SUPPLEMENTARY INFORMATION

TITLE COPD is accompanied by co-ordinated transcriptional perturbation in the quadriceps affecting the mitochondria and extracellular matrix.

AUTHORS
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SUPPLEMENTARY METHODS

Participants

Patients were recruited from clinics at the Royal Brompton Hospital whilst healthy controls were recruited by advertisement.

Physiological methods

Post-bronchodilator spirometry, lung volumes by plethysmography and carbon monoxide diffusion capacity were measured according to American Thoracic Society (ATS) guidelines and arterialized capillary earlobe blood gas tensions measured. Fat-free mass (FFM) was measured in participants, after a twenty-minute period resting supine, using the bioelectrical impedance technique (Bodystat 1500, UK). The FFM index (FFMI) was calculated using a disease-specific regression equation. Quadriceps strength was assessed by supine isometric Maximal Voluntary Contraction (MVC) as described by Edwards *et al* [1]. Post-bronchodilator exercise performance was assessed with a single 6-minute walk (6MW) performed according to ATS guidelines [2] and symptom-limited incremental cycle ergometry with metabolic testing as described previously [3].

Quadriceps sampling

Percutaneous biopsy of the *vastus lateralis* (quadriceps) was performed using the Bergstrom technique [4]. Samples for RNA and protein analysis were snap frozen in liquid nitrogen whilst samples for histology were frozen in melting isopentane prior to storage at –80°C.

Measurement of quadriceps fibre CSA and fibre proportions

Frozen 10μm transverse muscle cross-sections were incubated with primary antibodies against Type I and IIa myosin and laminin followed by fluorescent-labelled secondary antibodies. Epi-fluorescence (Nikon Eclipse 800, Nikon Instruments Europe BV, Netherlands) classified fibres as Type I, IIa (IIa and IIa/IIx), IIx and hybrid I/IIa and fibre CSA was determined using Lucia 4.81 software (Laboratory Imaging, Czech Republic) for each subject. Therefore, a subject's Type I and Type II fibre proportions and median Type I and Type II fibre CSAs could be calculated. At least 101 fibres were analysed per subject [5].

Western blotting and ELISA

Total protein homogenates from quadriceps specimens were prepared by homogenising muscle samples under liquid nitrogen and re-suspending the homogenate in lysis buffer (NaCl [300mM], Tris-Base [20mM], EDTA [10mM] 2% Igepal NP40 pH 7.3) supplemented with protease and phosphatase inhibitor cocktails (Sigma, UK). Protein concentrations were measured by Bradford assay and samples were mixed with sample buffer (loading buffer and betamercaptoethanol) at a 1:1 volume ratio and boiled for five minutes at 100°C. Total protein was analysed by gel electrophoresis using NuPAGE Novex 4-12% Bis-Tris Gel (Invitrogen) and the iBlot dry transfer technique to nitrocellulose membrane (Invitrogen). Membranes were blocked in 5% non-fat dry milk for 1 hour and then incubated in the relevant primary antibody at 4°C overnight. Following an hour long room temperature incubation in the relevant secondary antibody, proteins were visualised using ECLplus (GE Healthcare) and optical densities were determined using ImageJ (http://rsbweb.nih.gov/ij/index.html). Antibodies used were: rabbit anti-IDH2 (HPA007831, Sigma) 1/200 dilution, rabbit anti-AK1 (H-90) (sc-28785, Santa Cruz Biotechnology) 1/500 dilution, and horseradish peroxidise (HRP) conjugated pig anti-rabbit IgG antibody (P0399, Dako) 1/3000 dilution. Ponceau S was used as a loading control. Between blot comparisons were enabled by loading an equal amount of protein standard on each gel.

