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# *FGFR1* mutations cause Hartsfield syndrome, the unique association of holoprosencephaly and ectrodactyly

Nicolas Simonis,<sup>1</sup> Isabelle Migeotte,<sup>2,3</sup> Nelle Lambert,<sup>2,3</sup> Camille Perazzolo,<sup>2</sup> Deepthi C de Silva,<sup>4</sup> Boyan Dimitrov,<sup>5</sup> Claudine Heinrichs,<sup>6</sup> Sandra Janssens,<sup>7</sup> Bronwyn Kerr,<sup>8</sup> Geert Mortier,<sup>9</sup> Guy Van Vliet,<sup>10</sup> Philippe Lepage,<sup>6</sup> Georges Casimir,<sup>6</sup> Marc Abramowicz,<sup>2,3</sup> Guillaume Smits,<sup>3,6</sup> Catheline Vilain<sup>3,6</sup>

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For numbered affiliations see end of article.

## Correspondence to

Guillaume Smits, and Catheline Vilain, Department of Paediatrics, Hôpital Universitaire des Enfants Reine Fabiola (HUDERF), Université Libre de Bruxelles (ULB), Brussels, Belgium; [guillaume.smits@huderf.be](mailto:guillaume.smits@huderf.be) and [cavilain@ulb.ac.be](mailto:cavilain@ulb.ac.be)

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## ABSTRACT

**Background** Hartsfield syndrome is the rare and unique association of holoprosencephaly (HPE) and ectrodactyly, with or without cleft lip and palate, and variable additional features. All the reported cases occurred sporadically. Although several causal genes of HPE and ectrodactyly have been identified, the genetic cause of Hartsfield syndrome remains unknown. We hypothesised that a single key developmental gene may underlie the co-occurrence of HPE and ectrodactyly.

**Methods** We used whole exome sequencing in four isolated cases including one case-parents trio, and direct Sanger sequencing of three additional cases, to investigate the causative variants in Hartsfield syndrome.

**Results** We identified a novel *FGFR1* mutation in six out of seven patients. Affected residues are highly conserved and are located in the extracellular binding domain of the receptor (two homozygous mutations) or the intracellular tyrosine kinase domain (four heterozygous de novo variants). Strikingly, among the six novel mutations, three are located in close proximity to the ATP's phosphates or the coordinating magnesium, with one position required for kinase activity, and three are adjacent to known mutations involved in Kallmann syndrome plus other developmental anomalies.

**Conclusions** Dominant or recessive *FGFR1* mutations are responsible for Hartsfield syndrome, consistent with the known roles of FGFR1 in vertebrate ontogeny and conditional *Fgfr1*-deficient mice. Our study shows that, in humans, lack of accurate FGFR1 activation can disrupt both brain and hand/foot midline development, and that *FGFR1* loss-of-function mutations are responsible for a wider spectrum of clinical anomalies than previously thought, ranging in severity from seemingly isolated hypogonadotropic hypogonadism, through Kallmann syndrome with or without additional features, to Hartsfield syndrome at its most severe end.

## INTRODUCTION

Holoprosencephaly (HPE) results from impaired midline cleavage of the embryonic forebrain. It varies in severity from lobar HPE (a monoventricular cerebrum that lacks interhemispheric division) to microform HPE (such as single central maxillary incisor). Milder cerebral midline defects including isolated corpus callosum agenesis or arhinencephaly (the absence of olfactory bulbs and tracts) have also

been classified as falling within the HPE spectrum, at least in some instances.<sup>1</sup> Ectrodactyly, also known as split-hand/foot malformation, is a congenital limb malformation characterised by a median cleft of the hand and/or foot due to the absence of the central rays.<sup>2</sup> It encloses a broad spectrum of malformations, from shortening of the central digit to reduction of the hand/foot to a single ray, and may be variable even between the limbs of an affected individual. Several genes have been involved in non-syndromic HPE (including *SHH*, *ZIC2*, *SIX3*, *GLI2*, and *TGIF*) or ectrodactyly (such as *P63*, or the 10q24 duplication), with incomplete penetrance and variable expressivity.<sup>1–3</sup>

HPE and ectrodactyly can occur, separately, as part of numerous syndromes, but the co-occurrence of these two malformations, known as Hartsfield syndrome (OMIM 300571), has only been reported in 14 males and three females (see online supplementary table S1).<sup>4–9</sup> In addition to HPE and ectrodactyly, patients with Hartsfield syndrome show developmental defects of variable severity, ranging from one mildly affected individual with isolated hypogonadotropic hypogonadism (IHH), central diabetes insipidus, borderline low intelligence, and no facial dysmorphism to patients showing multiple congenital anomalies such as cleft lip and palate, malformed ears, hypo- or hypertelorism. There are also anecdotal reports of skull defects, vertebral anomalies, radial aplasia, eye anomalies or cardiac malformation (see online supplementary table S1). Targeted sequencing of HPE or ectrodactyly genes in selected patients has failed to identify mutations, and no convincing copy number variants were found.<sup>5–7,9</sup> Despite the variable expressivity of Hartsfield syndrome, we postulated that mutations in a single key developmental gene underlie the co-occurrence of HPE and ectrodactyly.

