Supplementary Materials

MATERIALS AND METHODS

HUMAN PLASMA METABOLOMICS ANALYSIS

Each sample was accessioned into the Metabolon Laboratory Information Management System (LIMS) and assigned a unique identifier associated with the original de-identified study code number. The identifier was used to track all sample handling, tasks and results.

Samples were prepared using the automated MicroLab STAR® system from Hamilton Company (Reno, NV, USA). Several recovery standards were added prior to the first step in the extraction process for quality control purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by HILIC/UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for UPLC-MS/MS analysis.

The UPLC-MS/MS platform utilised a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The dried sample extract was reconstituted in acidic or basic UPLC-compatible solvents, each of which contained 11 to 13 injection standards at fixed concentrations (14). Two aliquots were analysed using acidic positive ion conditions, chromatographically optimised for hydrophilic or hydrophobic compounds and the other using basic negative ion optimised conditions, using separate dedicated columns (Waters UPLC BEH C18-2.1x100 mm, 1.7 µm). Extracts reconstituted in acidic conditions were gradient eluted using water and methanol, containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA) for hydrophilic compounds or water, methanol, acetonitrile, 0.05% PFPA and 0.01% FA for hydrophobic compounds. Basic extracts were also gradient eluted using methanol and water, but with the addition of 6.5mM Ammonium Bicarbonate at pH 8. The fourth aliquot was analysed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1x150 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10 mM Ammonium Formate, pH 10.8. The MS analysis alternated between MS and data-dependent MS/MS scans using dynamic exclusion and the scan range covered 70-1000 m/z.

Raw data was extracted and peak-identified as per Metabolon's hardware and software. Biochemical identifications were based on three criteria: retention index within a narrow RI window of the proposed identification, accurate mass match to the library +/- 10 ppm, and the MS/MS forward and reverse scores between

the experimental data and authentic standards. The MS/MS scores were based on a comparison of the ions present in the experimental spectrum to the ions present in the library spectrum. More than 3300 commercially available purified standard compounds have been acquired and registered into LIMS for analysis on all platforms for determination of their analytical characteristics (REF).

Supplementary Table 1. Clinical description of CHM patients included in this study.

ID	DOB	Visual acuity OD	Visual acuity OS	FA_OD (mm2)	FA_OS (mm2)	Retinal Thickness OD (µm)	Retinal Thickness OS (µm)	Choroidal Thickness OD (µm)	Choroidal Thickness OS (µm)	EZ OD (µm)	EZ OS (µm)	CHM variant (NM_000390.4)
P1	1986	6_6	6_6	17.23	21.03	259	258	229	278	4075	4503	c.116+1G>A
P2	1983	6_6	6_6	7.95	7.17	335	346	141	148	2521	1951	c.759delA; p.(Tyr254Metfs*37)
P3	1979	6_5	6_5	0	0	221	245	252	263	5059	5234	c.-98C>T
P4	1980	6_6	6_6	28.1	32.02	312	254	254	224	4826	3684	c.116+1G>A
P5	1996	6_9	6_12	16.3	14.87	276	299	374	373	3950	2830	Deletion exon 3-intron 4
P6	1959	HM	6_15	4.74	0.99	80	241	76	83	259	647	c.1347C>G; p.(Tyr449*)
P7	1987	6_7.5	6-9.5	16.24	20.29	268	243	219	233	3308	3824	c.808C>T; p.(Arg270*)
P8	1989	6_6	6_6	35.2	33.08	246	257	286	282	4600	4781	c.808C>T; p.(Arg270*)
P9	1989	6_7.5	6_7.5	2.26	1.69	227	237	138	168	1567	2127	c.126C>G; p.(Tyr42*)
P10	1963	6_9	6_12	22.41	4.25	320	327	200	138	3597	2053	Deletion exons 3-15
P11	1955	6_5	6_19	9.32	3.86	294	240	225	148	2716	729	c.715C>T; p.(Arg239*)
P12	1965	LP	6_24	0	0	156	230	93	149	3047	828	c.495dupT; p.(Ala166Cysfs*8)

P13	1974	6_7.5	6_4.8	11.34	19.65	334	350	131	135	2455	2900	c.877C>T p.Arg293*
P14	1967	6_15	LP	0.41	0.16	221	157	114	60	716	400	c.698C>G; p.(Ser233*)
P15	1972	6_9.5	HM	1.99	0.5	238	190	166	140	633	0	Deletion exons 3-15
P16	1970	6_9	6_6	7.68	5.97	221	246	127	133	1177	740	c.649_652delTACT; p.(Tyr217Hisfs*14)
P17	1987	6_6	6_6	9.57	8.04	308	339	193	168	2969	2597	c.1245_1246delins14
P18	1976	6_6	6_4.8	20.65	22.88	255	263	193	210	1804	4020	c.715C>T; p.(Arg239*)
P19	1989	6_6	6_6	22.61	24.65	213	239	181	152	3090	2056	c.715C>T; p.(Arg239*)
P20	1997	6_12	6_12	18.73	14.39	268	281	230	243	3817	3542	c.877C>T p.(Arg293*)
P21	1994	6_9	6_9	11.76	12.14	272	268	244	213	2762	2174	Deletion exons 1-11
P22	1955	6_48	6_12	17.94	12.84	196	424	109	184	1824	3023	c.715C>T; p.(Arg239*)
P23	1980	6_9	6_9	30.79	38.27	291	292	248	211	5969	4979	Deletion exons 1-15
P24	1963	LP	HM	0	0	163	178	136	145	0	0	Deletion exons 10-11
P25	1968	6_6	6_6	17.44	12.46	231	275	141	145	2116	1356	c.799C>T; p.(Arg267*)

