

Supplementary Appendix
to
Expansion of Germline Variants in Primary Hyperparathyroidism: Krebs
Cycle Defects as a Cause of Parathyroid Adenomas

Endocrine Pathology

Hussam Alkaissi¹, Elias Chuki², Yi Liu³, James Welch², Lynn Bliss², Niharika Shah⁴, Sunita K. Agarwal², William F. Simonds², Martha Quezado⁴, Sanaz Sakiani¹, Thorkell Andresson⁵, Karel Pacak⁶, Naris Nilubol⁷, Lee S. Weinstein², Christopher A. Febres-Aldana⁴, Smita Jha²

¹National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA 20892

²Metabolic Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA 20892

³Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA 20892

⁴Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland, USA 20892

⁵Frederick National Laboratory for Cancer Research, National Institutes of Health, Frederick, Maryland, USA 21701

⁶Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda,
Maryland, USA 20892

⁷Endocrine Surgery Section, Surgical Oncology Program, National Cancer Institute, Bethesda,
Maryland, USA 20892

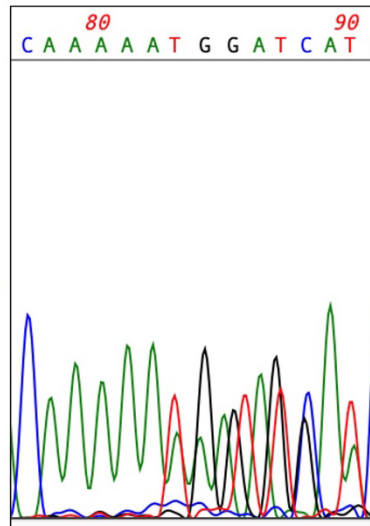
Supplementary Table 1: Metabolites measured in the study	
Metabolite name	Abb
Pyruvate	PYR
Lactate	LAC
Acetyl-coenzyme A	AcCoA
Citrate	CIT
Isocitrate	ISOCIT
Cis-aconitate	ACT
2-oxoglutarate	AKG
Succinate	SUC
Fumarate	FUM
Malate	MAL
Glutamine	GLN
Glutamic acid	Glu
Glucose-6-phosphate	G6P
Fructose-6-phosphate	F6P
Fructose-1,6-diphosphate	FBP
Dihydroxy-acetone-phosphate	DHAP
Glyceraldehyde-3-phosphate	GAP
3-phosphoglycerate	3PG
Phosphoenolpyruvate	PEP
6-phosphogluconate	6PG
Ribose-5-phosphate	R5P
Xylulose-5-phosphate	XYLU5P
Sedoheptulose-7-phosphate	S7P
Adenosine monophosphate	AMP
Adenosine diphosphate	ADP
Adenosine triphosphate	ATP
Guanosine triphosphate	GTP
Nicotinamide adenine dinucleotide	NAD
Nicotinamide adenine dinucleotide (reduced)	NADH
Nicotinamide adenine dinucleotide phosphate	NADP
Nicotinamide adenine dinucleotide phosphate (reduced)	NADPH
Flavin adenine dinucleotide	FAD
Glutathione	GSH

Supplementary Table 2: Concentration (μM) of metabolites normalized to protein (Batch 1)						
Metabolite	MEN1-PTH1	MEN1-PTH2	MEN1-PTH3	DK-2231-1	FHd-PCC1	FHd-PCC2
PYR	6.78	61.19	34.2	208	5.04	0.8
LAC	817.49	1985.49	3709.48	17445	696.52	381.38
AcCoA	0.06	0.42	0.76	2	0.07	0.06
CIT	47.5	37.26	92.71	203	12.3	6.19
ACT	12.13	4.49	24.57	51	2.73	1.6
AKG	0.3	3.31	5.24	27	2.22	0.88
SUC	7.62	30.76	108.05	803	37.79	21.19
FUM	25.64	56.76	66.87	4311	104.5	59.76
MAL	53.4	126.3	118.76	982	47.32	24.88
2HG	1.71	3	2.36	3	0.13	0.05
GLN	79.11	30.55	51.85	106	7.99	3.9
GLU	99.71	175.1	454.28	972	75.52	41.12
G6P	44.79	9.12	15.58	68	2.32	1.43
F6P	3.69	7.61	3.35	33	0.5	0.16
FBP	6.88	4.76	9.57	228	2.03	0.65
DHAP	1.24	2.43	3.67	8	0.14	0.06
GAP	14.2	7.68	22	42	1.4	0.9
3PG	118.94	71.88	145.85	440	7.06	3.83
PEP	15.69	10.91	19.73	110	1.21	0.42
6PG	1.82	4.12	6.68	84	0.52	0.52
R5P	3.79	2.94	2	7	0.31	0.05
XYLU5P	4.42	11.68	18.27	38	0.85	0.5
S7P	0.54	4.95	2.82	23	1.36	0.58
AMP	113.18	143.67	206.68	701	72.65	45.15
ADP	61.78	65.45	120.85	367	56.42	30.51
ATP	32.47	26.05	44.3	290	45.33	22.15
GTP	4.61	2.31	3.39	33	5.76	1.7
NAD	48.15	67.7	186.17	523	23.6	11.98
NADH	2.72	0.94	3.3	5	0.07	0.04
NADP	5.89	6.03	11.13	62	1.74	1.16
NADPH	1.03	0.11	0.19	1	0.01	0.01
FAD	2.02	2.34	6.22	6	0.29	0.26
GSH	4.96	182.83	408.59	772	64.73	34.57
FMR	0.48	0.45	0.56	4.39	2.21	2.4

