

# Natural helix 9 mutants of PPAR $\gamma$ differently affect its transcriptional activity

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## Supplemental Methods

R script to determine the association of common genetic variants with partial lipodystrophic phenotype.

```
library("ggplot2")
setwd("53SNPs")

# Read relevant data
dosage <- read.table("UKHLS.dosage.txt.assoc.dosage", sep=",", header=F, stringsAsFactors=F,
col.names=c("CHR", "SNP", "BP", "A1", "A2", "FRQ", "INFO", "OR", "SE", "P"), skip=1 )

head(dosage)

gts <- read.table("plink.ped", sep=' ', header=F, stringsAsFactors=F)
head(gts[,1:10])

snpinfo <- read.table("SNPdata.txt", sep='\t', header=T, stringsAsFactors=F)
colnames(gts) <- c("sample", "family", "pat", "mat", "gender", "phenotype", as.character(snpinfo$SNP))

# Selecting females only
gts <- subset(gts, gender=="2")

# Determine where reference allele != non-risk allele
snpinfo$flip <- snpinfo$Effect.Allele!=dosage$A1

# Convert genotypes to risk scores
scores <- gts[,7:ncol(gts)]
scores[scores==0]<-NA
scores[scores==11]<-0
scores[scores==12]<-1
scores[scores==21]<-1
scores[scores==22]<-2

# Adjust risk scores for 'flipped' positions
scores[,snpinfo$SNP[snpinfo$flip]] <- scores[,snpinfo$SNP[snpinfo$flip]]-2
scores <- abs(scores)

# Determine missing genotypes to prevent over scoring
liponas <- apply(scores, 1, function(x) sum(is.na(x)))
liporisk <- rowSums(scores, na.rm=T)
liporiskfrac <- liporisk/((53-liponas)*2)
rownames(scores) <- gts$sample
```

```

scores$liporiskfrac <- liporiskfrac
scores$riskalleles <- scores$liporiskfrac*106
# Plot results of patient relative to population
pdf(file="LipoRiskDistribution_Paper.pdf",width=6,height=10,useDingbats=F, pointsize=10 )
aplot <- ggplot(scores, aes(x=riskalleles)) + geom_histogram(binwidth=1, fill="#4396F0",
color="#4396F0", alpha=.5)
bplot <- aplot + ylab("UKHLS controls, n") + xlab("Number of risk alleles") + theme_bw() +
theme(panel.border = element_blank(), panel.grid.major = element_blank(), panel.grid.minor =
element_blank(), axis.line = element_line(colour = "black")) +
scale_x_continuous(breaks=round(seq(40,80,by=5),1)) +
scale_y_continuous(breaks=round(seq(0,900,by=100),1))
cplot <- bplot + theme(axis.text = element_text(size=15)) + theme(axis.title = element_text(size=20))
print(cplot + geom_vline(xintercept=53, color="red"))
dev.off()
# Calculate z-score
pop_sd <- sd(scores$riskalleles)*sqrt((length(scores$riskalleles)-1)/(length(scores$riskalleles)))
pop_mean <- mean(scores$riskalleles)
zscore <- (53 - pop_mean)/pop_sd
print(zscore)

