Online Supplementary Methods

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Table 1: Details for the proband's insulin requirement and diabetes control

Treatment and glycaemic control- Proband		**HbA1c (normal range HbA1C 4.4-6.4%)**
Initially	Actrapid 12/15/12/-	
1 year later	Actrapid 18/22/14/- Insulatard -/ - / - /8	HbA1c 6.4%
During pregnancy	Actrapid 6/6/6/- Insulatard -/ - / - /9	HbA1C 8.3%
Post-partum	no insulin required for 2 months	HbA1C 4.8 %
Current	Actrapid 4/4/4/- Insulatard -/ - / - /-	HbA1C 5.9 %

The proband was initiated on insulin therapy for diabetes from the age of 15 years. She has required variable insulin dosage. Notably, requiring less insulin throughout pregnancy and dietary treatment alone for 2 months post-natally and she is currently managed with gradually increasing insulin doses.

The substantial reduction in insulin requirement is at least in part due to the fact that the proband was initially diagnosed with type 1 diabetes and accordingly treated with replacement dose insulin (1.24IU/Kg/day). This resulted in recurrent hypoglycaemia and her insulin dosage was accordingly gradually reduced. A likely compounding factor is increased insulin requirements peri-puberty [1, 2] which coincided with the time of the probands diagnosis with diabetes.

Because of the pre-prandial hypoglycemia and postprandial hyperglycemia after delivery, low doses of short acting analogue are administrated, achieving good glycaemic control with HbA1c 5.9 % (DCCT).

FISH analysis

FISH was performed on patient metaphase chromosomes using labeled BACs and fosmids covering the entire genomic sequence and known promoters of genes within the break point regions. Probes were

selected using the University of California Santa Cruz (UCSC) genome browser (http://genome.ucsc.edu/) and obtained from the Children's Hospital Oakland Research Institute (CHORI) (http://bacpac.chori.org/).

Chromosome paints were used as per manufacturer's instructions. Labeled probes and chromosome paint were added onto slides of patient metaphase arrested chromosomes and incubated overnight to hybridise. Following hybridisation, the slides were washed and a secondary antibody added. Hybridisation efficiencies were >90%. Slides were mounted with Vectashield containing DAPI for chromosome counterstaining. A minimum of 20 metaphase spreads were analyzed by microscopy for each hybridisation experiment.

Gene dosage investigations

Multiplex Ligation-dependent Probe Amplification (MLPA) reactions were carried out according to the manufacturer's instructions using the EK1 reagent kit (MRC-Holland, Amsterdam, The Netherlands). Synthetic probes for *INSR* exons 11-17 (details available on request) and a set of previously described control probes were used at a concentration of 1.33 fmol/µl [3] and data analyzed as previously described [4]. SYBR green analysis was used to quantify *CHN2* gene dosage. To calculate dosage of *CHN2* exons 5-10 we used the absolute Blue QPCR SYBR green *ROX mix (Thermo Scientific, UK)* with oligonucleotides designed to *CHN2* exons 5-10 and GCK exon 1 (normal control). Real time PCR was carried out on the ABI 9700 and dosage quotients were calculated from average crossing points of triplicate samples, using the comparative Ct (ΔΔCt) method (3).

Table 2: Genomic *INSR* sequencing-primers

INSR fragment	Primer sequence
INSR_Exon 1- F	gtatttgtagctggcgaagc
INSR_Exon 1- R	cttgggtggggtcctctc
INSR_Exon 2- F	cccctgatccttctgatgc
INSR_Exon 2- R	gaacaaggcacgagacactg
INSR_Exon 3- F	gcatatgcagacaggaattgg
INSR_Exon 3- R	agttttaacaagcggcatcg
INSR_Exon 4- F	ccctccccttctctttctc
INSR_Exon 4- R	cagctcagagggacatggag
INSR_Exon 5- F	caccatggagaatcatgagaag
INSR_Exon 5- R	atgctgggattacaggcatc
INSR_Exon 6- F	aagatcaactccgagcatgg
INSR_Exon 6- R	caccagtccatggaaaaacc
INSR_Exon 7- F	cccaggctttggtctgaaac
INSR_Exon 7- R	tggagcacaaacgtagcaag
INSR_Exon 8- F	acttcccaggcctcatctg
INSR_Exon 8- R	tacaacctcactgcatcagc
INSR_Exon 9- F	ccagctttctttgcacactg