Analysis of gene expression data

Raw data quality was assessed using arrayQualityMetrics (3.22.1). Technical batch effects were identified via Principal Variance Component Analysis (PVCA 1.6.0) and the data adjusted accordingly using ComBat (SVA Version 3.12.0) [6]. Transcript clusters (TC) forming part of the main array design were retained (n = 28,869) for analysis. Universally low expressed TC were removed. Together these criteria yielded a total of 18,625 transcript clusters for analysis. Remaining outlying samples (n = 1) were specified through hierarchical clustering and also removed resulting in 95 samples (79 COPD, 16 Healthy controls) for analysis. Differential expression between healthy and COPD samples was estimated using Limma (Version 3.22.7). *P*-values were adjusted for multiple testing using the Benjamini & Hochberg method [7] to control the False Discovery Rate (FDR) below 5%. Occult patterns of transcriptional co-ordination were sought amongst differentially expressed genes through Weighted Gene Co-expression

Network Analysis (WGCNA Version1.46) [8], employing a soft-threshold power of 6 and minimum module size of 30. The relationship between modules and measured traits was assessed through robust biweight midcorrelation, with the exact number of observations taken into account in *P*-value calculation. Enrichment analysis was performed on unique entrez IDs using the Bioconductor packages topGO (Version 2.20.0) for Gene Ontology and DOSE (Version 3.0.4) for Disease-Gene Associations [9]. Annotations were made using packages annotate (Version 1.46.1) and hugene11sttranscriptcluster.db (Version 8.3.1). In order to estimate the relative importance of fibre type and disease state in predicting transcript abundance, we applied the R^2 decomposition based on the methods of Zuber and Strimmer [10]. Microarray data for samples used in this analysis have been deposited in the NCBI Gene Expression Omnibus (GEO) repository with Accession Number GSE100281.

References

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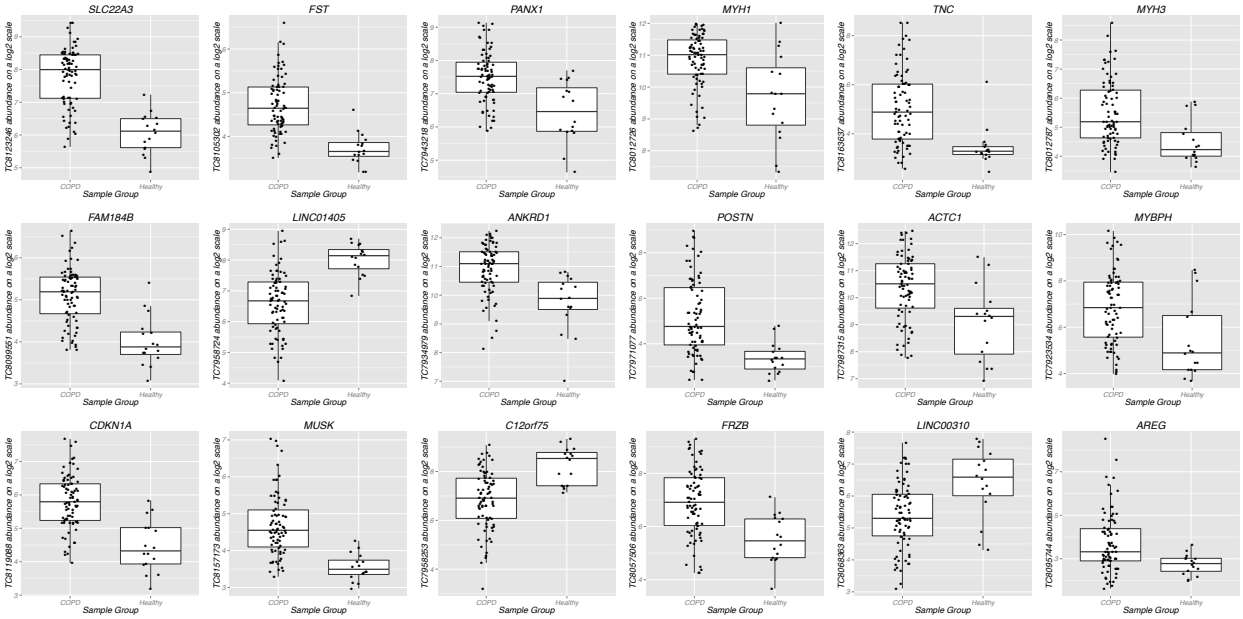
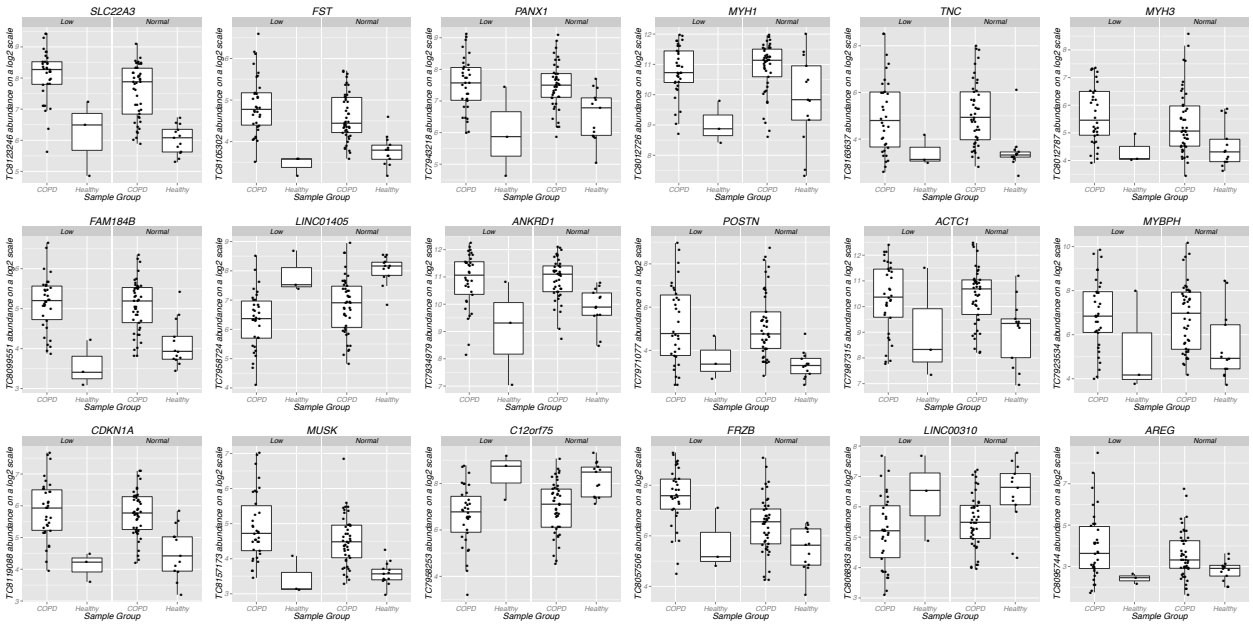
SUPPLEMENTARY FIGURES AND TABLES