## METHODS

### Patients

We selected six patients with Hartsfield syndrome (patients 1–6), and one female fetus with the association of HPE, ectrodactyly, and additional severe malformations. Patients 1, 3, 5, and 6 were previously described.<sup>5</sup> Patients 1–7 detailed phenotypes are described in table 1. Pictures of faces and hands

**Table 1** Detailed phenotypic description of the seven tested patients

Patient	1	2	3	4	5	6	7
FGFR1 mutation	c.494T>C p.L1655*	c.572T>C p.L1915*	c.1468G>C p.G490R	c.1867G>T p.D623Y	c.1884 T>G p.N628K	c.2174 G>A p.C725Y	–
Sex	M	M	M	F	M	M	F
Previous report <sup>5</sup>	Patient 3	–	Patient 5	–	Patient 2	Patient 4	–
Consanguinity	+	–†	–	–	–	–	–
Brain							
HPE	AL	L	SL	L	SL	L	AL
CCA	+	+	nr	Partial	Partial	Partial	nr
Pituitary	Normal	nr	nr	nr	Normal	Normal	nr
Diminished cortical thickness	+	+	–	–	–	–	+‡
Face							
CLP	Median	–	Bilateral	–	Bilateral	–	Cleft palate only
Eye	Hypotelorism	Hypotelorism	hypertelorism	Normal	Normal	Normal	Hypertelorism§
Hands							
Ectrodactyly	+	+	+	–	+	+	+
Digit number (right/left)	2/2	3/3	3/3	5/5	4/4	5/5	2/3
Other		6 metacarpal bones on the left side, with partial fusion of the 4th and 5th	Bifurcation of the thumbs		Fused 2nd and 3rd metacarpal bones		Forearm hypoplasia
Feet							
Ectrodactyly	+	+	+	+¶	+	+	–
Digit number (right/left)	1/1	2/2	2/2	5/5	2/3	4/3	5/5
Other			Equinovarus deformity				
Pituitary insufficiency	nr	nr	nr	CDI, HH, normal GH secretion, low response to TRH	CDI, HH	CDI, HH, normal GH secretion	nr
Genitalia	Normal	nr	Micropenis, cryptorchidism	Normal	Micropenis, cryptorchidism	Micropenis, cryptorchidism	Normal
Growth retardation	+	+	+	+ Good response to GH treatment	+ 160 cm (target: 176.5±8.5 cm)	+ 161.6 cm (target: 172.5±8.5 cm)	nr
DD/ID other	Severe Generalised hypertonia, no smile, seizures (grand mal)	Severe No language, spasticity	Severe No language, wheelchair bound	Mild	Moderate Wheelchair bound (spastic paraplegia)	Mild IQ 63 (Stanford-Binet score), at 6 years 8 months	na
Follow-up	Died at the age of 5 years	Died at the age of 4 years (respiratory infection)		Mainstream school with support	Lives in an institution	Works in a sheltered workshop	TOP

Positions of the mutations refer to coding DNA reference sequence CCDS6107.2 and Uniprot protein sequence P11362-1.

\*Homozygous mutations.

†Low level consanguinity could not be assessed, the parents being lost to follow-up.

‡Patient 7 has severe microcephaly (head circumference of 15 cm at 20 weeks), hydrocephaly, and severe disruption of the telencephalic architecture.

§Patient 7 has severe facial anomalies: absence of nasal wing on the right side, right microphthalmia and eye defect.

¶Patient 4: Left foot: fusion of first and second toes, large gap between second and third rays, syndactyly of toes 3–5, absence of the third phalange of digits 3 and 4. Right foot: central large gap with partial syndactyly of toes 3–5, absence of the third phalange of digits 2 and 3.

AL, alobar; CCA, corpus callosum agenesis; CDI, central diabetes insipidus; CLP, cleft lip and palate; DD, developmental delay; F, female; GH, growth hormone; HH, hypogonadotropic hypogonadism; HPE, holoprosencephaly; ID, intellectual disability; L, lobar; M, male; na, not applicable; nr, not reported; SL, semilobar; TOP, termination of pregnancy; TRH, thyrotropin releasing hormone.

of patients 1, 5, and 6 demonstrating the phenotypic range of facial dysmorphism and ectrodactyly found in patients with Harstfield syndrome are shown in figure 1. All patient samples were obtained and handled in agreement with the guidelines set out by the Université Libre de Bruxelles Hôpital Erasme ethics committee. Written informed consent was obtained from all participants (or guardians), except for patient 7, for whom parents gave verbal consent.

### Exome sequencing

Exomes were captured using the TruSeq capture kit (Illumina) and paired-end sequenced over 100 bp on a Illumina HiSeq2000 sequencer by a third party provider (AROS applied biotechnology). For exome analysis, we followed the guidelines from the Genome Analysis ToolKit (GATK) best practice recommendations v3 to process an average of 118.7 million paired-end reads per sample.<sup>10</sup> We aligned the reads to the



GATK. SNPs were processed with 'variant quality score recalibration', a Gaussian mixture model using Hapmap and 1000 genomes Omni 2.5 M SNP chip arrays as a training. Indels were processed with direct filtering. Third, we selected de novo variants in the trio by restricting to variants with heterozygous genotype in the patient and homozygous reference in the parents, and unknown in dbSNP v135. Additional filtering was performed by adapting a described procedure.<sup>14</sup> Specifically, we removed heterozygous variants in the child when >70% of reads were reference, discarded cases where >10% non-reference reads in a parent matched the child's call, removed calls where the offspring depth was <10% of the parents total depth, and retained only variants with genotype quality  $\geq 20$  for the three samples. Fourth, we searched for genes where variants were present in all four patients, and for which the variant found in the patient of the case-parents trio was de novo. Parameters used in the programmes are listed in online supplementary table S6.

