

Aberrant expression of agouti signaling protein (*ASIP*) as a cause of monogenic severe childhood obesity

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1 **Supplementary Information**

2 **1. Supplementary Tables**

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4 **Table S1. Clinical characteristics of the index patient and control children with adipose**
5 **tissue samples**

available	Adipose tissue samples						Gene expression data from PBL					PBL RNA sequencing	
	Patient	Ctr 1	Ctr 2	Ctr 3	Ctr 4	Ctr 10	Patient	Ctr 5	Ctr 6	Ctr 7	Ctr 8	Patient	Ctr 9
Anthropometric data													
Age (years)	12.4	17.4	13.3	11.8	12.7	14.7	14.1	16	12.8	12.3	15.0	15.0	46.2
Sex	female	female	male	female	female	male	female	female	female	female	female	female	female
Height (cm) / Height SDS	181 / 3.4	176 / 1.3	156 / 0.0	158 / 0.7	160 / 0.3	163 / -1.0	183 / 2.9	167 / 0.1	186 / 3.8	163 / 1.0	183 / 2.7	183 / 2.8	173
BMI (kg/m ²) / BMI SDS	47.6 / 3.7	40.5 / 3.7	25.5 / 1.8	31.4 / 2.7	29.7 / 2.4	22.2 / 0.8	37.8 / 3.2	33.1 / 2.8	44.7 / 3.6	31.0 / 2.6	38.6 / 3.3	39.7 / 3.4	19.0
Surgery	sleeve gastrectomy	sleeve gastrectomy	removal 8P	implan- tation 8P	implan- tation 8P	implan- tation 8P	---	---	---	---	---	---	---
Location	abdomen	abdomen	femur	knee	knee	knee	---	---	---	---	---	---	---

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7 Ctr, control; SDS, standard deviation score; BMI, body mass index; 8P, eight plate; PBL, peripheral blood
8 leukocytes

10 **Table S2. RNA sequencing results from SVF cells from the patient and control children**

Subject	Patient	Patient	Ctr 1	Ctr 2	Ctr 3	Ctr 4
SVF cell passage (p)	p15	p28	p16	p19	p19	p14
<i>ASIP</i> (FPKM)	5.07	4.89	0.20	0.36	0.18	0.22
<i>AHCY</i> (FPKM)	14.17	11.94	9.25	7.93	12.74	10.19
<i>ITCH</i> (FPKM)	19.24	25.77	21.31	18.67	21.92	24.46

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12 SVF, stromal vascular fraction; Ctr, control; SDS, standard deviation score; FPKM, fragments per kilobase

13 of transcript per million fragments mapped. *ASIP*, agouti signaling protein; *AHCY*,

14 adenosylhomocysteinase; *ITCH*, itchy E3 ubiquitin protein ligase

15 **Table S3. Variants strongly associated with red hair according to Morgan *et al.*¹ in the**
16 **patient and her father**

Gene	Variant ID	Effect	Associated with red hair	Patient	Father
<i>MC1R</i>	rs1805006	D84E	A	C/C	C/C
<i>MC1R</i>	rs1805007	R151C	T	C/C	C/C
<i>MC1R</i>	rs1110400	I155T	C	T/T	T/T
<i>MC1R</i>	rs1805009	D294H	C	G/G	G/G
<i>MC1R</i>	rs1805008	rg160Trp or R160W	T	C/C	C/C
<i>MC1R</i>	rs34357723	97kb from the 5' end of <i>MC1R</i>	T	C/C	C/C
<i>MC1R</i>	rs368507952	R306H	A	G/G	G/G
<i>MC1R</i>	rs200000734	R213W	T	C/C	C/C
<i>MC1R</i>	rs201326893	Y152X	A	C/C	C/C
<i>MC1R</i>	rs11547464	R142H	A	G/G	G/G
<i>MC1R</i>	rs3212379	upstream	T	C/C	C/C
<i>MC1R</i>	rs34474212	S83P	C	T/T	T/T
<i>MC1R</i>	rs34158934	T95M	T	C/C	C/C
<i>MC1R</i>	rs555179612	I182Hfs	TC	C/C	C/C
<i>RALY/ASIP</i>	rs6059655	non-coding	A	G/G	G/G

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18 The affected gene, variant ID, effect and the allele associated with red hair is shown, together with the
19 genotypes of the patient and her father. *MC1R*, melanocortin receptor 1; *RALY*, RALY heterogeneous
20 nuclear ribonucleoprotein; *ASIP*, agouti signaling protein

