

## Supplemental Material for

### Specific regions of the brain are capable of fructose metabolism

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*VNB predicts the brain as a site for fructose metabolism.*

Prediction of tissues other than the liver and kidneys that express genes required for fructose metabolism *via* the Fru-1-P pathway was performed using a bioinformatics approach aimed at physiomic assessment of gene expression. The VNB (Funari et al., 2010) was used to compile qualitative and quantitative expression profiles of each gene. By entering the RefSeq sequences of each human cDNA (and its aligned paralogs) for the genes in the Fru-1-P pathway (fructose transporters *gluts 2, 5, 7, 9, and 11*; *khk, aldoB, and aldoC*) into the VNB program, a list of tissues expressing this set of genes was compiled. The total number of EST sequences in dbEST for each gene was tallied for each tissue. This generated a qualitative profile of gene expression per tissue (Table S1), and revealed brain and nervous tissue as potentially major sites capable of fructose metabolism. A more stringent quantitative gene expression profile was generated by VNB, which tallied EST sequences from only those cDNA libraries in dbEST that have not been manipulated. In this quantitative profile, the level of expression of each gene was calculated as a percent of the total mRNA population in each tissue (Table S2). Tissues that had a quantitative level for each gene of >0.001% of total transcripts, with at least two hits in the qualitative profile, were considered for an analysis of tissues that have the key enzymes in

fructose metabolism via Fru 1-P at significant levels. The results are tabulated in Table S3, where the VNB correctly identified the liver and kidney as sites for expression of all the genes necessary for this pathway. Liver was the top ranked tissue in terms of expression levels for each of these genes, or set of genes (as in the case of the *aldos* and *gluts*). Kidney was among the top 19 tissues for these same genes. Notably, spleen/lymphoreticular tissue and brain/nervous tissue were also in the top four tissues predicted to have high expression of *khk*, with expression levels higher than or comparable to kidney for the other genes (*aldos* and *gluts*). By mass, the brain is the largest of these organs, and was the focus of this study. The percentage level of *khk* expression in the brain/nervous tissue was one-third of the liver and nearly that of the kidney. Among the fructose transporters, *glut5* was quantitatively predicted to be in the brain, while *gluts* 2, 7, and 11 were not (Table S2). Overall, there was generally little to no expression of *glut7* in most tissues, whereas *glut9* showed low expression levels in the brain and other tissues (Table S1). Both Table S1 and Table S2 show the full gene-specific qualitative and quantitative patterns, respectively, obtained from VNB.

**Table S1. Qualitative Data from VNB**

Top Seven Tissues for KHK Expression:										
	KHK	AldoC	AldoB	DAK	Glut2	Glut5	Glut6	Glut7	Glut9A	Glut9B
liver	37	80	392	31	46	1	3			
kidney	20	47	48	22	11	36	18	1	24	25
Spleen/lymphoreticular/lymphnode	12	136	42	77	3	50	25	2	1	
lymph node	2	16	11	1	1	24	3		1	
spleen	3	3	16	6	2	1	12			
lymphoreticular	3	53	25	34		12	1	1		
bone marrow	4	64		36		13	9	1		
Brain/cerebellum/cerebrum/nervous	55	2138	118	84		75	75	3	1	2
brain	23	649	61	60		47	37	1	1	2
cerebellum	4	401	2	6		3	9			
cerebrum	10	172	18	12		20	18			
nervous	18	107	33	5		5	10	2		
peripheral nervous system		16	4	1			1			
testis	2	34	18	51	2	75	7	6		
placenta	2	127	73	18	1	6	11	1	4	5
uterus	2	61	30	10	4	1			3	3
<b>Other Tissues:</b>										
adipose tissue		14								
adrenal cortex		9	4							
bone	1	8	9	1	2	1	2		2	2
cartilage		5	5	7	1		3		1	1
cervix	2	56	28	9	1		2			
colon	2	71	68	21	1	7		1		
ear					1					
embryonic tissue	1	101	49	12	4	8	2			
endocrine		1		5		1				
esophagus		4		2			1			
eye	2	117	55	10		4	11		1	
gastrointestinal tract	4	15	35	36		8	2			
genitourinary		15	11	1		1				
head and neck	1	576	605	3		1			1	2
heart	1	26	18		1	13	2			
lung	2	150	79	14	2	10	28		3	3
mammary gland	3	90	53	9		3	4		1	1
muscle	1	33	14	3	4	9	2		1	3
ovary		40	30	2		4	2		5	7
pancreas	1	62	33	8		1	5			
pancreatic islet	3	13	39	1	1		6		1	
parathyroid			1							
pineal gland		1	1			1				
pituitary gland		3								
prostate	3	70	36	35		21	7		1	3
retina	2	71	10			13	1		1	1
salivary gland		34	33	1						
skin		197	105	18	5	1	3			
stomach	7	19	8	5	4	2	1			
synovium				5		9	5			
thymus		3		5		3	3		1	
thyroid	1	29	25	3	1	1	2			
vascular		12	1	1			3		1	