INSR_Exon 9- R	caaagtgcatcagacacacg
INSR_Exon 10- F	gtggggtgtgtgtgtgtg
INSR_Exon 10- R	gaccttccaccaacaccaag
INSR_Exon 11- F	gtggcctccaagtgtcagag
INSR_Exon 11- R	aagcatctgctctccagcac
INSR_Exon 12- F	ctcctgcgctctgatctttc
INSR_Exon 12- R	cttggtcagccttgatgtcc
INSR_Exon 13- F	atggacaggtggcagaagtg
INSR_Exon 13- R	aaggggcatgctgaagtg
INSR_Exon 14- F	acactcccagatgtgcaaag
INSR_Exon 14- R	taagcacagccccagtcag
INSR_Exon 15- F	ggagtggatgtgatttttgatg
INSR_Exon 15- R	gcatgttttcccccagag
INSR_Exon 16- F	gccttggggagtcttgtatg
INSR_Exon 16- R	gaaggcaaaggaagctgatg
INSR_Exon 17- F	agtgttttcgaggggtttg
INSR_Exon 17- R	aggaggcagagaaagggaag
INSR_Exons 18&19- F	gaggagaaccctggtgagtc
INSR_Exons 18&19- R	caacttccttctgaaatcaaacc
INSR_Exon 20- F	tgctaggaccaaggctgaag
INSR_Exon 20- R	cgctcttggctctcactagc
INSR_Exon 21- F	tgtgttggcatgtgtgtttg
INSR_Exon 21- R	acccttttcaacgaacacctc
INSR_Exon 22-1- F	cagactcacccaggacgtg
INSR_Exon 22-1- R	TGGACATGGTAGAGTCGTGAG
INSR_Exon 22-2- F	CCTTCCTAACAGTGCCTACCG
INSR_Exon 22-2- R	tgaaagcagcagctattggtc

Table 3: Primers designed to amplify *INSR* cDNA in 9 overlapping fragments

Fragment	Sequence
HIR1_F (INSR cDNA)	TGCGCACACGAGAAGGAC
HIR1_R (INSR cDNA)	TGACCAGCGCGTAGTTAAAG
HIR2_F (INSR cDNA)	CCCGAAGATTTCCGAGACC
HIR2_R (INSR cDNA)	TGCAGCCGTGTGACTTACAG
HIR3_F (INSR cDNA)	TCGAACGATGTTGGACTCATAG
HIR3_R (INSR cDNA)	GATTGTTGCCTCCTCGAATG
HIR4_F (INSR cDNA)	AAGACCATCGACTCGGTGAC
HIR4_R (INSR cDNA)	TCGAACTCCGTCACATTCTG
HIR5_F (INSR cDNA)	ACATTCGGACATCTTTTGAC
HIR5_R (INSR cDNA)	GGCCGAATCCTCATACTCAC
HIR6_F (INSR cDNA)	ATTGCCTCAAAGGGCTGAAG
HIR6_R (INSR cDNA)	GCCACATCAAGTGGACGAC
HIR7_F (INSR cDNA)	CTGAAGCCAAGGCTGATGAC
HIR7_R (INSR cDNA)	GCATGGAAACACATCACTGG
HIR8_F (INSR cDNA)	GAGAAAGAGGCAGCCAGATG
HIR8_R (INSR cDNA)	CCAAAGTCTCCAATTTTGACAG
HIR9_F (INSR cDNA)	GGTCGCCCATGATTTTACTG
HIR9_R (INSR cDNA)	AGAATCCGCCCGTTTTTC

Table 4: Primers for *CHN2*

Primer name	SNP included	Sequence
CHN2_Intron 6F	rs3750103	GGTGAAGGTGGGTGTCAAAG
CHN2_Intron 7R		TCCACCTGCCCTTTTGTAAC
CHN2_Intron 12F	rs3750099 and rs34971642	TGCAATTTCCTGAACCAGAG
CHN2_Exon 13R		TCTGCACAATCAGCTTTTGG

Primer sequences for *CHN2* used to amplify regions of patient genomic DNA containing potentially informative single nucleotide polymorphisms. SNPs with a minor allele frequency of above 0.1 were selected for investigation using HapMap.

