Supplementary Data

FGFR1 mutations cause Hartsfield syndrome, the rare association of holoprosencephaly and ectrodactyly.

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Table S1

Reference	Sex	Age	HPE	Ectrodactyly	CLP	Face	Eye	Ears	Endocrine	Additional
Hartsfield[1]	M	Died at 7 days	Lobar	Ectrodactyly, with oligodactyly of both hands	CP	Absence of right nostril, marked hypertelorism	Microphtalmia	Malformed ears	-	Radial aplasia, abnormal skull, abnormal sutures
Young[2]	M	TOP at 18w	Lobar	RH: polysyn-dactyly with central cleft LH: syndactyly F: ectro-syndactyly	Bilateral CLP	Hypertelorism	-	Rudimentary low set	-	-
Imaizumi[3]	M	8m	Lobar	RH: absence of 3rd digit, syndactyly LH: hypoplastic 3rd digit, syndactyly F: 2 digits, syndactyly	Bilateral CLP	Hypertelorism	-	Low set, dysmorphic	CDI	-
Corona-Rivera[4]	M	3y	Semilobar + pachygyria	Ectrodactyly of feet	Lateral cleft of the labial commisure	Hypertelorism	Microphtalmia coloboma	Low set	-	Prominent metopic suture, abnormal skull, abnormal hair pattern
Abdel-Meguid[5]	M	1y	Lobar	Ectrodactyly	-	Hypotelorism, synophris	-		CDI	
Konïg[6]	M	9y	Lobar	RH: hypoplastic 3rd phalanges LH: ectrodactyly F: 4 digits syndactyly	Bilateral CLP		-	-	CDI, HH, GH deficiency	Micropenis, cryptorchidism
Zechi-Ceide[7]	M	3y	Semilobar	Ectrodactyly, hypoplastic 2nd (R and LH) and 3rd digits (RH). F: 3 digits, partial 4-5 syndactyly	Bilateral CLP	Nl	-	-	-	Micropenis, cryptorchidism
Vilain[8]	M	TOP at 36w	Arhinencephaly, vermian hypoplasia	Ectrodactyly with 5 fingers on both hands, 3 toes on both feet.	Left labial and gingival cleft.	-	-	-	-	-
Vilain*[8]	M	19y	Lobar	Ectrodactyly of 4 limbs	Bilateral CLP	-	-	-	CDI, HH, anosmia	Micropenis, cryptorchidism
Vilain*[8]	M	1m	Alobar	Severe oligodactyly of hands and feet	Median CLP	Hypotelorism	-	Low set, small	-	Abnormal skull with prominent and widely patent sutures
Vilain*[8]	M	29y	Lobar	Ectrodactyly (hands mildly affected)	-	-	-	-	CID, HH	Micropenis, cryptorchidism
Vilain*[8]	M	12y	Semilobar	Ectrodactyly, camptodactyly, bifurcation of thumbs	Bilateral CLP			Pre-auricular tags		Micropenis, cryptorchidism
Keaton[9]	M		Semilobar	Bilateral ectrodactyly of hands, absence of central ray	Bilateral CLP			Bilateral microtia		Sacral dysgenesis, micropenis
Keaton[9]	F	TOP	Lobar	RH ectrodactyly with absent middle ray	Midline facial cleft, absence of nose	-	-	-	-	Hemi-vertebrae, lordosis, kyphosis, interrupted aortic arch, VSD
Keaton[9]	F		Lobar	Ectrodactyly of 4	High palate	Hypotelorism	-	Dysplastic	CDI	

				limbs with absence of digits 2-4				protruding ears		
Metwalley Kalil[10]	F	11m	Lobar	Ectrodactyly	Right CL	-	-	-		Sparse hypo-pigmented hairs
Takenouchi[11]	M	8y	Lobar	Ectrodactyly. H : 4 fingers, gap between the 2nd and 3rd digits	CLP	-	-	-	CDI, HH	

Table S1. Description of the previously reported patients with Harstfield syndrome. M: male, F: female. HPE: holoprosencephaly. CLP: cleft lip and palate, CL: cleft lip, CP: cleft palate. Nl: normal. TOP: termination of pregnancy. RH: right hand, LH: left hand, RF: right foot, LF: left foot. HH: hypogonadotropic hypogonadism, CDI: central diabetes insipidus, GH: growth hormone. VSD: ventricular septal defect, * present report.

