SUPPLEMENTARY DATA

Supplemental Table 1: List of 203 genes encoding proteins containing FN3-domains (Prosite database, PS50835, index in 09 Feb. 2017)

Entry	Gene	Protein names
Q8N957	*ANKF1*	Ankyrin repeat and fibronectin type-III domain-containing protein 1
O75129	*ASTN2*	Astrotactin-2
Q9BWV1	*BOC*	Brother of CDO (Protein BOC)
Q4KMG0	*CDON*	Cell adhesion molecule-related/down-regulated by oncogenes
Q8N3K9	*CMYA5*	Cardiomyopathy-associated protein 5
P26992	*CNTFR*	Ciliary neurotrophic factor receptor subunit alpha
Q12860	*CNTN1*	Contactin-1
Q02246	*CNTN2*	Contactin-2
Q9P232	*CNTN3*	Contactin-3
Q8IWV2	*CNTN4*	Contactin-4
O94779	*CNTN5*	Contactin-5
Q9UQ52	*CNTN6*	Contactin-6
P12111	*CO6A3*	Collagen alpha-3(VI) chain
Q02388	*CO7A1*	Collagen alpha-1(VII) chain
Q99715	*COCA1*	Collagen alpha-1(XII) chain
Q05707	*COEA1*	Collagen alpha-1(XIV) chain
Q9P218	*COKA1*	Collagen alpha-1(XX) chain
O75462	*CRLF1*	Cytokine receptor-like factor 1
Q9HC73	*CRLF2*	Cytokine receptor-like factor 2
Q8IUI8	*CRLF3*	Cytokine receptor-like factor 3
P15509	*CSF2R*	Granulocyte-macrophage colony-stimulating factor receptor subunit alpha
Q99062	*CSF3R*	Granulocyte colony-stimulating factor receptor
P43146	*DCC*	Netrin receptor DCC
O60469	*DSCAM*	Down syndrome cell adhesion molecule
Q8TD84	*DSCL1*	Down syndrome cell adhesion molecule-like protein 1
Q63HQ2	*EGFLA*	Pikachurin
P0C7U0	*ELFN1*	Protein ELFN1
P21709	*EPHA1*	Ephrin type-A receptor 1
P29317	*EPHA2*	Ephrin type-A receptor 2
P29320	*EPHA3*	Ephrin type-A receptor 3
P54764	*EPHA4*	Ephrin type-A receptor 4
P54756	*EPHA5*	Ephrin type-A receptor 5
Q9UF33	*EPHA6*	Ephrin type-A receptor 6
Q15375	*EPHA7*	Ephrin type-A receptor 7
P29322	*EPHA8*	Ephrin type-A receptor 8
Q5JZY3	*EPHAA*	Ephrin type-A receptor 10
P54762	*EPHB1*	Ephrin type-B receptor 1
P29323	*EPHB2*	Ephrin type-B receptor 2
P54753	*EPHB3*	Ephrin type-B receptor 3
P54760	*EPHB4*	Ephrin type-B receptor 4
O15197	*EPHB6*	Ephrin type-B receptor 6
P19235	*EPOR*	Erythropoietin receptor