**Table S1. Quantitative Data from VNB (part 1)**

Top Seven Tissues for KHK Expression:		KHK		AldoC		AldoB		ALDOS		DAK		
	rank	ratio (hits/picks)	%	rank	ratio (hits/picks)	%	rank	ratio (hits/picks)	%	rank	ratio (hits/picks)	
liver	1	10/64904	0.0154	7	63/64904	0.0970	2	117/64904	0.1802	2	19/64904	0.0292
kidney	2	6/73096	0.0082	24	19/73096	0.0259	16	17/73096	0.0232	19	2/73096	0.0027
Spleen/lymphoreticular/lymphnode	3		0.0058	18		0.0347	9	4/39066	0.0419	16	1	0.0303
spleen		2/20451	0.0098		4/39066	0.102		16/20451	0.0782		2/20451	0.0097
lymphoreticular		3/66695	0.0045		3/20451	0.146		25/66695	0.0374		3/66695	0.0509
bone marrow		1/32258	0.0031		53/66695	0.0794						
Brain/cerebellum/cerebrum/nervous	4		0.0048	1		0.1870	11		0.0374	6		0.0067
brain		2/126222	0.0016		143/126222	0.1132		48/126222	0.0380		11/126222	0.0087
cerebellum					27/5235	0.5157						
cerebrum					5/6049	0.0826						
nervous		3/37709	0.0080		56/37709	0.1485		21/37709	0.0556			
peripheral nervous system					16/21313	0.0750		4/21313	0.0187		1/21313	0.0046
testis	5	1/83915	0.0012	21	25/83915	0.0297	19	16/83915	0.0190	20	0.0487	0.0035
placenta	6	1/98545	0.0010	15	55/98545	0.0558	14	31/98545	0.0314	15	0.0872	0.0071
uterus	7	1/136246	0.0007	16	56/136246	0.0411	18	27/136246	0.0198	18	0.0609	0.0058
<b>Other Tissues:</b>												
adipose tissue												
adrenal cortex				10	9/10935	0.0823	12	4/10935	0.0365	11		
bone	9	1/52917	0.0019	26	8/52917	0.0151	20	9/52917	0.0170	25	0.1188	
cartilage				29	1/24072	0.0041	24	3/24072	0.0124	28	0.0165	
cervix				3	49/30200	0.1622	5	22/30200	0.0728	5	5/30200	0.0165
colon				22	2/173165	0.0287	21	12/173165	0.0164	22	0.0451	0.0095
ear												
embryonic tissue	8	1/49165	0.0020	9	42/49165	0.0854	7	26/49165	0.0528	9	0.1382	0.0061
endocrine												
esophagus												
eye				8	7/175064	0.0945	8	36/75064	0.0479	8	0.1424	0.0093
gastrointestinal tract				14	9/15486	0.0581	1	29/15486	0.1872	4	0.2453	0.0064
genitourinary				25	6/27087	0.0221	22	4/27087	0.0147	23	0.0368	
head and neck												
heart				23	7/26005	0.0269	26			26	0.0269	
lung				11	104/142743	0.0728	13	52/142743	0.0364	13	0.1092	0.0063
mammary gland				4	65/41448	0.1568	4	37/41448	0.0892	3	0.2460	
muscle				19	24/70896	0.0338	23	10/70896	0.0141	21	0.0479	0.0028
ovary				17	34/88281	0.0385	17	20/88281	0.0226	17	0.0611	0.0011
pancreas	10	1/87367	0.0011	13	62/87367	0.0709	10	33/87367	0.0377	14	0.1086	0.0091
pancreatic islet												
parathyroid												
pineal gland				27	1/8778	0.0113	25	1/8778	0.0113	27	0.0226	
pituitary gland				20	3/9234	0.0324	24			24	0.0324	
prostate				12	60/83999	0.0714	9	32/83999	0.0380	12	0.1094	0.0261
retina				5	33/27400	0.1204	26	1/27400	0.0036	10	0.1240	
salivary gland				2	34/20874	0.1628	3	33/20874	0.1580	1	0.3208	0.0047
skin				6	196/171024	0.1146	6	105/171024	0.0613	7	0.1759	0.0105
stomach				28	1/22787	0.0043				29	0.0043	
synovium												
thymus												
thyroid												
vascular				29	1/23827	0.0041				30	0.0041	

