Supplementary

Original Research.

Molecular mechanism biomarkers predict diagnosis in schizophrenia and schizoaffective psychosis, with implications for treatment

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Contents

S 1. Inclusion criteria..... 2
S 2. Exclusion criteria 2
S 3. Rating measures..... 3
S 4. Laboratory Assays including details of urine riboflavin analysis. 4
S 5. Data characteristics for MTHFR C677T data sets. 8
S 6. Significant Spearman's correlate relationships within the MTHFR 677 TT genotype. 10
S 7. Significant Spearman's correlate relationships within the MTHFR 677 CC genotype. 11
S 8. Cross tab analysis for MTHFR 677 TT dependent variables 13
S 9. Logistic regression analysis for MTHFR 677 CC dependent biochemical variables 14
S 10. Significant case-correlates within the MTHFR 677 CT genotype 15

S 1. Inclusion criteria.

Inclusion Criteria	Method
Orientated in time, place and person. Non-detained under Mental Health Act Capacity to consent	Clinical examination and case-note examination
Diagnosis of schizophrenia or schizoaffective disorder, made by a consultant psychiatrist in the ward or community satellite clinic setting, according to the DSM-IV-R classification criteria and checked against DSM-IV-R symptom checklist at the recruitment stage	American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders. (4th Ed. Rev) (DSM IV-R). American Psychiatric Association 1994. Washington DC. 17. Simpson GM, Angus JWS. A rating scale for extrapyramidal side effects. Acta Psychiatry Scand. 1970; 212 (44):11–9.
Absence of rigidity, dyskinesia, tremor or instability in ocular-muscle dysfunction, hand, forearm and shoulder.	Clinical examination: Simpson GM, Angus JWS. (1970) A rating scale for extrapyramidal side effects. *Acta Psychiatrica Scandinavica*, **212** (44), 11-19.
Zuclopenthixol, Modecate, Amisulpride Risperidone, Quetiapine, sodium valproate.	Treatment chart and case-note examination
Risk factors of interest: such as family history of mental illness, history of emotional or physical abuse, developmental disorder, learning disorder or subclinical head injury.	Case-note examination and personal inquiry.

S 2. Exclusion criteria

Exclusion Criteria	Method
Lack of orientation in time, place and person. Detained under Mental Health Act Lacking consent capacity	Clinical examination and case-note examination
Alternative diagnosis	American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders. (4th Ed. Rev) (DSM IV-R). American Psychiatric Association 1994. Washington DC. 17. Simpson GM, Angus JWS. A rating scale for extrapyramidal side effects. Acta Psychiatry Scand. 1970; 212 (44):11–9.
Unstable pharmacotherapy over assessment period.	Treatment chart and case-note examination
Extra-pyramidal side effects in ocular, hand, forearm, and shoulder	Clinical examination; Simpson GM, Angus JWS. (1970) A rating scale for extrapyramidal side effects. Acta Psychiatrica Scandinavica, 212 (44), 11-19.

Clozapine, Olanzapine, antihistamines, or vitamin therapy	Treatment chart, case-note examination, and personal questioning.
Recent or unresolved history or positive test for alcohol or other substance abuse.	Treatment chart, case-note examination, and personal questioning
Upper respiratory tract infections	Treatment chart, case-note examination, and personal questioning
Intellectual disability or clinically documented or descriptive history of head injury with unconsciousness or hospitalisation.	Treatment chart, case-note examination, and personal questioning.