Q8TC84	*FANK1*	Fibronectin type 3 and ankyrin repeat domains protein 1
P02751	*FINC*	Fibronectin
Q9NZU1	*FLRT1*	Leucine-rich repeat transmembrane protein FLRT1
O43155	*FLRT2*	Leucine-rich repeat transmembrane protein FLRT2
Q9NZU0	*FLRT3*	Leucine-rich repeat transmembrane protein FLRT3
F2Z333	*FND10*	Fibronectin type III domain-containing protein 10
Q9BVV2	*FND11*	Fibronectin type III domain-containing protein 11
Q9Y2H6	*FND3A*	Fibronectin type-III domain-containing protein 3A
Q53EP0	*FND3B*	Fibronectin type III domain-containing protein 3B
Q4ZHG4	*FNDC1*	Fibronectin type III domain-containing protein 1
Q9H6D8	*FNDC4*	Fibronectin type III domain-containing protein 4
Q8NAU1	*FNDC5*	Fibronectin type III domain-containing protein 5
Q5VTL7	*FNDC7*	Fibronectin type III domain-containing protein 7
Q8TC99	*FNDC8*	Fibronectin type III domain-containing protein 8
Q8TBE3	*FNDC9*	Fibronectin type III domain-containing protein 9
Q9BTV5	*FSD1*	Fibronectin type III and SPRY domain-containing protein 1
Q9BXM9	*FSD1L*	FSD1-like protein
A1L4K1	*FSD2*	Fibronectin type III and SPRY domain-containing protein 2
P10912	*GHR*	Growth hormone receptor
P51610	*HCFC1*	Host cell factor 1 (HCF)
Q9Y5Z7	*HCFC2*	Host cell factor 2
Q08334	*I10R2*	Interleukin-10 receptor subunit beta
Q14626	*I11RA*	Interleukin-11 receptor subunit alpha
P42701	*I12R1*	Interleukin-12 receptor subunit beta-1
Q99665	*I12R2*	Interleukin-12 receptor subunit beta-2
P78552	*I13R1*	Interleukin-13 receptor subunit alpha-1
Q14627	*I13R2*	Interleukin-13 receptor subunit alpha-2
Q9UHF4	*I20RA*	Interleukin-20 receptor subunit alpha
Q6UXL0	*I20RB*	Interleukin-20 receptor subunit beta
Q8N6P7	*I22R1*	Interleukin-22 receptor subunit alpha-1
Q969J5	*I22R2*	Interleukin-22 receptor subunit alpha-2
Q6UWB1	*I27RA*	Interleukin-27 receptor subunit alpha
Q8IVU1	*IGDC3*	Immunoglobulin superfamily DCC subclass member 3
Q8TDY8	*IGDC4*	Immunoglobulin superfamily DCC subclass member 4
P08069	*IGF1R*	Insulin-like growth factor 1 receptor
Q86VF2	*IGFN1*	Immunoglobulin-like and fibronectin type III domain-containing protein 1
Q8N9C0	*IGS22*	Immunoglobulin superfamily member 22
P29460	*IL12B*	Interleukin-12 subunit beta
Q9HBE5	*IL21R*	Interleukin-21 receptor
Q5VWK5	*IL23R*	Interleukin-23 receptor
Q14213	*IL27B*	Interleukin-27 subunit beta
P14784	*IL2RB*	Interleukin-2 receptor subunit beta
P31785	*IL2RG*	Cytokine receptor common subunit gamma
Q8NI17	*IL31R*	Interleukin-31 receptor subunit alpha
P32927	*IL3RB*	Cytokine receptor common subunit beta
P24394	*IL4RA*	Interleukin-4 receptor subunit alpha
Q01344	*IL5RA*	Interleukin-5 receptor subunit alpha
P08887	*IL6RA*	Interleukin-6 receptor subunit alpha
P40189	*IL6RB*	Interleukin-6 receptor subunit beta

P16871	*IL7RA*	Interleukin-7 receptor subunit alpha
Q01113	*IL9R*	Interleukin-9 receptor
P17181	*INAR1*	Interferon alpha/beta receptor 1
P15260	*INGR1*	Interferon gamma receptor 1
P38484	*INGR2*	Interferon gamma receptor 2
Q8IU57	*INLR1*	Interferon lambda receptor 1
P06213	*INSR*	Insulin receptor
P14616	*INSRR*	Insulin receptor-related protein
P16144	*ITB4*	Integrin beta-4
P23352	*KALM*	Anosmin-1
O60229	*KALRN*	Kalirin
P32004	*L1CAM*	Neural cell adhesion molecule L1
P48357	*LEPR*	Leptin receptor
P42702	*LIFR*	Leukemia inhibitory factor receptor
Q9P244	*LRFN1*	Leucine-rich repeat and fibronectin type III domain-containing protein 1
Q9ULH4	*LRFN2*	Leucine-rich repeat and fibronectin type-III domain-containing protein 2
Q9BTN0	*LRFN3*	Leucine-rich repeat and fibronectin type-III domain-containing protein 3
Q6PJG9	*LRFN4*	Leucine-rich repeat and fibronectin type-III domain-containing protein 4
Q96NI6	*LRFN5*	Leucine-rich repeat and fibronectin type-III domain-containing protein 5
Q9P2V4	*LRIT1*	Leucine-rich repeat, immunoglobulin-like domain and transmembrane domain-containing protein 1
A6NDA9	*LRIT2*	Leucine-rich repeat, immunoglobulin-like domain and transmembrane domain-containing protein 2
Q3SXY7	*LRIT3*	Leucine-rich repeat, immunoglobulin-like domain and transmembrane domain-containing protein 3
Q8ND94	*LRN4L*	LRRN4 C-terminal-like protein
Q6UXK5	*LRRN1*	Leucine-rich repeat neuronal protein 1
Q9H3W5	*LRRN3*	Leucine-rich repeat neuronal protein 3
Q8WUT4	*LRRN4*	Leucine-rich repeat neuronal protein 4
A2RUH7	*MBPHL*	Myosin-binding protein H-like
Q6VMQ6	*MCAF1*	Activating transcription factor 7-interacting protein 1
Q5U623	*MCAF2*	Activating transcription factor 7-interacting protein 2
Q8NFP4	*MDGA1*	MAM domain-containing glycosylphosphatidylinositol anchor protein 1
Q7Z553	*MDGA2*	MAM domain-containing glycosylphosphatidylinositol anchor protein 2
Q12866	*MERTK*	Tyrosine-protein kinase Mer
Q13203	*MYBPH*	Myosin-binding protein H
Q15746	*MYLK*	Myosin light chain kinase, smooth muscle
P52179	*MYOM1*	Myomesin-1
P54296	*MYOM2*	Myomesin-2
Q5VTT5	*MYOM3*	Myomesin-3
Q00872	*MYPC1*	Myosin-binding protein C, slow-type
Q14324	*MYPC2*	Myosin-binding protein C, fast-type
Q14896	*MYPC3*	Myosin-binding protein C, cardiac-type
P13591	*NCAM1*	Neural cell adhesion molecule 1
O15394	*NCAM2*	Neural cell adhesion molecule 2
O00533	*NCHL1*	Neural cell adhesion molecule L1-like protein
Q8TB73	*NDNF*	Protein NDNF
Q92859	*NEO1*	Neogenin
O94856	*NFASC*	Neurofascin