```

## Supplemental figures

**Supplemental figure 1. Structural based sequence alignment of the amino acid residues surrounding PPAR $\gamma$  L451P between human PPARs and other members of the nuclear hormone superfamily.** Human PPAR $\gamma$  (NP\_056953.2), human PPAR $\alpha$  (CAG30433), human PPAR $\delta$  (Q03181), chimpanzee PPAR $\gamma$  (XP\_001153669.1), mouse PPAR $\gamma$  (NP\_035276.2), rat PPAR $\gamma$  (NP\_037256.1), cow PPAR $\gamma$  (NP\_851367.1), dog PPAR $\gamma$  (NP\_001019803.1), chicken PPAR $\gamma$  (NP\_001001460.1), and zebrafish PPAR $\gamma$  (NP\_571542.1). THRA, thyroid receptor alpha (NP\_003241.2); THRB, thyroid receptor beta (AAI06931.1); GR, glucocorticoid receptor (NP\_000167.1); MR, mineralocorticoid receptor (NP\_000892.2); AR, androgen receptor (P10275.2); FXR, farnesoid X receptor (NP\_001193908.1); VDR, vitamin D receptor (AAH60832.1); HNF $\alpha$ , Hepatocyte nuclear factor 4A (CAA61133.1). Residues analogous L451 and V318 are designated in yellow and bold. Residues analogous to 314, 316, S317, K347, and K422 are indicated in pink and bold. PPAR $\gamma$ 2 nomenclature. Dashes (-) represent the gaps included for appropriate alignment of the nuclear hormone receptors. The boundaries of the  $\alpha$ -helices 9, and 10 are also shown.

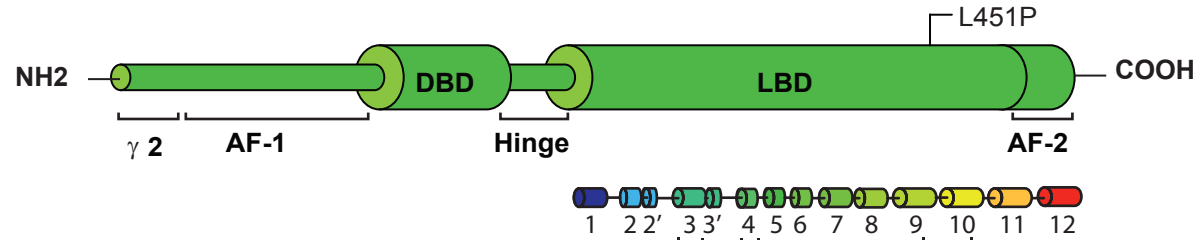
**Supplemental figure 2. Proline substitutions in helix 9 impair transcriptional activity of PPAR $\gamma$ .** U2OS cells were transiently cotransfected with expression vectors encoding PPAR $\gamma$  WT or PPAR $\gamma$  mutants respectively, and 3x peroxisome proliferator response element (PPRE)-Tk-Luc reporter. Activation of the luciferase reporter, in the absence or presence of 1  $\mu$ M 15d-PGJ2, is expressed as fold induction over that with empty vector (EV). Results are averages of at least three independent experiments assayed in duplicate  $\pm$  SEM. \*P<0.05 cells transfected with mutant vs. WT. Expression levels of the different proteins were confirmed by Western blot analysis using a PPAR $\gamma$  specific antibody. The arrow indicates PPAR $\gamma$  and the asteriks indicates an unknown non-specific band. WT, wildtype.

**Supplemental figure 3. Cofactor peptide binding profiles for GST-PPAR $\gamma$  LBD WT and L451P.** GST-proteins were incubated with DMSO or 10  $\mu$ M rosiglitazone. Images of all arrays were quantified and binding (AU; arbitrary units) was plotted. The experiments were performed in triplo.