Table S2

Exon	Product size	Forward primer	Reverse Primer
1	242	CCTTCTATTTGGGGACTCCG	GCAACTTAAAAGGAGCACAGAAC
2	403	CTCCCTGTCTTCCTCTCTCG	CACCTTCCTCTGAAACTGGC
3	227	GGCTTCCAGGACACACCTC	GTGCACCTGGGTTCCTCTC
4	231	TCCGTGTTCATCTGGAACTG	CTCTTAAACCCAATGCCCAG
5	290	CTGACCAGCTGCTCCTCTC	GGACTTCCTAACTCGGCCTC
6	264	GGCCTGCATTTTCCTCTG	CCTAAGAAACCTGGACACCC
7	561	GTGAGCCCACCCCTCTTTAG	GTCCAAATGCCTTCCTTGTG
8	284	AAAGTTACACGGGAGCAACG	CAGCTTGGGGCCTACATC
9	457	TTTCTGTCTCCTTCCCTTGC	GAGGCAGGTGTACGGGTG
10	341	TTGCTTTTCTAATGGAGCGG	AGGGCATTAGAGGCCCAG
11	279	AGAATGGGAAGGAGTCACCC	CACACCTTCCACCACTAGAATAG
12	259	ACAGAGAGGTGGAGATGGGG	CTGTTTGCTTGGAATGGGAC
13	249	CCTTAGCCTTTATCCTGCCC	GAGGCCTTGGGACTGATACC
14	349	CTTTGAGGTGAAGCCAAACC	CGCCACCACAAGATGATAAG
15	240	AGAGCCTTCCAGCTCCCTC	ACCCCACTCCTTGCTTCTC
16-17	606	GTCCCTTCCCACCTGTGC	CTCAAGCCCACTCTTGCC
18	255	AAGAGTGGGCTTGAGGGG	GCTCAGGGAGGTGCGTG
19	522	TGACCTCCAACCAGGTAAGG	GATCTGCCTCTTTGCACCTC

Table S2. List of primers for FGFR1 Sanger sequencing.

Table S3

Pos	Ref AA	Mut AA	Disease	Additional clinical features	Reference	Comments
48	G	S	IHH		Trarbach[12]	Sporadic IHH and normal olfactory sense
77	N	K	KS		Dode[13]	Present in controls too
78	R	C	KS	Cryptorchidism, micropenis	Pitteloud[14]	
97	G	D	KS		Dode[15]	
99	Y	C	IHH	HH reversal	Raivio[16]	
99	Y	C	KS		Dode[15]	
101	C	F	KS	CP, facial dysmorphism, ASD	Dode[13]	
102	V	fs	KS		Dode[15]	c.303-304insCC
102	V	I	KS		Albuisson[17]	
102	V	I	KS	Cryptorchidism, micropenis	Pitteloud[14]	
107	S	X	KS		Sato[18]	
112	T	T	SOD-like	HH, GH deficiency, CCA, eye defects	Raivio[19]	
117	N	S	IHH		Raivio[16]	Digenic GNRHR
129	D	A	KS	CP	Albuisson[17]	
165*	L	S	Hartsfield		This study	
167*	A	S	KS	CP, CCA, unilateral hearing loss, fusion of the fourth and fifth metacarpal bones	Dode[15]	
178	C	S	KS	Cryptorchidism, micropenis, hypoplasia of olfactory bulbs, CP, dental agenesis, external ear agenesis, right mandibular hypoplasia, thoracic dystrophia, failure to thrive, unique central incisor	Zenaty[20]	
191*	L	S	Hartsfield		This study	
197	F	L	KS		Sykiotis[21]	
224	D	H	KS	Cryptorchidism, micropenis	Pitteloud[14]	
228	Y	D	IHH	Osteoporosis	Raivio[16]	
237	G	D	KS	Occulo-motor apraxia, dental agenesis, bilateral cryptorchidism, synkinesia.	Pitteloud[14]	
237	G	S	IHH/KS	Bilateral cryptorchidism	Pitteloud[22]	One proband with IHH, brother with KS and bilateral cryptorchidism, father with anosmia only
237	G	S	KS	Bilateral cryptorchidism, occulo-motor apraxia, dental agenesis, synkinesia	Pitteloud[14]	
239	I	T	IHH	HH reversal	Raivio[16]	Digenic PROKR2
245	L	P	KS	CLP	Trarbach[12]	KS "+"