**Table S1. Quantitative Data from VNB (part 2)**

	Glut2			Glut5			Glut6			Glut7			Glut9A			Glut9B			FRU GLUTS		
	rank	ratio	%	rank	ratio	%	rank	ratio	%	rank	ratio	%	rank	ratio	%	rank	ratio	%	rank	ratio	%
<b>Top Seven Tissues for KHK Expression:</b> (hits/picks)																					
liver	2	13/64904	0.0200	15	1/64904	0.0015	14	1/64904	0.0015												
kidney				8	2/73096	0.0027	6	4/73096	0.0054												
Spleen/lymphoreticular/lymphnode	3	0.0097	0.0097	4	0.0128	0.0128	8		0.0045												
lymph node					3/39066	0.0076		1/39066													
spleen		2/20451	0.0097					2/20451	0.0025												
lymphoreticular					12/66695	0.0179		1/66695	0.0014												
bone marrow									0.0097												
Brain/cerebellum/cerebrum/nervous				12	0.0019	0.0019	5		0.0055												
brain					2/116754	0.0017		8/126222	0.0063												
cerebellum																					
cerebrum																					
nervous					1/47089	0.0021															
peripheral nervous system																					
testis				9	2/83915	0.0023		1/21313	0.0046												
placenta				11	2/98545	0.0020	4	7/98545	0.0071												
uterus	6	4/136246	0.0029																		
<b>Other Tissues:</b>																					
adipose tissue																					
adrenal cortex																					
bone				13	1/52917	0.0018	13	1/52917	0.0018												
cartilage							9	1/24072	0.0041												
cervix																					
colon	9	1/73165	0.0013	17	1/73165	0.0013															
ear	4	1/14805	0.0067																		
embryonic tissue	5	2/57982	0.0034	14	1/57984	0.0017															
endocrine							1	1/2408	0.0400												
esophagus																					
eye							16	1/75064	0.0013												
gastrointestinal tract				3	2/15486	0.0129															
genitourinary				7	1/27076	0.0036															
head and neck																					
heart				2	5/26005	0.0192															
lung	8	2/142743	0.0014	16	2/142743	0.0014															
mammary gland							7	7/142743	0.0049												
muscle							3	3/41448	0.0072												
ovary	1	3/106323	0.0028	6	8/70896	0.0112	15	1/70896	0.0014												
pancreas				10	2/88281	0.0022	12	2/88281	0.0022												
pancreatic islet				18	1/87367	0.0011	10	3/87367	0.0034												
parathyroid							2	1/5868	0.0170												
pineal gland				5	1/8778	0.0113															
pituitary gland																					
prostate				18	1/83999	0.0011	11	2/83999	0.0023												
retina				1	9/27400	0.0328															
salivary gland																					
skin	7	4/171035	0.0023				17	1/171024	0.0005												
stomach																					
synovium																					
thymus																					
thyroid																					
vascular							1	1/23827	0.0042												