### Sanger sequencing

For the patients analysed through whole exome sequencing (WES) (patients 1, 3, 5, 6), the known mutated exons were amplified and sequenced using the Sanger method to verify the exome results. Whole sequencing of *FGFR1* was performed for the three additional patients (patients 2, 4, 7) (table 1). To include all potential splice variants, a 'merged transcript' was considered, containing all coding positions on the 19 *FGFR1* protein coding transcripts described in Ensembl release 66 (Ensembl gene ENSG00000077782). Primers have been designed with ExonPrimer (see online supplementary table S2). The PCR reaction was performed with 50 ng DNA, 3 pmol primers F and R, 2 mM MgCl<sub>2</sub>, 0.2  $\mu$ L Taq and H<sub>2</sub>O to 20  $\mu$ L. The PCR programme comprised 94°C for 3 min, 94°C for 30 s, 20 touchdown cycles 65°C to 55°C, 20 cycles 55°C for 30 s, 72°C for 1 min, and 72°C for 5 min. Purification of PCR product for sequencing was realised with ExoSap-IT (USB products, Affymetrix) and Sanger sequencing was initiated with the PCR primers of the corresponding amplicons.

### RESULTS

We performed WES in a series of four unrelated patients (patients 1, 3, 5, 6, table 1). Given the sporadic occurrence of cases of Hartsfield syndrome, we included one case-parent trio (patient 5), to focus our analysis on de novo variants. *FGFR1* was selected as the best candidate, being the only gene where unknown variants were identified for the four patients, and patient 5's *FGFR1* variant being a de novo mutation (ie, not found in his parents' exome sequence). We used Sanger sequencing of PCR products from genomic DNA to confirm the *FGFR1* variants identified through exome sequencing and to sequence the parents when available (figure 1). To support the above results, we performed Sanger sequencing of all coding exons and exon-intron boundaries of *FGFR1* for two other patients with Hartsfield syndrome (patients 2 and 4) and for a fetus with Hartsfield syndrome and severe additional features (patient 7) (table 1). We identified mutations in patients 2 and 4, bringing to six the number of Hartsfield patients carrying an *FGFR1* mutation (figure 1). For patient 7, we could not find any *FGFR1* coding or splice site mutation using Sanger sequencing, or pathogenic copy number variations (CNVs) using an Agilent 60K comparative genomic hybridisation (CGH) array.

*FGFR1* (Uniprot P11362) is a member of the receptor tyrosine kinase superfamily. It is composed of an extracellular ligand binding domain that contains three immunoglobulin (Ig)-like

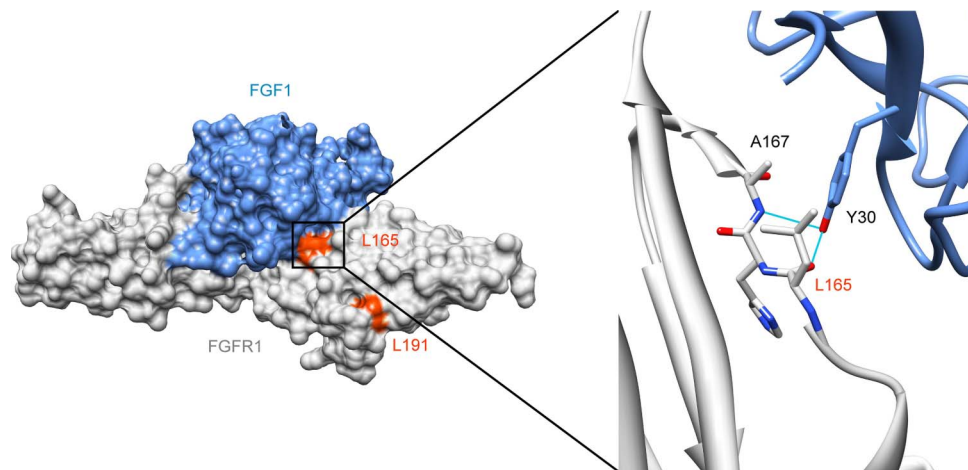
domains (D1–D3), a single transmembrane helix, and a cytoplasmic domain responsible for tyrosine kinase activity (figure 1).<sup>15</sup> The fibroblast growth factor (FGF) signalling pathway is a major player in embryonic development. Notably, in mice, the conditional lack of *Fgfr1* expression in developing telencephalon results in loss of cerebral commissures,<sup>16</sup> and also in the absence of olfactory bulbs,<sup>17</sup> as early emergence of gonadotropin releasing hormone (GnRH) neurons from the embryonic olfactory placode is dependent on *FGFR1* activation by FGF8.<sup>18</sup> During mouse autopod patterning, depending on the time and localisation of conditional gene inactivation, an *Fgfr1* insufficiency results in a variety of limb defects including loss restricted to the central digit or monodactyly.<sup>19</sup>

A wide phenotypic spectrum is observed in humans with *FGFR1* loss-of-function mutations, ranging from apparently asymptomatic carrier, IHH, typical Kallmann syndrome (KS) (the association of hypogonadotropic hypogonadism and anosmia, the latter due to the absence or hypoplasia of olfactory bulbs and tracts), to KS with associated features, mainly cleft lip and palate, and oligodontia (see online supplementary table S3). Identical *FGFR1* mutations may vary in phenotypic severity.<sup>20–22</sup> *FGFR1* mutations have also been reported in patients with phenotypes reminiscent of Hartsfield syndrome, such as the association of combined pituitary hormone deficiency, mild expression of HPE (corpus callosum agenesis, single central incisor), hand anomalies (brachydactyly, or fusion of metacarpal bones), and eye defects.<sup>20 22 23</sup>

In two of our patients with Hartsfield syndrome, we identified homozygous mutations affecting amino acid residues located in the extracellular ligand binding domain D2 of *FGFR1*: L165S, in patient 1, with a severe phenotype, and L191S, in patient 2, with a moderate phenotype (table 1, figure 1). Both parents of patient 1 were heterozygous for the L165S mutation, and were reported to be asymptomatic and spontaneously fertile. Parents of patient 2 were not available for testing. Mapping of these mutations on available *FGFR1* structures from the RCSB Protein Data Bank shows that L165S is likely to affect FGF binding; the effect for L191S is less clear (figure 2). One previous KS patient has been described with a homozygous *FGFR1* A167S mutation.<sup>20</sup> This patient had KS, cleft palate, corpus callosum agenesis, vertebral anomalies, unilateral fusion of fourth and fifth metacarpal bones, and bilateral oligodactyly of feet (four digits).<sup>24</sup>