250	R	Q	IHH	Micropenis	Falardeau[23]	Digenic FGF8
250	R	W	KS	Mental deficiency, epilepsy	Trarbach[12]	2 unrelated cases: familial and sporadic Kallmann syndrome respectively, only one with mental deficiency and epilepsy
250	R	W	KS		Dode[13]	
254	R	Q	KS		Pitteloud[14]	
254	R	W	IHH		Koika[24]	
270	G	D	KS		Dode[13]	
273	V	M	KS	CP	Albuisson[17]	
273	V	M	KS		Pitteloud[14]	
274	E	G	KS	Cryptorchidism, micropenis, CL, synkinesia	Pitteloud[14]	
277	C	Y	KS		Dode[15]	
281|282	SD	del	KS	Dental agenesis	Bailleul-Forestier[25]	
283	P	R	KS	Dental agenesis	Dode[13]	
285	P	R	KS		Sykiotis[21]	
312	K	K_splice	KS	Multiple dental agenesis	Dode[15]	c.936G>A, exon 7 (donor splice site), synonymous effect, pathogenicity not formally shown
324	E	X	KS	CP	Dode[13]	
332	S	C	KS		Dode[13]	
339	Y	C	KS		Pitteloud[14]	
341	C	W	KS	Dental agenesis, Low testicular volume, Osteopenia of lumbar vertebrae and femoral neck hyperkalemia	Bailleul-Forestier[25]	
342	L	S	KS	CLP, micropenis, clinodactyly	Pitteloud[26]	
343	A	V	KS		Trarbach[12]	
346	S	C	KS		Pitteloud[14]	
348	G	R	KS	CLP, dental agenesis	Bailleul-Forestier[25]	
365	R	fsX41	KS	Dental agenesis	Albuisson[17]	
361	A	P_splice	KS		Dode[13]	c.1081G>C
366	P	L	KS	Obesity, sleep disorder	Trarbach[12]	case + paternal aunts with Kallmann syndrome, and his normosmic father
429*	V	E	KS		Sykiotis[21]	Not in reviewer's comments
439	S	fs	KS	CLP, hearing loss, coarctation of the aorta	Dode[13]	1317_1318delTG
450	S	F	SOD-like	HH, CDI, CCA, central incisor, brachydactyly, pre-auricular tags, ASD, VSD	Raivio[19]	
470	R	L	IHH		Pitteloud[26]	Oligogenic GNRHR

483	P	S	SOD-like	CPD, CLP, microphtalmia coloboma	Raivio[19]	
490	G	R	Hartsfield		This study	
520	A	T	KS		Albuisson[17]	
538	I	V	KS	Bilateral cryptorchidism	Pitteloud[14]	
585	Y	X	KS		Pitteloud[14]	
604	A	T	KS	Facial dysmorphy, criptorchidism, micropenis	Sarfati[27]	Oligogenic *PROKR2*
607	V	M	KS	Bimanual synkinesia	Dode[15]	
609	R	X	KS	CLP	Riley[28]	
613	Y	fsX42	KS		Albuisson[17]	
618	K	fsX654	KS	Cubitus valgus	Trarbach[12]	1852_1853delAA
618	K	N	IHH	Frontal bossing	Raivio[16]	Oligogenic GNRHR
621	H	R	KS	CP, 6 toes (right foot) + 4 toes (left foot), corpus callosum agenesis	Dode[13]	
622	R	G	KS	Cryptorchidism, multiple fusion of metacarpal bones on both hands and feet, dental agenesis, unilateral external ear hypoplasia, Bartter syndrome	Zenaty[20]	
622	R	Q	KS	Cryptorchidism, cleft palate, micropenis, dental agenesis	Zenaty[20]	
622	R	X	KS	CL or CP	Dode[15]	
622	R	X	KS		Pitteloud[14]	Partial puberty and a subsequent reversal of HH
623	D	Y	Hartsfield		This study	Required for catalysis[29]
628	N	K	Hartsfield		This study	
657	T	fs	KS		Dode[15]	c.1970-1971delCA
659	N	splice	KS		Dode[13]	c.1977+1G>A
661	R	X	KS		Dode[13]	
666	W	R	KS	CP	Dode[15]	
671	A	P		Clinodactily, osteopenia	Raivio[16]	
680	Q	X	IHH	father has CP	Pitteloud[22]	Brother with nIHH, and his father with delayed puberty, cleft lip palate and dental agenesis
684	W	splice	KS		Dode[15]	IVS15+1G>A , intron 15 (donor splice site)
685	S	F	KS	CLP	Dode[13]	
687	G	R	KS		Sato[30]	
690	L	P	KS	Dental agenesis, micropenis, microtestes Osteoporosis of vertebrae	Bailleul-Forestier[25]	
693	I	F	KS		Dode[13]	

700	P	L	IHH		Sykiotis[21]	
703	G	R	KS		Pitteloud[14]	
703	G	S	KS		Pitteloud[14]	
719	M	R	KS		Dode[15]	
722	P	S	KS	CL, bimanual synkinesia	Trarbach[12]	
722|724	P|N	H|K	IHH	Dental agenesis, unilateral cryptorchidism	Pitteloud[22]	Mother with isolated hyposmia
725	C	Y	Hartsfield		this study	
730	Y	X	KS		Albuisson[17]	
745	P	R	KS		Sykiotis[21]	Not in reviewer's comments, oligogenic
745	P	S	KS		Sato[18]	
764|768	Q|D	H|H	IHH		Falardeau[23]	Oligogenic FGF8
768	D	H	IHH		Sykiotis[21]	
772	P	S	KS	CP, unilateral absence of nasal cartilage, iris coloboma	Dode[15]	
772	P	S	KS	bimanual synkinesia	Dode[13]	
795	V	I	KS		Trarbach[12]	
822	R	C	KS		Dode[13]	Present in controls too

