Supplement 1:

Material and Methods

Oligonucleotides used to amplify PCSK1's 14 exons:

Ex. 1 (232bp): (F) ACTCTTGTTCAAGCGAGTG; (R) GTTTCTTGAAAGTGGAAACT
Ex. 2 (510bp): (F) CTCAACCAATTCAACCCAATC; (R) CCCGTGACACAAGTTTACCTATG
Ex. 3 (196bp): (F) GAGGAAGTATACAGAGGTAG; (R) CATAGTCCTTCTGTAGGTAC
Ex. 4 (598bp): (F) TTATTTCAATGCCCACTGGTAC; (R) GTAGTTCAAAGAGAAGGAGCACAG
Ex. 5 (265bp): (F) AATGCTGCCACAGTGTTATA; (R) ACATTTCCTGAGCACTGGA
Ex. 6 (199bp): (F) ACCTATGCCCCATTAATTCA; (R) GCTATAGGGACAATCCTCTG
Ex. 7 (526bp): (F) TTCCTTCTGTGGTGTCAGTAGC; (R) AGCCTTAACTCCCATCCCTC
Ex. 8 (669bp): (F) GATTGAAGCAGAAAGAAAGAGAGG; (R) TCAGTCGTACCAAAGGTCAGTTA
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Ex. 10 (339bp): (F) TCCCTGAATGGAGATGCT; (R) AGGAGATACTTACCTGGGCTC
Ex. 11 (268bp): (F) CGAAGGAAGTTTGGATATAC; (R) TTGAATCATTCAACTTACAC
Ex. 12 (224bp): (F) ATCAGATGCTAGAGTGTATC; (R) TCATCCTCTCATTTACACTT
Ex. 13 (545bp): (F) CAGCTTTCCAAGAACACATCC; (R) CCATGTTTGACTTATTTCCTGC
Ex. 14 (596bp): (F) GCTCCCAGTCTTGAACTCTCTC; (R) CAACCACTTCAGACACAGGC

PCR was performed using ~10ng of genomic DNA by standard methods: denaturation: 95°C/5:00 (denaturation: 95°C/0:30; annealing: T_A°C/0:30; extension: 72°C/0:40) for 35 cycles, and a final extension: 72°C/7:00. Run exons #2, 4, 7, 8, 13, 14 at T_A = 60°C; exons #1, 5, 6, 11 at T_A = 53°C; and exons #3, 9, 10, 12 at T_A = 55°C.DNA was sequenced bidirectionally using standard methods.

Transient transfection of expression clones. cDNAs encoding the various PC1/3 mutant variants were transfected into HEK293 cells, plated the previous day at a density of 2 x 10^5 cells per well in 24-well plates. Cells were transfected in quadruplicate with 200ng/well of each plasmid DNA using Lipofectamine 2000 (Invitrogen). Five hours post-transfection, 1ml of growth medium was added to each well and incubation continued for an additional 24h. The medium was then replaced with 300μl of OptiMEM (Invitrogen) containing 100μg/ml bovine aprotinin (Desert Biologicals). Cells were incubated for an additional 16-24h before conditioned medium and cells were harvested. Conditioned medium was analyzed first by enzyme assay. Both cells and medium were then subjected to SDS-PAGE followed by Western blotting using primary antiserum against the amino terminus of mature mouse PC1/3 followed by goat anti-β-actin antiserum (Santa Cruz Biotechnology) as a loading control [12]. Western blots were then probed with horseradish peroxidase-coupled secondary antiserum. Visualization of immunoreactive protein was accomplished using the SuperSignal Dura Substrate kit (Thermo Scientific). All transfections were performed independently at least three times.

Results

Database assessment of identified PCSK1 variants:

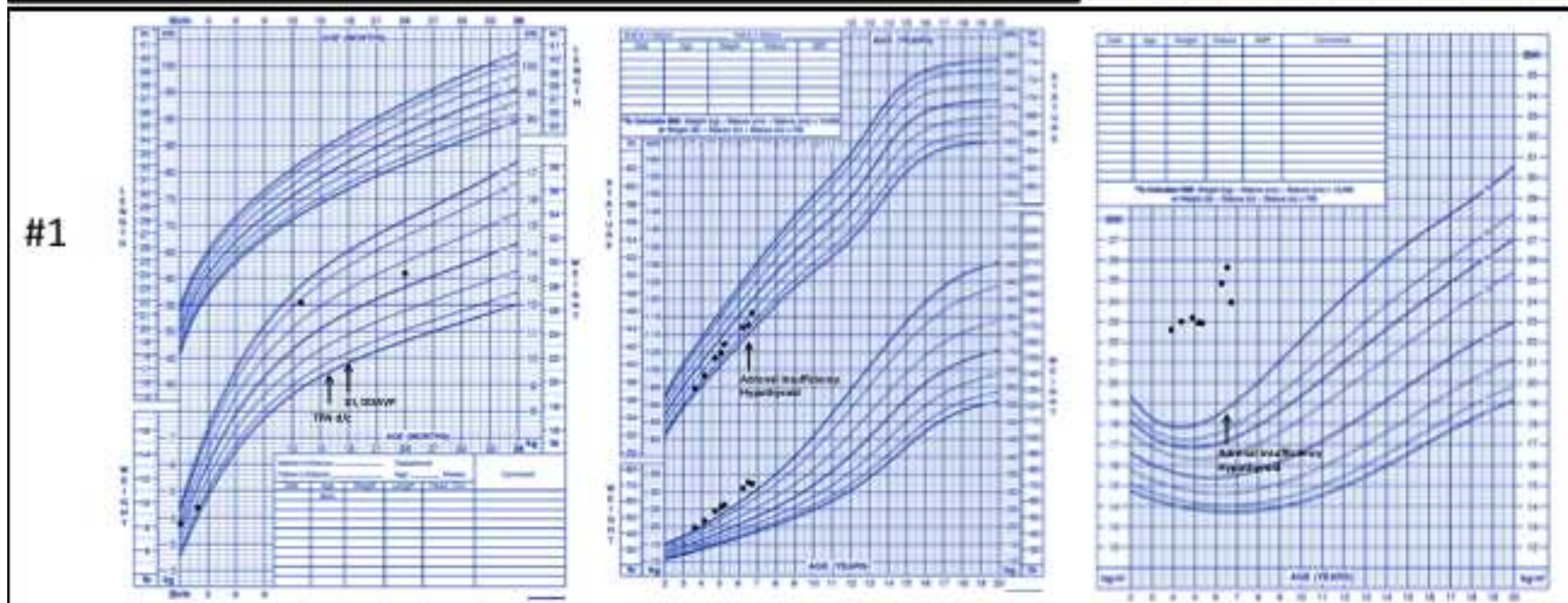
None of the variants were present in 1092 individuals of diverse ethnicities sequenced by the 1000 Genomes project (Phase 1 Integrated Variant Call Set, supp table XX). Furthermore, none of the variants were present in the 6500 NHLBI exomes representing individuals in the United States of European and African ancestry (NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) ESP6500SI accessed 2012-11-26). None of the variants were found as homozygous in 218 exomes sequenced at UCLA, which included 8 Turkish and 20 Arab individuals from ethnic groups not represented in 1000 Genomes or NHLBI. (In the UCLA exomes, a single Caucasian individual was heterozygous for p.G593R and a single Arab individual was heterozygous for p.R405X.)

The ethnic groups and number of samples in 1000 Genomes:

population	description	samples
ASW	Americans of African Ancestry in SW USA	61
CEU	Utah Residents w/ Northern & Western European ancestry	85
CHB	Han Chinese in Bejing, China	97
CHS	Southern Han Chinese	100
CLM	Colombians from Medellin, Colombia	60
FIN	Finnish in Finland	93
GBR	British in England and Scotland	89
IBS	Iberian population in Spain	14
JPT	Japanese in Tokyo, Japan	89
LWK	Luhya in Webuye, Kenya	97
MXL	Mexican Ancestry from Los Angeles USA	66
PUR	Puerto Ricans from Puerto Rico	55
TSI	Toscani in Italia	98
YRI	Yoruba in Ibadan, Nigera	88

#11

#9



Supplement 2: Growth chart of three subjects (#1, #9 and #11)

PROTEIN	Processed by PC1/3	Hormone Gene Symbol & Gene ID #	Mortality (Y/N)	PMID#	Hormone Gene Symbol & Gene ID #	Mortality (Y/N)	PMID#
Hormone - peptide					**Hormone - receptor**		
Cholecytokinin	X	Cck: 12424	N	10330022	Cckar(1): 12425	N	15314689
Gastrin	X	Gast: 14459	N	9287997	Cckbr(2): 12426	N	8978369; 8876222; 12688382
Ghrelin	X	Ghrl: 58991	N	14585959	Ghsr: 208188	N	16322794
Glucose-dependent insulinotropic polypeptide	X	Gip: 14607	N/D	N/D	Gipr: 381853	N	10611300
Guanylin	N/D	Guca2a:14915	N	12466132; 15026148	Gucy2c	N	9344852; 21865642
Uroguanylin	N/D	Guca2b:14916	N	14561709	Gucy2c	N	9344852; 21865642
Peptide YY	X	Pyy: 217212	N	16680491	Npy1r: 18166, Npy2r: 18167, Npy6r: 18169	N	12167864
Neurotensin/neuromedin N	X	Nts: 67405	N	11427716	Ntsr1: 18216, Ntsr2: 18217	N	15030383
GLP-1	X	Gcg: 14526	N	22928026	Gcgr: 14527, Glp1r: 14652	N	8898756
GLP-2	X	Gcg: 14526	N	22928026	Glp2r: 93896	N	20546737
Secretin	X	Sct: 20287	N	18534766	Sctr: 319229	N	17283064
Somatostatin	X	Sst: 20604	N	11430867	Sstr1: 20605, Sstr2: 20606, Sstr5: 20609	N	12752788
Galanin	X	Gal: 14419	N	22698811	Galr1 14427:, Galr2: 14428, Galr3: 14429	N	21179451, 18554714,17974627
Endocrine secreted protein							
Chromogranin A	X	Chga: 12652	N	16556729	N/A		
Chromogranin B	X	Chgb: 12653	N/D		N/A		
Chromogranin C	X	Scg2: 20254	N/D		N/A		
Others							
Neurogenin 3	N	Neurog3: 11925	Y	12456641	N/A		
PC1/3	X	Pcsk1: 18548	Y	12145326	N/A		

Supplement 3: Gut hormone/receptor null models and the risk of early postnatal mortality

Supplement 1:

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