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Congenital hypogonadotropic hypogonadism/Kallmann Syndrome is associated with statural gain in both men and women: a monocentric study

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Abstract (280 words)

Context: Congenital hypogonadotropic hypogonadism/Kallmann Syndrome (CHH/KS) is a rare condition characterized by gonadotropin deficiency and pubertal failure. Adult height (AH) in patients with CHH/KS has not been well studied.

Objective: To assess AH in a large cohort of patients with CHH/KS.

Patients: A total of 219 patients (165 males, 54 females). Parents and siblings were included.

Methods: AH was assessed in patients and family members. AH was compared to the general French population, mid parental target height (TH) and between patients and same-sex siblings. Delta height (ΔH) was considered the difference between AH and parental TH. ΔH was compared between patients and siblings, normosmic CHH and KS (CHH with anosmia/hyposmia), and according to underlying genetic defect. We examined correlations between ΔH and age at diagnosis and therapeutically-induced individual statural gain.

Results: Mean AH in men and women with CHH/KS was greater than in the French general population. Patients of both sexes had AH>TH. Males with CHH/KS were significantly, albeit moderately, taller than their brothers. ΔH was higher in CHH/KS compared to unaffected siblings (+6.2±7.2 cm versus +3.4±5.2 cm, p<0.0001). ΔH was positively correlated with age at diagnosis. Neither olfactory function (normosmic CHH *vs* KS) nor specific genetic cause impacted ΔH. Individual growth during replacement therapy inversely correlated with the age at initiation of hormonal treatment (p<0.0001).

Conclusions: CHH/KS is associated with higher AH compared to the general population and mid-parental TH. Greater height in CHH/KS than siblings indicates that those differences are in part independent of an intergenerational effect.

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Introduction

Congenital hypogonadotropic hypogonadism (CHH) with normal olfactory function (normosmic CHH) or with olfactory impairment (Kallmann Syndrome, KS) is a rare cause of disrupted puberty and infertility caused by deficient secretion (or action) of gonadotropin releasing hormone (GnRH) [1,2]. The estimated prevalence is approximately 1/4,000 and 1/10,000 respectively[1,3]. Clinically, CHH/KS is characterized by absent (or incomplete) pubertal development. In males, testosterone deficiency is evidenced by undervirilization while females typically present with primary amenorrhea and variable breast underdevelopment in the setting of estradiol deficiency [1]. Accordingly, the vast majority of cases are not diagnosed until late adolescence or early adulthood [1,4,5]. Diagnostic delay and thus initiation of replacement therapy have negative psychological consequences in both genders [1,2,4,5].

Puberty in both sexes is associated with accelerated skeletal growth and bone maturation. Long bone growth ends when the hyaline cartilage comprising the epiphyseal plates (so-called 'growth plates') fuse[6]. There is a paucity of data on pubertal growth and adult height of men and women born with CHH/KS[7-9]. A study of 36 patients suggests that the sex steroid deficiency associated with CHH/KS neither accelerates nor delays growth[9]. Only one study has examined the effect of sex steroid treatment on height parameters in this population[7]. Moreover, intergenerational effects (i.e. how height changes in successive generations) has yet to be taken into account in this patient population[10,11]. To date, much of the literature on growth and height in endocrine disorders of puberty has focused on growth hormone deficiency/multiple pituitary hormone deficiency and Turner syndrome[12, 13].

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This study aims to assess adult height in a sizeable cohort of patients with CHH/KS and compare height with the general population, mid-parental target height and same sex siblings. Secondarily, we aimed to examine adult height according to clinical presentation (i.e. normosmic CHH vs. KS) and identified rare genetic variants (mutations) underlying CHH/KS. In addition, we examine the effects of treatment (i.e. sex steroid replacement) on stature.

Patients and Methods

This study was conducted at a single large tertiary academic medical center (Department of Endocrinology and Reproductive Diseases, Bicêtre University Hospital, France). The National Agency of Medical Safety reviewed the study protocol and provided ethics approval (RCB 2017-A01584-49). All participants provided written informed consent in accordance with French Bioethics Law and the principles of the Declaration of Helsinki.

