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Supplementary Appendix

7 **I) Introduction**8 **Table 1.** Causes Of Increasing High Bone Mass In Adults

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Dysplasias

Osteopetrosis
 Engelmann disease
 Endosteal hyperostosis
 Van Buchem disease
 Sclerosteosis
 Craniodiaphyseal dysplasia
 Craniometaphyseal dysplasia
 Melorheostosis
 Mixed-sclerosing-bone-dysplasia

Metabolic

Skeletal fluorosis
 Heavy metal poisoning
 Hypervitaminosis A, D
 Hyper- and hypoparathyroidism
 Renal osteodystrophy
 Hypophosphatemic osteomalacia
 LRP5 activation
 Milk-alkali syndrome

Other

Myelofibrosis
 Mastocytosis
 Sarcoidosis
 Skeletal metastases
 Fibrogenesis imperfecta ossium
 Hepatitis C-associated osteosclerosis
 Ionizing radiation
 Lymphoma
 Multiple myeloma
 Osteonecrosis
 Paget bone disease

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11 Reproduced from reference #1-3

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13 II) Material and Methods

14 A) Serum Multiplex Biomarker Profiling:

15 We have used SMBP to characterize the biochemical disturbances of heritable metabolic
16 bone diseases.⁽¹⁰⁾ Here we contrasted our patient's values to values from 36 control sera
17 acquired at Shriners Hospital for Children, St. Louis, MO, USA. Batch assays were used to
18 examine aliquots of serum from healthy children and adults. At Amgen, the aliquots were
19 thawed once for the patient together with sera multiple assays (**Supplementary Table 2**) that
20 were completed within 72 hours using the manufacturer's protocols. Sclerostin (SOST) and
21 dickkopf1 (DKK-1) were measured using custom ELISAs developed at Amgen Inc. (Thousand
22 Oaks, CA, USA). Commercial or custom-made Luminex-based microbead multiplex kits
23 (Millipore/Linco, St. Charles, MO, USA) were used for osteopontin (OPN), osteocalcin (OCN),
24 receptor activator of NF- κ B ligand (RANKL), osteoprotegerin (OPG), transforming growth
25 factor- β (TGF β), adiponectin, fibroblast growth factor 23 (FGF-23), seven major cytokines
26 including interleukin (IL) 6, and matrix metalloproteinase (MMP) 1, 3, 7, 8, and 13. ELISA kits
27 were used for prostaglandin E₂ (PGE₂), insulin-like growth factor 1 (IGF1), and bone turnover
28 markers (BTMs) including bone-specific alkaline phosphatase (BAP), tartrate-resistant acid
29 phosphatase (TRACP5b), and collagen formation/degradation products such as CICP/CTX1,
30 respectively. The raw data were acquired in mean fluorescence intensity units using FlexMap 3D
31 (Luminex Corp, Austin, TX, USA). For the ELISAs, the initial measurements were collected as
32 optical density units at a specific wave length using a SpectraMax M5 plate reader (Molecular
33 Devices, Sunnyvale, CA, USA). Conversion of the results to concentration units was performed
34 using SoftMax Pro software (Molecular Devices) for the regression analysis of corresponding
35 standard curves.

36 To create the SMBP reference ranges, we considered control results for < 18 years-of-age
37 "pediatric" values, and \geq 18 years-of age "adult" values. To assess our patient's results, we
38 regarded the normal range as the 10th – 90th percentile using the information from the eight
39 pre-menopausal women with matching ages of 33 – 45 years.

40

41 **Table 2.**

42 Assays For Serum Multiplex Biomarker Profile

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Test	Kit	Manufacturer or Distributor	Location	Catalog #	Limit of Detection
1	Human MMP-3 Panel	Affymetrix	Santa Clara, CA	PC1001M	0.4 ng/ml
2	Human MMP 4-Plex Panel	“	“	PC1004M	15-30 pg/ml
3	Cathepsin K	ALPCO	Salem, NH	04-BI-20432	26 pg/ml
4	PGE Metabolite Kit (all species)	Cayman Chemical	Ann Arbor, MI	514531	20 pg/ml
5	Human C2C (CTXII)	IBEX	Montreal, Quebec, Canada	60-1001-001	22 ng/ml
6	Serum CrossLaps (CTXI)	IDS	Fountain Hills, AZ	AC-02F1	0.04 ng/ml
7	Bone TRAP (Human)	“	“	SB-TR201R	1.0 U/L
8	Human CICP (c-terminal propeptide of collagen type I)	Quidel	San Diego, CA	8003	2.4 ng/ml
9	Human BAP (bone specific ALP)	“	“	8012	1.4 U/L
10	Human Adiponectin MagPlex	Millipore	Billerica, MA	HADK1MAG-61K-01	22 ng/ml
11	Human RANKL	“	“	HBN-51K-1RANKL	9.6 pg/ml
12	Human MCP-1 MagPlex	“	“	HCYTOMAG-60K-01	1.9 pg/ml
13	Human SOST	“	“	SPR348	5 pg/ml
14	TGFB1	“	“	TGFB-64K-01	0.3 ng/ml
	Human MagPlex 6-Plex OPG/OC/OPN/PTH/FGF23/DKK-1	“	“	HBNMAG-51K-06	See Below
15	OPG	“	“	“	3.8 pg/ml
16	OC	“	“	“	0.137 ng/ml
17	OPN	“	“	“	0.076 ng/ml
18	PTH	“	“	“	3.9 pg/ml
19	FGF-23	“	“	“	16 pg/ml
20	DKK-1	“	“	“	0.003 ng/ml