S 3. Rating measures

Functional Rating Scale	Citation
The Brief Psychiatric Rating Scale (BPRS)	Overall JE and Gorham DR (1962) The Brief Psychiatric Rating Scale. Psychological Report 10(3): 799-812.
Positive and Negative Syndrome Scale for schizophrenia (PANSS)	Kay SR, Fiszbein A and Opler LA (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophrenia Bulletin 13(2): 261-276.
Clinical Global Impression of Severity (CGI)	Guy W (1976) Clinical global impressions [CGI]. ECDEU assessment manual for psychopharmacology. National Institute of Mental Health, Early Clinical Drug Evaluation, Psychopharmacology Research Branch, Rev. Rockville, MD, U.S.
Global assessment of Function (GAF)	Frances A, Incus HA, and First MB (1994) Diagnostic and Statistical Manual of Mental Disorders, 4th edition, American Psychiatric Association, Washington, DC.
Social and Occupational Functioning Assessment Scale (SOFAS)	Goldman HH, Skodol AE and Lave TR (1992) Revising axis V for DSM-IV: a review of measures of social functioning. American Journal Psychiatry 149(9): 1148-1156.

S 4. Laboratory Assays including details of urine riboflavin analysis.

Neuro-Biochemistry	**All fasting blood samples were collected between 9 and 11 am daily. By arrangement, transported directly to the laboratory with no storage.**	**Laboratory/Reference**
Serum Vitamin D (25-OH)	Diasorin Liason assay kit, for use on the Liaison platform. (nmol/L)	Clinpath Laboratories, 19 Fullarton Rd, Kent Town. South Australia 5067 +61 8 8366 2000.
Serum total Vitamin B12	Competitive Electrochemiluminescent Immunoassay. Roche Modular E 170 Automated Immunoassay Analyser, using Roche Vitamin B12 Reagent. (nmol/L)	Clinpath Laboratories. As above.
Plasma Red Cell Folate	Competitive Electrochemiluminescent Protein Binding Assay, using Roche Modular E 170, using Roche Elecsys Folate III assay on Automated Immunoassay Analyser. (nmol/L) Pfeiffer *et al.* (2004) and Roche indications are that for majority of patient samples with Total Folate of < 50 nmol/L, consisted of average, 93.3% 5-Methyl-THF, 2.3% Folic Acid, and 4.4% 5-Formyl-THF while samples with Total Folate of >50 nmol/L had, on average, 81.7% 5MeTHF, 15.7% FA, and 2.5% 5FoTHF.	Clinpath Laboratories.
Serum Vitamin B6 (Pyridoxal-5′-phosphate coenzyme form)	Whole blood High Pressure Liquid Chromatography with fluorescent detection. Chromsystems Vitamin B6 in Whole Blood High pressure Liquid Chromatography Reagent Kit. Waters Alliance 2695 Separations Module. Waters 474 Fluorescence Detector(nmol/L)	Sullivan Nicolaides Pathology 143 Whitmore St, Taringa. Queensland 4068. Australia. +61 7 337 8666
Serum Copper	Flame Atomic Absorption Spectrophotometry. Varian AA-240FS. (umol/L)	Douglass Hanly Moir Pathology 14 Griffnock Avenue, Macquarie Park. New South Wales 2113. +61 2 98555222
Plasma Red Cell Zinc	Inductively coupled plasma mass spectroscopy (ICP-MS), using 6% n-Butanol reagent and Agileny ICP-MS 7500ce analyser. (umol/L)	Sullivan Nicolaides Pathology.

Serum Ceruloplasmin	Immunoturbidimetric method, using 6K91-30 Multignet Caeruloplamin Kit and Abbott Architect ci16000 analyser. (g/L)	Douglass Hanly Moir Pathology.
Percentage Free Copper/Red Cell Zinc	Percentage of free copper in the serum calculated by an equation based on the molecular and atomic weights of ceruloplasmin and copper (one ceruloplasmin molecule binds to six copper atoms). The ratio of the percentage free copper to red cell zinc was calculated as "percentage free copper" / "Red cell zinc umol/L".	Calculated by authors
Intermediate substrates and enzymes		
MTHFR Ala222Val (C677T) methyl tetrahydrofolate reductase polymorphism	Peripheral blood samples were collected to assay for MTHFR Ala222Val (C677T) methyl tetrahydrofolate reductase polymorphism, from which red cells were lysed and DNA was isolated from residual lymphocyte fraction. Real time PCR analysis method. Roche Diagnostics Light-Cycler 480 kit. Using TecnoBiol reagents, Sigma probes and primers on Roche LC480 analyser	Douglass Hanly Moir Pathology.
Plasma homocysteine	Ice transported EDTA sample. Competitive Chemiluminescent Immunoassay, using Siemens Homocysteine reagent on Siemens Advia centaur Automated Immunoassay (umol/L).	SA Pathology. +61 8 8222 3000
Serum histamine	Beckman Coulter Radio Immunoassay, using Beckman Coulter R.I.A. Kit on Perkin Elmer Wizard 1470 Automated Gamma Counter. (umol/L)	Sullivan and Nicolaides.

