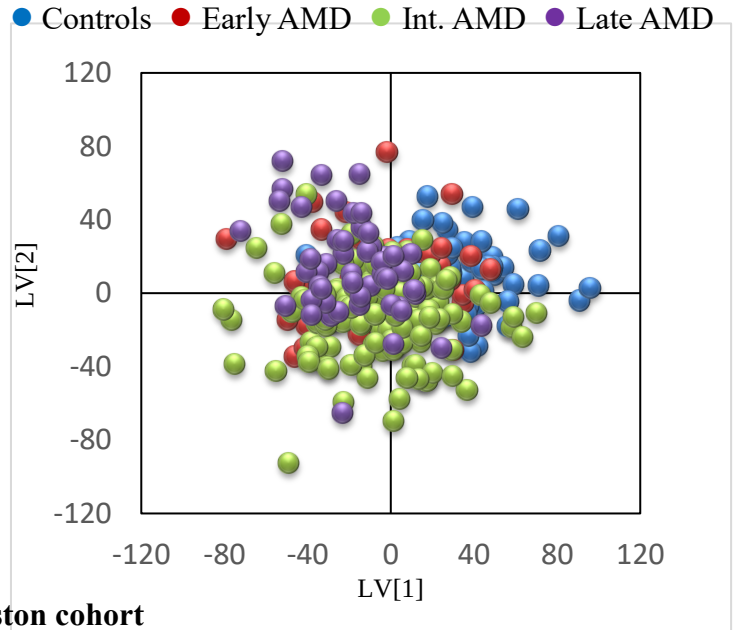


Figure S3. a) PCA and b) PLS-DA scores obtained for all controls (Coimbra and Boston cohorts) compared to all AMD patients (Coimbra and Boston, all disease stages).

Figure S4

a) Coimbra cohort



b) Boston cohort

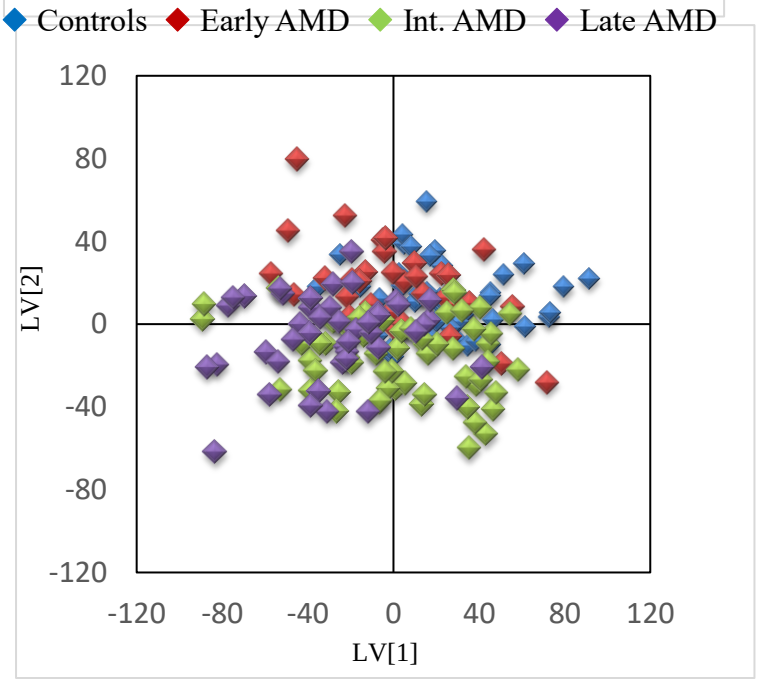


Figure S4. PLS-DA scores for variable-selected spectra of urine from Controls *vs* Late AMD in the a) Coimbra cohort, circles (blue, Controls, n=52; purple, Late AMD, n=53) and b) Boston cohort, diamonds (blue, Controls, n=46; purple, Late AMD, n=48). Q^2 value and MCCV results are shown for each model.