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48 **Table 3.**

49 Patient's Serum Multiplex Biomarker Profiling

Test #	Marker	Units	Control Range			Patient ⁺
			10 th -90 th Percentile	Mean	SD	
1	CICP	ng/ml	27.3 – 144.3	82.7	40.4	152.5
2	Bone ALP	U/L	21.3 – 48.8	31	10.1	32.7
3	TRACP5b	U/L	1.4 – 5.0	2.9	0.4	3.9
4	CTXI	ng/ml	0.25 – 0.67	0.46	0.14	0.61
5	CTSK	pg/ml	10.0 – 349	60.9	118.8	590
6	TGFβ1	ng/ml	38.0 – 73.2	46.5	11.8	30.2
7	RANKL	pg/ml	4.0 – 10.7	4.8	2.4	44.9
8	SOST	pg/ml	181 – 756	435	169	1242
9	OC	ng/ml	13.7 – 31.5	19.5	6.0	17.1
10	OPN	ng/ml	3.4 – 25.5	11.4	7.2	29.9
11	OPG	ng/ml	199 – 480	317	108	271
12	DKK-1	ng/ml	2.0 – 4.6	3.0	0.9	1.9
13	PTH	pg/ml	28.7 – 92.0	51.2	22.1	89.0
14	FGF-23	pg/ml	25.6 – 46.3	35.4	7.4	29.7
15	MCP-1	pg/ml	335 – 1724	927	446	638
16	C2C	ng/ml	169 – 322	248	58	251
17	PGE2	pg/ml	10.0 – 82.5	46.6	26.3	74.2
18	Adiponectin	ug/ml	10.3 – 218.1	83.5	73.2	38.3
19	MMP-1	pg/ml	282 – 878	570	205	284
20	MMP-7	pg/ml	211.4 – 834.5	514	219	2126
21	MMP-8	pg/ml	26.4 – 70.5	45.2	17.7	47.2
22	MMP-3	ng/ml	5.1 – 10.5	7.1	1.9	52.0

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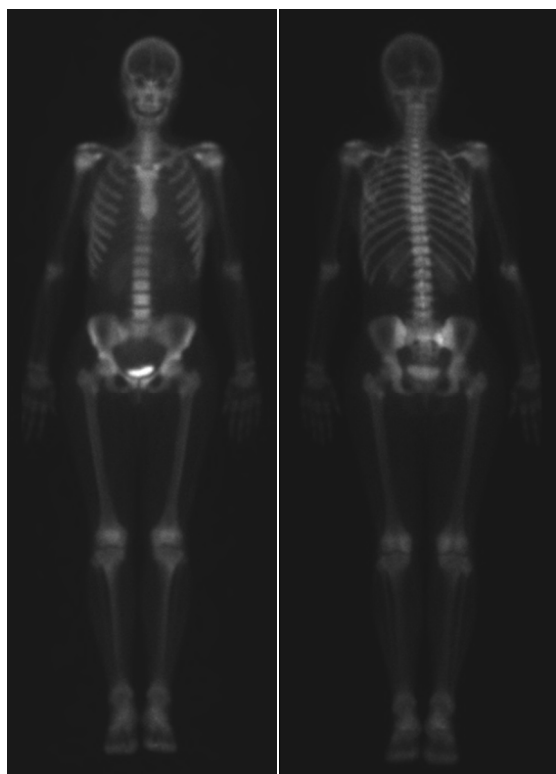
51 Controls = eight 33 to 45 year old women with normal range of 10th – 90th percentile values.52 ⁺Abnormal results are highlighted in bold italics.

53

54 **Figure 1.**

55 Bone scan.

56 Anterior and posterior whole-body bone scintigraphy images demonstrate increased
57 radionuclide uptake in several thoracic vertebral bodies, and more focally in the right sacrum,
58 seen best on the posterior image. No abnormal appendicular skeleton radionuclide uptake was
59 noted to correlate with the long bone findings on radiographs.