Urine Tests Neurotransmitters	**Method, Analyzer, Reagents.**	**Laboratory/Reference**
Biogenic amines: Dopamine, Noradrenaline and Adrenaline,	Spot-baseline (fasting) urinary neurotransmitter testing (second void morning), snap-frozen to minus 30 degrees and analysed by mass spectrometry, using nanomoles per millimole of urinary creatinine as a standard.	SA Pathology, Adelaide, South Australia. Whiting MJ. 2009. Simultaneous measurement of urine metanephrines and catecholamines by liquid chromatography with tandem mass spectrometric detection. *Annals of Clinical Biochemistry*, **46**:129-136
Creatinine	Spot urine specimen from the same void as biogenic amines, expressed in (millimoles per Litre)	SA Pathology, Adelaide SA.
Oxidative stress: Urinary hydroxyhemopyrroline-2- one	Fasting urine sample collected whilst patient at rest, separated from blood drawing by minimum of 2 hours.	Applied Analytical laboratories, Meadowbrook, Queensland.
((Urine Riboflavin ug/L	Ultra performance liquid chromatography (HPLC), with fluorometric detection of the eluted riboflavin Peak (1) riboflavin metabolites and peak (2) = riboflavin.	Lipid analysis laboratory Waite Agricultural Research Institute Adelaide SA.

<u>Urine analysis Method for riboflavin and its metabolites</u>

Phosphorylation of riboflavin maintains its metabolic trapping in tissues where it is mostly enzyme bound as flavoproteins like FMN and FAD. Gastaldim G.; Ferrari, G.; Verri, A.; Casirola, D.; Orsenigo, M.N.; Laforenza, U. Riboflavin phosphorylation is the crucial event in riboflavin transport by isolated rate enterocytes. *J Nutr* **2000**,*130*,2556–61. Unbound flavins are rapidly hydrolysed to free riboflavin, which diffuses from cells and is excreted in the urine as riboflavin or other metabolites, such as 7-hydroxymethylriboflavin (7-α-hydroxy riboflavin). There is a linear relationship between urinary recovery of riboflavin and its metabolites and riboflavin absorption (bioavailability) and urinary riboflavin recovery can serve as an index of riboflavin absorption (Morrison, A.B.; Campbell, J.A. Vitamin absorption studies. I Factors influencing the excretion of oral test doses of thiamine and riboflavin by human subjects. *J Nutr*. **1969**, *72*, 435-440. West. D/W.; Owen, E.C. The urinary excretion of metabolites of riboflavine by man. *Brit. J. Nutrition*. **1960**, *23*,889-898). Some urinary metabolites reflect bacterial activity in the gastrointestinal tract (Singer, T.P.; Kenney, W.C.; Biochemistry of covalently bound flavins. *Vitam Horm* **1974**,*32*,1–45. Chastain, J.L.; McCormick, D.B. Flavin catabolites: identification and quantitation in human urine. *Am J Clin Nutr* **1987**,46,830–4).