We identified three heterozygous mutations affecting amino acid residues located in the ATP binding pocket of the intracellular tyrosine kinase domain (TKD, amino acids 478–767): G490R (patient 3, moderate phenotype), D623Y (patient 4, mild phenotype), and N628K (patient 5, moderate phenotype). Analysis of the available crystallographic structures shows that the mutated amino acids are in close proximity to the ATP and the coordinating magnesium, suggesting impairment of *FGFR1* kinase activity (figure 3). In support of this hypothesis, D623 is known to be required for catalysis.<sup>25</sup> A tyrosine substitution would prevent it from fulfilling its role as a proton acceptor for the substrate. Adjacent mutations H621R, R622G, R622Q, and R622X provoke syndromic KS, some patients having corpus callosum agenesis (H621R), digit number anomalies (H621R) or fusion of metacarpal bones (R622G) (figure 4, see online supplementary table S3).<sup>20–22 26</sup>

We located one other heterozygous mutation, C725Y, in the intracellular C-terminal loop of the TKD (patient 6, mild phenotype). Mutations of neighbouring residues (P722S, P722H, and N724K) have been previously suggested to alter the conformation of this region (figures 3 and 4, see online supplementary table S3) and shown to decrease kinase activity.<sup>27 28</sup>



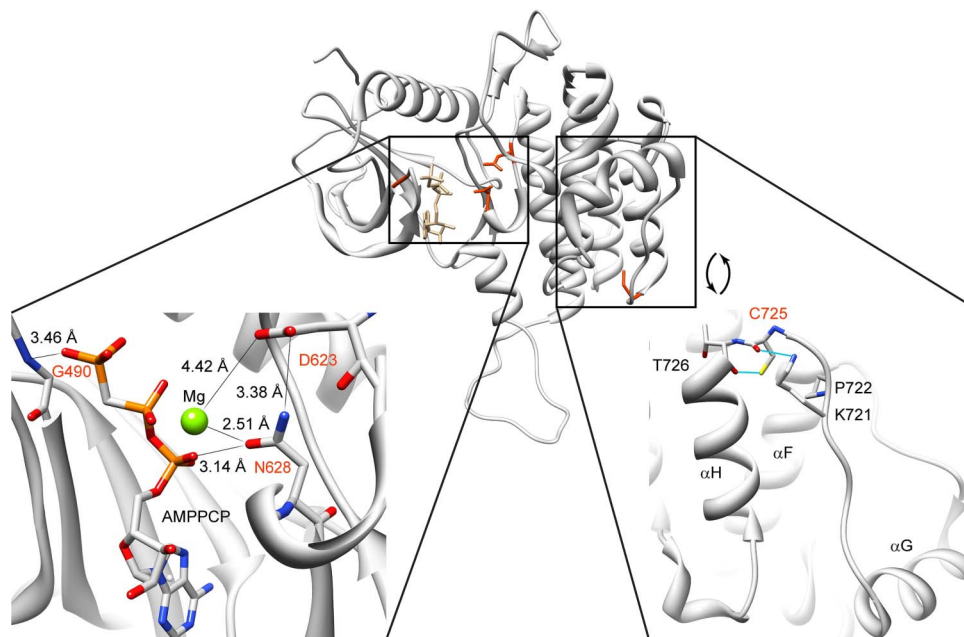
**Figure 2** Mapping of mutations L165S and L191S on crystal structure. Protein Data Bank structure 3OJV<sup>38</sup> showing the extracellular Ig-like domains 2 and 3 of FGFR1 (amino acids 147–359) bound to FGF1 in surface representation, and detail around leucine 165 in ribbon representation. FGFR1 is shown in grey and FGF1 in blue. Leucines 165 and 191 are coloured in orange red. The detailed view is highlighting the interface between FGFR1 and FGF1 around leucine 165. Tyrosine 30 on FGF1 forms hydrogen bonds with leucine 165 and alanine 167.<sup>39</sup> Substitution of the leucine 165 by a serine should affect FGF binding. These pictures were made using UCSF Chimera.<sup>40</sup>

To our knowledge, none of these *FGFR1* mutations have been previously reported in dbSNP, Exome Variant Server (<http://evs.gs.washington.edu/EVS/>) or the scientific literature, and all heterozygous mutations have occurred de novo. The substitutions involve amino acids highly conserved in mammals (L191), vertebrates (L165, C725) and eukaryotes (G490, D623, and N628) (see online supplementary figure S1). All mutations are predicted to be deleterious by SIFT and Polyphen 2.<sup>29 30</sup>