Patients

Patients with CHH/KS were enrolled in the study. Period of recruitment of the study population ranged from 1975 to 2018. A definitive diagnosis of isolated GnRH deficiency (CHH/KS) was based on the following[1,2]: 1) absent or incomplete puberty; 2) low serum sex steroid levels (males: testosterone [T] <1.5 ng/mL, females: estradiol [E2], <35 pg/mL) in the setting of low or inappropriately normal (<5.5 IU/L) serum gonadotropin levels (luteinizing hormone [LH, < 5.6 IU/L], follicle stimulating hormone [FSH, < 7.5 IU/L]); 3) otherwise normal anterior pituitary function evidenced by normal basal (>80 ng/mL) /stimulated (>180 ng/mL) cortisol, normal basal prolactin (>8.0 and <20 ng/mL) and normal thyroid function (free T4: 12.0-22 pmol/L, TSH 0.8-4.3 mIU/L) and normal stimulated growth hormone (GH peak >10 ng/mL 2005 either before and after 2005 via insulin tolerance test[14]; 4) normal serum DHEAS (males: >640 ng/mL, females: >412

ng/mL), iron/ferritin concentrations and age-adjusted insulin-like growth factor-1 (IGF-1) levels (before 1995: > 172 ng/mL after 2005 > 198 ng/mL)[15-17]; 5) normal magnetic resonance imaging (MRI) of the hypothalamic-pituitary region; and 6) no functional causes of hypogonadotropic hypogonadism (i.e. eating disorders, low BMI, malabsorption, chronic diseases). In cases when patients were diagnosed with CHH/KS between 13-17 years of age, the diagnosis was confirmed with repeat hormone profiling following a treatment washout after age 18 (total testosterone <1.5 ng/mL, LH <5.6 IU/L and FSH <7.6 IU/L in males, and persistent amenorrhea associated with estradiol <35 pg/mL, LH <6.5 IU/L and FSH <8.5 IU/L in females after a three-month withdrawal of previous sex steroid replacement therapy). Patients were considered normosmic CHH or having Kallmann syndrome (CHH + anosmia or hyposmia) based on olfactometry testing and cranial imaging (olfactory bulb hypoplasia/aplasia) as previously described[18,19]. In patients in whom CHH or KS was diagnosed between 13-17 yrs, the definitive diagnosis was made at 18 yrs in order to exclude a constitutional delay of growth and puberty, and after a 3-month withdrawal of a sex steroid replacement therapy. After washout, all male CHH/KS patients had total serum testosterone levels below 1.5 ng/mL, and all CHH/KS females had serum estradiol levels below 35 pg/mL associated with persistent amenorrhea.

CHH/KS patients without available parental height measures were excluded from the study. We also excluded affected parents (ie: with nCHH or KS) from the study.

Anthropometric measures

Height (in cm) was measured using a wall-mounted stadiometer at first visit and before introduction of hormonal replacement therapy. Patients were defined as reaching adult height (AH) by two or more stable height measurements (<1.5 cm difference between two measurements performed at least 6-months apart). Whenever available, parents and siblings were

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measured using a wall-mounted stadiometer. When not present in hospital consultation, in a subgroup we asked for parent and siblings' heights by telephone survey. As a reference population, we used publicly available data on the general French population (1980-2010) - French National Institute of Statistics (http://www.insee.fr/) and French National Institute of Health (http://invs.santepubliquefrance.fr/). We used the French National Nutritional and Health Study (ENNS, CNIL no. 905481; Paris Cochin CPP no. 2264), funded by the French National Agency of Public Health (Agence nationale Santé publique France, formerly Institut de veille sanitaire (InVS)). The survey encompassed a sample from the French general population by using a 3-stage sampling: 1/ municipalities or groups of municipalities (n=190) stratified by urban unit size and 8 major regions; 2/ households from phone lists (landlines, red lists, mobile phones and unbundled); 3/ 1 individual per household, adult or child, using the birthday method, see also https://epidemiologie-france.aviesan.fr). We used in our study the latest database published in 2011, July, 7th. This study included 3115 healthy adults from French general population aged 18-74. Data are consistent with less recent figures from other French National Health public databases (https://cress-umr1153.fr/index.php/courbes-carnet-de-sante/; https://www.epsilon.insee.fr/jspui/bitstream/1/19240/1/estat_1981_132_3.pdf).

Mid parental target height (TH) for each patient was determined (in cm) using Tanner's formulas (male TH = [father's height + mother's height +13 cm] X 0.5), female TH = [father's height + mother's height -13 cm] X 0.5)[20]. Delta height (ΔH) was calculated as the difference (in cm) between adult and mid parental target height (AH-TH=ΔH)[21]. A ΔH equal to zero indicates adult and target height are equivalent. A positive ΔH value indicates that AH is superior to TH while negative ΔH values indicate that AH is inferior to TH. Because delta height (ΔH = AH-TH), were not statistically different in male and female (see Results section) we were able to compare ΔH data of male CHH/KS from siblings of both genders. The individual growth during replacement therapy

was calculated as the difference between the AH and the basal height measured prior to initiating hormonal therapy (i.e. sex steroid replacement).