O60500	*NPHN*	Nephrin
Q92823	*NRCAM*	Neuronal cell adhesion molecule
Q5VST9	*OBSCN*	Obscurin
O75147	*OBSL1*	Obscurin-like protein 1
Q99650	*OSMR*	Oncostatin-M-specific receptor subunit beta
Q96FC7	*PHIPL*	Phytanoyl-CoA hydroxylase-interacting protein-like
Q92561	*PHYIP*	Phytanoyl-CoA hydroxylase-interacting protein
Q8NAT1	*PMGT2*	Protein O-linked-mannose beta-1,4-N-acetylglucosaminyltransferase 2
Q5R3F8	*PPR29*	Protein phosphatase 1 regulatory subunit 29
P16471	*PRLR*	Prolactin receptor
Q2VWP7	*PRTG*	Protogenin
P23467	*PTPRB*	Receptor-type tyrosine-protein phosphatase beta
P08575	*PTPRC*	Receptor-type tyrosine-protein phosphatase C
P23468	*PTPRD*	Receptor-type tyrosine-protein phosphatase delta
P10586	*PTPRF*	Receptor-type tyrosine-protein phosphatase F
P23470	*PTPRG*	Receptor-type tyrosine-protein phosphatase gamma
Q9HD43	*PTPRH*	Receptor-type tyrosine-protein phosphatase H
Q12913	*PTPRJ*	Receptor-type tyrosine-protein phosphatase eta
Q15262	*PTPRK*	Receptor-type tyrosine-protein phosphatase kappa
P28827	*PTPRM*	Receptor-type tyrosine-protein phosphatase mu
Q16827	*PTPRO*	Receptor-type tyrosine-protein phosphatase O
Q9UMZ3	*PTPRQ*	Phosphatidylinositol phosphatase PTPRQ
Q13332	*PTPRS*	Receptor-type tyrosine-protein phosphatase S
O14522	*PTPRT*	Receptor-type tyrosine-protein phosphatase T
Q92729	*PTPRU*	Receptor-type tyrosine-protein phosphatase U
P23471	*PTPRZ*	Receptor-type tyrosine-protein phosphatase zeta
Q9UFD9	*RIM3A*	RIMS-binding protein 3A
A6NNM3	*RIM3B*	RIMS-binding protein 3B
A6NJZ7	*RIM3C*	RIMS-binding protein 3C
O95153	*RIMB1*	Peripheral-type benzodiazepine receptor-associated protein 1
O15034	*RIMB2*	RIMS-binding protein 2
Q9Y6N7	*ROBO1*	Roundabout homolog 1
Q9HCK4	*ROBO2*	Roundabout homolog 2
Q96MS0	*ROBO3*	Roundabout homolog 3
Q8WZ75	*ROBO4*	Roundabout homolog 4
P08922	*ROS1*	Proto-oncogene tyrosine-protein kinase ROS
Q7Z5N4	*SDK1*	Protein sidekick-1
Q58EX2	*SDK2*	Protein sidekick-2
Q8TER0	*SNED1*	Sushi, nidogen and EGF-like domain-containing protein 1
Q92673	*SORL*	Sortilin-related receptor
Q15772	*SPEG*	Striated muscle preferentially expressed protein kinase
Q7Z7G0	*TARSH*	Target of Nesh-SH3
P24821	*TENA*	Tenascin
Q9UQP3	*TENN*	Tenascin-N
Q92752	*TENR*	Tenascin-R
P22105	*TENX*	Tenascin-X
Q16473	*TENXA*	Putative tenascin-XA
P35590	*TIE1*	Tyrosine-protein kinase receptor Tie-1
Q02763	*TIE2*	Angiopoietin-1 receptor