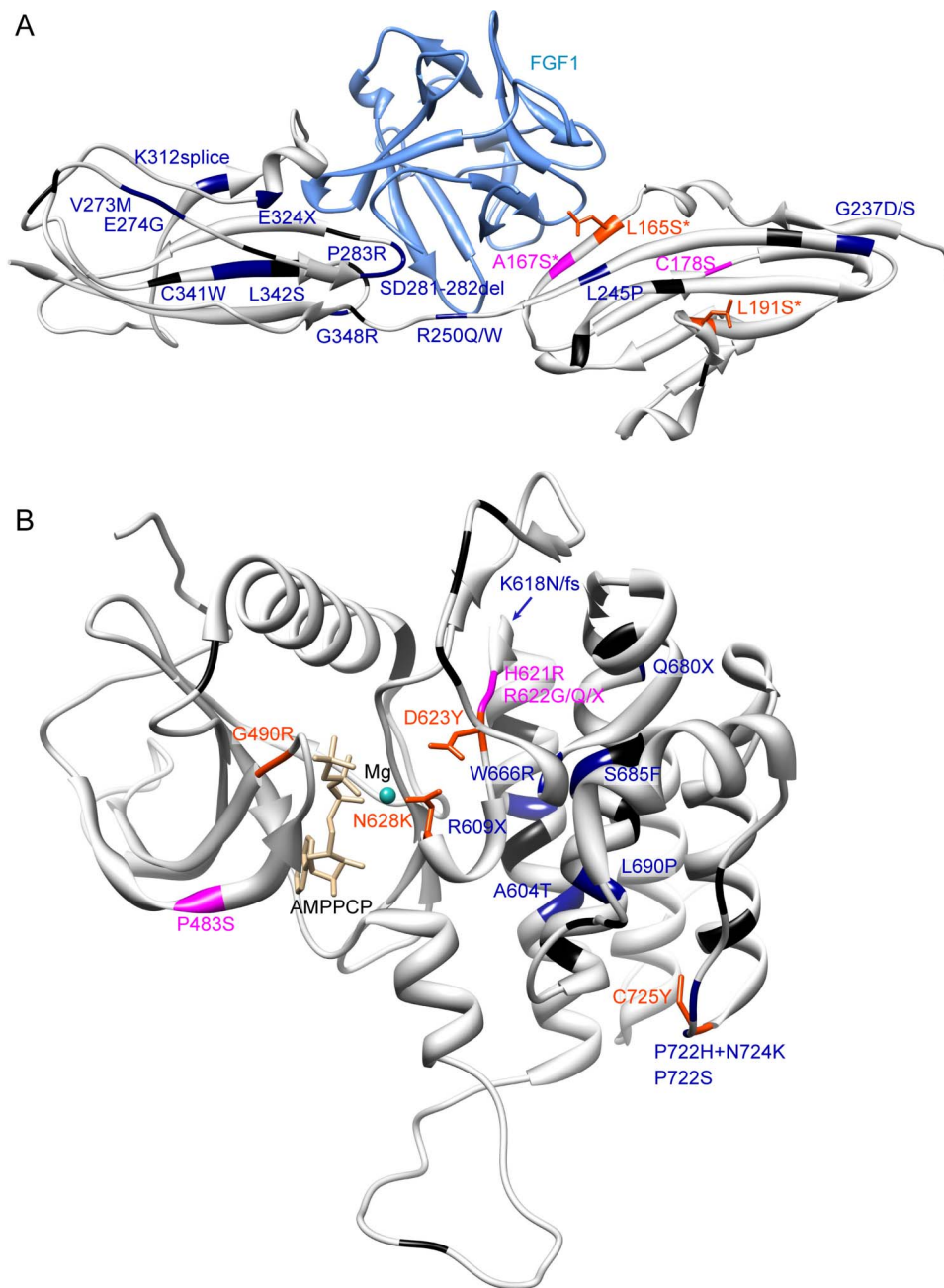
## DISCUSSION

Our study shows that *FGFR1* is responsible for Hartsfield syndrome, which is consistent with the known roles of FGFR1 in vertebrate ontogeny, human diseases, and observations of brain and digits anomalies in conditional *Fgfr1* deficient mice.

FGFR1 mutations also cause KS. From the cases reported in literature and in this study (see online supplementary table S4), it is not possible to confirm that KS is systematically part of



**Figure 3** Mapping of mutations G490R, D623Y, N628K, and C725Y on FGFR1 tyrosine kinase domain crystal structure. Protein Data Bank structure 3GQI<sup>41</sup> showing the intracellular kinase domain of FGFR1 (residues 464–770) in ribbon representation. The lower left part shows the details of the crystal structure surrounding the ATP binding pocket in the intracellular kinase domain of FGFR1. G490, D623, and N628 are in close proximity to the ATP's phosphates or coordinating magnesium. The lower right part shows the involvement of cysteine 725 in the positioning of the  $\alpha$ G-containing segment, along with T726, P722, and K721. Substitution of the cysteine 725 by a tyrosine will likely affect the conformation of this region.<sup>27</sup> The ATP analogue (AMPPCP) and wild-type residues of positions 490, 623, 628 and 721, 722, 725 and 726 are pictured in stick representation. Nitrogen, oxygen, phosphorus, and magnesium atoms are coloured blue, red, orange, and green, respectively. These pictures were made using UCSF Chimera.<sup>40</sup>



**Figure 4** Mapping of *FGFR1* mutations on known crystallographic structures. Idiopathic hypogonadotropic hypogonadism and Kallmann syndrome (KS) variants are depicted in black, KS with orofacial features variants are depicted in dark blue, more syndromic KS variants are depicted in magenta, and Hartsfield syndrome variants are depicted in orange red with the wild-type side chain in stick representation. Variants from the phenotypic three most severe categories are labelled. The most severe phenotype was considered if the same mutation was identified in several patients. Asterisk indicates a homozygous mutation. (A) Protein Data Bank (PDB) structure 3OJV,<sup>38</sup> showing extracellular immunoglobulin (Ig)-like domains 2 and 3, from amino acids 147–359. (B) PDB structure 3GQI,<sup>41</sup> showing the intracellular kinase domain, from residue 464–770. These pictures were made using UCSF Chimera.<sup>40</sup>

Hartsfield syndrome. Nevertheless, we suggest performing arhinencephaly, endocrinology and olfactory evaluations in patients with ectrodactyly, with or without additional malformations.