Method: Ultra performance liquid chromatography (HPLC) with fluorometric detection of the eluted riboflavin peak (1, 2)

Standardization procedure: Riboflavin standard was from Sigma Aldrich. Stock riboflavin standard solution (1 mg/mL) was prepared in 10% acetonitrile and stored in -80 °C freezer. The stock solution was diluted periodically as needed from which a new standard curve freshly made for each batch. Working solution with range between range between 4-20 mg/L, was prepared monthly from the stock and store in fridge. Standard curve was obtained by independently diluted levels of riboflavin from one stock solution, and the resulting peak area were then plotted against riboflavin concentrations.

Sample procurement and preparation procedure: 15-50 ml urine was collected in the early morning after overnight fast and immediately frozen at -20 degrees C and stored at this temperature with protection from light. 170 μL thawed urine was added to 30 μL of acetonitrile in a 1.5 mL Eppendorf tube and incubated at 4 °C for 30 mins. The tube was then centrifuged at 13000 rpm, 10 °C for 10 mins. The supernatant was transferred into a 96-well plate (Greiner, Australia), and 5 μL were injected on to HPLC to be analysed.

Details of HPLC procedure: The HPLC used Agilent 1260 Infinity system equipped with Agilent ZORBAX Eclipse Plus C18 column (RRHD 3.0×50mm, 1.8 μm) with guard column with a flow rate of 0.5 mL/min. The chromatographic separation was performed at 25 °C and used gradient elution with mobile phase A consisting of 1% acetonitrile and B of 50% acetonitrile. The gradient started with 0% B and ramped up to 100% at 2 mins and maintained for 0.9 mins. The mobile phase was then equilibrated to initial condition (at 3 min) before the next injection. Riboflavin was measured with a fluorescence detector (λ_{Ex}: 445nm and λ_{Em}: 530 nm). Samples with a very high riboflavin level (N=2) required extra dilution. Agilent OpenLAB was used for instrument control and data analysis. \

Outcome: Two ranges of fractions were obtained which were separated at 2 mins. According to literature, the first range (at 0.4 mins to 1.5 mins) is riboflavin metabolites, with the main preceding peak being reported in literature as 7-7α-hydroxy riboflavin (Shodex HPLC Column homepage https://www.shodex.com/en/dc/05/02/06.html#!) After this peak, riboflavin was detected at 3.35 mins with FMN and FAD eluting at its leading edge. Identification of HPLC elution Peak 1, is by exclusion and from previously reported research identifying the molecule as 7-7α-hydroxy riboflavin on HPLC fractionation. Further identification was impeded by limitation of available standards. It is therefore unclear whether Peak 1 co-analyte represents reduced riboflavin through analog inhibition or degradation.

S 5. Data characteristics for MTHFR C677T data sets (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp)

	MTHFR TT homozygous			**MTHFR CT heterozygous**			**MTHFR CC**		
	Mean	N	Std. Deviation	Mean	N	Std. Deviation	Mean	N	Std. Deviation
5 HIAA	1.57	7	0.787	2.26	61	3.371	3.71	65	6.269
NA/DA	0.18	7	0.064	0.18	61	0.112	0.18	65	0.155
AD/NA	0.13	7	0.048	0.18	61	0.154	0.17	65	0.120
NA/MHMA	1978.57	7	1237.557	1481.11	60	751.035	1646.30	63	1017.576
% Free Cu/Zn	0.14	7	0.440	0.29	62	0.465	0.26	64	0.585
AD/MHMA	2.50	7	1.893	2.70	60	2.796	2.89	63	2.990
Vitamin B12 /vit. D	8.99	7	4.953	9.77	61	7.796	9.17	64	5.778
Vitamin B12/ folate	0.26	7	0.086	0.25	62	0.147	0.23	64	.101
Vitamin B12 / zinc	33.09	7	10.160	31.11	62	14.153	28.95	65	12.429
Vitamin B12 / zinc X folate	0.02	7	0.007	0.02	62	0.010	0.02	64	0.008
Vitamin B12 x Cu / Zn X folate	0.03	7	0.105	0.06	62	0.112	0.05	64	0.173
Vitamin B12 x Cu / zinc x folate x vit B6	0.00	7	0.001	0.00	59	0.001	0.00	62	0.004
Vitamin B12 x Cu X homocysteine / zinc x folate x vit B6	0.00	7	0.000	0.00	57	0.000	0.00	61	0.002
Plasma Homocysteine	10.87	7	4.816	9.64	61	1.869	9.70	64	2.426
Histamine	0.61	7	0.273	0.69	62	0.304	0.67	65	0.381
Vitamin D	59.00	7	21.510	52.43	61	20.015	51.78	64	24.280
Red Cell folate	1830.71	7	484.247	1794.52	62	517.123	1779.00	64	374.541
Serum B12	452.14	7	126.796	421.65	62	205.263	386.43	65	154.087
Vitamin B6	315.00	7	554.519	146.86	59	124.183	115.04	63	87.609
HPL/Creatinine	2.27	7	1.590	4.37	61	5.601	5.04	65	6.517
HPL/SG	15.91	7	12.948	34.15	61	41.714	45.50	65	79.182