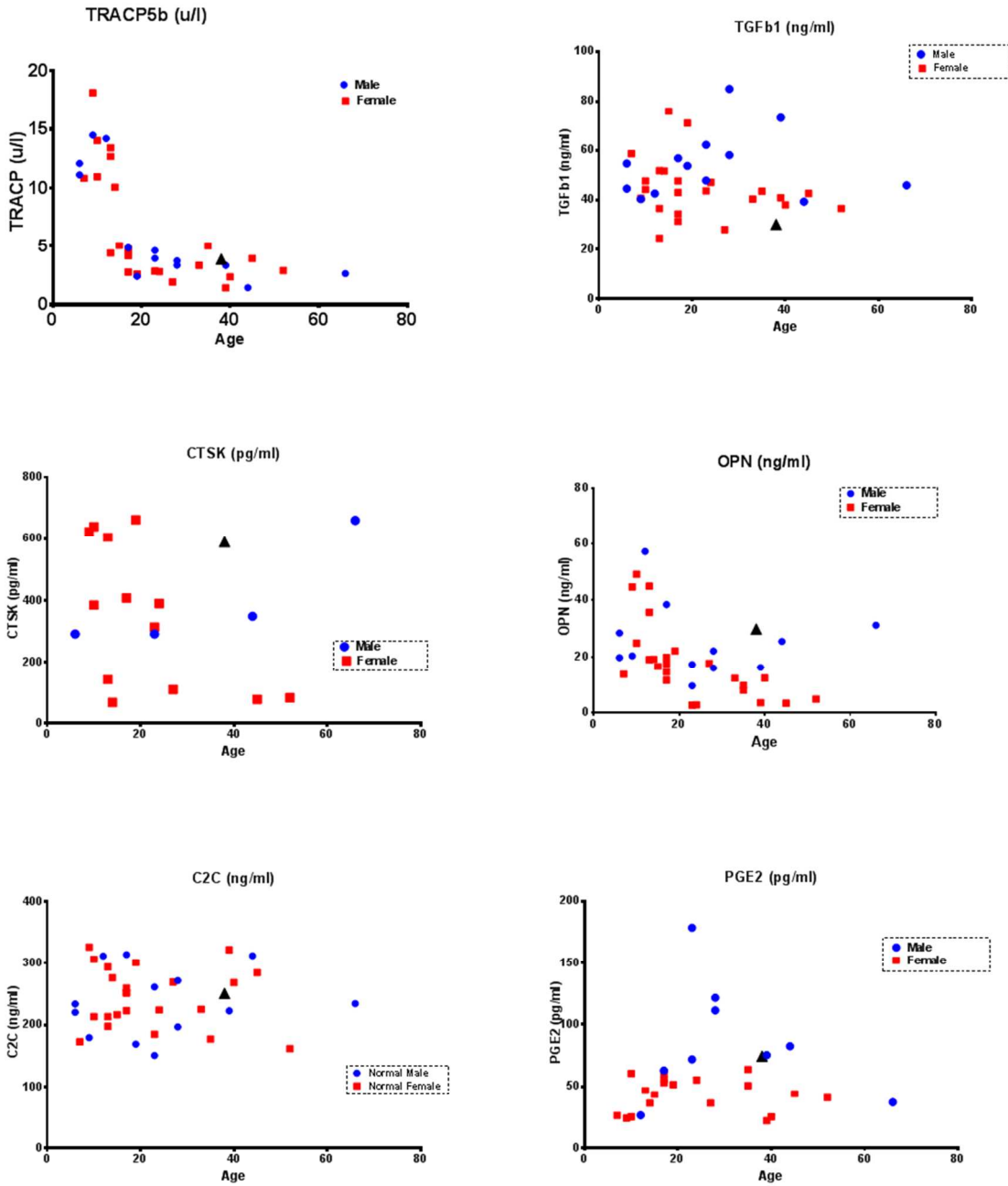
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63 **Figure 2.**

64 Serum Multiplex Biomarker Profiling.



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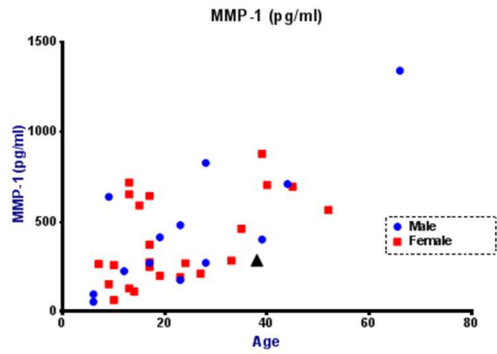
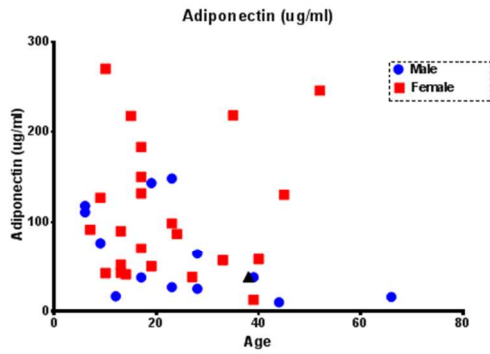
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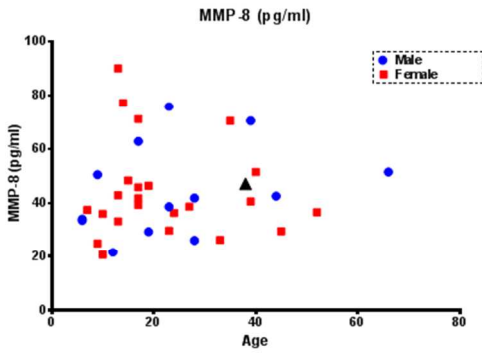
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70 **Figure 2 (continued)**



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