**Table S3**  
**Quantitative Expression Levels for Key Enzymes of the Fru-1-P Pathway**  
**in Tissues<sup>a</sup>**

Tissue <sup>d</sup>	<i>khk</i>		<i>aldos</i> <sup>b</sup>		<i>gluts</i> <sup>c</sup>	
	rank	%	rank	%	rank	%
Liver	1	0.015	1	0.277	1	0.022
Kidney	2	0.008	19	0.049	10	0.004
Spleen/lymphoreticular/lymph node	3	0.006	16	0.077	6	0.012
Brain/cerebellum/cerebrum/nervous	4	0.005	6	0.210	19	0.001
Testis	5	0.001	20	0.049	13	0.004
Placenta	6	0.001	15	0.087	12	0.004
Uterus	7	0.001	18	0.061	9	0.005

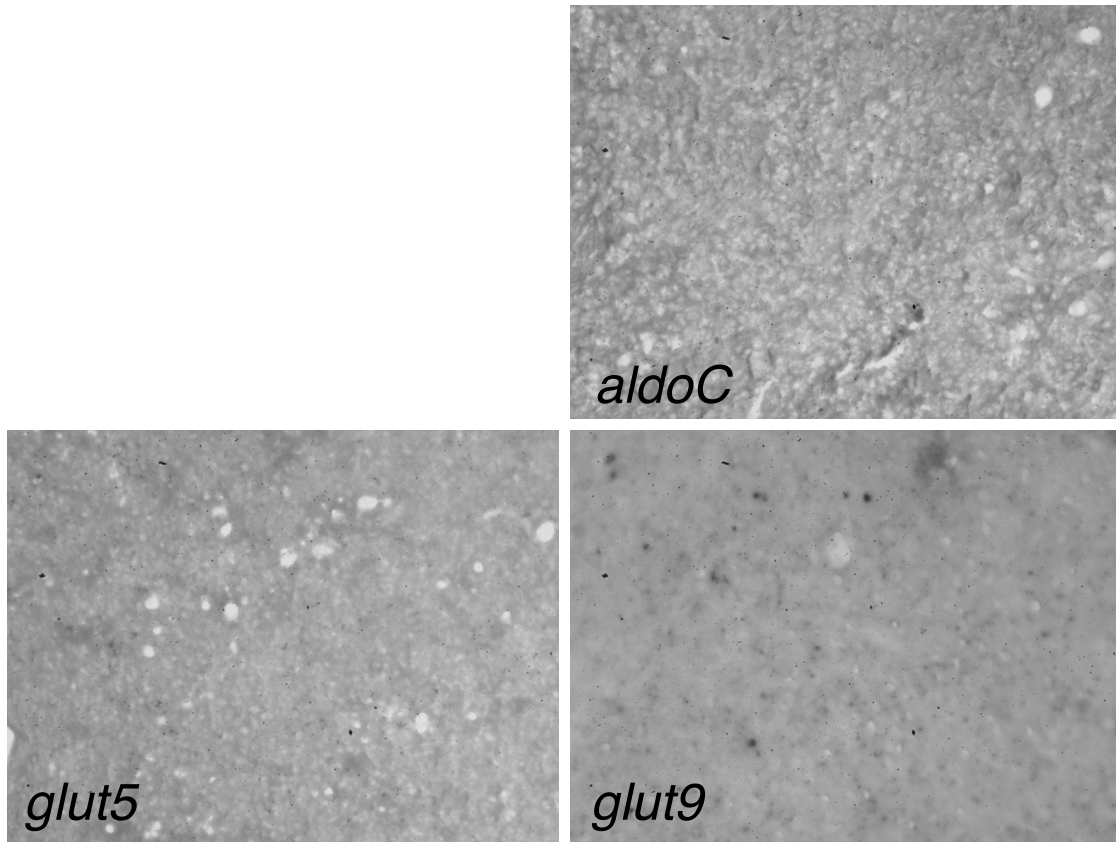
<sup>a</sup>Quantitative expression levels are expressed as a percentage of the total mRNA in different tissues generated from the VNB (Funari et al., 2010). Tissues are sorted in descending order by their predicted expression level of *khk*. The list includes only those tissues where the quantitative level was >0.001% with >2 hits in the qualitative/total profile for all genes (see *Supplemental Material*).

