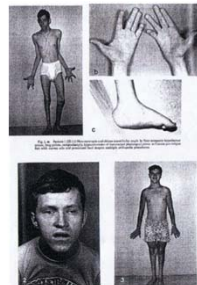


Investigating SNPs in the ZC4H2, FGD1, and MCT8 Genes in Humans

Kim Stauffer
Dr. Charles Schwartz, Melanie May
Greenwood Genetic Center

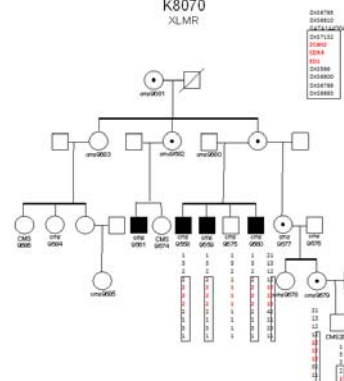
December 8, 2011

K8070

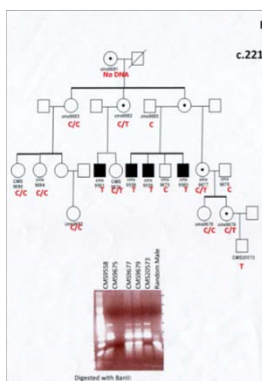


- Three genes in linkage region
 - ZC4H2
 - CDX4
 - ED1

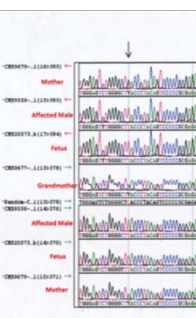
- Severe Mental Retardation
- Postnatal Retardation of growth
- Facial Features:
 - Midface hypoplasia
 - Medial eyebrow flare
 - Exotropia +/- ptosis
 - Obtuse mandibular angle
 - High narrow palate
 - Open mouth/drooling
- Hands
 - Camptodactyly of fifth finger
 - Hyperextensible metacarpal phalangeal joints
 - Long hands
 - Long palm
 - Syndactyly-soft tissue
 - Proximally placed thumbs
- Sidely Line
- Feet and Legs
 - Hallux valgus
 - Hypoplastic fifth nail
 - Open hallux pattern
 - Distal muscle wasting
- Orthopedic-Other
 - Thoracic scoliosis
- Exotropia
- Microcephaly
- Low finterip arches



K8070
XL MR



K8070
CDX4
c.221C>Tp.574L



*Tested 739 Normal Chromosomes

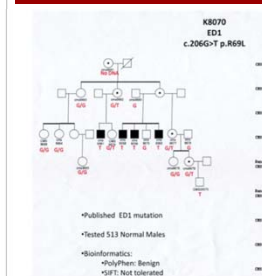
*Bioinformatics:
• PolyPhen: Damaging
• SIFT: Not tolerated
• Panther: position does not align
• Protran: difference in Alpha and flexible region