Figure S5

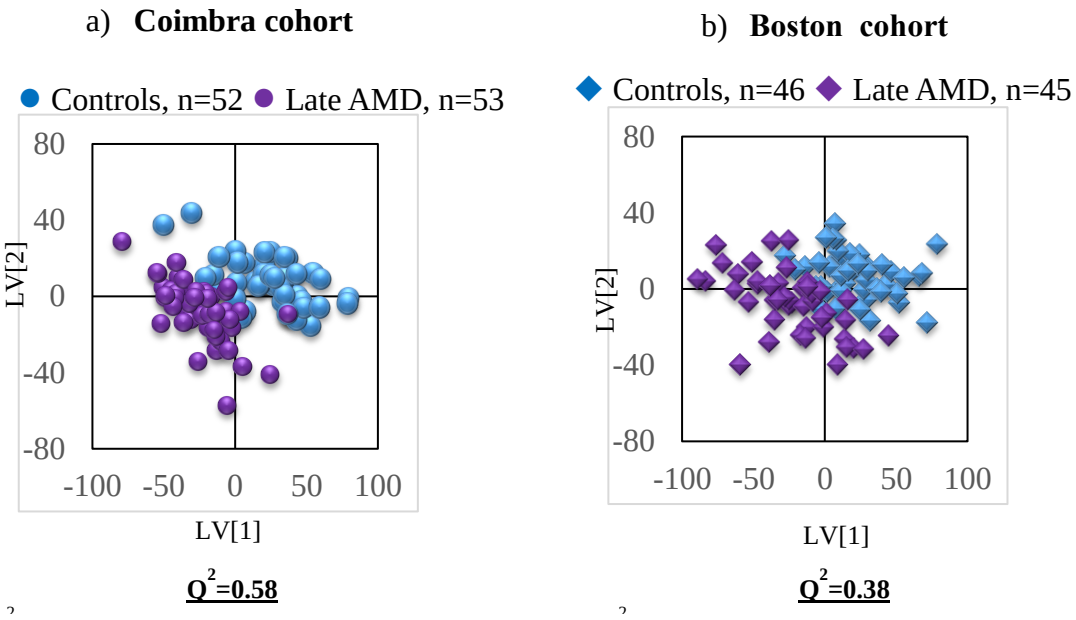


Figure S5. PLS-DA obtained with the full resolution spectra for both study cohorts: a) Coimbra cohort, circles (blue: controls, n=52; red: early AMD, n=56; green: intermediate AMD, n=141; purple: late AMD, n=54); b) Boston cohort, diamonds (blue: controls, n=46; red: early AMD, n=33; green: intermediate AMD, n=60; purple: late AMD, n=45).