Q8WZ42	*TITIN*	Titin
P40238	*TPOR*	Thrombopoietin receptor
O15344	*TRI18*	E3 ubiquitin-protein ligase Midline-1
Q9NQ86	*TRI36*	E3 ubiquitin-protein ligase TRIM36
Q8IWZ5	*TRI42*	Tripartite motif-containing protein 42
Q7Z4K8	*TRI46*	Tripartite motif-containing protein 46
Q6ZTA4	*TRI67*	Tripartite motif-containing protein 67
Q9UJV3	*TRIM1*	Probable E3 ubiquitin-protein ligase MID2
Q9C026	*TRIM9*	E3 ubiquitin-protein ligase TRIM9
Q9P2J2	*TUTLA*	Protein turtle homolog A
Q9UPX0	*TUTLB*	Protein turtle homolog B
Q06418	*TYRO3*	Tyrosine-protein kinase receptor TYRO3
P30530	*UFO*	Tyrosine-protein kinase receptor UFO
Q5DID0	*UROL1*	Uromodulin-like 1
O75445	*USH2A*	Usherin
Q6EMK4	*VASN*	Vasorin
Q6PCB0	*VWA1*	von Willebrand factor A domain-containing protein 1

Supplemental Table 2: List of 22 genes encoding proteins containing FN3-domains known to be involved in axon guidance (GO:0007411)

Candidate Gene	# rare variants	# probands (KS/nCHH)
ANOS1	3	3 (3/0)
BOC	6	6 (2/4)
CHL1	4	4 (3/1)
CNTN2	7	12 (6/6)
CNTN4	4	4 (3/1)
DCC	5	7 (6/1)
EPHA5	1	1 (1/0)
EPHA8	3	4 (3/1)
EPHB1	2	2 (2/0)
EPHB2	1	3 (3/0)
EPHB3	4	4 (3/1)
FLRT2	3	4 (2/2)
FLRT3	1	2 (1/1)
L1CAM	2	2 (2/0)
NCAM1	3	4 (0/4)
NEO1	6	6 (4/2)
NFASC	5	6 (4/2)
PTPRO	3	5 (4/1)
ROBO1	1	2 (1/1)
ROBO2	2	5 (5/3)
ROBO3	2	4 (1/3)
TNR	2	2 (0/2)

Case summaries

Family # 1, Patient II-2

***DCC* p.N176S (heterozygous)**

***PROKR2* p.L173R (heterozygous)**

The anosmic Caucasian male was born with bilateral cryptorchidism, right inguinal hernia, micropenis and glandular hypospadias. Bilateral orchidopexy and right inguinal herniotomy were performed at 10 months of age, but orchidopexies had to be repeated for right and left testes at age 7 and 9 years respectively. Penis size was normalized following 4 injections of Sustanon at age 2-3 years. Due to high risk of CHH, he continued being following by an endocrine specialist. Other phenotypes included delayed childhood motor milestones, delayed deciduous dentition (only 7 teeth at 2.3 years), mild facial asymmetry with hypoplastic right ear pinna and transverse palmar creases. Mild bilateral synkinesia was also observed. Renal ultrasound and head CT were normal at age 3 years. The patient was unable to tolerate a cranial MRI. By age 10 it became obvious that he was anosmic, which was confirmed later by formal testing (< 5th %ile). At age 13, he had absent puberty (testes 1 ml) and hormonal profiling compatible with hypogonadotropic hypogonadism (T<1.0 nmol/l, LH&FSH < 0.5 U/l). Anterior pituitary function was otherwise normal. Based on these findings he was diagnosed with Kallmann syndrome and was started on testosterone treatment shortly before his 14th birthday to induce virilization. Hormonal assessment at age 18 confirmed the diagnosis of CHH. His parents and brother have normal reproductive and olfactory phenotype. The proband harbors a mutation in *DCC*, inherited by his father, as well as in *PROKR2* inherited by his mother. His brother was found to also harbor the *DCC* and *PROKR2* mutations.

Family # 2, Patient II-1

***DCC* p.Gly470Asp (heterozygous)**

The Caucasian male proband was born in the context of perinatal asphyxia. Physical examination showed micropenis without cryptorchidism, hoarse cry, moderate hypotonia and a third left nipple. An MRI revealed a Rathke's cleft cyst. Gonadotropines levels were low for minipuberty (LH 0.5 U/l, FSH 0.7 U/l) without dysfunction of other pituitary axes. An hCG-stimulation test indicated adequate elevation of testosterone to 14.1 nmol/l. Micropenis was corrected by 3 testosterone injections at 4 months of age. Family history was unremarkable for puberty and reproductive status. Infancy was marked by retarded speech and psychomotor development. At 14 years of age, no signs of puberty were present. Testicular volume was 1.5 ml bilaterally. Diffuse obesity was noted with BMI of 28 kg/m^2. Laboratory assessment confirmed hypogonadotropic hypogonadism. An MRI (under general

anesthesia due to psychomotor agitation) revealed a reduced pituitary volume, a decrease in the size of the Rathke's cyst and an asymmetry of the olfactory bulbs (absence of the right one), though both olfactory sulcus were visible. A formal olfactory test was impossible to perform. Induction of puberty by testosterone administered transcutaneously was initiated at age 15.1 years. Evaluation at 1 year of treatment showed satisfactory progression of virilization with a testosterone level at 5.5 nmol/l. The proband harbors a mutation in *DCC* with no changes in known CHH genes. The patient's mother is carrier of the same mutation in *DCC*. Father's DNA was not available.