<sup>b</sup>The predicted expression values of *aldoB* and *aldoC* were summed (*aldos*) to give a total predicted expression of Fru-1-P-cleaving aldolases.

<sup>c</sup>The predicted expression values of *glut2*, *glut5*, *glut7*, and *glut9* were summed (*gluts*) to give a total predicted expression value of fructose-transporting *gluts*.

<sup>d</sup>The tissues that would have considerable overlap of cell types were grouped. Brain, cerebellum, cerebrum, peripheral nervous tissue, and nervous tissue were averaged. Likewise, spleen, lymph node, bone marrow, and the general lymphoreticular tissues were averaged.

**Figure S1.**



**Fig S1. Negative control for gene expression of genes in the Fru-1-P pathway** Coronal slices (14  $\mu$ m) of adult mouse brain were probed with DIG-conjugated sense mRNA with the same sequence as the mRNA to the genes for aldolase C (*aldoC*), GLUT5 (*glut5*), and GLUT9 (*glut9*). Expression was visualized by color development from horseradish peroxidase-conjugated anti-DIG antibody as described in Experimental Procedures.

**Table S4**

**Substrate Specificity Constant Values ( $k_{cat}/K_m$ ) of Vertebrate Aldolase**

**Isozymes toward both Fru 1,6-P<sub>2</sub> and Fru-1-P<sup>a</sup>**

Specie	Fru 1,6-P <sub>2</sub> $k_{cat}/K_m$ ( $\times 10^{-6} \text{ s}^{-1} \text{ M}^{-1}$ )			Fru 1-P $k_{cat}/K_m$ ( $\times 10^{-6} \text{ s}^{-1} \text{ M}^{-1}$ )			RATIO $\frac{\text{Fru 1,6-P}_2 k_{cat}/K_m}{\text{Fru 1-P } k_{cat}/K_m}$			Fold difference in RATIOS		
	AldA	AldB	AldC	AldA	AldB	AldC	AldA	AldB	AldC	A/B	C/B	A/C
Rabbit <sup>b</sup>	3	0.67	1.2	0.000048	0.0025	0.000068	63000	270	18000	23	7	4
Human <sup>c</sup>	1.1	1.8	0.49	0.000016	0.0019	0.00018	69000	950	2700	73	3	26
Human <sup>d</sup>	1.1	1.1	1.6	0.000037	0.0029	0.00082	30000	380	2000	80	5	15

<sup>a</sup>These values were derived from the literature for studies in which all three isozymes were assayed from the same source and the kinetic parameters toward both substrates were reported. <sup>b</sup>From Penhoet and Rutter, 1971. <sup>c</sup>From Pezza *et al.*, 2003. <sup>d</sup>From Kusakabe *et al.*, 1994.

**References**

- Funari, V.A., Voevodski, K., Leyfer, D., Yerkes, K., Cramer, D., Tolan, D.R., 2010. Quantitative gene-expression profiles in real time from expressed sequence tag database. *Gene Expression*. 14, 321-336.
- Kusakabe, T., Motoki, K., Hori, K., 1994. Human aldolase C: characterization of the recombinant enzyme expressed in *Escherichia coli*. *Journal of Biochemistry (Tokyo)*. 115, 1172-1177.
- Penhoet, E.E., Rutter, W.J., 1971. Catalytic and immunochemical properties of homomeric and heteromeric combinations of aldolase subunits. *Journal of Biological Chemistry*. 246, 318-323.
- Pezza, J.A., Choi, K.H., Berardini, T.Z., Beernink, P.T., Allen, K.N., Tolan, D.R., 2003. Spatial clustering of isozyme-specific residues reveals unlikely determinants of isozyme specificity in fructose 1,6-bisphosphate aldolase. *Journal of Biological Chemistry*. 278, 17307-17313.