University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student
   Surname: Turcanu Forename: Mihnea Vlad
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2. Supervisor
   Surname: Dawson Forename: Jesse
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3. Research Project Report
   3.1 Project Title (maximum 20 words):

   A metabolomic analysis of patients with suspected stroke.

   3.2 Project Lay Summary (copied from application):

   Stroke incidence is rising and there is need for better diagnosis of suspected stroke, better detection of stroke subtypes and better understanding of heterogeneity in outcomes after stroke. This project will perform detailed analysis of the serum of patients presenting with suspected stroke. We will assess the levels of hundreds of small molecules called metabolites, which are products of normal and abnormal function of cells. By comparing these levels between those with stroke and those without and those with severe and less severe stroke we hope to provide pathophysiological insights and improve care for patients with suspected stroke.

   3.3 Start Date: 10th June 2013 Finish Date: 15th July

   3.4 Original project aims and objectives (100 words max):
   The project will assess the potential for metabolomic data to meet specific areas of clinical need. For example, more accurate early diagnosis in suspected stroke may obviate need for repeated imaging and reduce use of expensive therapies when they are not required, while improved risk prediction may allow these to be better targeted. Our hypothesis is that

evaluation of metabolic profiles in patients presenting with suspected stroke will identify differences in metabolic pathways in patients with definite stroke compared to those without and more importantly will provide useful information in those with possible stroke, where current clinical evaluation leaves uncertainty. We also hypothesise that metabolomic derangement will be demonstrably different across the range of stroke severity.

3.5 Methodology: Summarise and include reference to training received in research methods etc. (250 words max):

This project involved analysis and interpretation of a large dataset recently developed using serum samples from patients with suspected stroke. The dataset incorporates data on 110 patients and measurements of over 800 metabolites. The student gained exposure to;

- Analysis / handling of large data-set (using Excel and SPSS)
- Statistical analysis
- Literature review
- Generic skills in preparation of samples for laboratory analysis (centrifuge / pipetting)
- Preparation of samples for metabolomic analysis (methanol extraction)
- Exposure to mass spectrometry techniques (LC-MS)

In this study we performed metabolomic analysis using LC-MS on the serum of patients with suspected stroke. We compared metabolite levels in patients who turned out to have stroke to those who were found to have an alternate diagnosis.

3.6 Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

We found that abundance of 64 metabolites was different in patients with stroke compared to controls (table 1). Of these, 23 were fatty acids and 4 of these were from the fatty acid biosynthesis metabolic pathway: dodecanoic acid, hexadecanoic acid, octadecanoic acid, octadecenoic acid (figure 1). These levels were increased in stroke patients.

Levels of 13 metabolites were related to stroke severity (table 2).

3.7 Discussion (500 words max):

Our results demonstrate differences in the metabolome of patients with suspected stroke who turn out to have suffered stroke compared to those who do not have stroke. Some of these metabolites are also related to stroke severity. These differences may allow us to better identify stroke in the future and may help up predict outcome. Our findings require further validation.

The most notable difference was in levels of fatty acids and 4 metabolites from the fatty acid biosynthesis metabolic pathway were increased in abundance in stroke patients. Many of these metabolites are known to be vasoactive so the importance of this finding warrants further evaluation.

An individual patient level analysis in those who had possible stroke (where we could not be certain one way or another will now be performed using the metabolomic data.

4. Reflection by the student on the experience and value of the studentship (300 words max):

This experience had a great impact on me. It helped me broaden my horizon and consider research as a future career. I found it very exciting and rewarding working on a project which improves medicine and patients' health. By reading many articles on stroke I got more familiar with scientific writing, stroke terminology and research methods. Through this internship I gained invaluable skills and knowledge which I will be able to use for the final year research project. Now I have a much better insight into large data-set analyses, preparation of samples for metabolomic analyses, LC-MS and statistics.

I am grateful to the Head of College Scholars List Scheme and my supervisor for the opportunity to take part in this studentship. I would highly recommend students to undertake summer internships.

5. Dissemination: (note any presentations/publications submitted/planned from the work):

   We will submit data from this study to an international stroke conference.