Family # 3, Subject III-1

***DCC* p.P645S (heterozygous)**

The anosmic proband of Indian subcontinent descent was born without cryptorchidism nor micropenis and first presented to medical attention at age 22 years for evaluation of absent puberty despite a normal stature (170 cm). At that time he was unvirilized, markedly obese (BMI 40 kg/m^2) and had prepubertal testes (3 mL). Serum measurement of reproductive hormones showed hypogonadotropic hypogonadism (T < 1 nmol/L, LH < 1 IU/L, FSH < 1 IU/L). Anterior pituitary function was otherwise normal. Anosmia was confirmed using readily-available odorants but not by quantitative smell testing. He returned to India before either renal ultrasound or cranial MRI could be performed. These findings were consistent with Kallmann syndrome and he initiated testosterone replacement therapy to induce virilization, but which without any change in testis volume when reassessed at age 28 years. Notably, both his father and paternal grandfather had delayed puberty, whereas a maternal uncle is hyposmic.

Family # 4, Patient II-2

***DCC* p.G649E (heterozygous)**

***CHD7* p.Y1616C (heterozygous)**

***SEMA3a* p.R66W (heterozygous)**

The anosmic Caucasian female first presented to medical attention for evaluation of primary amenorrhea at age 16. Her exam was notable for lack of pubertal development and hormonal profiling revealed undetectable gonadotropins (LH/FSH < 1.0 U/L) in the setting of low estradiol levels (< 60 pmol/L). Formal smell testing confirmed anosmia (UPSIT: 13/40, <5th %ile) and she was diagnosed with Kallmann syndrome. A renal ultrasound showed normal anatomy while a cranial MRI revealed absent olfactory bulbs. DEXA bone density revealed osteopenia of the hip which subsequently normalized with ongoing estrogen therapy. Her history is notable for mild bilateral sensorineural hearing loss identified on audiology testing. Her father has anosmia that is thought to be secondary to nasal polyps. Her brother has cerebral palsy and displayed bilateral cryptorchidism, but was biochemically

eugonadal when tested. The patient harbors mutations in *DCC, CHD7 and SEMA3a* inherited by her mother. Her brother is also carrier of the *CHD7* mutation.

Family # 5, Patient II-1

DCC p.Ser876Tyr

The female normosmic proband of mixed origin (mother from Haiti, father from Switzerland) was brought to medical attention at age 17. She presented menses once at age 16, then secondary amenorrhea. Her physical status was notable for diffuse obesity (BMI 27.07 kg/m^2), moderate facial acne and absent puberty (Tanner II, breast development). Laboratory data showed markedly low estradiol (<0.04 nmol/l) associated with undetectable gonadotropines (LH & FSH < 0.5 U/l). Polycystic ovaries syndrome was excluded as well as a non-classical form of congenital adrenal hyperplasia. GnRH stimulation testing revealed a weak FSH response (<0.4 to 1.2 U/L) and a flat LH response (< 0.5 U/l). No anomaly of other pituitary axes was present. Cranial MRI revealed reduced size of the pituitary gland with significant thinning of the distal portion of the pituitary stalk. DEXA bone density revealed marked osteoporosis of the lumbar spine that responded favorably to subsequent estrogen supplementation. Family history is characterized by delayed puberty in the mother (menarche at age 16) with difficulty conceiving, delayed puberty in a maternal aunt (menarche at age 18) and normal puberty in the father. Familial heights are low at the maternal side with tendency to obesity (mother: 85 kg, 150 cm). The patient harbors a DCC mutation without any changes in known CHH genes. Mother's DNA was tested and found negative for the DCC mutation, whereas the father did not agree to provide blood for genetic testing.

Family # 6, Patient II-1

DCC Gly470Asp

NTN1 Thr525Arg

The Caucasian male proband and his brother (Patient # II-2) were born with bilateral cryptorchidism requiring surgical correction. He presented at age 18 for evaluation of absent pubertal development. He was unvirilized with prepubertal testes (< 3mL) and was noted at that time to be anosmic (confirmed via formal smell testing, <5th %ile). Hormone measurement revealed low gonadotropin levels (LH and FSH both 0.8 IU/L) in the setting of a frankly low serum testosterone (1.0 nmol/L). GnRH stimulation testing revealed a weak LH response (0.8 to 3.5 IU/L) and a flat FSH response (FSH remained 0.8 IU/L). Ultrasound showed both kidneys were present and cranial MRI indicated a normal pituitary yet shallow olfactory sulci and intact olfactory bulbs. Based on these findings he was diagnosed with Kallmann syndrome and started on testosterone to induce virilization and subsequently was transitioned to hCG (and later pulsatile GnRH *via* pump) to stimulate testicular development. Besides

his twin brother (Patient #7) there is no other family history of hypogonadism, delayed puberty or midline defects. The proband and his brother both harbor the same mutations in *DCC* and *NTN1*. There are no changes in known CHH genes.