CDX4 EST Profile

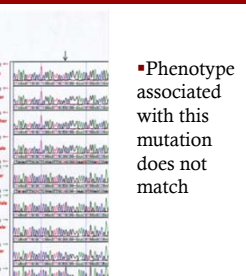
EST Profile	Accession	Gene	Species	Chromosome	Start	End	Orientation
AF040401	U14641	CDX4	Human	10	111,111,111	111,111,111	+
AF040402	U14642	CDX4	Human	10	111,111,111	111,111,111	+
AF040403	U14643	CDX4	Human	10	111,111,111	111,111,111	+
AF040404	U14644	CDX4	Human	10	111,111,111	111,111,111	+
AF040405	U14645	CDX4	Human	10	111,111,111	111,111,111	+
AF040406	U14646	CDX4	Human	10	111,111,111	111,111,111	+
AF040407	U14647	CDX4	Human	10	111,111,111	111,111,111	+
AF040408	U14648	CDX4	Human	10	111,111,111	111,111,111	+
AF040409	U14649	CDX4	Human	10	111,111,111	111,111,111	+
AF040410	U14650	CDX4	Human	10	111,111,111	111,111,111	+
AF040411	U14651	CDX4	Human	10	111,111,111	111,111,111	+
AF040412	U14652	CDX4	Human	10	111,111,111	111,111,111	+
AF040413	U14653	CDX4	Human	10	111,111,111	111,111,111	+
AF040414	U14654	CDX4	Human	10	111,111,111	111,111,111	+
AF040415	U14655	CDX4	Human	10	111,111,111	111,111,111	+
AF040416	U14656	CDX4	Human	10	111,111,111	111,111,111	+
AF040417	U14657	CDX4	Human	10	111,111,111	111,111,111	+
AF040418	U14658	CDX4	Human	10	111,111,111	111,111,111	+
AF040419	U14659	CDX4	Human	10	111,111,111	111,111,111	+
AF040420	U14660	CDX4	Human	10	111,111,111	111,111,111	+
AF040421	U14661	CDX4	Human	10	111,111,111	111,111,111	+
AF040422	U14662	CDX4	Human	10	111,111,111	111,111,111	+
AF040423	U14663	CDX4	Human	10	111,111,111	111,111,111	+
AF040424	U14664	CDX4	Human	10	111,111,111	111,111,111	+
AF040425	U14665	CDX4	Human	10	111,111,111	111,111,111	+
AF040426	U14666	CDX4	Human	10	111,111,111	111,111,111	+
AF040427	U14667	CDX4	Human	10	111,111,111	111,111,111	+
AF040428	U14668	CDX4	Human	10	111,111,111	111,111,111	+
AF040429	U14669	CDX4	Human	10	111,111,111	111,111,111	+
AF040430	U14670	CDX4	Human	10	111,111,111	111,111,111	+
AF040431	U14671	CDX4	Human	10	111,111,111	111,111,111	+
AF040432	U14672	CDX4	Human	10	111,111,111	111,111,111	+
AF040433	U14673	CDX4	Human	10	111,111,111	111,111,111	+
AF040434	U14674	CDX4	Human	10	111,111,111	111,111,111	+
AF040435	U14675	CDX4	Human	10	111,111,111	111,111,111	+
AF040436	U14676	CDX4	Human	10	111,111,111	111,111,111	+
AF040437	U14677	CDX4	Human	10	111,111,111	111,111,111	+
AF040438	U14678	CDX4	Human	10	111,111,111	111,111,111	+
AF040439	U14679	CDX4	Human	10	111,111,111	111,111,111	+
AF040440	U14680	CDX4	Human	10	111,111,111	111,111,111	+
AF040441	U14681	CDX4	Human	10	111,111,111	111,111,111	+
AF040442	U14682	CDX4	Human	10	111,111,111	111,111,111	+
AF040443	U14683	CDX4	Human	10	111,111,111	111,111,111	+
AF040444	U14684	CDX4	Human	10	111,111,111	111,111,111	+
AF040445	U14685	CDX4	Human	10	111,111,111	111,111,111	+
AF040446	U14686	CDX4	Human	10	111,111,111	111,111,111	+
AF040447	U14687	CDX4	Human	10	111,111,111	111,111,111	+
AF040448	U14688	CDX4	Human	10	111,111,111	111,111,111	+
AF040449	U14689	CDX4	Human	10	111,111,111	111,111,111	+
AF040450	U14690	CDX4	Human	10	111,111,111	111,111,111	+
AF040451	U14691	CDX4	Human	10	111,111,111	111,111,111	+
AF040452	U14692	CDX4	Human	10	111,111,111	111,111,111	+
AF040453	U14693	CDX4	Human	10	111,111,111	111,111,111	+
AF040454	U14694	CDX4	Human	10	111,111,111	111,111,111	+
AF040455	U14695	CDX4	Human	10	111,111,111	111,111,111	+
AF040456	U14696	CDX4	Human	10	111,111,111	111,111,111	+
AF040457	U14697	CDX4	Human	10	111,111,111	111,111,111	+
AF040458	U14698	CDX4	Human	10	111,111,111	111,111,111	+
AF040459	U14699	CDX4	Human	10	111,111,111	111,111,111	+
AF040460	U14700	CDX4	Human	10	111,111,111	111,111,111	+
AF040461	U14701	CDX4	Human	10	111,111,111	111,111,111	+
AF040462	U14702	CDX4	Human	10	111,111,111	111,111,111	+
AF040463	U14703	CDX4	Human	10	111,111,111	111,111,111	+
AF040464	U14704	CDX4	Human	10	111,111,111	111,111,111	+
AF040465	U14705	CDX4	Human	10	111,111,111	111,111,111	+
AF040466	U14706	CDX4	Human	10	111,111,111	111,111,111	+
AF040467	U14707	CDX4	Human	10	111,111,111	111,111,111	+
AF040468	U14708	CDX4	Human	10	111,111,111	111,111,111	+
AF040469	U14709	CDX4	Human	10	111,111,111	111,111,111	+
AF040470	U14710	CDX4	Human	10	111,111,111	111,111,111	+
AF040471	U14711	CDX4	Human	10	111,111,111	111,111,111	+
AF040472	U14712	CDX4	Human	10	111,111,111	111,111,111	+
AF040473	U14713	CDX4	Human	10	111,111,111	111,111,111	+
AF040474	U14714	CDX4	Human	10	111,111,111	111,111,111	+
AF040475	U14715	CDX4	Human	10	111,111,111	111,111,111	+
AF040476	U14716	CDX4	Human	10	111,111,111	111,111,111	+
AF040477	U14717	CDX4	Human	10	111,111,111	111,111,111	+
AF040478	U14718	CDX4	Human	10	111,111,111	111,111,111	+
AF040479	U14719	CDX4	Human	10	111,111,111	111,111,111	+
AF040480	U14720	CDX4	Human	10	111,111,111	111,111,111	+
AF040481	U14721	CDX4	Human	10	111,111,111	111,111,111	+
AF040482	U14722	CDX4	Human	10	111,111,111	111,111,111	+
AF040483	U14723	CDX4	Human	10	111,111,111	111,111,111	+
AF040484	U14724	CDX4	Human	10	111,111,111	111,111,111	+
AF040485	U14725	CDX4	Human	10	111,111,111	111,111,111	+
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AF040488	U14728	CDX4	Human	10	111,111,111	111,111,111	+
AF040489	U14729	CDX4	Human	10	111,111,111	111,111,111	+
AF040490	U14730	CDX4	Human	10	111,111,111	111,111,111	+
AF040491	U14731	CDX4	Human	10	111,111,111	111,111,111	+
AF040492	U14732	CDX4	Human	10	111,111,111	111,111,111	+
AF040493	U14733	CDX4	Human	10	111,111,111	111,111,111	+
AF040494	U14734	CDX4	Human	10	111,111,111	111,111,111	+
AF040495	U14735	CDX4	Human	10	111,111,111	111,111,111	+
AF040496	U14736	CDX4	Human	10	111,111,111	111,111,111	+
AF040497	U14737	CDX4	Human	10	111,111,111	111,111,111	+
AF040498	U14738	CDX4	Human	10	111,111,111	111,111,111	+
AF040499	U14739	CDX4	Human	10	111,111,111	111,111,111	+
AF040500	U14740	CDX4	Human	10	111,111,111	111,111,111	+