6. Signatures: Supervisor Date Student Date

Table 1 – Metabolites that differed in patients with stroke compared to non storke.

PeakID	Mass	RT	FORMULA	Putative metabolite	Fold Change in Definite Stroke	P value
n1404	256.6382	3.696774	C26H43NO7S	[ST hydrox] N-(3alpha-hydroxy-5beta-cholan-24-oyl)-glycine 3-sulfate	2.21	0.00034
p292	163.0846	8.003971	C6H13NO4	D-Fucosamine	2.28	0.000412
p2565	146.058	7.315571	C6H10O4	(S)-2-Aceto-2-hydroxybutanoate	1.79	0.000462
p2225	156.0536	10.51378	C6H8N2O3	4-Imidazolone-5-propanoate	1.54	0.000479
n3272	289.627	4.072554	C26H45NO9S2	Taurochenodeoxycholate-3-sulfate	2.10	0.000562
p2202	345.2669	3.512817	C22H35NO2	[FA (20:5)] N-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-ethanolamine	2.36	0.000737
n51	332.2716	3.548058	C22H36O2	[FA (22:4)] 7Z,10Z,13Z,16Z-docosatetraenoic acid	1.65	0.003221
n314	104.0474	8.582797	C4H8O3	(R)-3-Hydroxybutanoate	2.98	0.003611
n1722	109.0197	14.47662	C2H7NO2S	Hypotaurine	1.46	0.005047
n42	330.2561	3.547574	C22H34O2	Taxa-4(20),11(12)-dien-5alpha-yl acetate	1.87	0.005075
p3980	202.1321	16.42139	C9H18N2O3	Ile-Ala	1.94	0.005103
n3170	110.0367	5.935001	C6H6O2	p-Benzenediol	0.81	0.005142
n7	214.1571	3.850677	C12H22O3	3-Oxododecanoic acid	1.80	0.005786
n34	284.2714	3.569657	C18H36O2	Octadecanoic acid	1.36	0.007711
p1795	237.085	18.27989	C8H15NO7	N-Acetyl-D-glucosaminate	1.68	0.007779
n2220	238.1569	3.681438	C14H22O3	[FA oxo(5:1/5:0/4:0)] (1R,2R)-3-oxo-2-(2'Z-pentenyl)-cyclopentanebutanoic acid	1.54	0.008194
p3372	300.1111	4.576597	C16H16N2O4	5-Nitro-2-(3-phenylpropylamino)benzoic acid	1.53	0.008517
n304	286.2145	4.089625	C16H30O4	[FA (16:0/2:0)] Hexadecanedioic acid	1.97	0.008804
p2126	368.3443	4.929174	C27H44	[ST] (5Z,7E)-9,10-seco-5,7,10(19)-cholestatriene	0.55	0.009041

n38	304.2404	3.569439	C20H32O2	[FA (20:4)] 5Z,8Z,11Z,14Z-eicosatetraenoic acid	1.45	0.012334
n2026	141.0427	6.037787	C6H7NO3	Gentianaine	0.49	0.012467
n50	296.2716	3.570251	C19H36O2	[FA methyl(18:0)] 11R,12S-methylene-octadecanoic acid	1.64	0.013143
n48	308.2719	3.560793	C20H36O2	Icosadienoic acid	1.65	0.013511
p1153	180.0648	7.168124	C7H8N4O2	Theophylline	0.56	0.014721
n1415	132.0786	4.732377	C6H12O3	[FA hydroxy(6:0)] 4-hydroxy-hexanoic acid	1.42	0.015118
n2834	174.1255	4.05087	C9H18O3	[FA hydroxy(9:0)] 2-hydroxy-nonanoic acid	1.27	0.015387
n52	252.209	3.568309	C16H28O2	[FA (16:2)] 9,12-hexadecadienoic acid	1.55	0.015907
n30	256.2403	3.611908	C16H32O2	Hexadecanoic acid	1.52	0.016273
p2787	166.0492	8.087622	C6H6N4O2	7-Methylxanthine	0.55	0.017057
p2855	181.0682	7.289512	C19H22O7	Nagilactone C	0.57	0.018062
p1560	103.0633	13.12262	C4H9NO2	L-3-Amino-isobutanoate	1.38	0.020451
n2439	265.0811	9.760153	C10H11N5O4	5'-Dehydroadenosine	1.57	0.022102
n232	104.0473	7.446724	C4H8O3	4-Hydroxybutanoic acid	1.49	0.022364
p2935	297.2669	3.550819	C18H35NO2	[SP (2:0)] sphinga-4E,14Z-dienine	1.67	0.023941
n58	298.287	3.566503	C19H38O2	Nonadecanoicacid	1.35	0.024183
n458	200.1777	3.738205	C12H24O2	Dodecanoic acid	1.87	0.024684
p2704	141.0193	15.67077	C2H8NO4P	Ethanolamine phosphate	2.12	0.025428
n2965	366.3499	3.523279	C24H46O2	[FA (24:0)] 15Z-tetracosenoic acid	1.30	0.026426
n1751	302.2247	3.578252	C20H30O2	[FA (20:5)] 5Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid	1.83	0.0281
n2064	392.2929	3.769827	C24H40O4	[ST hydrox] 3alpha,19-Dihydroxy-5beta-cholan-24-oic Acid	0.47	0.028846
n1166	160.1099	4.175261	C8H16O3	Ethyl (R)-3-hydroxyhexanoate	1.55	0.032021