Figure E1a: Transcript clusters differentially expressed at a 5% false discovery threshold accompanied by a minimum absolute fold change of 2.



Abbreviations: Transcript Cluster (TC). Abundance is shown on a log2 scale.

Figure E1b: Transcript clusters differentially expressed at a 5% false discovery threshold accompanied by a minimum absolute fold change of 2 split by FFMI in the low and normal range.



Abbreviations: Transcript Cluster (TC), Fat Free Mass Index (FFMI). Abundance is shown on a log2 scale. A low FFMI and normal FFMI was defined using the Dutch criteria (low if less than 15 kg/m^2 for women and less than 16 kg/m^2 in men).

Figure E2: Full-length IDH2 Western blot image corresponding to Figure 2 A

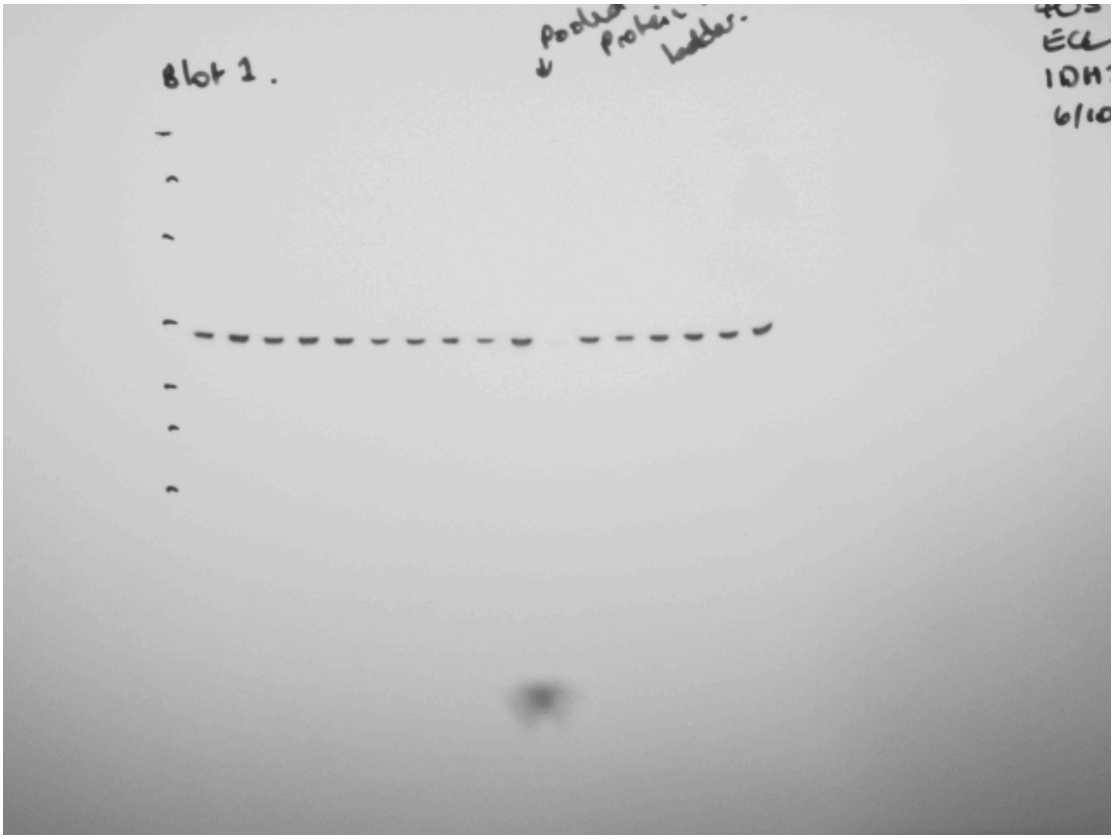


Figure E3: Full-length Ponceau S loading control image corresponding to Figure 2 A

