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Supplemental Data

Identification and Characterization of an Inborn Error of Metabolism Caused by Dihydrofolate Reductase Deficiency

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Table S1. Summary of Known Disorders Related to Folate Transport and Metabolism

Disorder	Gene	OMIM	Important clinical features	Ref
Hereditary folate malabsorption	*PCFT*	611672	Low blood and CSF folate levels in infancy with anaemia, diarrhoea, immune deficiency, infections and neurological deficits.	1
Cerebral folate transport deficiency	*FOLR1*	136430	Low CSF folate levels causing neurodegeneration characterized by developmental regression, movement disturbance and leukodystrophy from early childhood.	2
Methylene-tetrahydrofolate reductase deficiency	*MTHFR*	607093	Variable age of onset (infancy to adulthood) and severity. Common features are developmental delay, motor and gait abnormalities, seizures, psychiatric manifestations, hyperhomocystinemia, low or normal methionine and absence of megaloblastic anaemia.	3
Methionine synthase deficiency (CblG)	*MTR*	156570	Developmental delay, megaloblastic anaemia with homocystinuria and without methylmalonic aciduria	4
Methionine synthase reductase deficiency (CblE)	*MTRR*	602568	Developmental delay, megaloblastic anaemia with homocystinuria and without methylmalonic aciduria	5
Glutamate forminotransferase deficiency	*FTCD*	606806	Severe form with developmental delay, seizures, elevated folate levels and presence of FIGLU in urine following administration of histidine. A mild form has also been reported.	6

Table S2. Results of Proband's Investigations that Were Found to Be Normal

Hemoglobinopathy screen
Transferrin isoelectric focusing
Sialic acid content
Mucopolysaccharides
Very long chain fatty acids
Cholesterol and 7-dehydrocholesterol levels
Plasma glucose and lactate
Activities of respiratory chain enzyme complexes I, II, III and IV in skeletal muscle
Analysis of common *POLG1* mutations
Urine organic acids
Urine, plasma and CSF amino acids
Plasma ammonia
Urine urate, hypoxanthine, xanthine, pseudouridine, uracil, thymine and succinyl adenosine levels
Immunoglobulin levels and sub-classes
FOLR1 and *FOLR2* sequencing

Table S3. Regions of Homozygosity Unique to the Proband

The genomic regions of homozygosity unique to the proband that were identified by autozygosity are shown below. Nucleotide numbers in the table are based on NCBI build 36. The 3Mb region at chromosome 5q14.1 flanked by rs4521453 and rs10059759, containing the *DHFR* gene is highlighted in bold. Importantly, the loci containing potential candidate genes, *TCN2, PCFT, MTHFR, MTR, MTRR, FOLR1* and *FTCD* were not homozygous in the proband.

Chromosome	Nucleotide number start	Cytoband Start	Nucleotide number end	Cytoband End	Size (kbp)
1	238,424,796	q43	246,877,270	q44	8,452
1	171,630,183	q25.1	173,286,248	q25.1	1,656
2	114,881,944	q14.1	129,097,050	q14.3	14,215
2	169,755,268	q31.1	180,484,267	q34	10,729
2	206,487,607	q31.1	211,614,325	q34	5,127
2	23,481,816	p24.1	26,747,938	p23.3	3,266
2	232,175,438	q37.1	235,340,542	q37.3	3,165
3	190,926,256	q28	197,922,334	q29	6,996
3	117,992,882	q13.31	121,010,467	q13.33	3,017
3	72,248,225	p13	74,138,367	p13	1,890
3	95,025,696	q11.2	96,552,879	q11.2	1,527
5	151,883,873	q33.1	169,885,305	q35.1	18,001
5	**77,664,850**	**q13.2**	**81,292,374**	**q14.1**	**3,628**
5	26,401,350	p15.2	29,885,156	p13.3	3,484
7	118,286,776	q31.31	120,492,215	q31.31	2,205
7	156,716,799	q36.3	158,623,513	q36.3	1,906
8	121,887,852	q22.3	128,382,683	q24.21	6,495
8	105,849,484	q22.3	106,838,246	q24.21	989
9	36,587	p24.3	10,457,284	p23	10,420
10	2,175,839	p15.3	11,457,616	p14	9,281
11	27,843,969	p14.1	34,309,803	p13	6,465
11	121,538,364	q23.2	122,758,013	q24.1	1,220
12	45,834,102	q13.11	52,587,311	q15	6,753
14	67,404,339	q24.1	75,840,910	q24.3	8,436
16	26,038,708	p12.1	31,567,929	p11.2	5,529
16	45,092,478	q11.2	49,564,860	q21	4,472
17	62,176,152	q24.2	65,719,223	q24.3	3,543
18	3,587,003	p11.31	10,303,579	p11.21	6,717
19	3,049,245	p13.3	6,673,045	p13.3	3,623
22	25,300,902	q12.1	41,427,458	q13.31	16,127
				Total size	**179,332**