Urine creatinine	7.11	7	4.346	9.67	61	5.872	9.29	65	5.944
Vitamin B2	2.9748	6	3.37918	10.3920	55	27.67252	4.3641	61	7.43990
Peak Region 1 amplitude	0.5677	6	0.29610	0.9870	54	1.14692	0.8631	61	0.64584
Peak Region 1 area under peak	6.3711	6	3.09404	11.3988	54	12.45771	9.0003	61	5.27581
Peak Region 2 amplitude	4.8302	6	3.00026	14.0674	54	20.87696	8.4740	61	10.46451
Peak Region 2 area under peak	19.0015	6	9.33328	51.7247	54	70.62112	30.8188	61	31.23222
Dilution Corrected B2	5.6226	6	3.51032	21.2474	54	36.09666	10.1939	61	13.19762
Creatinine ROC	0.29	7	0.488	0.08	61	0.277	0.18	65	0.391
B2/creatinine	0.3791	6	0.33655	1.1955	55	2.22057	0.6143	61	0.90479
Vitamin B2 ROC	0.3333	6	0.51640	0.4364	55	0.50050	0.3115	61	0.46694
B2/creatinine ROC	0.3333	6	0.51640	0.4364	55	0.50050	0.6230	61	0.48867
Peak 2 area / Peak 1 area	3.3471	6	1.59285	5.2637	54	7.48945	4.1196	61	4.86627
Peak 2 amplitude / Peak 1 amplitude	12.6634	6	12.61957	17.0468	54	24.52610	14.4725	61	29.08680
Peak 2 amplitude - Peak 1 amplitude	4.2625	6	3.03072	13.0804	54	20.57116	7.6109	61	10.32019
Peak 2 area - Peak 1 area	12.6304	6	8.27341	40.3259	54	66.85722	21.8185	61	29.97784
(Peak 2 amplitude - Peak 1 amplitude) / creatinine	0.8096	6	0.72408	2.5139	54	5.11354	1.5433	61	3.67045
Peak 1 amplitude / Peak 2 amplitude	0.3294	6	0.55104	0.2914	54	0.58291	0.3724	61	0.53113
Peak 1 area / Peak 2 area	0.4381	6	0.39856	0.4438	54	0.38774	0.5567	61	0.47255
Peak 1 amplitude - Peak 2 amplitude	-4.2625	6	3.03072	-13.0804	54	20.57116	-7.6109	61	10.32019
Peak 1 area - Peak 2 area	-12.6304	6	8.27341	-40.3259	54	66.85722	-21.8185	61	29.97784
(Peak 1 amplitude - Peak 2 amplitude) / creatinine	-0.8096	6	0.72408	-2.5139	54	5.11354	-1.5433	61	3.67045
(Peak 1 area - Peak 2 area) / creatinine	-3.3411	6	4.82111	-7.5742	54	15.35162	-4.3593	61	10.18464
(Peak 1 area + Peak 2 area) / creatinine	6.3855	6	8.44647	11.5180	54	19.37069	7.0372	61	11.92654
Peak1 amplitude + Peak2 amplitude	5.3979	6	2.99887	15.0544	54	21.24037	9.3372	61	10.64612
(Peak 2 area - Peak 1 area) / creatinine	3.3411	6	4.82111	7.5742	54	15.35162	4.3593	61	10.18464