Table S3. Known FGFR1 mutations involved in isolated hypogonadotropic deficiency (IHH) / Kallman syndrome (KS)/ Septo-optic-like dysplasia (SOD-like)/ Hartsfield syndrome. Each line shows 1 case. Pos: Position on the amino acid sequence of Uniprot P11362 isoform 1. Asterisk indicates a homozygous mutation. Ref AA: reference amino acid. Mut AA: mutant amino acid. X for stop codon, splice for splicing variant, fs for frameshift. Disease: KS: Kallman syndrome, IHH: isolated hypogonadotropic hypogonadism, SOD-like: septo-optic-like dysplasia, CCA: corpus callosum cgenesis, CDI: central diabetes insipidus, ASD: atrium septum defect, VSD: ventricular septal defect, CPD: combined pituitary deficiency, CP: cleft palate, CLP: cleft lip and palate, CL: cleft lip. This list has been compiled starting from the mutations referenced in Uniprot entry P11362-1 to which we added the missing IHH/KS/SOD *FGFR1* mutations we could find in the literature and the Harstfield mutations found in this study.

Table S4

	HH	CDI	Pituitary other	Genitalia	Anosmia	Puberty	Adult	Arhinen-cephaly	
Patient 1* (Vilain3) [8]	NE	NE	NE	?	NA	NA	NA	?	Died at 5y
Patient 2 *	NE	NE	NE	Normal	NE	NA	NA	NE	Died at 4y
patient 3* (Vilain5) [8]	NE	-	NE	Small	NA	?	NA	?	
Patient 4*	+	+	Normal GH secretion, low response to RH	Female	?	Oestrogen therapy at 15y due to absent menarche, despite early signs of puberty	NA	?	
Patient 5* (Vilain 2) [8]	+	+	-	Bilateral cryptorchidism Small penis	Suspected	Induced at 13.3y	Tanner P 3	?	
Patient 6* (Vilain4) [8]	+	+	-	Small penis	NE	Induced at 14.9y	Tanner P5G5 (except for 2 ml testis volume)	?	
Patient 7*	NA	NA	NA	Female	NA	NA	NA	NR	TOP
Vilain 1[8]	NA	NA	NA	Normal	NA	NA	NA	+	TOP
Hartsfield[1]	NA	NA	NA	NR	NA	NA	NA	Absence of olfactory bulbs & tracts	Died at 7d
Young[2]	NA	NA	NA	NR	NA	NA	NA	NR	TOP
Imaizouni[3]	NR**	+	NR	NR	NR	NA	NA	NR	
Corona-Rivera[4]	NR	NR	NR	NR	NR	NA	NA	NR	
Abdel-Meguid[5]	NR	+	NR	Nl	NA	NA	NA	NR	Died at 1w
Konig[6]	+	+	SmC/GH extrem low, TSH nl	Small penis-hypospadias-cryptorchidism	NE	NR	NR	NR	
Zeichi[7]	NR	NR	NR	Cryptorchidism, small penis	NR	NA	NA	NR	
Keaton 13[9]	NR	NR	NR	Micropenis	NR	NR	NA	NR	
Keaton 14[9]	NR	NR	NR	Female nl	NR	NR	NR	NR	
Keaton 15[9]	NR	+	NR	Female	NR	NR	NR	NR	
Metwalley[10]	-***	-	-	Female	NR	NA	NA	NR	
Takenouchi[11]	+	+	Nl except HH	Micropenis, cryptorchidism	NR	NR	NR	Absence of olfactory bulbs & tracts	

Table S4. Kallmann Syndrome symptoms observed in Hartsfield syndrome patients. HH: hypogonadotropic hypogonadism, CDI: central diabetes insipidus NA : not applicable, NR : not reported, NE :not evaluated, Nl :normal,* present report,**not reported : but authors say *« endocrinal evaluation performed because of a High serum sodium level and he was diagnosed with diabetes insipidus»* but did not further detail, *** anterior and posterior pituitary functions tests showed no abnormalities.