**Supplemental Table 1. SNP genotype of PPAR $\gamma$  L451P index patient.**

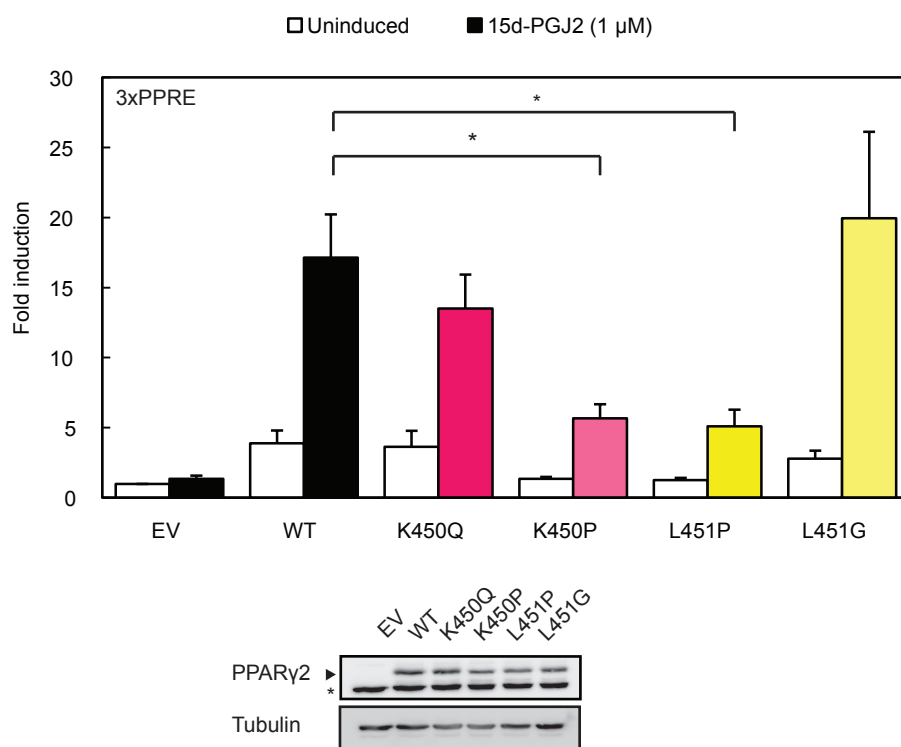
PCR was performed in the index patient to genotype the lead SNPs in 53 genomic regions that were previously identified to impair peripheral adipose tissue storage (51). The index patient harbors 53 risk alleles.

Supplemental figure 1. Structure sequence alignment of the amino acid residues surrounding PPAR $\gamma$  L451P between human PPARs and other members of the nuclear receptor superfamily