Family # 6, Patient II-2

DCC Gly470Asp

NTN1 Thr525Arg

Like his twin brother (Patient #6), the Caucasian anosmic patient was born with bilateral cryptorchidism and underwent orchidopexy. He also presented at age 18 with absent pubertal development (TV < 3mL) and was confirmed to be anosmic on formal testing. His serum gonadotropins and testosterone levels were undetectable and he exhibited a flat response to GnRH stimulation (LH 0.8 to 1.0 IU/L, FSH remained 0.8 IU/L). He was diagnosed with Kallmann syndrome and underwent a similar treatment regimen as his brother (testosterone then hCG then pulsatile GnRH). His medical history is also notable for pulmonary stenosis. Both brothers harbor identical mutations in *DCC* and *NTN1*.

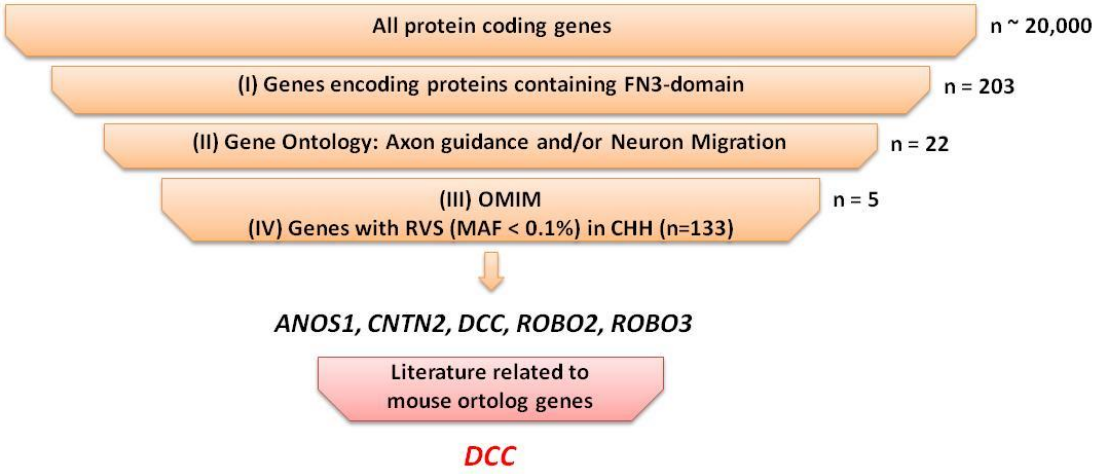
Family # 7, Patient II-1

***NTN1* p.Arg362Cys (heterozygous)**

***GnRH* p.Leu30fs (homozygous)**

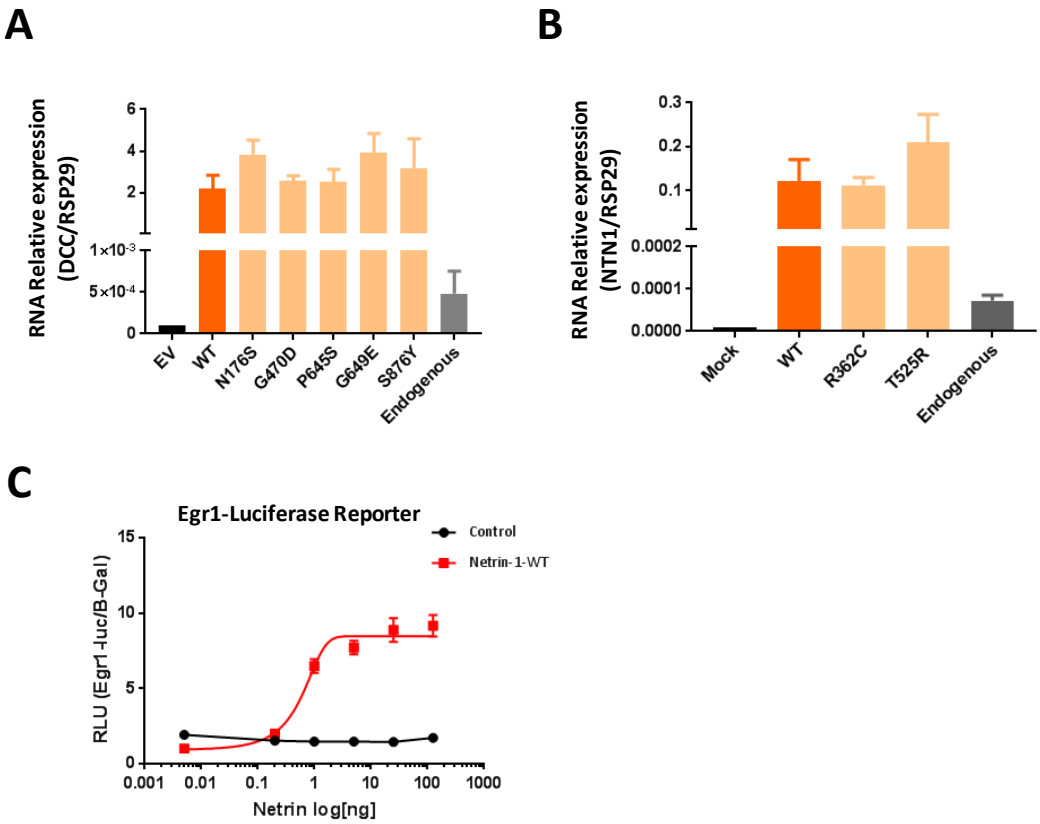
The male CHH proband was first evaluated at age 8 years for bilateral cryptorchidism and micropenis (< 3 cm). His parents come from the same village in Armenia and deny consanguinity. Repeated questioning of the father revealed delayed puberty. Ultrasound identified inguinal testes with calculated volume of approximately 0.1 ml. Bilateral orchidopexy was performed shortly afterwards. At age 13 years and 6 months, there were no signs of puberty. A GnRH stimulation test showed undetectable baseline gonadotropines (FSH, LH < 0.5 IU/l) that responded minimally to 1.3 and 0.8 IU/l respectively. Formal smell testing indicated normal sense of smell. Puberty induction by testosterone injections was initiated and resulted in linear growth and development of secondary sexual characteristics. Tanner V pubic hair was seen at age 15 years and 6 months. On request of the father, testosterone treatment was stopped at age 16 years. The patient developed clinical and biochemical hypogonadism. The patient harbors a *de novo* mutation in *NTN1*. An additional homozygous frameshift mutation of *GNRH1* has been previously described *(Chan et al, PNAS 2009)* with both parents being heterozygous.