Table S5

Gene	Patient 1	Patient 3	Patient 5	Parents of patient 5		Patient 6
ABCA1	0	0	0	0	0	0
APOE	0	0	0	0	0	0
B9D1	0	0	0	0	0	0
BMP1	0	0	0	0	0	0
BMP10	0	0	0	0	0	0
BMP15	0	0	0	0	0	0
BMP2	0	0	0	0	0	0
BMP2K	0	0	0	0	0	0
BMP3	1	0	0	0	0	0
BMP4	0	0	0	0	0	0
BMP5	0	0	0	0	0	0
BMP6	0	0	0	0	0	0
BMP7	0	0	0	0	0	0
BMP8A	0	0	0	0	0	0
BMP8B	0	0	0	0	0	0
BMPER	0	0	0	0	0	0
BMPR1A	0	0	0	0	0	0
BMPR1B	0	0	0	0	0	0
BMPR2	0	0	0	0	0	0
BOC	0	0	0	0	0	0
BTRC	0	0	0	0	0	0
CC2D2A	0	0	0	0	0	0
CDC42	0	0	0	0	0	0
CDO	0	0	0	0	0	0
CDON	0	0	0	0	0	0
CHD7	0	0	0	0	0	0
CHRD	0	0	0	0	0	1
DHCR7	1	0	0	0	0	0
DISP1	0	0	0	0	0	0
DISP2	0	0	0	0	0	0
DKK1	0	0	0	0	0	0
DLX1	0	0	0	0	0	0
DLX2	0	0	0	0	0	0
DLX5	0	0	0	0	0	0
DLX6	1	0	1	0	0	1
DSS1	0	0	0	0	0	0
FAM123B	0	0	0	0	0	0
FBXW4	0	0	0	0	0	0
FGF1	0	0	0	0	0	0
FGF10	0	1	0	0	0	0
FGF11	0	0	0	0	0	0
FGF12	0	0	0	0	0	0
FGF13	0	0	0	0	0	0
FGF14	0	0	0	0	0	0

FGF16	0	0	0	0	0	0
FGF17	0	0	0	0	0	0
FGF18	0	0	0	0	0	0
FGF19	0	0	0	0	0	0
FGF2	0	0	0	0	0	0
FGF20	0	0	0	0	0	0
FGF21	0	0	0	0	0	0
FGF22	0	0	0	0	0	0
FGF23	0	0	0	0	0	0
FGF3	0	0	0	0	0	0
FGF4	0	0	0	0	0	0
FGF5	0	0	0	0	0	0
FGF6	0	0	0	0	0	0
FGF7	0	0	0	0	0	0
FGF8	0	0	0	0	0	0
FGF9	0	0	0	0	0	0
FGFBP1	0	0	0	0	0	0
FGFBP2	0	0	0	0	0	0
FGFBP3	0	0	0	0	0	0
FGFR1	1	1	1	0	0	1
FGFR1OP	0	0	0	0	0	0
FGFR1OP2	0	0	0	0	0	0
FGFR2	0	0	0	1	0	0
FGFR3	0	0	0	0	0	0
FGFR4	0	0	0	0	0	0
FGFRL1	0	0	0	0	0	1
FMN	0	0	0	0	0	0
FOXH1	0	0	0	0	0	0
GAS1	0	0	0	0	0	0
GLI1	0	0	0	0	1	0
GLI2	0	0	0	0	1	2
GLI3	0	0	0	0	0	0
GNRH1	0	0	0	0	0	0
GNRHR	0	0	0	0	0	0
GRE	0	0	0	0	0	0
HIP	0	0	0	0	0	0
HOXA1	0	0	0	0	0	0
HOXA10	0	0	0	0	0	0
HOXA11	0	0	0	0	0	0
HOXA13	0	0	0	0	0	0
HOXA2	0	0	0	0	0	0
HOXA3	0	0	0	0	0	0
HOXA4	0	0	0	0	0	0
HOXA5	0	0	0	0	0	0
HOXA6	0	0	0	0	0	0

HOXA7	0	0	0	0	0	0
HOXA9	0	0	0	0	0	0
HOXB1	0	0	0	0	0	0
HOXB13	0	0	0	0	0	0
HOXB2	0	0	0	0	0	0
HOXB3	0	0	0	0	0	0
HOXB4	0	0	0	0	0	0
HOXB5	0	0	0	0	0	0
HOXB6	0	0	0	0	0	0
HOXB7	0	0	0	0	0	0
HOXB8	0	0	0	0	0	0
HOXB9	0	0	0	0	0	0
HOXC10	0	0	1	0	0	1
HOXC11	0	0	0	0	0	0
HOXC12	0	0	0	0	0	0
HOXC13	0	0	0	0	0	0
HOXC4	0	0	0	0	0	0
HOXC5	0	0	0	0	0	0
HOXC6	0	0	0	0	0	0
HOXC8	0	0	0	0	0	0
HOXC9	0	0	0	0	0	0
HOXD1	0	0	0	0	0	0
HOXD10	0	0	0	1	0	0
HOXD11	0	0	0	0	0	0
HOXD12	0	0	0	0	0	0
HOXD13	0	0	0	0	0	0
HOXD3	0	0	0	0	0	0
HOXD4	0	0	0	0	0	0
HOXD8	0	0	0	0	0	0
HOXD9	0	0	0	0	0	1
HS6ST1	0	4	0	1	1	2
HUWE1	0	0	1	0	1	1
JAG1	0	0	0	0	0	0
JAG2	0	0	0	0	0	0
KAL1	0	0	0	0	0	0
KISS1	0	0	0	0	0	0
KISS1R	0	0	0	0	0	0
LRP2	1	1	1	1	0	0
LRP6	0	0	0	0	0	0
MSX1	0	0	0	0	0	0
MSX2	0	0	0	0	0	0
NELF	0	0	0	0	0	0
NKX2.1	0	0	0	0	0	0
NODAL	0	0	0	0	0	0
NOG	0	0	0	0	0	0