Genetic analyses

Following informed consent, genomic DNA was extracted from peripheral blood leukocytes using standards protocols and direct sequencing was performed using traditional Sanger sequencing. From a total of 215 patients with available genetic analysis we found mutations in the coding regions of the following genes underlying CHH/KS: *ANOS1* (formerly *KAL1*, n=20/215, 9.3%), *FGFR1* (n=36/215, 16.8%), *PROK2* (n=5/215, 2.3%), *PROKR2* (n=13/215, 6.1%), *CHD7* (n=8/215, 3.7%), *SEMA3A* (n=11/215, 5.1%), *GNRH1* (n=2/215, 0.9%), *GNRHR* (n=4/215, 1.9%), *KISS1* (n=0/215, 0%), *KISS1R* (n=7/215, 3.3%), *TAC3* (n=2/215, 0.9%), *TACR3* (n=2/215, 0.9%) and *WDR11* (n=0/215, 0%). These genes were sequenced as previously reported[22-29]. We did not found any deleterious genetic mutation in 103 cases out of 215.

Statistical analyses

Patient and sibling statural parameters were compared using Student T- tests. Chi-square or Fisher's exact tests were employed for categorical comparisons between groups as appropriate. We used linear regression to examine the relationship between ΔH and age at diagnosis/start of hormonal substitution and therapeutically-induced growth respectively. Sample size for paired tests was calculated with Sample Size Calculator (https://clincalc.com/stats/samplesize.aspx) and Statulator Beta (http://statulator.com/SampleSize/ss2PM.html). By analyzing previous pertinent literature, a number of 34 pairs is needed to detect a statistic significant difference (alpha 0.05, power 0.8). For comparison of height measures according to genetic variants, we performed Kruskal-Wallis and ANOVA tests. Graphics and statistical analyses were conducted using GraphPad

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Prism, version 5.0f (GraphPad Software, Inc., San Diego, CA). Data are reported as individual values or using mean and standard deviation unless otherwise noted. A *p* value less than 0.05 was considered statistically significant.

Results

A total of 219 patients (165 males, 54 females) were included in the study (Table 1). Of these patients, 56 males and 16 females were initially diagnosed between 13-17 years of age – all of whom had CHH/KS confirmed after age 18. Cases were equally divided between normosmic CHH (n=109) and Kallmann syndrome (n=110). A total of 172/219 (79%) of patients had siblings.

Median adult height (AH) in male and female CHH/KS patients was above the median height reported in the French reference population (Figure 1A and Figure 2A). Parental AH of the patients did not differ from the reference population - neither 1980 nor 2010 (Supplementary Figure 1).

In male CHH/KS patients, mean AH was 179.0 ± 8.2 cm (median: 179 cm, interquartile range [IQR]: 174-185 cm, Figure 1A). Comparing AH to mid-parental target height (TH) revealed that on average, male patients with CHH/KS surpassed their mid-parental TH (179.0 ± 8.2 vs. 175.0 ± 5.6 cm, $p<0.0001$, Figure 1A). Interestingly, male CHH/KS patients were significantly taller than their brothers (181.3 ± 7.9 vs. 179.0 ± 6.9, $p = 0.045$)(Figure 1B).

In female patients mean AH was 166.0 ± 6.7 cm (median: 166 cm, IQR: 162-170.5 cm, Figure 2A). Similarly, female patients with CHH/KS surpassed their mid-parental TH (166 ± 6.2 vs. 162 ± 4.6 cm, $p<0.0001$, Figure 2A). Female CHH/KS patients (n=16) did not differ from their sisters (n=16) (166.1 ± 6.2 vs. 165.0 ± 5.8 cm, $p = 0.26$) (Figure 2B).

Examining delta height (ΔH = AH-TH), showed that values were similar in male and female patients (4.0 ± 7.2 vs. 4.3 ± 6.1 cm, $p = 0.8$). Among unaffected siblings, mean ΔH was also positive (3.4 ± 5.2 cm) - consistent with progressive generational statural gain[10, 11]. However, patient ΔH (n=172)

was significantly greater than unaffected siblings (6.2 ± 7.2 vs. 3.4 ± 5.2 cm, p <0.0001, Figure 3A). We examined the ΔH distribution and found the vast majority of CHH/KS patients 161/219 (73.5%) had a positive ΔH (Figure 3B). Further, patients were more likely to have positive ΔH compared to their unaffected siblings (n=172, 88.4% vs. 73.8%, *p* = 0.0009, see Supplementary Figure 2).

Patient's age at diagnosis was correlated with ΔH (Figure 3C).