p2504	729.5312	3.889077	C40H76NO8P	[PC (14:0/18:2)] 1-tetradecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phosphocholine	0.76	0.032361
n1267	116.0473	7.234431	C5H8O3	3-Methyl-2-oxobutanoic acid	1.42	0.033134
n631	214.1934	3.702881	C13H26O2	CAI-1	1.38	0.033729
n1719	102.0681	7.279265	C5H10O2	Pentanoate	1.39	0.034746
n3244	379.2489	4.694031	C18H38NO5P	[SP] Sphing-4-enine-1-phosphate	1.20	0.035913
p2475	248.116	5.160522	C13H16N2O3	6-Hydroxymelatonin	0.86	0.036423
n2836	432.3241	4.00458	C27H44O4	[ST (2:0)] (7E)-(1S,3R,6R)-6,19-epidioxy-9,10-seco-5(10),7-cholestadiene-1,3-diol	1.28	0.037137
n41	270.256	3.59406	C17H34O2	[FA (17:0)] heptadecanoic acid	1.37	0.037832
p3347	183.0896	7.304883	C9H13NO3	L-Adrenaline	0.83	0.038822
p3604	96.02122	14.2221	C5H4O2	Furfural	1.24	0.039013
p3910	130.0743	14.75684	C5H10N2O2	Casein K	0.42	0.039483
n3741	350.2459	3.885871	C21H34O4	[ST trihydrox] 3alpha,11beta,21-5alpha-trihydroxy-pregnane-20-one	1.37	0.039695
p2051	205.0741	7.429687	C11H11NO3	Indolelactate	1.23	0.042335
n163	230.1519	4.747722	C12H22O4	Dodecanedioic acid	1.72	0.043316
p3645	259.1208	7.318178	C15H17NO3	Norsanguinine	0.81	0.043458
n1818	189.0096	7.220675	C6H7NO4S	2-Pyridyl hydroxymethane sulfonic acid	0.63	0.043604
n29	282.2558	3.578951	C18H34O2	[FA (18:1)] 9Z-octadecenoic acid	1.55	0.044077
p1066	179.0795	14.08791	C6H13NO5	D-Glucosamine	1.24	0.044836
p1645	72.02106	9.730565	C3H4O2	Methylglyoxal	2.97	0.045337
p542	479.1686	10.50482	C17H29N5O9S	Asn-Met-Thr-Asp	0.34	0.046999
n2532	130.0994	4.289724	C7H14O2	[FA (7:0)] heptanoic acid	1.16	0.047114
p2310	159.0896	7.404717	C7H13NO3	5-Acetamidopentanoate	0.72	0.048744

n1715	202.157	3.831277	C11H22O3	[FA hydroxy(11:0)] 2-hydroxy-undecanoic acid	1.40	0.049465