Table 5: Primer sequences to amplify the entire genomic sequence of *JAZF1*

Primer name	Sequence
JAZF1_Exon 1F-1	GCAGGCCTAGCGAGATAAAG
JAZF1_Exon 1R-1	CCTGTCATGGTGCTACATCG
JAZF1_Exon 1F-2	AGCCCCTCCTCTCCTCAC
JAZF1_Exon 1R-2	ACCCGGGCCAACTAACTC
JAZF1_Exon 2F	CTGCTTAGCCTCTGTTACTTTGTG
JAZF1_Exon 2R	TCAATAAGCAGCAGATATAAGGTTG
JAZF1_Exon3F	CTATACATGGCCCAGGTTCC
JAZF1_Exon3R	GAGAAGGAGGAGAGGGGAGA
JAZF1_Exon 4F	GCTCCTGACAGTCCTTGCAC
JAZF1_Exon4R	CCCTGTTTCCATGTGGTTATG
JAZF1_Exon 5F-1	GGTTTCCGAAGTTTGACAGG
JAZF1_Exon 5R-1	GGGAAATGTGCTGAAGAACC
JAZF1_Exon 5F-2	TTGCACATGTAATCATCATACCC
JAZF1_Exon 5R-2	GCAGGCACTCAATCAATGTG
JAZF1_Exon 5F-3	GCTGAAAATCTTGCCATCTG
JAZF1_Exon 5R-3	TTTCCAATGAGAATGCAGACC
JAZF1_Exon 5F-4	GACCCCTATTGCCTCTTCTTC
JAZF1_Exon 5R-4	GGAAATTTTTAGAGGGCAGTG
JAZF1_Exon 5F-5	GGATGACAGTTCTGTTTGTTAGATG
JAZF1_Exon 5R-5	CAAAATATTCCCATCCTTACCTG
JAZF1_Exon 5F-6	TTCAATGCTTTCAAAGATAAATTCC
JAZF1_Exon 5R-6	TTTCCATGGTAAACTTGATACTGG

Table 6: Primer sequences for *GHRHR*

primer name	SNP included	Sequence
GHRHR_Exon 2F	rs4988496	ACAAGCAGCAGAGGAGATGC
GHRHR_Exon 3R		GAGCTGAAGTGAGAGAAGAAATCC
GHRHR_Exon 6F	rs740366	CCGGAACTACGTCCACACC
GHRHR_Exon 6R		CTGCAGTGGTCAGTGTCGTC
GHRHR_Exon 4F	rs4988498	GGCTGTGAAACGGGATTG
GHRHR_Intron 4R		GCTCTCTGCTGGAAAACTGC

Primer sequences for *GHRHR* used to amplify regions of patient genomic DNA containing potentially informative single nucleotide polymorphisms

Table 7: Primer sequences for *GRB10*

Primer Name	SNP included	Sequence
GRB10_Ex3F	rs1800504	TTTGGGTTTGATTGCTCTCC
GRB10_Ex3R		CATCCTTGGAGAAGGCTCTG
GRB10_Ex6F	rs1800506 and rs1800505	AGTTTCCTTCCTGACCTCATCTT
GRB10_Ex6R		TCTGATTCTCAAACCCATATGCT
GRB10_Ex11F	rs4947710	TCTGCCTTATTTCCCTGCAT
GRB10_Ex11R		TTCCTCTGGGAGAAGACCAC
GRB10_Ex15F	rs3807550	CTGAGGTGGCAGCCTCTAAC
GRB10_Ex15R		AGTCTTCAGCCGAGAGGACA

Primer sequences for *GRB10* used to amplify regions of patient genomic DNA containing potentially informative single nucleotide polymorphisms

Adipose Tissue Biopsies

Adipose tissue biopsies of subcutaneous adipose tissue were taken under local anaesthesia (1% lignocaine) using a 12-gauge needle under aseptic conditions. The biopsies were then washed and placed in pre-weighed eppendorfs containing trizol (Invitrogen, Paisley, UK) and homogenised in an automated homogeniser (Retsch-MM301) prior to storage in a -80 freezer for analysis at a later date.