		Helix 3		Helix 4		Helix 9		Helix 10							
Homo sapiens	PPAR $\gamma$	...	319	FQGGQ <b>Q</b> RSV <b>E</b> AVQ	331	...	339	LNDQV <b>T</b> LL <b>K</b> Y	348	...	445	LELQ <b>I</b> KL <b>N</b>	HP <b>E</b> SSQ	L <b>F</b> AKLL	464
Homo sapiens	PPAR $\alpha$	...	273	FHCC <b>Q</b> TSV <b>E</b> TVT	285	...	275	LNDQV <b>T</b> LL <b>K</b> Y	284	...	408	LRLH <b>I</b> Q <b>S</b> N	HP <b>D</b> DI <b>F</b>	L <b>F</b> PKLL	427
Homo sapiens	PPAR $\delta$	...	246	FYRC <b>Q</b> TV <b>E</b> TVR	258	...	302	LNDQV <b>T</b> LL <b>K</b> Y	311	...	381	LE <b>F</b> HI <b>O</b> AN	HP <b>D</b> AQ <b>Y</b>	L <b>F</b> PKLL	400
Pan troglodytes	PPAR $\gamma$	...	319	FQGG <b>Q</b> RSV <b>E</b> AVQ	331	...	339	LNDQV <b>T</b> LL <b>K</b> Y	348	...	445	LELQ <b>I</b> KL <b>N</b>	HP <b>E</b> SSQ	L <b>F</b> AKLL	464
Mus musculus		...	319	FQGG <b>Q</b> RSV <b>E</b> AVQ	331	...	339	LNDQV <b>T</b> LL <b>K</b> Y	348	...	445	LELQ <b>I</b> KL <b>N</b>	HP <b>E</b> SSQ	L <b>F</b> AKVL	464
Rattus norvegicus		...	319	FQGG <b>Q</b> RSV <b>E</b> AVQ	331	...	339	LNDQV <b>T</b> LL <b>K</b> Y	348	...	445	LELQ <b>I</b> KL <b>N</b>	HP <b>E</b> SSQ	L <b>F</b> AKVL	464
Bos taurus		...	319	FQGG <b>Q</b> RSV <b>E</b> AVQ	331	...	339	LNDQV <b>T</b> LL <b>K</b> Y	348	...	445	LELQ <b>I</b> KL <b>N</b>	HP <b>E</b> SSQ	L <b>F</b> AKLL	464
Canis lupus		...	319	FQGG <b>Q</b> RSV <b>E</b> AVQ	331	...	339	LNDQV <b>T</b> LL <b>K</b> Y	348	...	445	LELQ <b>I</b> KL <b>N</b>	HP <b>E</b> SSQ	L <b>F</b> AKLL	464
Gallus gallus		...	289	FQRC <b>Q</b> RSV <b>E</b> AVQ	301	...	309	LNDQV <b>T</b> LL <b>K</b> Y	318	...	415	LELQ <b>I</b> KL <b>N</b>	HP <b>E</b> SSQ	L <b>F</b> AKLL	434
Danio rerio		...	341	FHS <b>V</b> Q <b>R</b> SA <b>E</b> AIS	353	...	361	LNDQV <b>T</b> LL <b>K</b> Y	370	...	467	LELQ <b>I</b> KL <b>N</b>	HP <b>D</b> SL <b>Q</b>	L <b>F</b> AKVL	486
PPAR $\gamma$		...	319	FQGG <b>Q</b> RSV <b>E</b> AVQ	331	...	339	LNDQV <b>T</b> LL <b>K</b> Y	348	...	445	LELQ <b>I</b> KL <b>N</b>	HP--ESSQ	L <b>F</b> AKLL	464
THR $\alpha$		...	224	FSE <b>F</b> TR <b>I</b> IT <b>P</b> AIT	236	...	244	CE <b>D</b> Q <b>I</b> ILL <b>K</b> G	253	...	349	FE <b>H</b> Y <b>V</b> NR	KH--NIP <b>H</b>	F <b>W</b> PKLL	468
THR $\beta$		...	278	FS <b>H</b> ET <b>R</b> IIT <b>P</b> AIT	290	...	298	CE <b>D</b> Q <b>I</b> ILL <b>K</b> G	307	...	403	FE <b>H</b> Y <b>I</b> NY <b>R</b>	KH--HV <b>T</b> H	F <b>W</b> PKLL	422
GR		...	569	MT <b>T</b> LN <b>L</b> GG <b>R</b> QVI	581	...	589	L <b>D</b> DQ <b>M</b> TLL <b>Q</b> Y	598	...	696	LG <b>K</b> A <b>I</b> V <b>K</b> R	EG--N <b>S</b> SQ	N <b>W</b> Q <b>R</b> F <b>Y</b>	716
MR		...	775	L <b>S</b> T <b>I</b> LN <b>L</b> AG <b>K</b> QMI	787	...	795	L <b>E</b> DQ <b>I</b> TLL <b>Q</b> Y	804	...	903	L <b>R</b> K <b>M</b> V <b>T</b> K <b>C</b>	PN--N <b>S</b> GQ	S <b>W</b> Q <b>R</b> F <b>Y</b>	922
AR		...	710	L <b>S</b> S <b>I</b> LN <b>L</b> GE <b>R</b> QLV	722	...	730	V <b>D</b> DQ <b>M</b> AVI <b>Q</b> Y	739	...	838	L <b>D</b> R <b>I</b> L <b>A</b> CK	R <b>K</b> --N <b>P</b> TS	C <b>S</b> R <b>R</b> F <b>Y</b>	857
FXR		...	297	FL <b>I</b> L <b>T</b> EM <b>A</b> T <b>N</b> HVQ	309	...	317	H <b>E</b> DQ <b>I</b> ALL <b>K</b> G	326	...	419	L <b>Q</b> K <b>L</b> Q <b>K</b> I <b>H</b>	Q <b>P</b> --E <b>N</b> PQ	H <b>F</b> AC <b>L</b> L	438
VDR		...	236	L <b>P</b> H <b>L</b> AL <b>L</b> V <b>S</b> YSIQ	248	...	256	S <b>E</b> DQ <b>I</b> VLL <b>K</b> S	265	...	363	L <b>Q</b> TY <b>I</b> RC <b>R</b>	H <b>P</b> PPG <b>S</b> HL	L <b>Y</b> AK <b>M</b> I	384
HNF4 $\alpha$		...	184	-- <b>V</b> C <b>E</b> SM <b>K</b> EQLL	196	...	204	L <b>D</b> DQ <b>V</b> ALL <b>R</b> A	213	...	310	L <b>E</b> DY <b>I</b> ND <b>R</b>	Q <b>Y</b> --D <b>S</b> RG	R <b>F</b> G <b>E</b> LL	329