ΔH did not differ between patients with normosmic CHH and those with KS (4.4 ± 6.4 vs. 4.4 ± 7.3 cm, *p* = 0.9, Figure 4A). Similarly, no significant differences in ΔH values were identified based on genetic causes in the 214 patients who had genetic screening (Figure 4B).

Anthropometric measures prior to hormonal replacement initiation were available for 118 patients. As shown in Figure 5, we observed an inverse relationship between the age of treatment initiation and individual statural gain during replacement therapy (r = -0.57, *p* <0.0001). In other words, patients had lower statural growth potential when the treatment was initiated later. It should be noted that when analyzing data about period recruitment, we found a negative correlation between the year of birth and the age at treatment initiation, indicating that age at treatment initiation was earlier in recent decades (negative correlation: r= 0.35; p<0.0001).

We dichotomized the data and observed that patients starting treatment before the age of 16 exhibited significantly greater individual statural growth potential compared to their counterparts who started treatment later (16.6±5.9 versus 4.1±5.2 cm, p<0.0001 Figure 5, inset). Important and significant individual statural growth differences were also observed when dichotomizing patients starting hormonal treatment before or after the age of 14 (18.1±8.1 versus 6.8±7.1 cm, p<0.0001), and before or after the age of 18 (13.1 ± 7.3 vs. 2.5 ± 3.9 cm, *p* <0.0001).

Post-therapeutic growth was greater in male (+9.2±8.8 cm, n=98) than in female CHH/KS (+4.4±6.1 cm, n=33, p= 0.0051).

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Bone age was assessed in a subgroup of 59 CHH/KS patients (15 females, 44 males) before hormonal treatment. Bone age was significantly lower than chronological age (females: 16.7±1.8 versus 13.2±1.0, p<0.0001; males: 16.3±2.9 versus 13.3±1.2, p<0.0001, Supplemental Figure 3A). A positive correlation was found between chronological and bone age (R=0.73, p<0.0001, Supplementary Figure 3B).

Discussion

Herein we report adult height (AH) in a single-center cohort of men and women with CHH/KS who were identified using rigorous, well-defined criteria. We found that mean AH of patients exceeded the general population, mid-parental target height (TH) and sibling adult height. Few studies have examined height in patients with CHH/KS. These studies are quite dated, limited by small sample sizes and are confounded by the fact that some studies include patients with other causes of pubertal delay[7,8,12,30] . Our findings of increased height among CHH/KS patients is consistent with the previous study by Uriarte and colleagues[8]. These investigators analyzed AH in 41 males with CHH/KS and found that compared to a reference group of young American males, male CHH/KS patients were 3 cm taller on average. To our knowledge, the present work is the first to compare AH in women with CHH/KS with a same sex reference population.

Distinct from prior studies, we accounted for genetic background by comparing patient AH to mid-parental target height (i.e. target height [TH]) as well as to AH in sex-matched siblings. This is relevant, as familial genetic component is known to have a major effect on AH[31]. In the present study, both male and female patient AH significantly exceeded TH. These observations indicate CHH/KS does not have a deleterious genetic effect on height potential – as has been previously suggested[32]. Rather, patients with CHH/KS are likely to have higher growth potential compared to their unaffected relatives. The present study is the first to use healthy, sex-matched siblings as

genetic controls to examine AH in patients with CHH/KS. This is particularly relevant due to the observed generational effect on AH in which offspring are progressively taller than previous generations. Thus, even in healthy populations, the AH in offspring of both sexes exceeds mid-parental target height[10]. Indeed, we observed a positive delta height (ΔH) in CHH/KS unaffected siblings, consistent with this generational effect. We further controlled for this in the present study by including same sex siblings. We revealed that male CHH/KS patients were significantly taller than their healthy brothers. We failed to detect a significant difference between female CHH/KS patients and their sisters, probably because of an insufficient sample size. However, by analyzing ΔH differences and thus by pooling males and females together, CHH/KS patients were significantly taller than their siblings. Our findings suggest that part of the difference between the patients' adult height and their mid-parental target height could be explained by a secular trend[10]. However when compared to their siblings, a modest but significant increase in height was also observed. Thus, the increased statural growth potential in CHH/KS is not solely due to a generational effect, but could be explained by the slow and delayed maturation and welding of growth cartilage secondary to low endogenous sex steroids levels associated with this condition.

It is worthwhile to note that a minority of patients had a negative ΔH (AH below mid-parental target height (TH)). However, this was not unique to patients. Indeed, brothers/sisters were more likely than their affected siblings (CHH/KS) to have negative ΔH. The relative low stature observed in a minority of patients thus seems unrelated to the disease and its treatment. Growth and AH are multifactorial and contributors other than genetic potential play a part. It is widely acknowledged that environmental and nutritional factors play a role in adult height[33].