Our six *FGFR1* mutated patients show mild to severe Hartsfield syndrome. Variable expressivity and incomplete penetrance is well known in HPE, ectrodactyly and KS suggesting the role of additional factors and tissue specific sensitivity.<sup>31–32</sup> We explored the possibility of oligogenic inheritance<sup>33–36</sup> in the four patients screened by WES, as well as the potential role of distinct *FGFR1* isoforms, but no obvious pattern could be found (see

online supplementary table S5, supplementary figure S2). These questions should be addressed by the study of a large cohort of patients with IHH, KS, and Hartsfield syndrome.

We observe that *FGFR1* mutations responsible for Hartsfield syndrome occur in several clusters in important functional domains (figure 4): homozygous mutations in the ligand binding domain D2; heterozygous substitutions in the TKD core. Only the C725Y mutation lies alone at the TKD C-terminal extremity, among mutations reported in patients with IHH/KS with orofacial features. It will be interesting

to see if this clustering will resist the addition of new Hartsfield mutations.

The above work represents substantial evidence that *FGFR1* is the most prevalent, if not the sole gene causing Hartsfield syndrome. The six *FGFR1* mutated patients described here represent a homogeneous phenotype of HPE, ectrodactyly, with or without cleft lip and palate, and pituitary deficiency, although each of the features observed vary in severity. We however found no *FGFR1* mutation or large *FGFR1* deletion in a female fetus with severe brain malformation (HPE with severe disruption of the telencephalic architecture, heterotopies and diminished cortical thickness), ectrodactyly, bilateral forearm hypoplasia, cleft palate, hypertelorism, eye defect, and orbital hypoplasia on the right side (patient 7, table 1). This phenotype substantially deviates from the spectrum of clinical features observed in patients with Hartsfield syndrome and *FGFR1* mutations, and might represent another diagnostic entity. Whether Hartsfield syndrome is a genetically homogeneous affliction will need further study.

Depending on the localisation of the amino acid substitution, Hartsfield syndrome can have an autosomal dominant or autosomal recessive mode of inheritance. With no recurrence of Hartsfield syndrome having been reported so far, the intrafamilial variability in clinical manifestations is unknown. The main challenge to improve genetic counselling will be to decipher the genetic and environmental factors responsible for the wide variability of the *FGFR1* mutations disease spectrum.

In conclusion, our findings demonstrate that Hartsfield syndrome is part of a wide spectrum of developmental anomalies caused by *FGFR1* loss-of-function mutations. This spectrum included unaffected carrier, seemingly IHH, isolated KS, and KS with additional features (including anomalies of digits falling out of the definition of ectrodactyly, or mild expression of HPE, such as corpus callosum agenesis or central incisor), and septo-optic-like dysplasia.<sup>20–22</sup> The clinical entity known as Hartsfield syndrome now sits at its most severe end. In consequence, any patient with hand/foot midline defects (even mild ones) and affected by central diabetes insipidus, hypogonadotropic hypogonadism, anosmia or HPE should have their *FGFR1* gene sequenced.

#### Author affiliations

<sup>1</sup>Laboratoire de Bioinformatique des Génomiques et des Réseaux (BiGRé), Université Libre de Bruxelles (ULB), Brussels, Belgium

<sup>2</sup>Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Université Libre de Bruxelles (ULB), Brussels, Belgium

<sup>3</sup>ULB Center of Human Genetics, Hôpital Erasme, Université Libre de Bruxelles (ULB), Brussels, Belgium

<sup>4</sup>Department of Physiology, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka

<sup>5</sup>Department of Clinical Genetics, Guy's Hospital, London, UK

<sup>6</sup>Department of Paediatrics, Hôpital Universitaire des Enfants Reine Fabiola (HUDERF), Université Libre de Bruxelles (ULB), Brussels, Belgium

<sup>7</sup>Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium

<sup>8</sup>Manchester Academic Health Science Centre, University of Manchester, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK

<sup>9</sup>Center for Medical Genetics, Antwerp University Hospital and University of Antwerp, Antwerp, Belgium

<sup>10</sup>Endocrinology Service and Research Center, Hôpital Sainte-Justine and Department of Pediatrics, Université de Montréal, Montréal, Québec, Canada

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**Contributors** GS and CV designed the study strategy. NS performed analysis of next generation sequencing data, conservation and structure analysis. IM, NL and CP performed Sanger sequencing and analysis. CV, GS, DCdS, BD, CH, BK, GM, SJ, and GVV recruited subjects, gathered clinical data and contributed DNA samples. IM, PL,

GC and MA contributed technical support and discussions. NS, GS and CV wrote the manuscript. All authors reviewed the manuscript.

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**Patient consent** Obtained.

**Ethics approval** ULB Hôpital Erasme ethics committee.

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**Data sharing statement** Data used in this study are available upon request.

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## Supplementary Data

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

Nicolas Simonis,<sup>1</sup> Isabelle Migeotte,<sup>2,3</sup> Nelle Lambert,<sup>2,3</sup> Camille Perazzolo,<sup>2</sup> Deepthi C. de Silva,<sup>4</sup> Boyan Dimitrov,<sup>5</sup> Claudine Heinrichs,<sup>6</sup> Sandra Janssens,<sup>7</sup> Bronwyn Kerr,<sup>8</sup> Geert Mortier,<sup>9</sup> Guy Van Vliet,<sup>10</sup> Philippe Lepage,<sup>6</sup> Georges Casimir,<sup>6</sup> Marc Abramowicz,<sup>2,3</sup> Guillaume Smits,<sup>3,6,11,\*</sup> and Catheline Vilain,<sup>3,6,11,\*\*</sup>

<sup>1</sup> Laboratoire de Bioinformatique des Génomes et des Réseaux (BiGRe), Université Libre de Bruxelles (ULB), 1050 Brussels, Belgium.

<sup>2</sup> Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Université Libre de Bruxelles (ULB), 1070 Brussels, Belgium.