- No human EST profile
- Mouse Profile shows no expression of this gene in the brain

ED1 Profile



K8070
ED1
c.206G>T p.R69L



*Published ED1 mutation

*Tested 513 Normal Males

*Bioinformatics:
• PolyPhen: Benign
• SIFT: Not tolerated
• Panther: Weak

- Phenotype associated with this mutation does not match

ZCH42 Profile

**K8970
ZCH42
c.484T>A p.L43H**

- Confirmed by the Solids machine at GGC
- Conserved in chimpanzee, dog, cow, mouse, rat, chicken, zebrafish, fruit fly, mosquito

EST Profile of ZCH42

Breakdown by Body Sites

adipose tissue	0	0/13106
adipose gland	168	2/22186
airway	0	0/40089
blood	43	0/20472
bone	43	0/12347
bone marrow	0	0/80795
breast	71	78/13134
colon	28	1/50157
connective tissue	28	3/14326
esophagus	0	0/20221
esophageal tissue	27	0/21854
heart	17	0/20423
intestine	37	0/21788
kidney	17	0/21443
lung	0	0/20782
muscle	133	0/44279
muscle tissue	18	1/8181
pancreas gland	52	0/15226
pancreas	0	0/8767
skin	0	0/16763
stomach	19	2/10295
testis	0	0/24798
testis germinal	48	1/20325
uterus	0	0/41225
uterus gland	0	0/14891
uterus muscle	0	0/20579
uterus stroma	15	0/18420
vagina	0	0/20952
vagina muscle	18	1/13861
vagina stroma	19	1/84881
vein	151	2/138311
vein blood	292	22/81128
vein wall	0	0/47430
vein wall muscle	0	0/14888
vein wall stroma	0	0/52489

Breakdown by Health State

adipose tissue	0	0/13107
adipose gland	168	1/222029
blood	38	2/81774
bone	43	1/12758
breast carcinoma	0	0/14722
breast (mammary gland) tumor	21	2/19424
colorectal cancer	0	0/24252
colon carcinoma	24	2/82119
esophageal cancer	0	0/14224
esophageal tumor	0	0/17254
esophageal tumor	0	1/14332
genitourinary tumor	281	2/12849
glioma	64	0/16879
head and neck tumor	0	0/16320
kidney tumor	72	0/88873
leukemia	35	0/88873
liver tumor	20	2/18632
lymphoma	0	0/16298
lung cancer	138	1/16298
lymphoma	138	1/16298
lymphoma	20	0/87241
lymphoma	20	1/20180
lymphoma	20	2/17678
pancreatic tumor	19	2/104910
pancreatic neuroendocrine tumor	127	1/128810
prostate cancer	19	2/18294
retroplacental	0	0/16251
skin tumor	0	0/14428
soft tissue sarcoma	23	0/12189
uterine tumor	22	2/18228