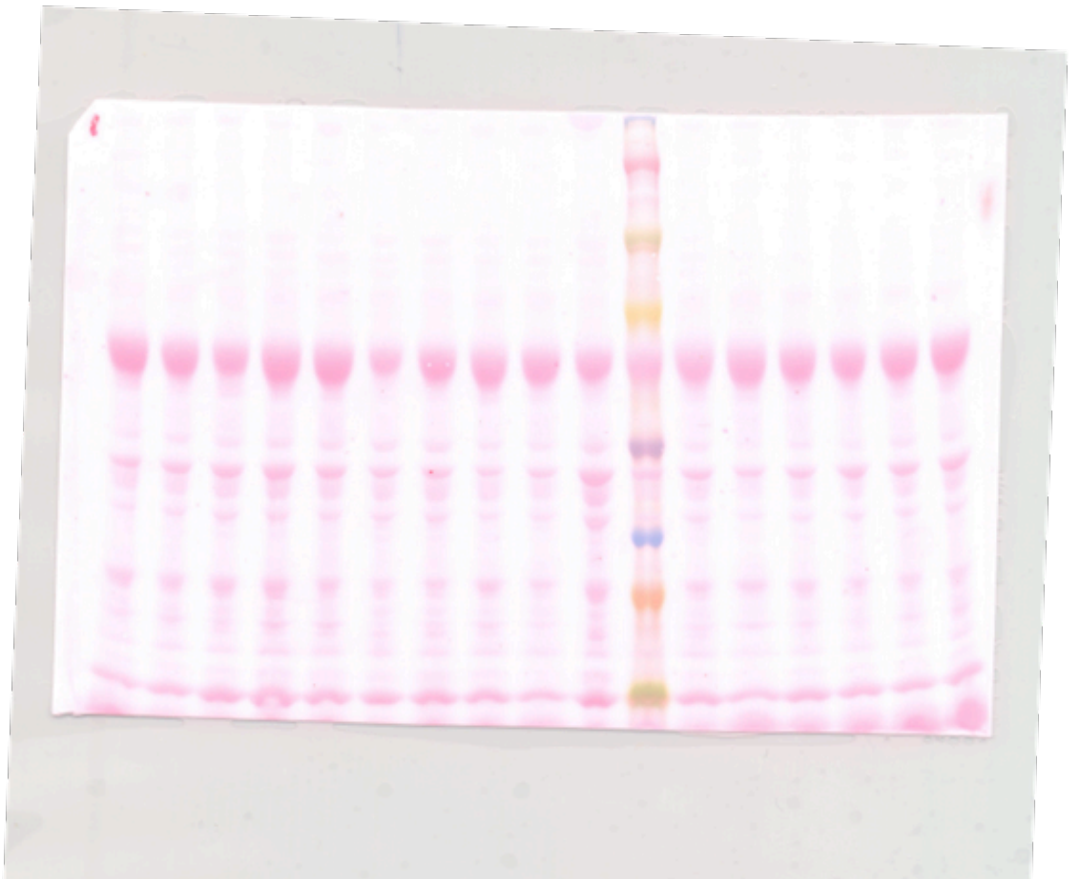


Table E1: The clinical, physiological and quadriceps fibre characteristics of COPD patients and healthy controls.

Group differences in categorical data are tested with Fisher's exact test (†). For quantitative traits, depending on the data distribution values are mean (Standard Deviation), or median (Inter Quartile Range), with the latter denoted by *. Group differences as assessed with the Welch two sample t-test or Mann Whitney U-test (*) depending on the data distribution.

Abbreviations: Forced Expiratory Volume in 1 second (FEV_1), carbon monoxide diffusion capacity (TL_{CO}), arterial partial pressure of oxygen (PaO_2), time for quadriceps force to drop to 80% of initial force in seconds (T_{80}), maximal voluntary contraction (MVC), 6 minute walk test (6MW), oxygen consumption on maximal incremental cycle ergometry (VO_2), cross-sectional area (CSA).

	COPD patients N=79	**Healthy controls** N=16	***P*-value**
Age (years)	67.08 (8.36)	66.56 (6.86)	7.95E-01
Sex (% males)†	64.56	50.00	3.97E-01
Smoking history (pack-years)*	45.00 (27.25)	3.00 (11.25)	**3.97E-07**
Current smokers (%)†	15.79	0.00	1.17E-01
% on oral prednisolone†	10.13	0.00	3.44E-01
FEV_1 (l)*	1.01 (0.69)	2.92 (0.69)	**2.72E-09**
FEV_1(% predicted)*	42.80 (25.20)	105.50 (10.85)	**3.61E-10**
TL_{CO} (% predicted)	42.87 (17.23)	89.69 (16.10)	**3.94E-10**
PaO_2 (kPa)	9.23 (1.23)	10.58 (1.30)	**9.62E-04**
$PaCO_2$ (kPa)	5.07 (0.51)	5.09 (0.44)	8.95E-01
Body mass index (kg/m^2)*	24.50 (5.60)	24.80 (3.85)	7.58E-01
Fat-free mass index (kg/m^2)*	16.00 (2.75)	16.10 (2.30)	4.86E-01
% with a low fat-free mass index†	43.04	18.75	9.31E-02
Quadriceps MVC (kg)*	27.90 (16.70)	33.50 (10.20)	1.31E-01
Quadriceps twitch force (Kg)*	7.55 (2.40)	8.60 (2.60)	1.98E-01
Quadriceps endurance (T_{80};s)*	80.00 (27.5)	105.00 (97.50)	1.60E-01
Locomotion time (min/12 h)*	40.00 (37.50)	90.50 (52.75)	**8.81E-06**
6MW distance (m)	392.62 (115.15)	612.31 (70.78)	**1.27E-11**
Peak VO_2 (ml/kg/min)*	11.40 (5.85)	22.00 (7.73)	**2.43E-08**
Type I fibre proportion (%)	28.95 (12.80)	54.44 (15.74)	**7.19E-06**
Type I/IIa fibre proportion (%)*	3.00 (5.00)	1.50 (4.50)	5.41E-02
Type IIa fibre proportion (%)	60.11 (11.67)	40.13 (14.64)	**5.78E-05**
Type IIx fibre proportion (%)*	4.00 (9.00)	2.50 (4.75)	9.48E-02
Type I fibre CSA (μm^2)*	5036 (1741)	5120 (1478)	3.32E-01
Type I/IIa fibre CSA (μm^2)*	5135 (2240)	5487 (2012)	3.44E-01
Type IIa fibre CSA (μm^2)*	3980 (1748)	4440 (2129)	2.35E-01
Type IIx fibre CSA (μm^2)*	2909 (1869)	4963 (2953)	**1.42E-03**