PITX1	0	0	0	0	0	0
PITX2	0	0	0	0	0	0
PITX3	0	0	0	0	0	0
PROK2	0	0	0	0	0	0
PTCH1	0	0	0	0	0	0
PTCH2	0	0	0	0	0	0
REDD1	0	0	0	0	0	0
ROR2	0	1	0	0	0	0
SEMA3A	1	0	0	0	0	0
SHH	0	0	0	0	0	0
SIX3	0	0	0	0	0	0
SMAD1	0	0	0	0	0	0
SMAD2	0	0	0	0	0	0
SMAD3	0	0	0	0	0	0
SMAD4	0	0	0	0	0	0
SMAD5	0	0	0	0	0	0
SMAD6	0	0	0	0	0	0
SMAD7	0	0	0	0	0	0
SMAD9	0	0	0	0	0	0
SMO	0	0	0	0	0	0
SOX2	0	0	0	0	0	0
SUFU	0	0	0	0	0	0
TAC3	0	0	0	0	0	0
TACR3	0	0	0	0	0	0
TBX1	0	0	0	0	0	0
TBX10	0	0	0	0	0	0
TBX15	0	0	0	0	0	0
TBX18	0	0	0	0	0	1
TBX19	0	0	0	0	0	0
TBX2	0	0	0	0	1	0
TBX20	0	0	0	0	0	0
TBX21	0	0	0	0	0	0
TBX22	0	0	0	0	0	0
TBX3	0	0	0	0	0	0
TBX4	0	0	0	0	0	0
TBX5	0	0	0	0	0	0
TBX6	0	0	0	0	0	0
TBXA2R	0	0	0	0	0	0
TBXAS1	0	0	0	0	0	1
TCTN1	0	0	0	0	0	0
TDGF1	0	0	0	0	0	0
TGIF	0	0	0	0	0	0
TGIF1	0	0	0	0	0	0
TP63	0	0	0	0	0	0
TWSG1	1	0	0	0	0	0

WDR11	0	0	0	0	0	0
WNT1	0	0	0	0	0	0
WNT10A	0	0	0	0	0	0
WNT10B	0	0	0	0	0	1
WNT11	0	0	0	0	0	0
WNT16	0	0	0	0	0	0
WNT2	0	0	0	0	0	0
WNT2B	0	0	0	0	0	0
WNT3	0	0	0	0	0	0
WNT3A	0	0	0	0	0	0
WNT4	0	0	0	0	0	0
WNT5A	0	0	0	0	0	0
WNT5B	0	0	0	0	0	0
WNT6	0	0	0	0	0	0
WNT7A	0	0	0	0	0	0
WNT7B	0	0	0	0	0	0
WNT8A	0	0	0	0	0	0
WNT8B	0	0	0	0	0	0
WNT8C	0	0	0	0	0	0
WNT9A	0	0	0	0	0	0
WNT9B	0	1	0	0	0	0
ZIC2	0	0	0	0	0	0

Table S5. Rare SNPs found in candidate genes for IHH, Holoprosencephaly or split hand-foot malformation. To explore the potential for multiple genes to be involved in Hartsfield syndrome, we counted the number of rare non synonymous coding SNPs (GMAF unknown or < 0.01) in genes known to be involved in IHH, Holoprosencephaly (HPE) or split hand-foot malformation (SHFM). Genes were selected manually from the OMIM database and scientific literature, including genes involved in limb bud development.