S 6. Significant Spearman's correlate relationships within the MTHFR 677 TT genotype.

Admission frequency = admit no/DOI
Cost care burden = admission frequency + DSP
Correlation is generally significant at the 0.05 level (2-tailed).
Correlation is significant at the 0.01 level (2-tailed) (Bold text).
Red = over methylation markers
Blue – undermethylation markers
*The Benjamini-Hochberg procedure critical p values for Spearman correlations were 0.011 for the TT genotype, therefore all correlations in this table are significant after correction for multiple variables. DOI = duration of illness.

MTHFR 677 TT	A Dependent	A:B	B Independent Correlate A with B	B:C	C Correlate B with C Remarks
N 7, Prevalence 5.2% Mean DOI 5.71 years					
	case		**MTHFR 677 TT**		
	case	N6, rho 0.828, P0.042	Vitamin B2 ug/l	N 6, rho 0.829, P 0.042	Peak 2 amplitude / Peak 1 amplitude
	case	N6, rho 0.828, P0.042	**Vitamin B2/creatinine**	N6, rho 0.886, P 0.019	Peak 2 area / Peak 1 area
	case	N6, rho 0.828, P0.042	Vitamin B2/creatinine		Peak 2 amplitude / Peak 1 amplitude
	case	N 6, rho 1.000, P 0.000	**Vitamin B2/creatinine ROC**		
	case	N6, rho 0.828, P 0.042	**Peak2/Peak 1 amplitude**		
	case	N6, rho 0.828, P 0.042	**Peak2/Peak 1 area**		
	case	**N 7, rho 1.000, P 0.000**	**High DA ROC**		
	case	N 7, rho 0.750, P 0.052	AD/NA ROC		

S 7. Significant Spearman's correlate relationships within the MTHFR 677 CC genotype.

(P2/P1, P2-P1 = riboflavin synthesis and P1-P2 amplitude or area under peak = riboflavin (vitamin B2) metabolism products). In the interest of space, not statistically significant in the correlation matrix, have not been included and their absence as significant variables must be imputed from their absence.
Admission frequency = admit no/DOI
Cost care burden = admission frequency + DSP*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed) (Bold text).
Red = over methylation markers. Blue – undermethylation markers
(Benjamini-Hochberg critical p 0.039 for the CC genotype, DOI = duration of illness))

MTHFR 677 CC	**Dependent A**	**A:B**	**Independent B**	**B:C**	**C**
N 57-65, Prev 48.5%	**Mean DOI 7.18**		**Correlate A with B**		**Correlate B with C**
	case		**MTHFR 677 CC**		
	case	N64, rho -0.245, P 0.051	RC folate		
			RC folate	N 62, rho 0.287, P 0.024	Vitamin B6
	case	**N 64, rho 0.323, P 0.009**	**Low RC folate ROC**		
	case	**Nil sig**	**Vitamin B12/RC folate**		
	case	**N63, rho 0.432, P 0.000**	**Low Vit B6 ROC**		
	case	**N63, rho -0.335, P 0.007**	**Vitamin B6**		
			Vitamin B6	N 62, rho 0.287, P 0.024	RC folate
			Vitamin B6	N 61, rho -0.277, P 0.078	NA/MHMA
	case	**N 64, rho -0.344, P 0.005**	**Low Vitamin D ROC**		
			Vitamin D	N 63, rho 0.295, P 0.019	RC FOLATE
			Vitamin D	Nil sig	Vitamin B6
			Vitamin D	N 62, rho -0.322, P 0.011	NA/MHMA
	case	**N 60, rho 0.475, P 0.000**	**5HIAA**		
	case	**N65, rho 0.263, P 0.000**	**5HIAA ROC**		