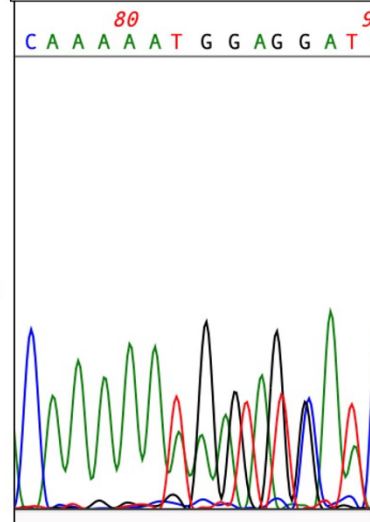
Supplementary Table 3: Concentration (μM) of metabolites normalized to protein (Batch 2)						
Metabolite	MEN1-PTH4	MEN1-PTH5	MEN1-PTH6	DK-2231-2	DK-2005	FHd-PCC3
PYR	1.04	3.38	6.58	2.87	0.46	10.32
LAC	40.84	89.58	1.48	150.13	36.71	431.92
AcCoA	0.01	0.01	0	0.01	0.01	0.03
CIT	1.43	0.95	0.3	1.4	1.61	11.1
ACT	0	0	0	0	0	2.93
AKG	0.05	0.14	0.74	0.2	0.11	1.41
SUC	0.74	1.84	0.2	5.41	1.13	23.44
FUM	1.17	1.6	0.61	27.2	1.62	83
MAL	3.74	4.8	0.43	7.67	5.21	28.24
2HG	0.08	0.14	0.08	0.07	0.08	0.14
GLN	0.87	1.42	8.72	1.68	1.08	6.65
GLU	4.09	8.18	1.22	5.14	8.2	45.1
G6P	0.3	0.52	0.03	0.73	0.34	0.86
F6P	0.13	0.23	0.01	0.21	0.14	0.3
FBP	0.13	0.35	0.03	0.46	0.33	0.61
DHAP	0.16	0.43	0.02	0.34	0.13	0.05
GAP	0.15	0.17	0.03	0.04	0.17	0.75
3PG	0.57	0.93	0.15	0.94	0.55	2.02
PEP	0.05	0.06	0.06	0.07	0.04	0.86
6PG	0.12	0.25	0.07	0.35	0.16	0.3
R5P	0.29	0.77	0.04	0.19	0.44	0.48
XYLU5P	0.24	0.69	0.03	0.21	0.14	0.11
S7P	0.34	0.4	0.02	1.02	0.42	1.73
AMP	4.24	4.39	0.15	4.8	3.47	45.61
ADP	1.05	2.04	0.19	1.65	0.88	33.14
ATP	0.37	1.68	0.12	0.83	0.34	31.21
GTP	0.02	0.07	0.01	0.04	0.03	2.92
NAD	1.69	2.2	0.08	2.52	1.6	11.44
NADH	0.03	0.02	0.01	0.03	0.02	0.06
NADP	0.09	0.11	0.02	0.15	0.09	0.64
NADPH	0.01	0.01	0.01	0.01	0.01	0.01
FAD	0.05	0.07	0.01	0.07	0.05	0.29
GSH	12.62	13.99	0.11	15.73	14.53	47.13
FMR	0.31	0.33	1.42	3.55	0.31	2.94

***FH*, p.K477dup
c.1431_1433dup**

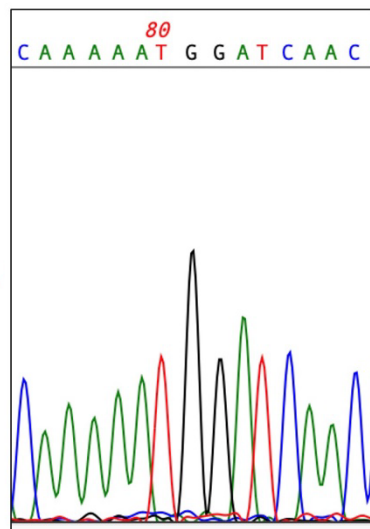
**DK-2005
Tumor DNA**



**DK-2005
Blood DNA**



Control DNA



Supplementary Figure 1:
Chromatograms showing sequence of *FH* variant p.K477dup in parathyroid tumor DNA and blood DNA of patient DK-2005, compared to a control DNA sample. Loss of heterozygosity is not observed in the tumor DNA due to the lack of predominance of variant allele peaks compared to blood DNA.