ZCH42 Bioinformatics

- A Zinc finger gene, c.484T>A in Exon 1
- Polyphen: **damaging**
- SIFT: tolerated
- ipTree: **destabilizing**

ZCH42 Polymorphism Study

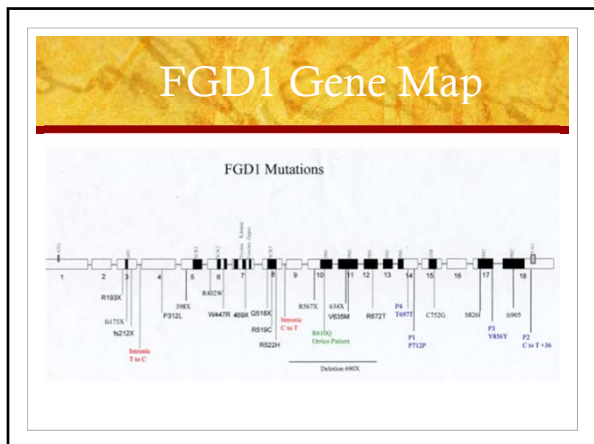
Plate	Patients that Amplified	Mutation Band present
Random 1	71	0
Lander Students	43	0
Random 3	76	0
UPD Males 1	58	0
Random 4	48	0
CEPH Males	84	0
Random Female Plate 1	132	0
Random Female Plate 2	118	0
Random Female Plate 3	122	0
Random Female Plate 4	68	0
Random Female Plate 5	122	0
Random Female Plate 6	180	0
Lander Female Plate 1	180	0
Lander Female Plate 2	180	0
SCS Black Females	180	0
Total:	1302	0

FGD1

- Looking at family K9195
- Defects in this gene are the cause of facio-genital dysplasia/Aarskog-Scott syndrome and XLID
- Short stature, hypertelorism, shawl scrotum, brachydactyly

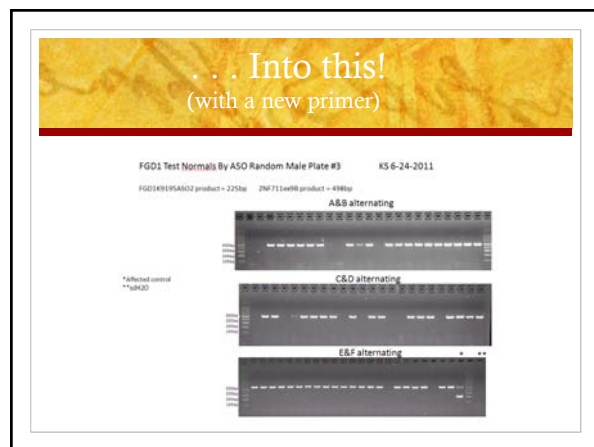
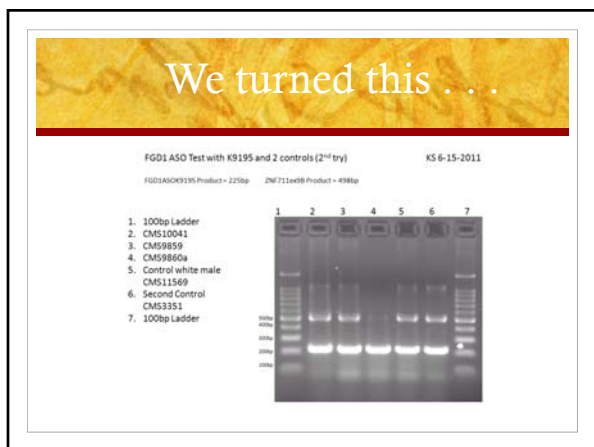
FGD1

- Exon 1
- c.81 A>G p.H94R
- Gene encodes a protein that binds to a GTPase
- This protein also stimulates a mitogen activated protein kinase cascade



FGD1 Bioinformatics

Protein Website	Amino Acid Change in Protein	Effect
IPTR3E-STAB	H94R	Negative(stabilizing)
Mupro	H94R	Delta delta G = increase stability
I-Mutant2.0 (gpcr)	H94R	Decrease stability
Panther	H94R	Position does not align to the HMM
PolyPhen	H94R	Benign