Table 2 – Correlation between metabolite levels and stroke severity

Peak	Putative ID	Correlation	P value
n1016	3-Methylbut-2-enal	-0.340	0.009
n163	Dodecanedioic acid	0.278	0.034
n1818	2-Pyridyl hydroxymethane sulfonic acid	-0.276	0.036
n232	4-Hydroxybutanoic acid	0.323	0.014
n2964	[FA (20:0)] eicosanoic acid	0.270	0.040
n2965	[FA (24:0)] 15Z-tetracosenoic acid	0.367	0.005
n314	(R)-3-Hydroxybutanoate	0.416	0.035
n314	(R)-3-Hydroxybutanoate	0.296	0.024
n34	dihydroartemisinic acid hydroperoxide	0.276	0.036
n50	[FA methyl(18:0)] 11R,12S-methylene-octadecanoic acid	0.288	0.028
n58	Nonadecanoicacid	0.274	0.037
p2126	[ST] (5Z,7E)-9,10-seco-5,7,10(19)-cholestatriene	0.326	0.013
p2234	Ergothioneine	-0.443	0.027

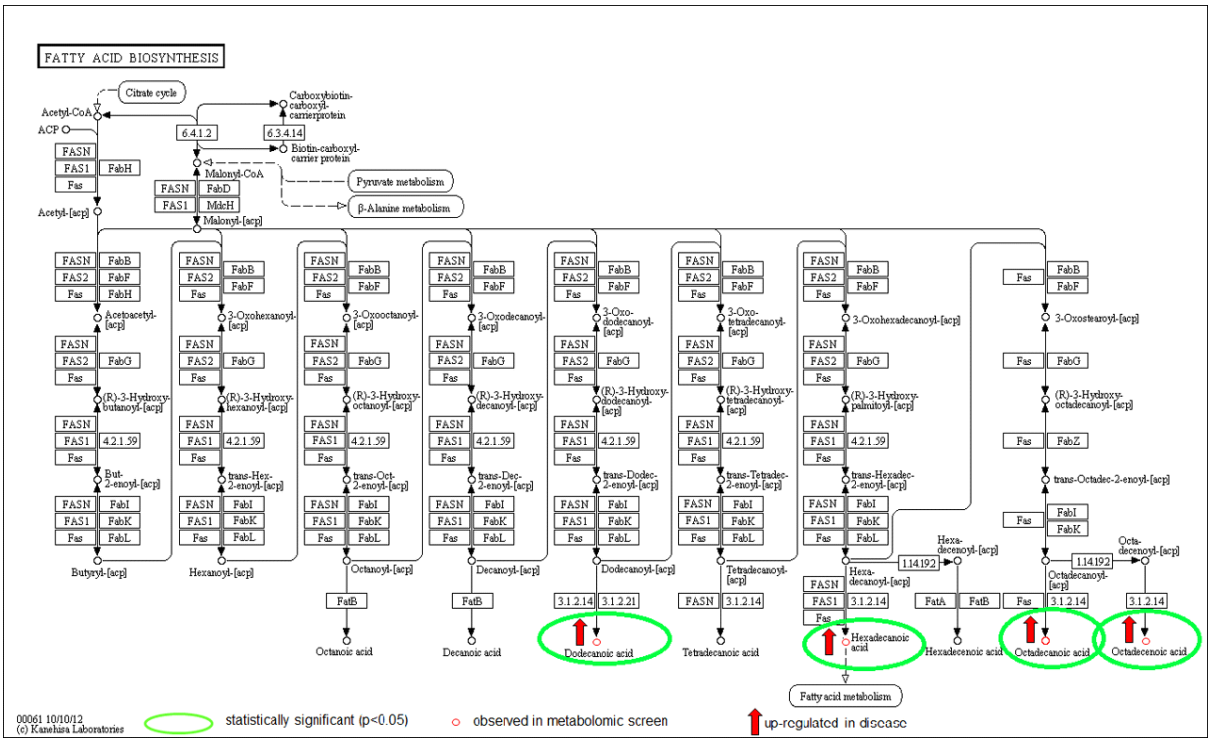

Fig.1 Fatty acid Biosynthesis Pathway