Table E2: Demographics of the cohort subset included in the IDH2 protein level analysis

Abbreviations: Forced Expiratory Volume in 1 second (FEV_1), carbon monoxide diffusion capacity (TL_{CO}), arterial partial pressure of oxygen (PaO_2).

	COPD low N=16	**COPD normal N=14**	**Healthy controls *N*=13**	***P*-value (between COPD groups)**
Age (years)	64([11])	66(10)	69(8)	0.56
Sex (% males)	69	43	50	0.27
Smoking history (pack-years)	38(28,75)	45(28,71)	4(0,9)	0.89
Current smokers (%)	0	14	0	0.21
FEV_1 (l)	0.75(0.58,1.3)	1.11(0.93,1.55)	2.92(2.17,3.14)	0.07
FEV_1(% predicted)	27(20,46)	47(34,61)	111(101,118)	0.02
TL_{CO} (% predicted)	26(18,44)	47(39,56)	92(81,100)	0.007
Body mass index (kg/m^2)	22.4(19.7,24.8)	24.3(21.1,26.4)	24.8(22.6,26.5)	0.35
Fat-free mass index (kg/m^2)	15.1(14.5,16.4)	15.8(14.1,16.9)	16.4(15.2,19.0)	0.76

Table E3: Enriched Disease Gene Associations (DGA) by Module.
Abbreviations: Unified Medical Language System (UMLS), *P*-value adjusted using the Benjamini and Hochberg method (Adj. *P*), Disease Gene Association (DGA).