<sup>3</sup> ULB Center of Human Genetics, Hôpital Erasme, Université Libre de Bruxelles (ULB), 1070 Brussels, Belgium.

<sup>4</sup> Department of Physiology, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka.

<sup>5</sup> Department of Clinical Genetics, Guy's Hospital, London SE1 9RT, United Kingdom.

<sup>6</sup> Department of Paediatrics, Hôpital Universitaire des Enfants Reine Fabiola (HUDERF), Université Libre de Bruxelles (ULB), 1020 Brussels, Belgium

<sup>7</sup> Center for Medical Genetics Ghent, Ghent University Hospital, 9000 Ghent, Belgium.

<sup>8</sup> Manchester Academic Health Science Centre, University of Manchester, Central Manchester University Hospitals NHS Foundation Trust, Manchester M139WL, United Kingdom.

<sup>9</sup> Center for Medical Genetics, Antwerp University Hospital and University of Antwerp, 2650 Antwerp, Belgium.

<sup>10</sup> Endocrinology Service and Research Center, Hôpital Sainte-Justine and Department of Pediatrics, Université de Montréal, Montréal, H3T 1C5 Québec, Canada.

<sup>11</sup>These authors jointly directed this work.

\*Correspondence: [guillaume.smits@huderf.be](mailto:guillaume.smits@huderf.be)

\*\*Correspondence: [cavilain@ulb.ac.be](mailto:cavilain@ulb.ac.be)

**Table S1**

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

				limbs with absence of digits 2-4				protruding ears		
Metwalley Kalil <sup>10</sup>	F	11m	Lobar	Ectrodactyly	Right CL	-	-	-		Sparse hypo-pigmented hairs
Takenouchi <sup>11</sup>	M	8y	Lobar	Ectrodactyly. H : 4 fingers, gap between the 2 <sup>nd</sup> and 3 <sup>rd</sup> 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. NI: 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	CACCTTCCTCTGAACTGGC
3	227	GGCTTCCAGGACACACCTC	GTGCACCTGGGTTCTCTC
4	231	TCCGTGTTTCATCTGGAAGT	CTCTTAAACCAATGCCAG
5	290	CTGACCAGCTGCTCCTCTC	GGAATTCCTAACTCGGCCTC
6	264	GGCCTGCATTTTCCTCTG	CCTAAGAAACCTGGACACCC
7	561	GTGAGCCCACCCCTCTTTAG	GTCCAAATGCCTTCCTTG
8	284	AAAGTTACACGGGAGCAACG	CAGCTTGGGGCCTACATC
9	457	TTTCTGTCTCCTTCCCTTGC	GAGGCAGGTGTACGGGTG
10	341	TTGCTTTTCTAATGGAGCGG	AGGGCATTAGAGGCCAG
11	279	AGAATGGGAAGGAGTCACCC	CACACCTTCCACCACTAGAATAG
12	259	ACAGAGAGGTGGAGATGGGG	CTGTTTGCTTGAATGGGAC
13	249	CCTTAGCCTTTATCCTGCCC	GAGGCCTTGGGACTGATACC
14	349	CTTTGAGGTGAAGCCAAACC	CGCCACCACAAGATGATAAG
15	240	AGAGCCTTCCAGCTCCCTC	ACCCCACTCCTTGCTTCTC
16-17	606	GTCCCTTCCCACCTGTGC	CTCAAGCCCACTTGTCC
18	255	AAGAGTGGGCTTGAGGGG	GCTCAGGGAGGTGCGTG
19	522	TGACCTCCAACCAGGTAAGG	GATCTGCCTCTTGCACCTC

**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 <sup>12</sup>	Sporadic IHH and normal olfactory sense
77	N	K	KS		Dode <sup>13</sup>	Present in controls too
78	R	C	KS	Cryptorchidism, micropenis	Pitteloud <sup>14</sup>	
97	G	D	KS		Dode <sup>15</sup>	
99	Y	C	IHH	HH reversal	Raivio <sup>16</sup>	
99	Y	C	KS		Dode <sup>15</sup>	
101	C	F	KS	CP, facial dysmorphism, ASD	Dode <sup>13</sup>	
102	V	fs	KS		Dode <sup>15</sup>	c.303-304insCC
102	V	I	KS		Albuisson <sup>17</sup>	
102	V	I	KS	Cryptorchidism, micropenis	Pitteloud <sup>14</sup>	
107	S	X	KS		Sato <sup>18</sup>	
112	T	T	SOD-like	HH, GH deficiency, CCA, eye defects	Raivio <sup>19</sup>	
117	N	S	IHH		Raivio <sup>16</sup>	Digenic GNRHR
129	D	A	KS	CP	Albuisson <sup>17</sup>	
165*	L	S	Hartsfield		This study	
167*	A	S	KS	CP, CCA, unilateral hearing loss, fusion of the fourth and fifth metacarpal bones	Dode <sup>15</sup>	
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 <sup>20</sup>	
191*	L	S	Hartsfield		This study	
197	F	L	KS		Sykiotis <sup>21</sup>	
224	D	H	KS	Cryptorchidism, micropenis	Pitteloud <sup>14</sup>	
228	Y	D	IHH	Osteoporosis	Raivio <sup>16</sup>	
237	G	D	KS	Occulo-motor apraxia, dental agenesis, bilateral cryptorchidism, synkinesia.	Pitteloud <sup>14</sup>	
237	G	S	IHH/KS	Bilateral cryptorchidism	Pitteloud <sup>22</sup>	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 <sup>14</sup>	
239	I	T	IHH	HH reversal	Raivio <sup>16</sup>	Digenic PROKR2
245	L	P	KS	CLP	Trarbach <sup>12</sup>	KS "+"