We observed a positive relationship between age at CHH/KS diagnosis and ΔH. Patients who are diagnosed earlier have relatively lower ΔH values. A possible explanation for this finding is that,

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before diagnosis and prior to any hormonal treatment, because of the very low concentrations of sex steroids[34,35] linear growth could continue without cartilage epiphyseal fusion until sex steroid replacement is initiated.

Neither form (i.e. normosmic CHH vs. KS) nor genetic cause had any specific observed effect on AH. Thus, the increased AH appears to be intrinsic to congenital hypogonadotropic hypogonadism – i.e. absence of increased sex steroids typical during puberty. It is also interesting that patients harboring mutations in *FGFR1* did not have impaired AH. This gene has known effects on bone development and is associated with skeletal phenotypes in CHH/KS[1,2,36,37] . However, the present findings are in agreement with those from a prior study that included five KS patients[38] suggesting that *FGFR1* mutations do not alter the development/maturation of the hyaline cartilage comprising the epiphyseal plates.

Clinically, height is a major consideration for patients. Abnormal growth, disrupted puberty and short stature are among the most common causes for endocrine consultation among adolescents[4,39]. Moreover, looking physically different from peers is a major concern at presentation[1,2,4,39]. Importantly, the AH prognosis specific to each disease influences clinical decision-making regarding the timing for initiating treatment and selected sex steroid doses. In the present study, as expected we show that individual statural growth during treatment was greatest when treatment was initiated earlier. An explanation is that younger CHH/KS patients just have more growth potential than older CHH patients. We must however emphasize that in CHH/KS patients even when replacement treatment is started after age 18, further growth can occur – albeit much less. In the handful of patients who were started on sex steroid treatment around the age of 30 had no observable change in height. Such observation suggests that the growth plates had fused[6] despite a severe (yet not complete[34]) sex steroid deficiency. Low

circulating sex steroids levels, mostly derived from adrenal precursors, are present in CHH/KS patients[34] and may contribute to slower bone maturation in these patients.

Conclusions

We demonstrate that CHH/KS does not negatively affect stature in adulthood regardless of sex, clinical form (normosmic CHH vs. KS) or genetic etiology. Men and women with CHH/KS are, on average, taller than the general population, exceed their mid-parental target height and male CHH/KS have greater adult stature than unaffected brothers.

Our data also suggest that an earlier treatment does not seem to negatively impact adult stature but solely make it closer to mid-parental target height. On the other side, late treatment could produce a taller than predicted adult stature but further studies are needed to definitively answer to this hypothesis. Finally, we can not exclude that the potential modest increase in adult stature associated with a late treatment could be obtained at the expense of a detriment of quality of life, lack of masculinization or feminization of CHH/KS adolescents, and suboptimal bone mass peak acquisition.