ID	Description	*P* value	Adj. *P*	Q value	Count	Module
umls:C0023264	Leigh Disease	3.73E-12	1.01E-08	9.28E-09	21	Turquoise
umls:C0878544	Cardiomyopathies	5.88E-09	7.99E-06	7.31E-06	46	Turquoise
umls:C2936907	NADH:Q(1) Oxidoreductase deficiency	1.79E-08	1.63E-05	1.49E-05	9	Turquoise
umls:C0949658	Cardiomyopathy, Hypertrophic, Familial	4.92E-08	3.34E-05	3.06E-05	13	Turquoise
umls:C0342776	Nicotinamide adenine dinucleotide coenzyme Q reductase deficiency	2.43E-07	1.32E-04	1.21E-04	11	Turquoise
umls:C0751651	Mitochondrial Diseases	2.99E-07	1.36E-04	1.24E-04	20	Turquoise
umls:C0011853	Diabetes Mellitus, Experimental	5.07E-07	1.97E-04	1.80E-04	42	Turquoise
umls:C1838979	MITOCHONDRIAL COMPLEX I DEFICIENCY	1.09E-06	3.70E-04	3.38E-04	9	Turquoise
umls:C0007193	Cardiomyopathy, Dilated	1.58E-06	4.77E-04	4.36E-04	36	Turquoise
umls:C0017924	Glycogen Storage Disease Type V	1.69E-05	4.61E-03	4.21E-03	6	Turquoise
umls:C0007194	Hypertrophic Cardiomyopathy	3.01E-05	7.45E-03	6.82E-03	20	Turquoise
umls:C0034345	Pyruvate Dehydrogenase Complex Deficiency Disease	5.20E-05	1.18E-02	1.08E-02	6	Turquoise
umls:C0026848	Myopathy	7.40E-05	1.53E-02	1.40E-02	26	Turquoise
umls:C0162670	Mitochondrial Myopathies	7.89E-05	1.53E-02	1.40E-02	10	Turquoise
umls:C1960469	Left ventricular non compaction	9.75E-05	1.68E-02	1.53E-02	6	Turquoise
umls:C0022548	Keloid	9.85E-05	1.68E-02	1.53E-02	18	Turquoise
umls:C0003811	Cardiac Arrhythmia	1.21E-04	1.93E-02	1.77E-02	10	Turquoise
umls:C0340489	Lone atrial fibrillation	1.70E-04	2.50E-02	2.29E-02	5	Turquoise
umls:C1271104	Blood pressure finding	1.75E-04	2.50E-02	2.29E-02	30	Turquoise
umls:C0940937	Precancerous lesions	1.88E-04	2.56E-02	2.34E-02	13	Turquoise
umls:C0342782	Depletion of mitochondrial DNA	2.20E-04	2.82E-02	2.58E-02	6	Turquoise
umls:C0242656	Disease Progression	2.28E-04	2.82E-02	2.58E-02	21	Turquoise
umls:C0085548	Autosomal Recessive Polycystic Kidney Disease	2.57E-04	3.04E-02	2.78E-02	8	Turquoise
umls:C0949857	Mitochondrial Respiratory Chain Deficiencies	2.80E-04	3.17E-02	2.90E-02	6	Turquoise
umls:C0268237	Cytochrome-c Oxidase Deficiency	3.01E-04	3.28E-02	3.00E-02	8	Turquoise
umls:C0029124	Optic Atrophy	3.52E-04	3.68E-02	3.37E-02	7	Turquoise
umls:C0018800	Cardiomegaly	3.90E-04	3.93E-02	3.59E-02	18	Turquoise
umls:C0020295	Hydronephrosis	4.38E-04	4.26E-02	3.89E-02	6	Turquoise
umls:C1511291	Breast Cancer Model	4.67E-04	4.36E-02	3.98E-02	10	Turquoise
umls:C0020615	Hypoglycemia	4.80E-04	4.36E-02	3.98E-02	15	Turquoise
umls:C0085584	Encephalopathies	5.36E-04	4.53E-02	4.14E-02	14	Turquoise
umls:C0162292	External Ophthalmoplegia	5.49E-04	4.53E-02	4.14E-02	4	Turquoise
umls:C0221155	Systolic hypertension	5.49E-04	4.53E-02	4.14E-02	4	Turquoise
umls:C0034069	Pulmonary Fibrosis	1.51E-08	1.82E-05	1.49E-05	13	Green
umls:C0024667	Animal Mammary Neoplasms	5.16E-07	3.11E-04	2.54E-04	9	Green
umls:C0042373	Vascular Diseases	2.98E-06	1.20E-03	9.78E-04	11	Green
umls:C1333768	Gastric Gastrointestinal Stromal Tumor	1.75E-05	5.08E-03	4.15E-03	3	Green
umls:C0030524	Paratuberculosis	2.11E-05	5.08E-03	4.15E-03	4	Green
umls:C0239946	Fibrosis, Liver	2.82E-05	5.58E-03	4.56E-03	9	Green
umls:C1800706	Idiopathic Pulmonary Fibrosis	3.65E-05	5.58E-03	4.56E-03	9	Green
umls:C0025286	Meningioma	4.09E-05	5.58E-03	4.56E-03	10	Green