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

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

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

**Table S4**

	HH	CDI	Pituitary other	Genitalia	Anosmia	Puberty	Adult	Arhinen- cephaly	
Patient 1* (Vilain3) <sup>8</sup>	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) <sup>8</sup>	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) <sup>8</sup>	+	+	-	Bilateral cryptorchidism Small penis	Suspected	Induced at 13.3y	Tanner P 3	?	
Patient 6* (Vilain4) <sup>8</sup>	+	+	-	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 <sup>8</sup>	NA	NA	NA	Normal	NA	NA	NA	+	TOP
Hartsfield <sup>1</sup>	NA	NA	NA	NR	NA	NA	NA	Absence of olfactory bulbs & tracts	Died at 7d
Young <sup>2</sup>	NA	NA	NA	NR	NA	NA	NA	NR	TOP
Imaizouni <sup>3</sup>	NR**	+	NR	NR	NR	NA	NA	NR	
Corona-Rivera <sup>4</sup>	NR	NR	NR	NR	NR	NA	NA	NR	
Abdel-Meguid <sup>5</sup>	NR	+	NR	NI	NA	NA	NA	NR	Died at 1w
Konig <sup>6</sup>	+	+	SmC/GH extrem low, TSH nl	Small penis- hypospadias- cryptorchidism	NE	NR	NR	NR	
Zeichi <sup>7</sup>	NR	NR	NR	Cryptorchidism, small penis	NR	NA	NA	NR	
Keaton 13 <sup>9</sup>	NR	NR	NR	Micropenis	NR	NR	NA	NR	
Keaton 14 <sup>9</sup>	NR	NR	NR	Female nl	NR	NR	NR	NR	
Keaton 15 <sup>9</sup>	NR	+	NR	Female	NR	NR	NR	NR	
Metwalley <sup>10</sup>	-***	-	-	Female	NR	NA	NA	NR	
Takenouchi <sup>11</sup>	+	+	NI 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, NI :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.

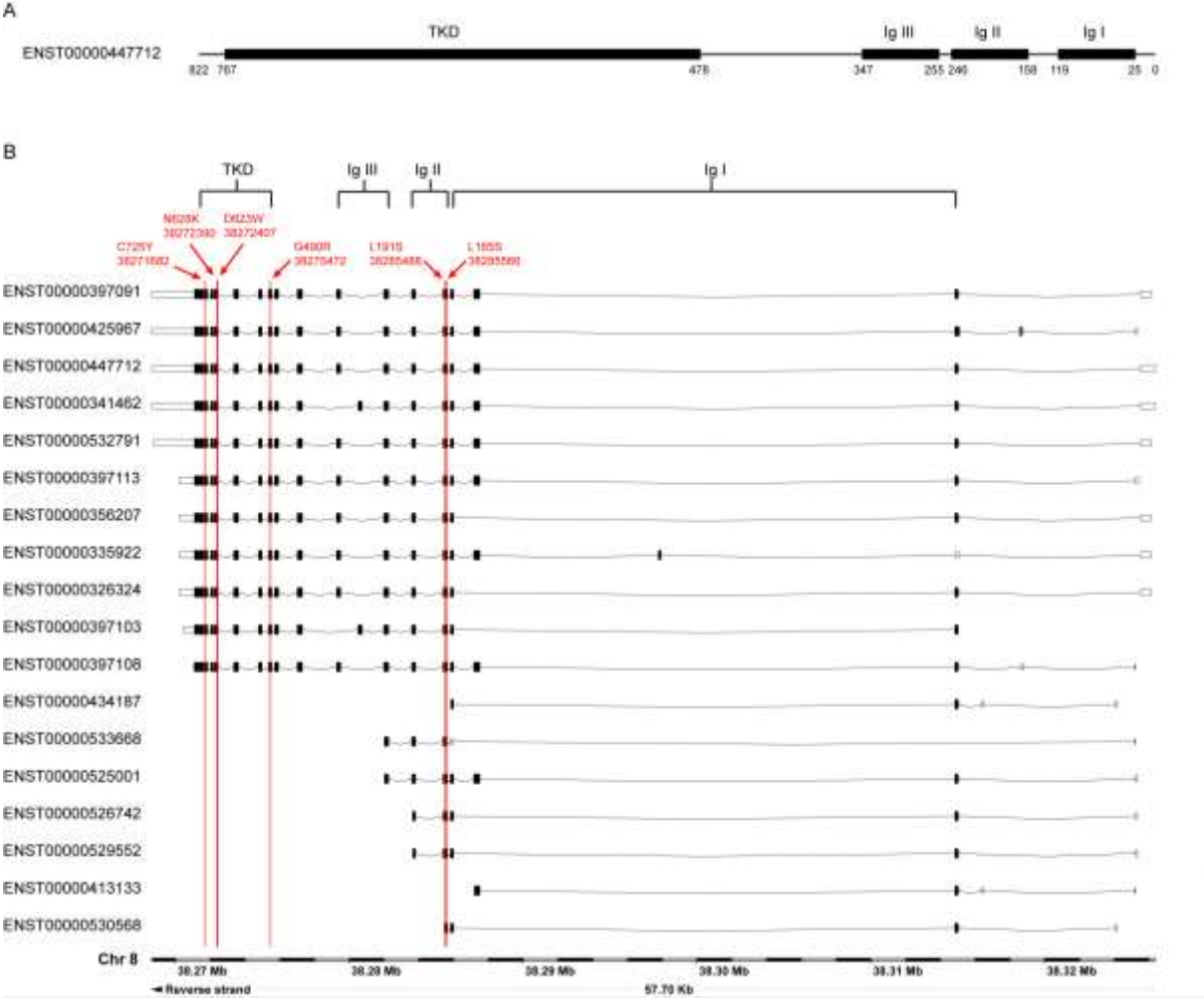
**Table S6**

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 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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