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References

1. Young J, Xu C, Papadakis GE, Acierno JS, Maione L, Hietamaki J, Raivio T & Pitteloud N. Clinical Management of Congenital Hypogonadotropic Hypogonadism. *Endocr Rev* 2019 **40** 669-710.
2. Boehm U, Bouloux PM, Dattani MT, de Roux N, Dode C, Dunkel L, Dwyer AA, Giacobini P, Hardelin JP, Juul A, Maghnie M, Pitteloud N, et al. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism--pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol* 2015 **11** 547-564.
3. Fromantin M, Gineste J, Didier A & Rouvier J. [Impuberism and hypogonadism at induction into military service. Statistical study]. *Probl Actuels Endocrinol Nutr* 1973 **16** 179-199.
4. Dwyer AA, Quinton R, Pitteloud N & Morin D. Psychosexual development in men with congenital hypogonadotropic hypogonadism on long-term treatment: a mixed methods study. *Sex Med* 2015 **3** 32-41.
5. Dzemaili S, Tiemensma J, Quinton R, Pitteloud N, Morin D & Dwyer AA. Beyond hormone replacement: quality of life in women with congenital hypogonadotropic hypogonadism. *Endocr Connect* 2017 **6** 404-412.
6. Bourguignon JP. Linear growth as a function of age at onset of puberty and sex steroid dosage: therapeutic implications. *Endocr Rev* 1988 **9** 467-488.
7. Dickerman Z, Cohen A & Laron Z. Growth in patients with isolated gonadotrophin deficiency. *Arch Dis Child* 1992 **67** 513-516.
8. Uriarte MM, Baron J, Garcia HB, Barnes KM, Loriaux DL & Cutler GB, Jr. The effect of pubertal delay on adult height in men with isolated hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 1992 **74** 436-440.
9. Varimo T, Hero M, Laitinen EM, Miettinen PJ, Tommiska J, Kansakoski J, Juul A & Raivio T. Childhood growth in boys with congenital hypogonadotropic hypogonadism. *Pediatr Res* 2016 **79** 705-709.
10. Cole TJ. The secular trend in human physical growth: a biological view. *Econ Hum Biol* 2003 **1** 161-168.
11. Ong KK, Ahmed ML & Dunger DB. Lessons from large population studies on timing and tempo of puberty (secular trends and relation to body size): the European trend. *Mol Cell Endocrinol* 2006 **254-255** 8-12.
12. Bourguignon JP, Vandeweghe M, Vanderschueren-Lodeweyckx M, Malvaux P, Wolter R, Du Caju M & Ernould C. Pubertal growth and final height in hypopituitary boys: a minor role of bone age at onset of puberty. *J Clin Endocrinol Metab* 1986 **63** 376-382.
13. Klein KO, Rosenfield RL, Santen RJ, Gawlik AM, Backeljauw PF, Gravholt CH, Sas TCJ & Mauras N. Estrogen Replacement in Turner Syndrome: Literature Review and Practical Considerations. *J Clin Endocrinol Metab* 2018 **103** 1790-1803.
14. Chanson P, Cailleux-Bounacer A, Kuhn JM, Weryha G, Chabre O, Borson-Chazot F, Dubois S, Vincent-Dejean C, Brue T, Fedou C, Bresson JL, Demolis P et al. Comparative validation of the growth hormone-releasing hormone and arginine test for the diagnosis of adult growth hormone deficiency using a growth hormone assay conforming to recent international recommendations. *J Clin Endocrinol Metab* 2010 **95** 3684-3692.
15. Ducos B, Cabrol S, Houang M, Perin L, Holzenberger M, Le Bouc Y. IGF type 1 receptor ligand binding characteristics are altered in a subgroup of children with intrauterine growth retardation. *J Clin Endocrinol Metab* 2001 **86** 5516-5524.

16. Galland F, Kamenicky P, Affres H, Reznik Y, Pontvert D, Le Bouc Y, Young J, Chanson P. McCune-Albright syndrome and acromegaly: effects of hypothalamopituitary radiotherapy and/or pegvisomant in somatostatin analog-resistant patients. *J Clin Endocrinol Metab* 2006 **91** 4957-4961.
17. Chanson P, Arnoux A, Mavromati M, Brailly-Tabard S, Massart C, Young J, Piketty ML, Souberbielle JC; VARIETE Investigators. Reference Values for IGF-I Serum Concentrations: Comparison of Six Immunoassays. *J Clin Endocrinol Metab* 2016 **101** 3450-3458.
18. Maione L, Benadjaoud S, Eloit C, Sinisi AA, Colao A, Chanson P, Ducreux D, Benoudiba F & Young J. Computed tomography of the anterior skull base in Kallmann syndrome reveals specific ethmoid bone abnormalities associated with olfactory bulb defects. *J Clin Endocrinol Metab* 2013 **98** E537-546.
19. Maione L, Cantone E, Nettore IC, Cerbone G, De Brasi D, Maione N, Young J, Di Somma C, Sinisi AA, Lengo M, et al. Flavor perception test: evaluation in patients with Kallmann syndrome. *Endocrine* 2016 **52** 236-243.
20. Tanner JM, Goldstein H & Whitehouse RH. Standards for children's height at ages 2-9 years allowing for heights of parents. *Arch Dis Child* 1970 **45** 755-762.
21. Straetemans S, De Schepper J, Thomas M, Verlinde F, Rooman R & Bespeed. Validation of Prediction Models for Near Adult Height in Children with Idiopathic Growth Hormone Deficiency Treated with Growth Hormone: A Belgian Registry Study. *Horm Res Paediatr* 2016 **86** 161-168.
22. Dode C, Levilliers J, Dupont JM, De Paepe A, Le Du N, Soussi-Yanicostas N, Coimbra RS, Delmaghani S, Compain-Nouaille S, Baverel F, et al. Loss-of-function mutations in FGFR1 cause autosomal dominant Kallmann syndrome. *Nat Genet* 2003 **33** 463-465.
23. Dode C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, et al. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet* 2006 **2** e175.
24. Francou B, Paul C, Amazit L, Cartes A, Bouvattier C, Albarel F, Maiter D, Chanson P, Trabado S, Brailly-Tabard S, et al. Prevalence of KISS1 Receptor mutations in a series of 603 patients with normosmic congenital hypogonadotrophic hypogonadism and characterization of novel mutations: a single-centre study. *Hum Reprod* 2016 **31** 1363-1374.
25. Hanchate NK, Giacobini P, Lhuillier P, Parkash J, Espy C, Fouveaut C, Leroy C, Baron S, Campagne C, Vanacker C, et al. SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with Kallmann syndrome. *PLoS Genet* 2012 **8** e1002896.
26. Kim HG, Ahn JW, Kurth I, Ullmann R, Kim HT, Kulharya A, Ha KS, Itokawa Y, Meliciani I, Wenzel W, et al. WDR11, a WD protein that interacts with transcription factor EMX1, is mutated in idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. *Am J Hum Genet* 2010 **87** 465-479.
27. Pingault V, Bodereau V, Baral V, Marcos S, Watanabe Y, Chaoui A, Fouveaut C, Leroy C, Verier-Mine O, Francannet C, et al. Loss-of-function mutations in SOX10 cause Kallmann syndrome with deafness. *Am J Hum Genet* 2013 **92** 707-724.
28. Salenave S, Chanson P, Bry H, Pugeat M, Cabrol S, Carel JC, Murat A, Lecomte P, Brailly S, Hardelin JP, et al. Kallmann's syndrome: a comparison of the reproductive phenotypes in men carrying KAL1 and FGFR1/KAL2 mutations. *J Clin Endocrinol Metab* 2008 **93** 758-763.
29. Marcos S, Sarfati J, Leroy C, Fouveaut C, Parent P, Metz C, Wolczynski S, Gerard M, Bieth E, Kurtz F, et al. The prevalence of CHD7 missense versus truncating mutations is higher in patients with Kallmann syndrome than in typical CHARGE patients. *J Clin Endocrinol Metab* 2014 **99** E2138-2143.