umls:C0278996	Cancer of Head and Neck	4.17E-05	5.58E-03	4.56E-03	10	Green
umls:C0024115	Lung diseases	5.58E-05	6.07E-03	4.96E-03	9	Green
umls:C0024668	Mammary Neoplasms, Experimental	6.40E-05	6.07E-03	4.96E-03	7	Green
umls:C0032927	Precancerous Conditions	6.69E-05	6.07E-03	4.96E-03	10	Green
umls:C0026640	Mouth Neoplasms	7.01E-05	6.07E-03	4.96E-03	7	Green
umls:C1335110	Oligodendroglial Neoplasm	7.05E-05	6.07E-03	4.96E-03	3	Green
umls:C1334015	High Grade Intraepithelial Neoplasia	1.17E-04	9.40E-03	7.68E-03	3	Green
umls:C0553692	Brain hemorrhage	2.05E-04	1.54E-02	1.26E-02	3	Green
umls:C0162871	Aortic Aneurysm, Abdominal	2.82E-04	2.00E-02	1.63E-02	6	Green
umls:C0206638	Giant Cell Tumor of Bone	3.27E-04	2.09E-02	1.71E-02	4	Green
umls:C0494165	Secondary malignant neoplasm of liver	3.29E-04	2.09E-02	1.71E-02	9	Green
umls:C1292772	Leukemia, Myeloid, Chronic, Atypical, BCR-ABL Negative	3.63E-04	2.15E-02	1.76E-02	3	Green
umls:C0023269	Leiomyosarcoma	3.75E-04	2.15E-02	1.76E-02	5	Green
umls:C0877008	Enzyme inhibition disorder	5.35E-04	2.93E-02	2.40E-02	3	Green
umls:C0022572	Keratoacanthoma	5.85E-04	3.06E-02	2.51E-02	3	Green
umls:C0282612	Prostatic Intraepithelial Neoplasias	6.24E-04	3.13E-02	2.56E-02	5	Green
umls:C0032285	Pneumonia	7.30E-04	3.52E-02	2.88E-02	8	Green
umls:C0010072	Coronary Thrombosis	8.78E-04	3.94E-02	3.22E-02	3	Green
umls:C0007786	Brain Ischemia	8.83E-04	3.94E-02	3.22E-02	8	Green
umls:C0699893	Skin carcinoma	1.07E-03	4.60E-02	3.76E-02	4	Green
umls:C1834674	Bethlem myopathy	1.23E-03	4.90E-02	4.01E-02	2	Green
umls:C0029456	Osteoporosis	1.27E-03	4.90E-02	4.01E-02	8	Green
umls:C0993582	Arthritis, Experimental	1.29E-03	4.90E-02	4.01E-02	5	Green
umls:C0000786	Spontaneous abortion	1.30E-03	4.90E-02	4.01E-02	6	Green
umls:C1519670	Tumor Angiogenesis	1.34E-03	4.90E-02	4.01E-02	8	Green
umls:C0349782	Ischemic cardiomyopathy	1.58E-07	3.29E-04	2.86E-04	9	Blue
umls:C0221505	Lesion of brain	1.47E-05	1.34E-02	1.16E-02	7	Blue
umls:C0600452	Hepatopulmonary Syndrome	1.94E-05	1.34E-02	1.16E-02	8	Blue
umls:C0026848	Myopathy	4.53E-05	2.35E-02	2.04E-02	13	Blue
umls:C0686353	Muscular Dystrophies, Limb-Girdle	1.13E-04	4.61E-02	4.00E-02	6	Blue
umls:C0006287	Bronchopulmonary Dysplasia	1.35E-04	4.61E-02	4.00E-02	10	Blue
umls:C0042345	Varicosity	1.65E-04	4.61E-02	4.00E-02	5	Blue
umls:C0026846	Muscular Atrophy	2.22E-04	4.61E-02	4.00E-02	9	Blue
umls:C1511789	Desmoplastic	2.30E-04	4.61E-02	4.00E-02	6	Blue
umls:C0007193	Cardiomyopathy, Dilated	2.40E-04	4.61E-02	4.00E-02	14	Blue
umls:C0011884	Diabetic Retinopathy	2.77E-04	4.61E-02	4.00E-02	10	Blue
umls:C0026850	Muscular Dystrophy	2.98E-04	4.61E-02	4.00E-02	10	Blue
umls:C0410179	Scleroatonic muscular dystrophy	3.10E-04	4.61E-02	4.00E-02	3	Blue
umls:C0877009	Muscle fibrosis	3.10E-04	4.61E-02	4.00E-02	3	Blue
umls:C0149721	Left Ventricular Hypertrophy	3.42E-04	4.74E-02	4.12E-02	10	Blue