RNA extraction, retro-transcription and gene expression studies

RNA extraction and DNAse treatment was performed using the PureLink™ Micro-to-Midi Total RNA Purification System (Invitrogen, Paisley, UK). RNA samples were retro-transcribed to produce cDNA using a high capacity cDNA retrotranscription kit (Applied Biosystems, Warrington, Cheshire, UK) as per manufacturer's instructions.

House keeping genes used for gene expression studies: For a panel of healthy tissues three housekeeping genes were used these were *GAPDH* (Glyceraldehyde-3-phosphate dehydrogenase), *PPIA* (Peptidylpropyl-isomeraseA gene) and *18S*. Similarly for adipose tissue the three housekeeping genes used were *18S*, *UBC* (Ubiquitin C) and *PPIA* and for EBV transformed lymphocytes the housekeeping genes used were *GAPDH*, *PPIA* and *18S*.

Probes used for gene expression studies: All probes used were inventoried assays on demand ordered from the Applied Biosystems website unless specifically stated otherwise. For *GRB10* expression the probe Hs00959293_m1 was used. For *JAZF1* expression the probe Hs00697776_m1 was used and for *GHRHR* the probe Hs00173457_m1 was used.

For *CHN2* expression we identified all transcripts likely to be affected by the break point. Three transcripts (EU732751.1, EU732752.1, EU732753.1) are covered by an inventoried probe Hs00906969_m1. One transcript (BQ446523.1) was discarded as it is not listed in UCSC genome browser as a human aligned mRNA and also neither its entire sequence nor any part of it match a sequence within the *CHN2* genomic sequence. We designed two assays to target the exon-exon boundary

between exons 5-6 and 9-10 to cover the AK124021and EU732750.1.1 transcripts respectively (details available on request).

For *INSR* gene expression studies the probe Hs00961561_m1 was selected. This covers all known transcripts and targets exons 9-10 i.e. upstream of the breakpoint. .

References

1. Hannon TS, Janosky J, Arslanian SA. Longitudinal study of physiologic insulin resistance and metabolic changes of puberty. *Pediatr Res* 2006; **60**:759-763.
2. Acerini CL, Williams RM, Dunger DB. Metabolic impact of puberty on the course of type 1 diabetes. *Diabetes Metab* 2001; **27**:S19-25.
3. Ellard S, Thomas K, Edghill EL, Owens M, Ambye L, Cropper J*, et al.* Partial and whole gene deletion mutations of the GCK and HNF1A genes in maturity-onset diabetes of the young. *Diabetologia* 2007; **50**:2313-2317.
4. Lai KK, Lo IF, Tong TM, Cheng LY, Lam ST. Detecting exon deletions and duplications of the DMD gene using Multiplex Ligation-dependent Probe Amplification (MLPA). *Clin Biochem* 2006; **39**:367-372.