Figure S6

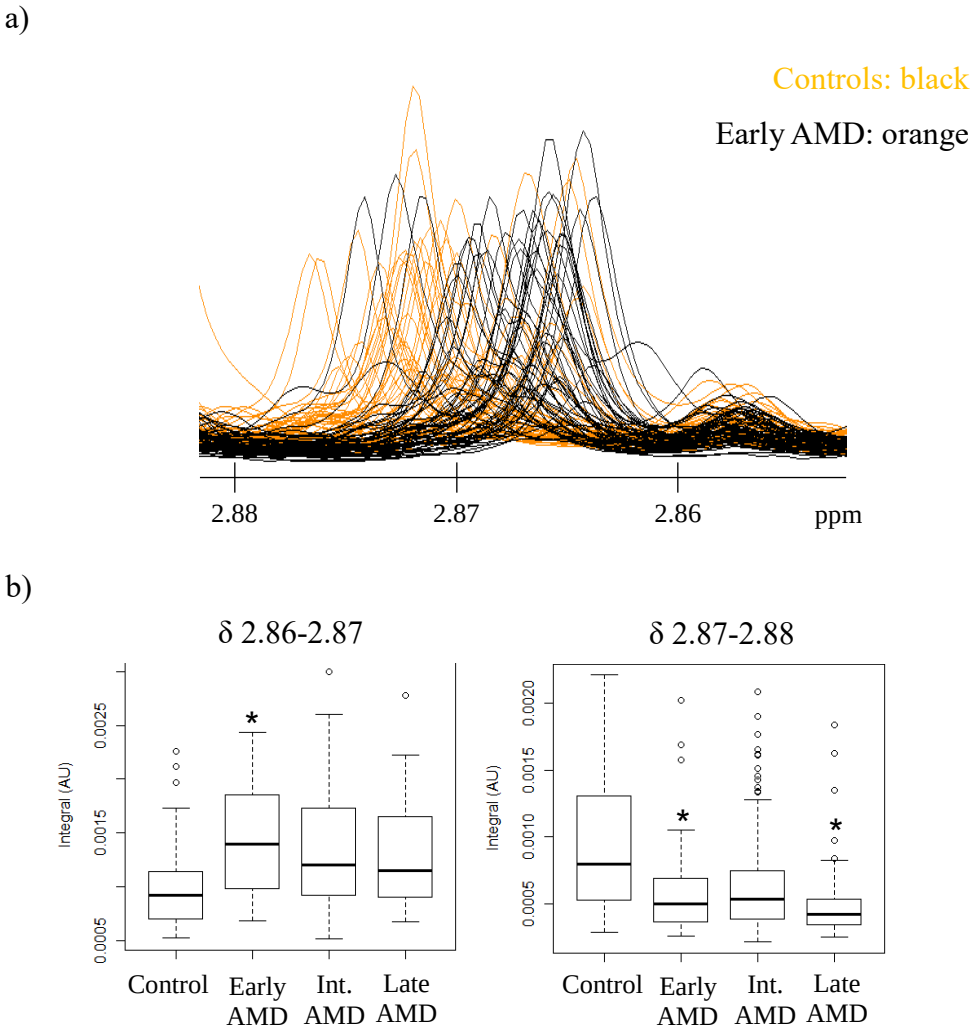


Figure S6. a) Spectra and b) boxplots for unassigned spectral region 2.86-2.88 ppm, which appears to separate controls and early AMD groups in the Coimbra cohort.

Table S1. Comorbidities characterizing each of the subject groups with corresponding percentages and statistical relevance. Statistical comparison (Person Chi^2 or Fisher Exact Test, according to Cochran Rules) between groups was performed (control *vs* early AMD; early *vs* intermediate AMD; intermediate *versus* late AMD. *: p-values < 0.05 corresponding to comparison with previously indicated group.

	Control	Early AMD	Intermediate AMD	Late AMD
Coimbra cohort	n = 53	n = 57	n = 141	n = 54
Hypertension, n (%)	26 (49)	30 (53)	81 (57)	35 (65)
Dyslipidemia, n (%)	26 (49)	32 (56)	63 (47)	28 (52)
Heart disease, n (%)	10 (19)	8 (14)	33 (23)	10 (19)
Blood disease, n (%)	1 (2)	0 (0)	5 (4)	4 (8)
Renal disease, n (%)	1 (2)	2 (4)	7 (5)	3 (6)
Liver disease, n (%)	0 (0)	1 (2)	1 (1)	3 (6)

Prior cancer, n (%)	5 (10)	4 (7)	12 (9)	4 (8)
Rheumatic disease, n (%)	0 (0)	1 (2)	1 (1)	3 (6)
Neurologic disease, n (%)	10 (19)	12 (21)	20 (15)	9 (17)
Thyroid disease, n (%)	9 (17)	4 (7)	12 (9)	0 (0) *
Boston cohort	n = 47	n = 33	n = 66	n = 48
Hypertension, n (%)	14 (30)	8 (24)	25 (37)	23 (47)
Dyslipidemia, n (%)	17 (36)	11 (33)	28 (42)	23 (47)
Heart disease, n (%)	2 (4)	7 (21) *****	8 (12)	7 (15)
Blood disease, n (%)	5 (11)	4 (12)	6 (9)	2 (4)
Renal disease, n (%)	2 (4)	2 (6)	2(3)	6 (13)
Liver disease, n (%)	2 (4)	1 (3)	1 (2)	1 (2)
Prior cancer, n (%)	14 (30)	9 (27)	30 (46)	14 (29)
Rheumatic disease, n (%)	4 (9)	4 (12)	11 (17)	10 (21)
Neurologic disease, n (%)	3 (7)	2 (6)	8 (12)	3 (6)
Thyroid disease, n (%)	8 (17)	2(6)	14 (21) *****	1 (2)

Table S2. Metabolite identification in the ^{1}H NMR spectrum of urine of a control subject (Coimbra cohort). The bottom section of the table lists the unassigned spin systems observed in this work to change according to AMD stage. 2-HIBA, 2-hydroxyisobutyrate; 2-KG, 2-ketoglutarate; 2-Py, *N*-methyl-2-pyridone-5-carboxamide; β-HBA: β-hydroxybutyrate; 3-HIVA: 3-hydroxyisovalerate; 4-DEA: 4-deoxyerythronic acid; 4-DTA: 4-deoxythreonic acid; 4-HPA: 4-

hydroxyphenylacetate; GAA: guanidoacetate; IS: indoxyl sulphate; *p*-CS: *para*-cresol sulphate; PAG: phenylacetylglutamine; TMA: trimethylamine; TMAO: trimethylamine-*N*-oxide. U*i*, unassigned resonances according to Table 2.