Table S6

Program	Version	Parameters
BWA	0.6.1	
Picard MarkDuplicates	1.54	REMOVE_DUPLICATES=true, VALIDATION_STRINGENCY=LENIENT, AS=true
GATK	1.6	
SNPEff	2.0.5	
BEDTools coveragebed	2.1.3	
SNPSift	1.3.4	
GATK RealignerTargetCreator	1.6	--known 1000G_phase1.indels --known Mills_and_1000G_gold_standard.indels
GATK CountCovariates	1.6	-knownSites dbsnp_135 -cov ReadGroupCovariate -cov QualityScoreCovariate -cov CycleCovariate -cov DinucCovariate
GATK UnifiedGenotyper	1.6	--max_alternate_alleles 12 -glm BOTH --dbsnp dbsnp_135 -stand_call_conf 30.0 -stand_emit_conf 10.0
GATK VariantRecalibrator (SNPs)	1.6	-an QD -an HaplotypeScore -an MQRankSum -an ReadPosRankSum -an FS -an MQ
GATK VariantFiltration (indels)	1.6	--filterExpression "QD < 2.0" --filterName QDFilter --filterName ReadPosRankSum --filterExpression "ReadPosRankSum < -20.0" --filterName FS --filterExpression "FS > 200.0"

Table S6. List of parameters for the programs used in the exome analysis.

Figure S1

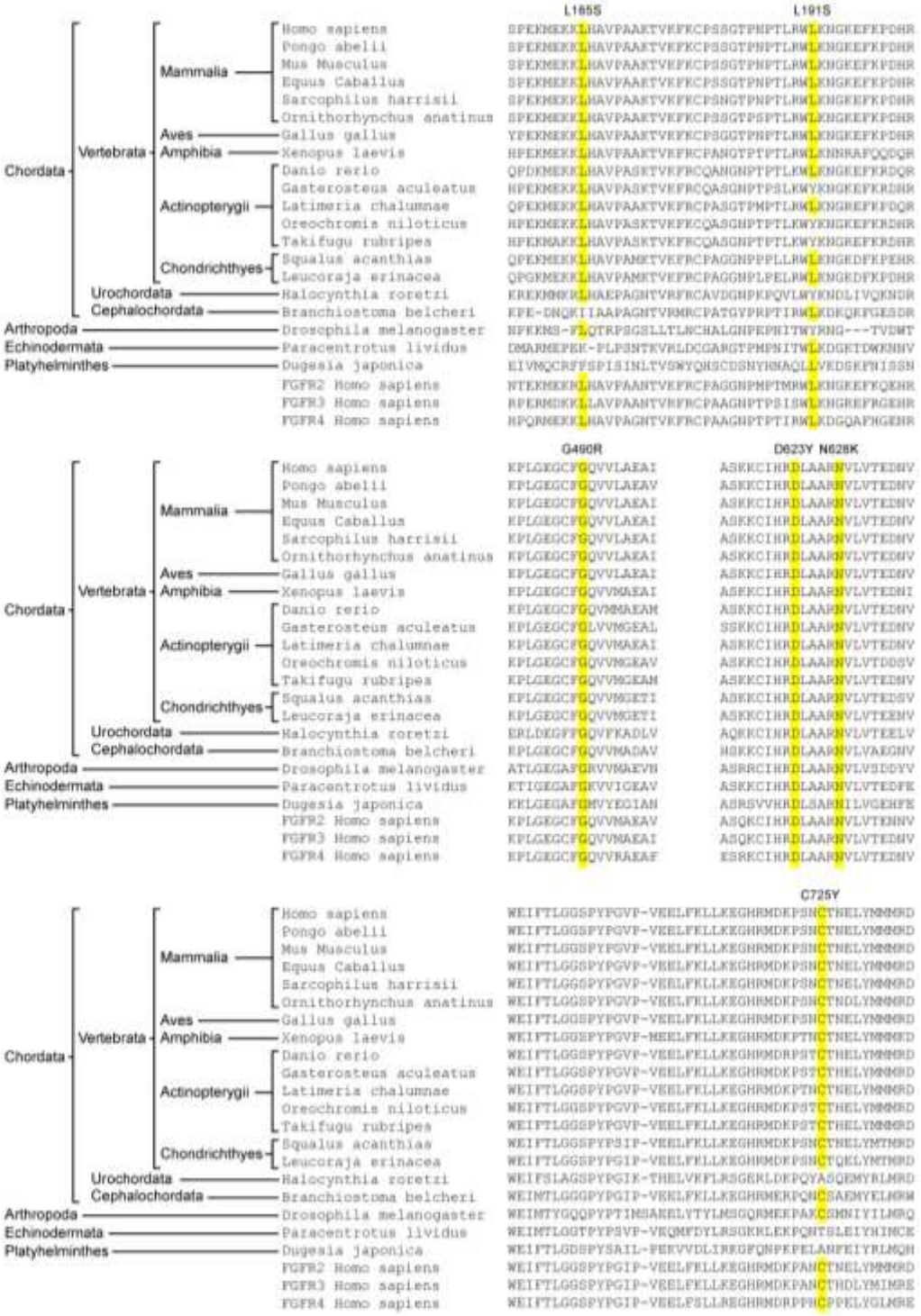

Figure S1. Conservation of FGFR1 residues. The positions of the mutations for Hartsfield patients involve amino acids highly conserved in mammals (L191), vertebrates (L165, C725) and eukaryotes (G490, D623 and N628). All 6 positions are conserved in FGFR2, 3 and 4. Sequences were selected from Uniprot to pick the closest sequence to FGFR1_human (P11362) for each species (best BLAST hit). Sequences were aligned with Clustal Omega.