21 **Table S4. Primer sequences for qPCR**

Target	Primer, forward	Primer, reverse	Probe/SYBR green
Gene expression			
<i>ACTB</i>	CGAGCGCGGCTACAGCTT	CCTTAATGTCACGCACGATTT	ACCACCACGGCCGAGCGG
<i>ADIPOQ</i>	GGC CGT GAT GGC AGA GAT	CCT TCA GCC CCG GGT ACT	CGATGTCTCCCTTAGGACC AATAAGACCTGG
<i>AGRP</i>	GGATCTGTTGCAGGAGGCTCA G	TGAAGAAGCGGCAGTAGCACG T	GCCCGCTCCTCACGTCGCT
<i>AHCY</i>	Taqman Assay Hs04183463_g1(4331182, Thermo Fisher Scientific Inc.		
<i>AP2</i>	GCTTTTGTAGGTACCTGGAAC TTG	ACACTGATGATCATGTTAGGTTT GG	CCTGGTGGCAAAGCCCACT CCTCAT
<i>ASIP</i>	CAACTCCTCTGTGAACCTACTG GAT	TGCCGATCTGTTGGATTCT	CCCTTCTGTCTCTATTGTGG CGCTGAAC
<i>CXCR4</i>	CACCGCATCTGGAGAACCA	GCCCATTTCTCGGTGTAGTT	SYBR green
<i>FOXA2</i>	GGGAGCGGTGAAGATGGA	TCATGTTGCTCACGGAGGAGTA	SYBR green
<i>HPRT</i>	GGCAGTATAATCCAAAGATGGT CAA	GTCTGGCTTATATCCAACACTT CGT	CAAGCTTGCTGGTGAAAAG GACCCC
<i>ITCH</i>	TaqMan Assay Hs00395201_m1 (4331182, Thermo Fisher Scientific Inc)		
<i>LEPR</i>	TTGTGCCAGTAATTATTTCTCT T	CTGATCAGCGTGGCGTATTT	SYBR green
<i>MC4R</i>	CTTATGATGATCCCAACCCG	GTAGCTCCTTGCTTGCATCC	TGCTTTCAATTGCAGTGGA CAGGTACT
<i>MESP</i>	CTGCCTGAGGAGCCCAAGT	GCAGTCTGCCAAGGAACCA	SYBR green
<i>MIXL</i>	CCGAGTCCAGGATCCAGGTA	CTCTGACGCCGAGACTTGG	SYBR green
<i>NANOG</i>	CCTTCCTCCATGGATCTGCTT	CTTGACCGGGACCTTGCTTC	SYBR green
<i>Nestin</i>	GTCTCAGGACAGTGCTGAGCCT TC	TCCCCTGAGGACCAGGAGTCTC	SYBR green
<i>OCT4</i>	CAATTTGCCAAGCTCCTGAAG	AAAGCGGCAGATGGTCGTT	SYBR green
<i>PAX6</i>	TCTTTGCTTGGGAAATCCG	CTGCCCGTTCAACATCCTTAG	SYBR green
<i>POMC</i>	CAGCCAGTGTGAGGACCTC	AAGTGGCCCATGACGTAATT	GGAGTGCATCCGGGCGCTG CA
<i>PPARG</i>	GATCCAGTGTTGCAGATTACA A	GAGGGAGTTGGAAGGCTCTTC	TGACCTGAAACTTCAAGAG TACCAAAGTGCAA
<i>SOX1</i>	GAGAACCCCAAGATGCACAA	CCTCGGACATGACCTTCCA	SYBR green
<i>SOX2</i>	CCTCCGGGACATGATCAGCATG TA	GCAGTGTGCCGTTAATGGCCGT G	SYBR green
<i>SOX17</i>	GGCGCAGCAGAATCCAGA	CCACGACTTGCCGAGCAT	SYBR green
<i>T</i>	CAACCTCACTGACGGTGAAAAA	ACAAATTCTGGTGTGCCAAAGT T	SYBR green
<i>TBP</i>	TTGTAACTTGACCTAAAGACC ATTGC	TTCGTGGCTCTCTTATCCTCAT G	AACGCCGAATATAATCCCA AGCGGTTTG
gDNA copy numbers			
<i>ACTB</i>	CGAGCGCGGCTACAGCTT	CCTTAATGTCACGCACGATTT	SYBR green
<i>ITCH-ASIP fusion</i>	TGGAGAAAAGGAATCATCAGAG GA	GTGGTGAGCAAGGGACGTTTCA GC	SYBR green