case	N65, rho 0.263, P 0.034	DA
case	**N65, rho 0.362, P 0.003**	**High DA ROC**
case	**N65, rho 0.634, P 0.000**	**NA**
case	**N65, rho 0.643, P 0.000**	**High NA ROC**
case	**N63, rho 0.552, P 0.000**	**NA/MHMA**
case	**N63, rho 0.556, P 0.000**	**High NA/MHMA ROC**
case	**N 65, rho 0.669, P 0.000**	**AD**
case	**N65, rho 0.616, P 0.000**	**High AD ROC**
case	**N65, rho 0.634, P 0.000**	**AD/MHMA**
case	**N65, rho 0.601, P 0.000**	**NA + AD/MHMA**
case	**N65, rho 0.650, P 0.000**	**High AD/MHMA ROC**
case	**N65, rho 0.361, P 0.003**	**AD/NA**
case	**N65, rho 0.430, P 0.000**	**AD/NA ROC**

S8. Cross tab analysis for MTHFR 677 TT dependent variables.

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```
tab case B2ROC if mthfrpoly== homozygous = MTHFR 677 TT
           |         B2ROC
      case |      Pos.       Neg. |     Total
-----------+----------------------+----------
  Abnormal |         2           0 |         2
    Normal |         0           4 |         4
-----------+----------------------+----------
     Total |         2           4 |         6