Abbreviations: DOB, date of birth; FA, fluorescein angiography; OD, right eye; OS, left eye;; EZ, ellipsoid zone; LP, light perception; HM, hand movement.

Supplementary Table 2. Dietary intake of CHM patients and controls.

	Mean values Control	**Mean values CHM**	***p*-value**
Fat (g)	78.2	80.1	0.635
Energy (Kcal)	2091	2183	0.541
Protein (g)	89.7	91.5	0.621
NSP (g)	17.7	19.6	0.357
% energy from fats	33.3	32.9	0.977
% energy from saturated fatty acids	12.4	12.0	0.516
% energy from polyunsaturated fatty acids	5.5	5.6	0.541
% energy from monounsaturated fatty acids	11.8	11.7	0.869
Carotene (ug)	3877	4623	0.399
Vitamin C (mg)	132.2	140.5	0.528
Fruits (portions/day)	2.14	2.19	0.712
Vegetables (portions/day)	4.31	4.50	0.607
Leafy vegetables (portions/day)	0.85	1.13	0.264
Meat (portions/day)	1.11	1.23	0.541
Fish (portions/day)	0.36	0.42	0.565
Milk (pints/day)	0.57	0.47	0.968
Other dairy (portions/day)	0.87	0.89	0.46
Cereal foods (portions/day)	5.19	5.46	0.93

Supplementary Table 3. Top 30 metabolites with most separation potential between CHM and control groups. Figures in coloured boxes represent fold change (FC) values significantly increased (red) or decreased (green) in choroideremia patients compared to age-matched controls.

Metabolite	Sub Pathway	Mean Control	Mean CHM	CHM/ Control FC	*p* value
Sphingadienine	Sphingolipid Metabolism	0.8736	1.4326	1.64	3..6471E-06
Iminodiacetate (IDA)	Chemical	1.5682	0.9047	0.58	1.9034E-08
Cysteine S-sulphate	Methionine, Cysteine, SAM and Taurine Metabolism	8.2307	0.7902	0.10	2.7906E-10
Cysteine	Methionine, Cysteine, SAM and Taurine Metabolism	1.0930	0.8869	0.81	6.8246E-7
Sphinganine	Sphingolipid Metabolism	0.8402	1.1818	1.41	3.2126E-07
Cysteinylglycine	Glutathione Metabolism	1.6662	0.9251	0.56	5.9919E-06
Sarcosine	Glycine, Serine and Threonine Metabolism	1.1676	0.8454	0.72	3.8408E-06
1-stearoyl-GPS (18:0)*	Lysophospholipid	0.2791	1.0650	3.82	7.794E-05
Adenosine 5' monophosphate (AMP)	Purine Metabolism	0.8544	1.4386	1.68	0.0003
1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	Phosphatidylserine	0.1410	0.8234	5.84	2.274E-5
Phosphoethanolamine	Phospholipid Metabolism	0.9025	1.2660	1.40	0.0012
1-stearoyl-2-oleoyl-GPS (18:0/18:1)	Phosphatidylserine	0.8936	2.4976	2.79	0.0001
Aspartate	Alanine and aspartate Metabolism	0.8983	1.1075	1.23	0.0021
Cortisone	Corticosteroids	1.1314	0.8424	0.74	0.0201
Cys-gly oxidized	Glutathione Metabolism	0.8730	1.1796	1.35	0.0201
Serotonin	Tryptophan Metabolism	0.4484	1.7132	3.82	0.0082
Hypotaurine	Methionine, Cysteine, SAM and Taurine Metabolism	0.8833	1.3528	1.53	0.0001
3-phosphoglycerate	Glycolysis, Gluconeogenesis, and Pyruvate Metabolism	0.8156	1.2728	1.56	0.0018
Hexadecasphingosine (d16:1)*	Sphingolipid Metabolism	0.7790	1.0263	1.32	0.0043