Supplemental figure 2. Proline substitutions in helix 9 impair transcriptional activity of PPAR $\gamma$ .







Supplemental table 1: SNP genotype of PPAR $\gamma$  L451P index patient

SNP	Chromosome	Position	Effect Allele	Other Allele	Genotype patient	Risk Allele score
rs683135	1	39895460	A	G	GA	1
rs17386142	1	50815783	C	T	CC	2
rs11577194	1	110500175	T	C	CC	0
rs9425291	1	172312769	A	G	GG	0
rs4846565	1	219722104	G	A	GG	2
rs2249105	2	65287896	A	G	AG	1
rs10195252	2	165513091	T	C	CC	0
rs492400	2	219349752	T	C	TT	2
rs2943645	2	227099180	T	C	CT	1
rs308971	3	12116620	G	A	GG	2
rs3864041	3	15185634	T	C	CC	0
rs295449	3	47375955	A	G	AG	1
rs11130329	3	52896855	A	C	AA	2
rs9881942	3	123082416	A	G	AG	1
rs645040	3	135926622	T	G	TT	2
rs2699429	4	3480136	C	T	CT	1
rs3822072	4	89741269	A	G	GA	1
rs6822892	4	157734675	A	G	GG	0
rs4865796	5	53272664	A	G	AA	2
rs459193	5	55806751	G	A	AG	1
rs4976033	5	67714246	G	A	AG	1
rs6887914	5	112711486	C	T	CT	1
rs1045241	5	118729286	C	T	TT	0
rs2434612	5	158022041	G	A	AG	1
rs966544	5	173350405	G	A	AA	1
rs12525532	6	35004819	T	C	CT	1
rs6937438	6	43815364	A	G	AA	2
rs2745353	6	127452935	T	C	TT	2
rs9492443	6	130398731	C	T	CT	1
rs3861397	6	139828916	G	A	AG	1
rs17169104	7	15883727	G	C	CG	1
rs972283	7	130466854	G	A	AG	1
rs2126259	8	9185146	T	C	CC	0
rs1011685	8	19830769	C	T	CC	2
rs4738141	8	72469742	G	A	AA	0
rs7005992	8	126528955	C	G	CG	1
rs498313	9	78034169	A	G	AG	1
rs10995441	10	64869239	G	T	TT	0
rs11231693	11	63862612	A	G	GG	0
rs17402950	12	14571671	G	A	AA	0
rs718314	12	26453283	G	A	AG	1
rs7973683	12	124449223	C	A	CC	2
rs7323406	13	111628195	A	G	GG	0
rs7176058	15	39464167	A	G	AG	1
rs8032586	15	73081067	C	T	CG	1
rs754814	17	4657034	T	C	CC	0
rs7227237	18	47174679	C	T	CC	2
rs8101064	19	7293119	T	C	CC	0
rs4804833	19	7970635	A	G	GG	0
rs4804311	19	8615589	A	G	AA	2
rs731839	19	33899065	G	A	AG	1
rs6066149	20	45602638	G	A	GG	2
rs132985	22	38563471	C	T	CC	2