 Accepted Manuscript published as EJE-19-0537.R2. Accepted for publication: 26-Nov-2019

30. Raboch J & Reisenauer R. Analysis of body height in 829 patients with different forms of testicular pathology. *Andrologia* 1976 **8** 265-268.
31. Jelenkovic A, Hur YM, Sund R, Yokoyama Y, Siribaddana SH, Hotopf M, Sumathipala A, Rijsdijk F, Tan Q, Zhang D, et al. Genetic and environmental influences on adult human height across birth cohorts from 1886 to 1994. *Elife* 2016 **5**.
32. Kaushanski A & Laron Z. Growth pattern of boys with isolated gonadotropin deficiency. *Isr J Med Sci* 1979 **15** 518-521.
33. Perkins JM, Subramanian SV, Davey Smith G & Ozaltin E. Adult height, nutrition, and population health. *Nutr Rev* 2016 **74** 149-165.
34. Giton F, Trabado S, Maione L, Sarfati J, Le Bouc Y, Brailly-Tabard S, Fiet J & Young J. Sex steroids, precursors, and metabolite deficiencies in men with isolated hypogonadotropic hypogonadism and panhypopituitarism: a GCMS-based comparative study. *J Clin Endocrinol Metab* 2015 **100** E292-296.
35. Trabado S, Maione L, Salenave S, Baron S, Galland F, Bry-Gauillard H, Guiochon-Mantel A, Chanson P, Pitteloud N, Sinisi AA, et al. Estradiol levels in men with congenital hypogonadotropic hypogonadism and the effects of different modalities of hormonal treatment. *Fertil Steril* 2011 **95** 2324-2329, 2329 e2321-2323.
36. Maione L, Dwyer AA, Francou B, Guiochon-Mantel A, Binart N, Bouligand J & Young J. GENETICS IN ENDOCRINOLOGY: Genetic counseling for congenital hypogonadotropic hypogonadism and Kallmann syndrome: new challenges in the era of oligogenism and next-generation sequencing. *Eur J Endocrinol* 2018 **178** R55-R80.
37. Villanueva C, Jacobson-Dickman E, Xu C, Manouvrier S, Dwyer AA, Sykiotis GP, Beenken A, Liu Y, Tommiska J, Hu Y, et al. Congenital hypogonadotropic hypogonadism with split hand/foot malformation: a clinical entity with a high frequency of FGFR1 mutations. *Genet Med* 2015 **17** 651-659.
38. Hero M, Laitinen EM, Varimo T, Vaaralahti K, Tommiska J & Raivio T. Childhood growth of females with Kallmann syndrome and FGFR1 mutations. *Clin Endocrinol (Oxf)* 2015 **82** 122-126.
39. Dwyer AA, Phan-Hug F, Hauschild M, Elowe-Gruau E & Pitteloud N. TRANSITION IN ENDOCRINOLOGY: Hypogonadism in adolescence. *Eur J Endocrinol* 2015 **173** R15-24.