$\delta_{H\ ppm}$ (multiplicity, assignment/ $\delta_{C\ ppm}$)

(please see next 2 pages for full table)

1,6-anhydroglucose	3.54 (m, C2H), 3.69 (m, C3H,C4H), 3.76 (dd), 4.10 (dd, CH), 4.62 (dd,CH_2), 5.46 (br, C1H)
1-methyl-histidine	3.07 (dd,βCH_2); 3.16 (dd, $\beta' CH_2$); 3.72 (s, CH_3); 3.96 (dd, αCH_2); 7.05 (s, ring); 7.78 (s, ring)
2-HIBA	1.36 (s, CH_3)
2-KG	3.45 (t, βCH_2), 3.01 (t, γCH_2)
2PY	3.62 (s, CH_3); 6.67 (d, C3H ring/120.85); 7.97 (dd, C4H ring); 8.33 (d, C6H ring/145.46)
3-aminoisobutyricacid	1.19 (d, CH_3/17.87); 2.61 (m, αCH); 3.06 (dd, βCH_2)
β-HBA	1.20 (d, CH_3); 2.31(m, CH_2); 2.41 (m, CH_2); 4.15 (m, CH)
3-HIVA	1.27 (s, βCH_3/31.02); 2.37 (s, αCH_2)
3-methyl-histidine	3.28 (dd, βCH_2/28.05); 3.75 (s, NCH3/35.18); αCH/56.52); 7.15 (s, C6H/126.33); 8.12 (s, C2H/140.81)
4-DEA	1.11 (d, γCH_3/18.26); 4.08 (d, αCH/78.67); 4.10 (m, βCH/71.54)
4-DTA	1.23 (d, γCH_3/21.37); 3.84 (d, αCH/79.04); 4.12 (m, βCH/71.59)
4-hydroxyhippurate	3.95 (s, CH_2); 6.98 (d, C3H, C5H ring); 7.76 (d, C4,2H, C6H ring)
4-HPA	3.46 (s, CH_2/46.3); 6.86 (d, C3H, C5H ring/118.4); 7.17 (d, C2H, C6H ring/133.3)
Acetate	1.93 (βCH3)
Acetoacetate	2.29 (s, CH_2); 3.46 (s, CH_3)
Acetone	2.24 (s)
Alanine	1.49 (d,βCH3/18.99); 3.78 (q, αCH)
Allantoin	5.39 (s, CH/66.19)
Ascorbate	3.76 (m, CH2(OH)); 4.01 (m, CH (OH)); 4.52 (d,C1H)
Betaine	3.27 (s, CH3/56.13); 3.91 (s, CH2/69.11)
Carnitine	2.44 (dd, αCH2/45.74; 3.23 (s, N(CH3)3/72.80; 3.43 (m, γCH2/72.80; 4.57 (m, βCH2/66.88)
Choline	3.20 (s, N(CH3)3/56.52); 3.52 (m, NH/70.60); 4.07 (m, CH2(OH))
cis-aconitate	3.12 (d, CH/46.13); 5.79 (t, CH2/127.13)
Citrate	2.54 (d, α,β CH2/48.17); 2.69 (d, α×,β× CH2/48.17)
Creatine	3.04 (s, NCH3/39.66); 3.94 (s, NCH2/56.55
Creatinine	3.05 (s, NCH3/32.86); 4.06 (s, NCH2/59.05)
DMA	2.73 (s, CH3/37.41)
DMG	2.93 (s, $(CH3)_2$/46.21); 3.72 (s, CH)
Formate	8.47 (s, CH/173.97)
Fumarate	6.53 (s, CH)
Furoylglycine	3.93 (s, CH2); 6.65 (dd, C4H ring/114.98); 7.19(d, C3H ring); 7.70 (d, C5H ring)
Galactose	3.49 (dd, C4H), 3.64 (dd, C3H), 3.75 (m, C1H, C2H, CH2), 3.83 (m, C3H), 3.93 (d. C2H), 3.98 (d, C2H), 4.10 (t, C1H), 4.60 (d, CH2), 5.28 (d, C5H)
α-Glucose	3.23 (dd, C2H); 3.44 (m, C4H); 3.50 (t, C3H); 3.72 (dd, C6H'); 3.90 (m, C6H); 4.65 (d, C1H)
β-Glucose	3.42 (t, C4H); 3.54 (dd, CH); 3.71 (t, C3H); 3.77 (dd, C6H); 3.84 (m, C5H); 5.25 (d, C1H/94.97)
Glutamine	2.15 (m, βCH2/29.24); 2.47 (m, γCH2/33.67); 3.79(t, αCH/57.41)
Glycine	3.57 (s, αCH2/44.45)
GAA	3.80 (s, CH2/47.44)
Hippurate	3.97 (d, CH2/46.65); 7.56 (t, C4H, C6H ring/131.60); 7.64 (t, C3H, C5H ring/134.99); 7.83(d, C4H/129.97); 8.52 (br, NH)
Histidine	3.18 (dd, βCH2/30.40); 3.28 (dd, β'CH2/30.40);4.01(dd, αCH_2/57.64); 7.13 (s, C4H ring/120.05); 7.98 (s, C2H ring/138.74)
Hypoxanthine	8.20 (s, C2H ring); 8.22 (s, C8H)
IS	7.21 (dd, C8H/122.53), 7.28 (dd, C7H/125.01), 7.36 (s, C2H/119.08), 7.51 (d, C6H/115.04), 7.70 (d, C9H/120.57)
Isoleucine	0.94 (t, δCH_3); 1.01 (d, βCH_2); 1.26 (m, γCH_2); 1.47(m, $\beta' CH_2$); 1.98 (m, $\gamma' CH_2$); 3.62 (d, αCH)