Phenylacetate	Phenylalanine Metabolism	0.6640	1.4180	2.14	0.0053
3-methylcytidine	Pyrimidine Metabolism	1.1051	0.8807	0.80	0.0026
Bilirubin (Z,Z)	Hemoglobin and Porphyrin Metabolism	1.2231	0.9971	0.82	0.0053
Sphinganine 1-phosphate	Sphingolipid Metabolism	0.8836	1.1957	1.35	0.0007
Lactosyl-N-behenoyl-sphingosine (d18:1/22:0)*	Sphingolipid Metabolism	0.8854	1.0297	1.16	0.0078
Sphingosine 1-phosphate	Sphingolipid Metabolism	0.9226	1.1452	1.24	0.0156
3-(3-hydroxyphenyl) propionate	Benzoate Metabolism	1.7713	0.7427	0.42	0.0006
3-carboxy-4-methyl-5-propyl-2-furan propanoate (CMPF)	Fatty Acid, Dicarboxylate	1.7559	1.2924	0.74	0.0078
N-acetyl-2-aminooctanoate*	Fatty acid, Amino	1.5519	0.9568	0.62	0.0022
Beta-cryptoxanthin	Vitamin A Metabolism	1.6441	1.1354	0.69	0.003
Inosine 5'-monophosphate (IMP)	Purine Metabolism	0.5003	1.2011	2.40	0.0222

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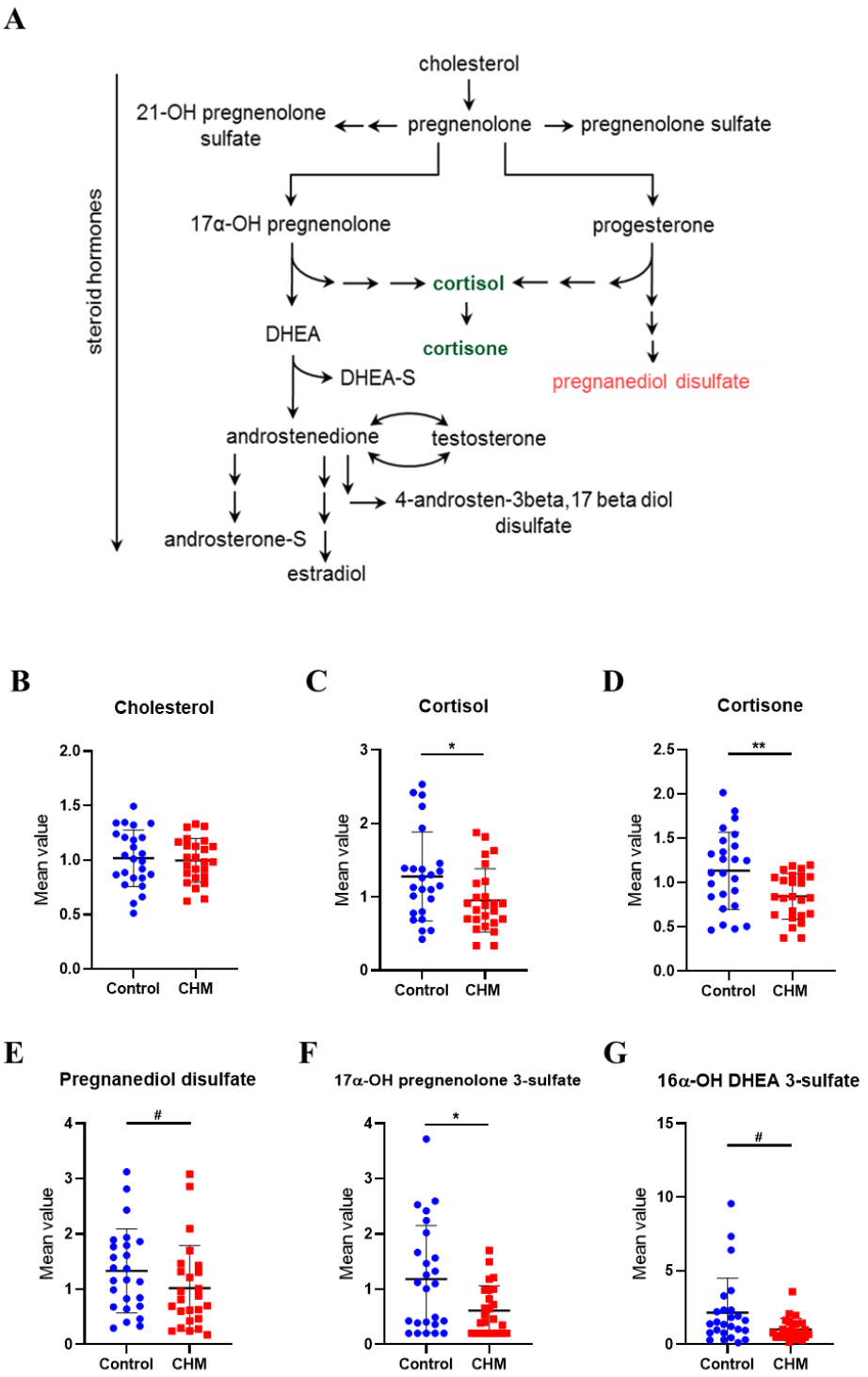

Supplementary Figure 1. Biochemicals from steroids metabolism pathway altered in CHM patients. (A) Schematics of steroid metabolism pathway. B-G) Selected metabolites altered in CHM vs control. Levels in CHM patient samples are represented in red and control samples in blue Values are shown in scatter dot plots with lines indicating mean ± SD (n=25). *p* value was determined using matched pair *t* tests: # $0.05 < p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$.