Online Supplementary Results

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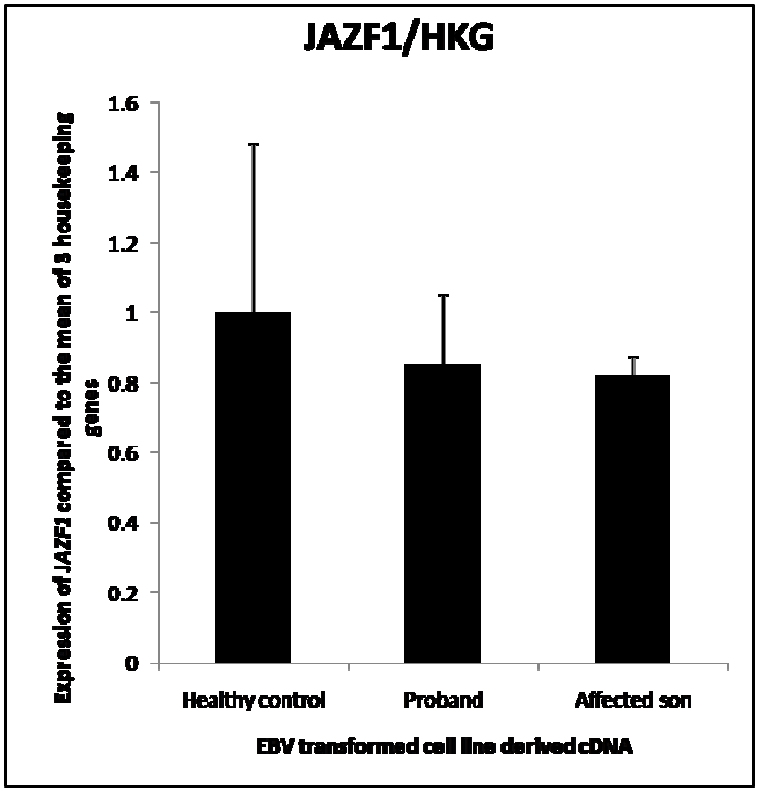
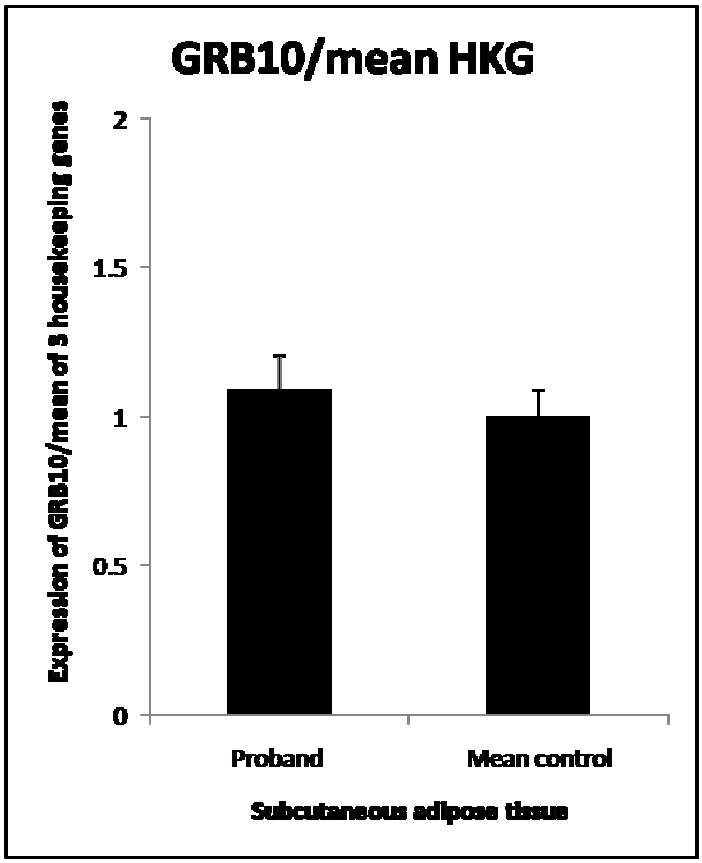
Figure 1: *JAZF1* expression was not altered in EBV-transformed lymphocyte patient cell line derived cDNA compared to a healthy male control.



JAZF1 gene expression relative to three housekeeping genes (HKG) (*GAPDH*, *PPIA* and *18S*) was not altered between patients and healthy control. The results are further normalised to the healthy control sample. Error bars reflect the 95% confidence intervals.

Figure 2: *GRB10* expression was not altered in patient adipose tissue derived cDNA compared to the mean of healthy control adipose tissue (n=3).



GRB10 gene expression relative to three housekeeping genes (HKG) (*UBC*, *PPIA* and *18S*) was not altered between patient adipose tissue (AT) and adipose tissue from healthy controls. Results were normalised to those of 3 matched healthy controls. The error bars reflect the 95% confidence intervals.

Table 1: Results of a GHRH-Arginine stimulation test performed on the proband showing a normal response (GH peak response >10ng/ml)

Time (Min)	IGF-1 (ng/ml)	hGH (ng/ml)	hGH (mIU/L)
0´	314.0 (117-329)	0.08	0.192
15´		2.6	6.24
30´		17.3	41.52
45´		26.8	64.32
60´		24.7	59.28
75´		18	43.2
90´		10.3	24.72
105´		4.8	11.52
120´		2.4	5.76

A growth hormone-releasing hormone (GHRH)-Arginine stimulation test (details available on request) was performed on the proband to exclude a defect in the Growth Hormone Releasing Hormone Receptor as the gene encoding GHRHR is adjacent to the breakpoint on chromosome 7. This showed a normal growth hormone response with a peak GH response above 10ng/ml, thereby excluding a defect in GHRHR as the cause of the pre- and post- natal growth retardation.