Lactate	1.34 (d, CH_3/22.43); 4.11 (q, CH/71.53)
Lactose	3.28 (dd, C2H); 3.55 (m, C'2H); 3.59 (dd, C2H); 3.66 (m, C'3H, C3H,C5H); 3.73 (m, C'6H, C'5H); 3.79 (m, C6H); 3.86 (m, C6H, C3H); 3.94 (m, C6H, C'4H, C4H); 4.46 (d, C'1H/ 105.8); 5.25 (d, C1H)
Leucine	0.96 (t, γCH_3); 1.7 (m, CH_2); 3.73 (t, αCH)
Lysine	1.48 (m, γCH_2/24.28); 1.73 (m, δCH_2/29.06); 1.92 (m, βCH_2/32.60); 3.03 (t, εCH_2)/42.02); 3.77 (t, αCH/57.28)
Malonate	3.11 (s, CH2/48.7)
NMND	4.48 (s, NCH3/51.30); 8.18 (m, C5H ring); 8.90 (d, C4H ring); 8.97 (d, C6H ring); 9.29 (s, C2H ring)
p-CS	2.35 (s, CH3); 7.21 (d, C2H, C6H ring/124.12); 7.29 (C3H, C5H ring/125.04)
PAG	1.93 (m, βCH2) ; 2.11 (m, β'CH2); 2.27 (t, γCH2/34.44); 3.67 (d, CH2); 4.18 (m, αCH); 7.36 (m, C2H, C4H, C6H ring/132.01); 7.43 (m, C3H, C5H ring/131.84)
Pyruvate	2.38 (s, CH3)
Scyllo-inositol	3.36 (s, CH)
Succinate	2.41 (s, CH2/36.85)
Sucrose	3.48 (t, C4H), 3.56 (dd, C2H), 3.63 (s, C1'H2), 3.77 (t, C3H), 3.83 (dd, CH2, C'6H2), 3.85 (m, C5H), 3.89 (m, C'5H), 4.06 (t, C'4H), 4.22 (d, C'3H), 5.41 (d, C1H)
Tartrate	4.35 (s, CH(OH))
Taurine	3.26 (t, CH2SO3); 3.43 (t, NCH2)
Threonine	1.33 (d, CH3/22.44); 3.61 (d, βCH/63.36); 4.26 (dd, αCH/68.90)
Trigonelline	4.44 (s, CH3/50.98); 8.09 (t, C3H ring); 8.84 (br, C2H, C4H ring); 9.12 (s, C6H ring/148.73)
TMA	3.89 (s, CH3/47.40)
TMAO	3.28 (s, CH3/62.31)
Tyrosine	3.06 (dd); 3.21 (dd); 3.95 (dd); 6.91 (d, C3H, C5H ring/118.83); 7.20 (d, C2H, C6H ring/124.15)
Urea	5.79 (br s, NH2)
Valine	0.99 (d, γCH3); 1.04 (d, γ'CH3); 2.27 (m, βCH); 3.61 (d, αCH)
Xylose	3.23 (dd, C3H), 3.33 (dd, C6H), 3.42 (t, C4H), 3.53 (dd, C3H), 3.63 (m, C6H, C5H, C4H), 3.93 (dd, C6H), 4.59 (d, C2H), 5.21 (d, C2H)
Unassigned spin systems varying with AMD evolution	
U1	6.58 (s)
U2	7.68 (d)
U3	4.40 (s), 8.79 (d)
U4	2.39 (d)
U5	8.03 (s)
U6	8.70 (d)
U7	2.19 (s), 9.05 (s)