FGD1 Polymorphism Study

Sex	Patients that amplified	Mutation Band present
Random Male 1	82	0
Random Male 3	56	0
Random Male 4	57	0
Random Male 6	14	0
Random Female 1	140	0
Random Female 2	158	0
Random Female 3	176	0
Random Female 4	188	0
Random Female 5	186	0
Total:	1057	0

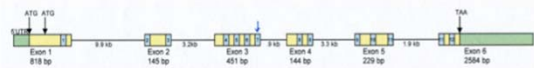
Allan-Herndon-Dudley Syndrome

K9591

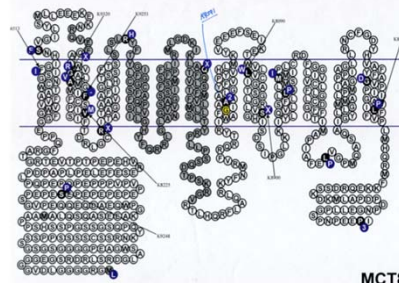
- Developmental Delay
- No Speech
- Inadequate head control
- truncal muscle hypotonia
- slightly spastic limbs
- Elevated T3 levels
- Decreased T4 level
- No clinical signs of hypothyroidism

MCT8- Monocarboxylate Transporter 8

- 7th Transmembrane Domain
- Very active and specific thyroid hormone transporter
- Exon 3
 - c.1201G>A p.G401R
 - Conserved in humans, pigs, dogs, etc.



MCT8 Protein Structure



MCT8

MCT8 Conservation Across Species

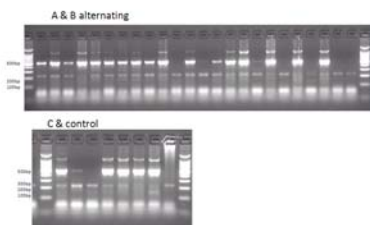
MCT8
c.1201G>A p.G401R
K9591

Homo Sapiens	MRVFFQRTYRINAFGI I AAAALGYFVYV
Sus scrofa (pig)	MRVFFQRTYRINAFGI I AAAALGYFVYV
Canis familiaris (dog)	MRVFFQRTYRINAFGI I AAAALGYFVYV
Pongo abelii (Sumatran orangutan)	MRVFFQRTYRINAFGI I AAAALGYFVYV
Macaca mulatta (rhesus monkey)	MRVFFQRTYRINAFGI I AAAALGYFVYV
Mus musculus (mouse)	MRVFFQRTYRINAFGI I AAAALGYFVYV
Rattus norvegicus (rat)	MRVFFQRTYRINAFGI I AAAALGYFVYV
Equus caballus (horse)	MRVFFQRTYRINAFGI I AAAALGYFVYV
Bos taurus (cow)	MRVFFQRTYRINAFGI I AAAALGYFVYV
Alliurotopoda melanoleuca (giant panda)	MRVFFQRTYRINAFGI I AAAALGYFVYV
Gallus gallus (chicken)	LRVFFRRTYRINAFGIATNVLGYFVFM
Taeniopygia guttata (zebra finch)	LRVFFRRTYRINAFGIATNVLGYFVFM
Drosophila persimilis	VEIWRKRFRVIVLQVPLALFGYFVYV

MCT8 Bioinformatics

Protein Website	Amino Acid Change in Protein	Effect
Panther	G401R	0.76208 probability of having a deleterious effect
PolyPhen	G401R	Probably damaging
IPTREE-STAB	G401R	Negative(destabilizing)
RESCUE-ESE	G401R	No discernible affect
Mupro	G401R	Delta delta G - increase stability
MUstab	G401R	Decreased protein stability, prediction confidence = 81.61%
I-mutant	G401R	Decrease in stability

MCT8 ASO Random Male #6 KS 6-20-2011
MCT8ASO#9591 product - 227bp 2N711e09# product - 498 bp



- After this gel, got all new buffer, water, etc. to eliminate possibility of contamination.
 - Still got bad results.
- Tried raising the temperature and adding more and less primer
- Nothing got rid of the mystery bands
 - Decided to sequence the samples for best accuracy

MCT8 Sequencing

Waltz	Patients that Amplified	Base change present
Random Male 1	80	No
Random Male 3	54	No
Random Male 4	47	No
Random Female 1	172	No
Random Female 2	170	No
Random Female 3	186	No
Random Female 4	186	No
Random Female 5	178	No
Total:	1073	0