Table S4. Primer Sequences for Amplification of *DHFR*

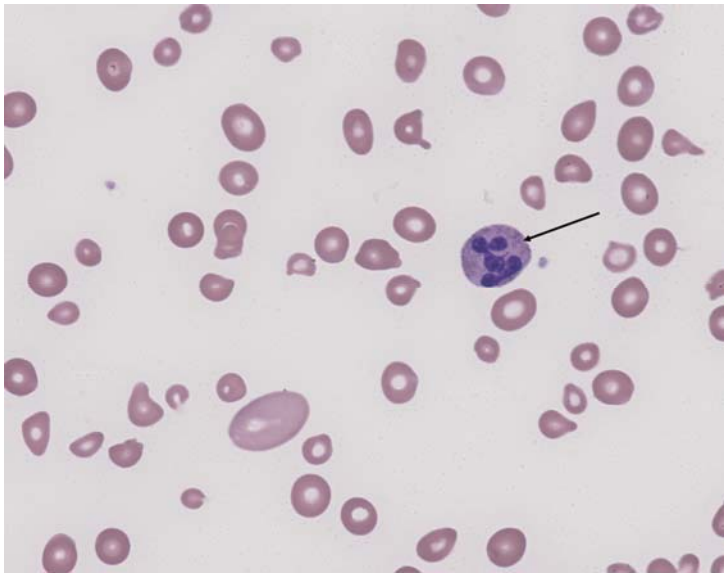
Exon	Primer	Product length
1	Forward – TTCGCGCCAAACTTGACCG	292
	Reverse – AAAAGGGGAATCCAGTCGG	
2	Forward – CGACTGGATTCCCCTTTTC	476
	Reverse – ATAATTTGCTCGTGCGTTG	
3	Forward – AGCATGCAGACTCCACACAG	373
	Reverse – GCAGCTTCATCAATAGCTCCTT	
4	Forward – GGTCAGAGGCCATACTGATG	434
	Reverse – CAGTACAGATAATGTGCTGCTTC	
5	Forward – GGCAGCACCAAGCATATTTT	351
	Reverse – GCACCCATCATCCTAGCAGT	
6	Forward – CCAACTTGACAGTGGCTTACC	394
	Reverse – GCAAGAATGTCTCATAAATGGTATC	

Note: Annealing temperature of 55°C was used for all the primer pairs. The same primers were used for bidirectional sequencing.