Supplemental Figure 1.



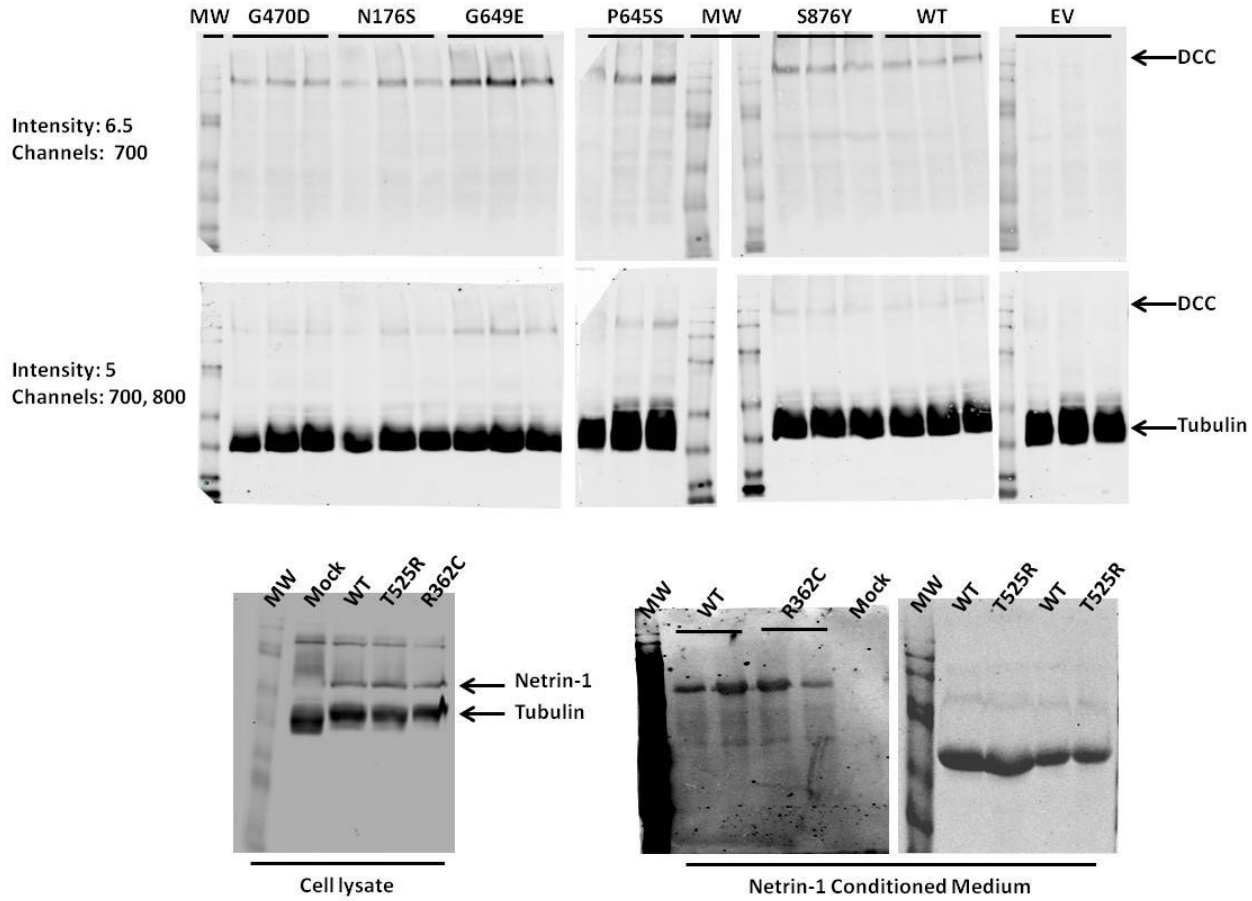
Supplemental figure 1. Bioinformatics workflow for filtering and prioritization of CHH candidate genes.