23 Sequences are given in 5' to 3' direction. As dye/quencher FAM/TAMRA or HEX/TAMRA was used. If
24 SYBR green was used this is indicated in the last column. *ACTB*, beta actin; *ADIPOQ*, adiponectin;
25 *AGRP*, agouti-related protein; *AHCY*, adenosylhomocysteinase; *AP2*, adipocyte protein 2; *ASIP*, agouti
26 signaling protein; *CXCR4*, C-X-C Motif Chemokine Receptor 4; *FOXA2*, Forkhead Box A2; *HPRT*,
27 hypoxanthine-guanine-phosphoribosyltransferase; *ITCH*, itchy E3 ubiquitin protein ligase; *ITCH-ASIP*
28 fusion, unique sequence of the *ITCH-ASIP* fusion gene; *LEPR*, leptin receptor; *MC4R*, melanocortin 4
29 receptor; *MESP*, Mesoderm Posterior BHLH Transcription Factor; *MIXL*, Mix Paired-Like Homeobox;
30 *NANOG*, Nanog Homeobox; *OCT4*, POU Class 5 Homeobox 1; *PAX6*, Paired Box 6; *POMC*,
31 proopiomelanocortin; *PPARG*, peroxisome proliferator-activated receptor gamma; *SOX1*, SRY-Box
32 Transcription Factor 1; *SOX2*, SRY-Box Transcription Factor 2; *SOX17*, SRY-Box Transcription Factor 17;
33 *T*, T-Box Transcription Factor T; *TBP*, TATA-box binding protein
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Table S5. Primer sequences for 5' RACE PCR and cloning experiments

5' RACE PCR	<i>ASIP</i> -specific prime	nested <i>ASIP</i> -specific primer
	TTGAGGCTGAGCACGCGGCAG GAGCAGG	CTGCGGAAGAAGCGGCACTGG CAGGAGG
Cloning	Forward	Reverse
<i>ITCH</i> - <i>ASIP</i> fusion gDNA	TGGAGAAAAGGAATCATCAGAG GA	GTGGTGAGCAAGGGACGTTTCA GC
For Vector construction:		
2,868 bp <i>ITCH</i> promoter sequence (NM031483.7)	CTGGAGCTCGAAGTGGTTTTGA AAGTACTTTGCT	ACTGCTCGAGAAAGCGCAGGC GCCTGAGCGCG.
<i>ASIP</i> mRNA sequence (NM001672.2)	ACTGCCATGGGCCTCCTGGGAT GGATGTCACCC	ACTGTCTAGATGGGGGCGCTCA GCAGTTGAGGC
<i>ITCH</i> - <i>ASIP</i> mRNA	ACTGCCATGGAGCTGTGGTCG GGGCTCGGGAC	ACTGTCTAGATGGGGGCGCTCA GCAGTTGAGGC

Sequences are given in 5' to 3' direction. 5' RACE PCR, 5' Rapid amplification of cDNA-ends and polymerase Chain Reaction; *ASIP*, agouti signaling protein; *ITCH*, itchy E3 ubiquitin protein ligase; *ITCH*-*ASIP* fusion, unique sequence of the *ITCH*-*ASIP* fusion gene.

2. Supplementary Figures

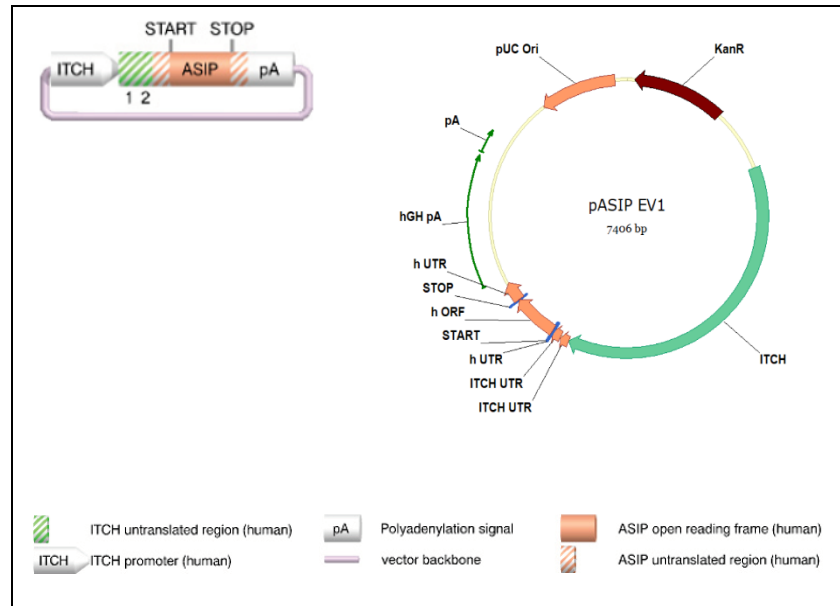


Figure S1. Taconic expression vector containing the *ITCH*-*ASIP* fusion mRNA sequence under control of the *ITCH* promoter

A scheme of the plasmid including the legend and the plasmid map of the construct is shown. *ITCH*, itchy E3 ubiquitin protein ligase; *ASIP*, agouti signaling protein

48 **3. Supplementary References**

- 49 1. Morgan MD, Pairo-Castineira E, Rawlik K, et al. Genome-wide study of hair colour in UK
50 Biobank explains most of the SNP heritability. Nat Commun 2018;9:5271.