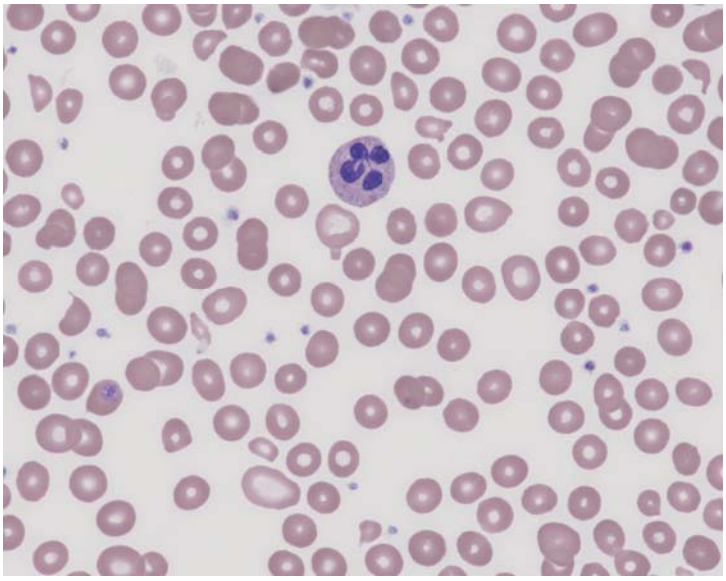
Table S5. Primers for QPCR of *DHFR, GUSB* and *PPIB*

Gene	Gene ID	Primer sequence
DHFR	NM_000791.3	Forward – GTTCCTGGGAGCACCTTTTC
		Reverse – ATGCAGACAGTGCCAGCTC
GUSB	NM_000181.1	Forward -AGAGTGGTGCTGAGGATTGG
		Reverse – CCCTCATGCTCTAGCGTGTC
PPIB	NM_000942.4	Forward -CGGAAAGACTGTTCCAAAAAC
		Reverse - GATTACACGATGGAATTTGCTG

Figure S1. Proband's Peripheral Blood Films



1A Proband's peripheral blood film at diagnosis with modified Wright's stain at 500x magnification showing a hypersegmented neutrophil (marked by arrow) with macrocytic red cells, red cell fragments, marked poikilocytosis and anisocytosis.



1B. Proband's peripheral blood film after treatment.

Figure S2. ClustalW Alignment of DHFR Protein Sequence from Human, Mouse, Chicken, Zebra-fish and *Drosophila*

Nucleotide phosphate binding sites are highlighted in green. Leucine (L) at position 80, the residue disrupted by mutations in our patients is highlighted in red demonstrating its conservation at least to *Drosophila.*