Supplemental Figure 2.



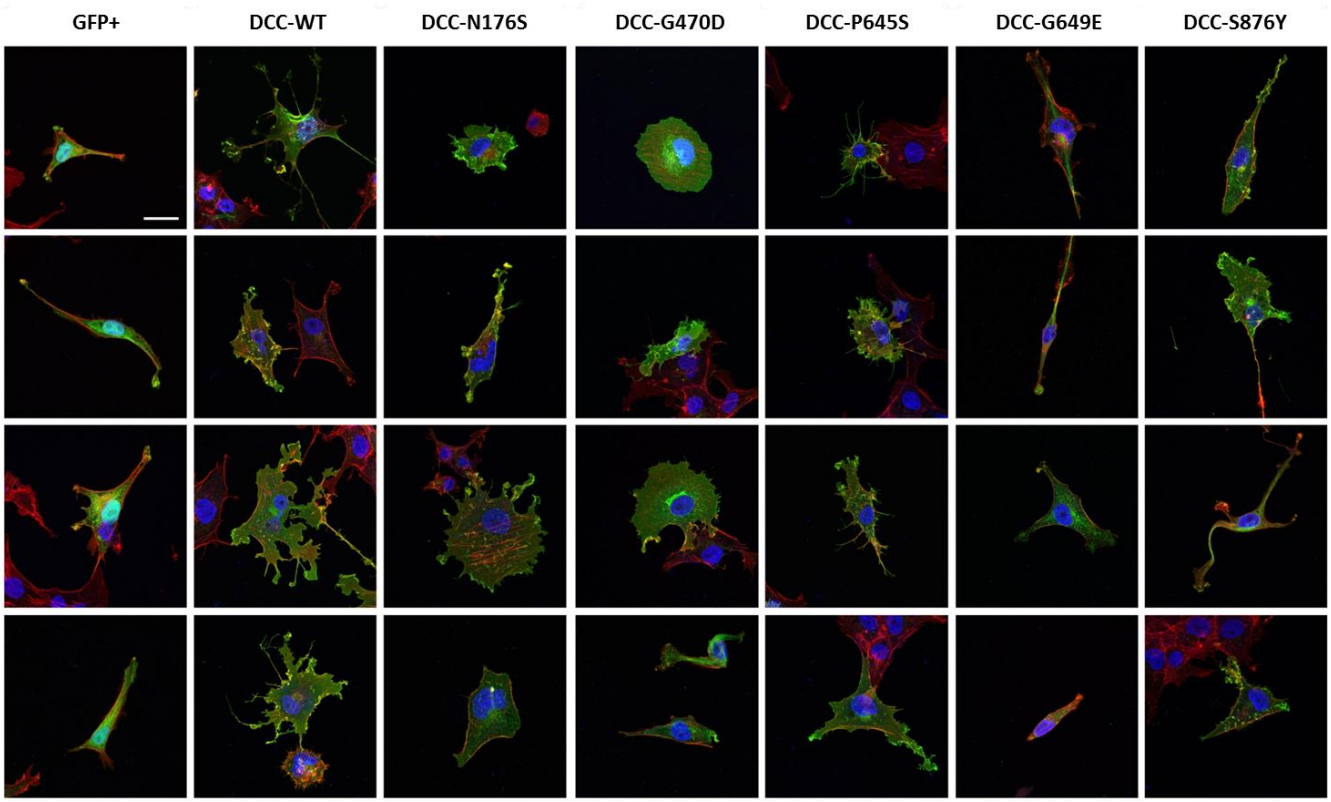
Supplemental figure2. **A.** Relative expression of DCC RNA after transient transfection in CHO cells. **B**. Relative expression of Netrin-1 RNA after transient transfection in CHO cells. **D**. Dose-response curve with increasing quantity of Netrin-1 WT transfected in CHO cells. Results are mean of 3 independent experiments each performed in triplicate. Error bars represent SEM.

Supplemental Figure 3.



Supplemental figure3. Full blot image of Figure 2. A-B, MW: Prism Ultra Protein Ladder (10-245 kDa) (Abcam, ab116028).

Supplemental Figure 4.



Representative of immunocytofluoresence of DCC in GN11 cells. GN11 cells are transfected with GFP or DCC plasmid, 24 hours post-transfection cells are plated onto poly-lysine coated coverslips for 24 hours. Cells are stained for DCC (green), phalloïdin (red), noyau (blue).