True abnormal diagnosis defined as case = 1
```

S9. **Logistic regression analysis for MTHFR 677 CC dependent biochemical variables** (taking into account population [prevalence of the disease (0.45%)).

Logistic regression

MTHFR 677 CC Obs 57, LR Chi2(4)45.72

Prob > chi2 0.0000, Pseudo R2 0.5797

. diagt case predcase10 if mthfrpoly==2

	RECODE of p10	
case	Pos.	Neg.	Total
Normal	2	25	27
Abnormal	28	2	30
Total	30	27	57

(Pr(case))

True abnormal diagnosis defined as case = 1

case	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf.	Interval]
Vitamin B6	.974799	.0122398	-2.03	0.042	.9511021	.9990863
Peak / Ratio	.1738954	.1643242	1.85	0.064	.0272858	1.108255
AD + NA/MHMA	1.25044	.0909248	3.07	0.002	1.084347	1.441974
5-HIAA	1.816841	.6930404	1.57	0.118	.8602464	3.837167
_cons	.2041357	.3062405	-1.06	0.290	.0107885	3.862558

Loglikelihood-16.572295 {95% Confidence Interval]

Prevalence		**Pr(A)**	**.45%**	**----- (given) -----**
Sensitivity	Pr(+|A)	93.3%	77.9%	99.2%
Specificity	Pr(-|N)	92.6%	75.7%	99.1%
ROC area	(Sens. + Spec.)/2	.93	.862	.997
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	12.6	3.31	48
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	.072	.0188	.276
Odds ratio	LR(+)/LR(-)	175	24.8	1234
Positive predictive value	Pr(A|+)	5.39%	1.47%	17.8% (lr)
Negative predictive value	Pr(N|-)	100%	99.9%	100% (lr)
Pre-test odds	prev/(1-prev)	.0045	----- (given) -----	
Post-test odds (+)	Pr(A|+)/(1-Pr(A|+))	.057	.015	.217 (lr)
Post-test odds (-)	Pr(A|-)/(1-Pr(A|-))	.00033	.00125	8.5e-05 (lr)

*z is coefficient/Standard error (Std. Err.), where OR (Odds Ratio) is exponential of the coefficient of predictor variables.
Pz = probability associated z factor at 95% confidence level. Peak ratio = Peak 1 amplitude / Peak 2 amplitude.

Logistic regression for biomedical variables, without HPLC data.

MTHFR 677 CC

Number of obs	=	59
LR chi2(4)	=	43.9
Prob > chi2	=	0
Pseudo R2	=	0.5369

Log likelihood = -18.934775

case certainty CC n 59	Odds Ratio (OR)	Std. Err.	z	P>|z|	[95% Conf.	Interval]
free % Cu/Zn ROC	9.975525	11.82251	1.94	0.052	0.9775362	101.7979
AD / MHMA ROC	25.40205	24.09701	3.41	0.001	3.957291	163.057
Vitamin B12 /vitanin D ROC	21.29819	25.73367	2.53	0.011	1.994681	227.4113
Low vitamin B6 ROC	12.17347	12.51401	2.43	0.015	1.623309	91.2909
_cons	0.0020782	0.0037968	-3.38	0.001	0.0000579	0.0746153

S 10. Significant case- correlates within the MTHFR 677 CT genotype

Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed) (Bold text). Red = overmethylation markers. Blue – undermethylation markers

(Critical Benjamini-Hochberg correction P value is 0.038 for the CT genotype).

MTHFR 677 CT	Dependent A	A:B	Independent B	B:C	Interdependent C
	case	40/42 significant symptoms	Symptoms		
	case	N 61, rho 0.384, P 0.002	DA		
	case	N 61, rho 0.377, P 0.003	High DA ROC		
	case	N61, rho0.686, P 0.000	NA		
	case	N 61, rho 0.642, P 0.000	High NA ROC		
	case	N 61, rho 0.591, P 0.000	AD		
	case	N 61, rho 0.607, P 0.000	High AD ROC		
	case	N 60, rho 0.545, P0.000	NA/MHMA		
	case	N 60, rho 0.567, P 0.000	NA/MHMA ROC		
	case	N 60, rho 0.427, P 0.001	AD/MHMA		
	case	N60, rho 0.528, P 0.000	AD/MHMA ROC		
	case	N 61, rho 0.498, P0.000	NA/DA		
	case	N 61, rho 0.481, P 0.000	NA/DA ROC		
	case	N 61, rho 0.339, P 0.007	AD/NA ROC		
	case	N 54, rho 0.382, P 0.004	Area under peak 2 ROC		
	case	N 54, rho 0.316, P 0.020	Peak 1-peak 2 area ROC		

case	Nil sig	% free Cu/Zn		
case	N 62, rho 0.323, P 0.011	Low folate ROC		
		Vitamin B12	Nils sig	RC folate
		Vitamin B12	N 54, rho 0.339, P 0.012	Peak 2 amp/peak 1 amp
		Vitamin B12	N 54, rho 0.329, P 0.015	Peak 2 area/peak1 area
case	N 62, rho 0.228, P 0.075	High vit B12 ROC		
case	N 62, rho 0.277, P 0.029	[Vitamin B12 X % free Cu X /[Zinc X folate]		
case	N 58, rho 0.294, P 0.025	[Vitamin B12 X % free Cu X homocysteine]/		
case	N 57, rho 0.277, P 0.037	[Vitamin B12 X %free Cu X homocysteine] / [Zn X folate X vitamin B6 X vitamin D]		
case	Nil sig (cancels)	Vitamin B6?		
		Vitamin B6	N 59, rho 0.373, P 0.004	RC folate
		Vitamin B6	N 53, rho 0.449, P 0.001	B2 ug/L
		Vitamin B6	N 53, rho 0.314, P 0.022	B2/creatinine
		Vitamin B6	N 52, rho 0.306, P 0.027	Peak 2 amp/peak 1 amp
		Vitamin B6	N 52, rho 0. 316, P 0.023	Peak 2 area/peak1 area
		Vitamin D	N 61, rho 0.252, P 0.050	RC folate