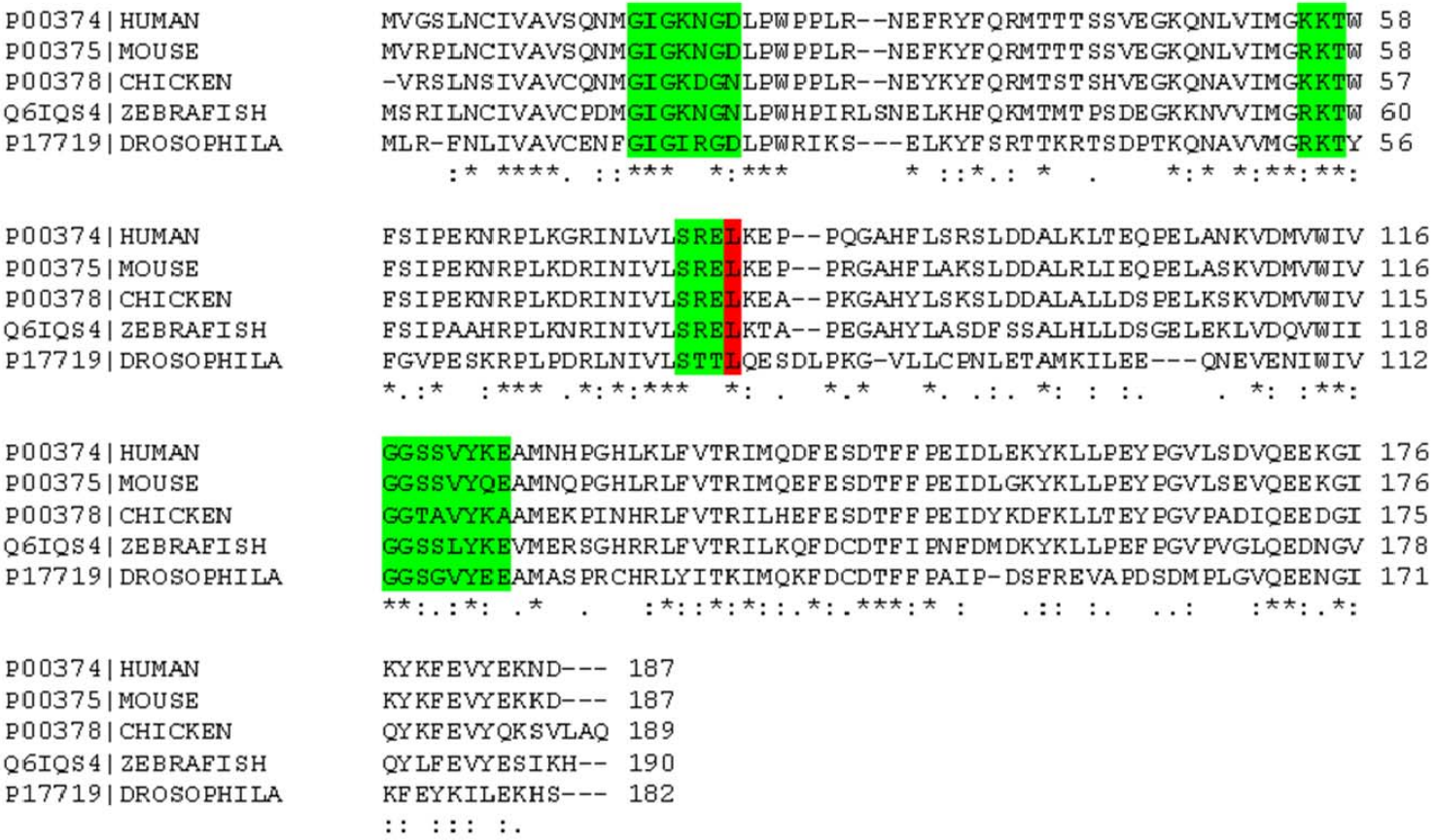
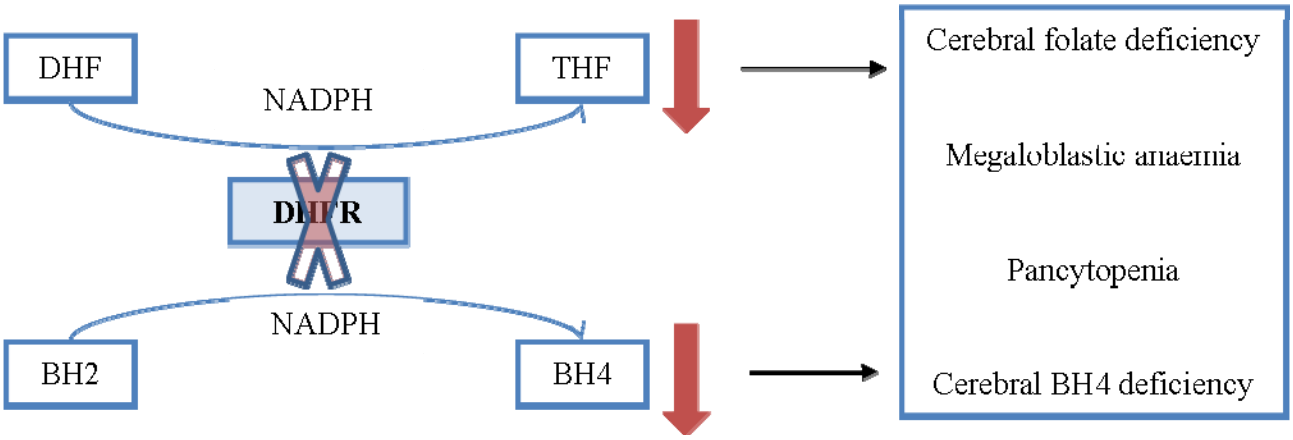


Figure S3. Summarised Representation of the Consequences of Loss of Activity of DHFR



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