Figure S2

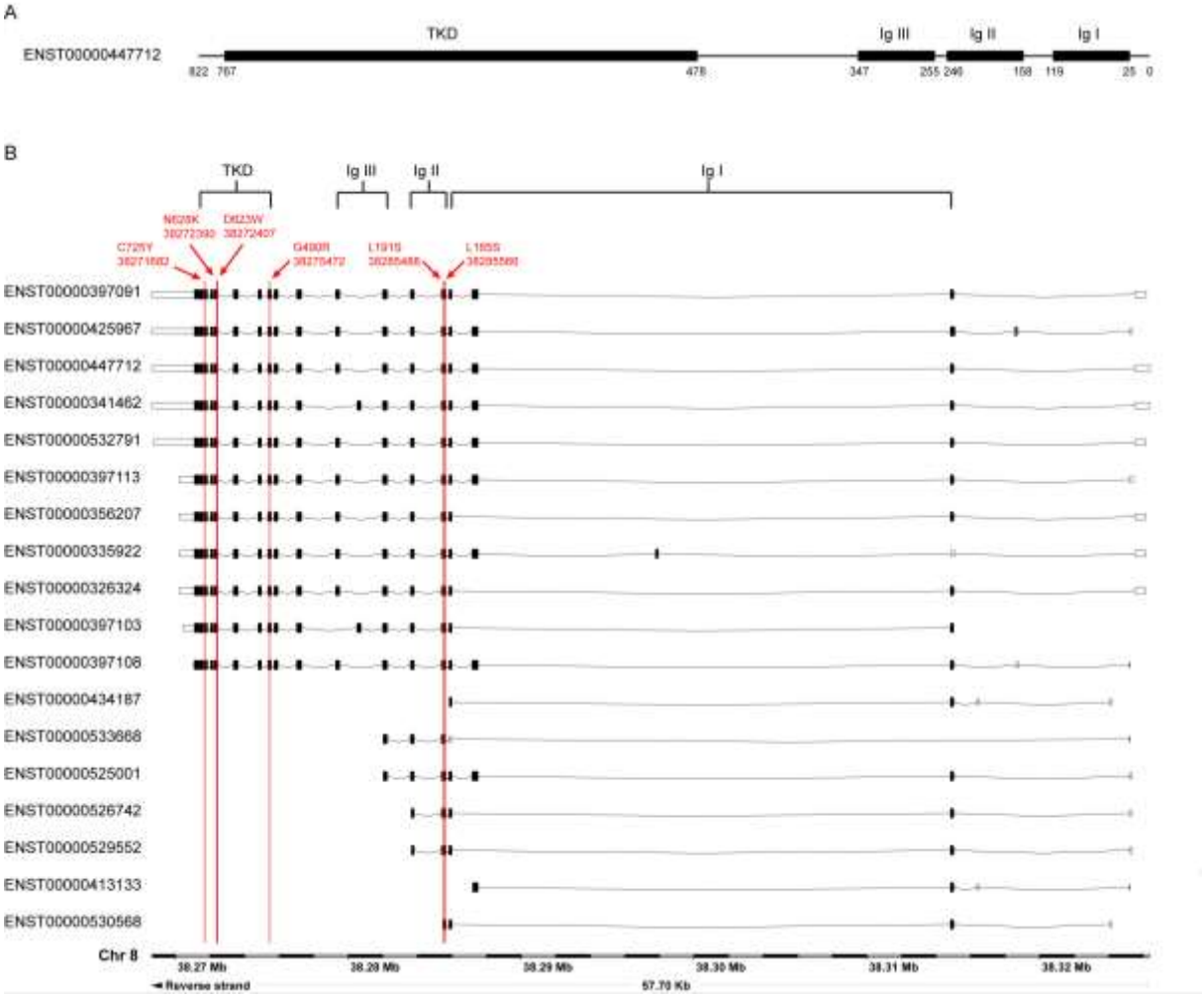

Figure S2. Positions of Hartsfield syndrome mutations relative to protein-coding FGFR1 isoforms. (A) Representation of the functional protein domains corresponding to transcript ENST00000447712, according to UNIPROT entry P11362-1. TKD: tyrosine kinase domain, Ig I,II,III: Immunoglobulin domains. (B) Alignment of all protein coding transcripts corresponding to Ensembl Gene ENSG00000077782, along with the corresponding positions of functional protein domains, mutations discovered in this study and position on chromosome 8. This figure was modified from the Ensembl genome browser.

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