Legends to Figures

Figure 1. **Adult and mid-parental target height in CHH/KS males and their brothers.**

Panel A. Individual values (n=165) and mean ± SE of male CHH/KS adult height versus mid-parental target height. Dark grey zone shows the 2.5 to the 97.5 centile of the French male general population. Light grey indicates the min and max limits of the French general population. Dotted line indicates median. General French population data are from the public database of the French National Institute of Health, http://invs.santepubliquefrance.fr/.

Mid-parental target height has been calculated by means of the parental height according to Tanner formula [20]. ****p<0.0001.

Panel B. Comparison between the adult height in a subgroup of CHH/KS males and that of their unaffected brothers (n=77). Individual values and mean ± SE are shown. *p<0.05.

Figure 2. **Adult and mid-parental target height in in CHH/KS females and their sisters**.

Panel A. Individual values and mean ± SE of female CHH/KS adult height versus mid-parental target height (n=54). Dark grey zone shows the 2.5 to the 97.5 centile of the French male general population. Light grey indicates the min and max limits of the French general population. Dotted line indicates median. General French population data are from the public database of the French National Institute of Health, http://invs.santepubliquefrance.fr/.

Mid-parental target height has been calculated by means of the parental height according to Tanner formula [16]. ****p<0.0001.

Panel B. Comparison between the adult height in a subgroup of CHH/KS females and that of their unaffected sisters. Individual values (n=16) and mean ± SE are shown.

ns: non-significant.

Figure 3. **Difference between adult and mid-parental target height (delta) in pooled male and female CHH/KS patients**.

Panel A. Delta height values between pooled male and female CHH/KS patients and their male and female siblings (n=172) (see text, Methods and Results section). Data are shown as box and whiskers (min to max). Red line indicates zero (no difference between adult and mid-

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parental target height). **** p<0.0001. Note that delta height in siblings is above zero (generational effect, see Results section).

Panel B. Distribution of positive (AH>TH, >0 cm) and negative (AH<TH, <0 cm) ΔH in CHH/KS population. Each vertical bar represents the number of patients. On x-axis numbers represent the delta (adult – mid parental target height, in cm). Vertical red line indicates zero (no difference between adult and mid parental target height).

Panel C. Individual values of delta height (ΔH) in male and female CHH/KS patients according to the age at first treatment (in years), indicating correlation between the delta height (in cm) and the age at first replacement therapy. Best fit and 95% confidence interval is shown.

Figure 4. **Delta height (ΔH) (difference between adult height and mid-parental target height) in pooled male and female CHH/KS patients according to olfactory status (KS versus nCHH) and genetic form.**

Panel A. Individual delta height values in Kallmann syndrome versus normosmic CHH patients (nCHH). Dotted line indicates zero (no difference between adult height and mid-parental target height).

Panel B. Individual delta height (ΔH) values in CHH/KS patients with and in those without identified genetic abnormalities. Dotted line indicates zero (no difference between adult and mid-parental target height).

Data are reported as individual ΔH values in cm; mean ± SE are also indicated in each group.

Figure 5. **Individual post-therapeutic statural growth according to the age at beginning of hormonal treatment in CHH/KS patients**.

In this figure, each individual point indicates the difference between the adult height and the height before the hormonal substitution. On y-axis, this growth gain is expressed in cm. On x-axis, the age at the beginning of treatment is expressed in years. Blue line shows the nonlinear fit. Inset: individual post-replacement therapy growth gain in CHH/KS patients in whom hormonal substitution started before or after the age of 16 solely for indicating more growth potential in younger than older CHH/KS patients; ****p<0.0001.

Table 1 – Clinical and biochemical characteristics of patients with CHH/KS at diagnosis.

	Male CHH/KS (n=165)	Normal range in males	Female CHH/KS (n=54)	Normal range in females
Age at diagnosis (yrs.)	17.5±6.0	-	16±3.3	-
Age at treatment initiation (yrs)	17.8±6.2	-	16.3±3.2	-
Weight (kg)	76±23	-	63±12	-
BMI (kg/m^2)	24.8±6	20-25	23±4	21-25
Testicular volume (mL)	3.3±2.4	>12	-	-
Ovarian volume (mL)	-	-	1.8±1.5	>2.6 - <10
normosmic CHH/KS (n)	76/91	-	33/19	-
Hormonal profile				
Total testosterone (ng/mL)	0.48±0.7	3.4-8.5	0.27±0.1	0.17-0.59
Total estradiol (pg/mL)	9.1±8.9	10-38	14±11	13-97
LH (IU/L)	0.8±1.1	1.6-5.6	1.6±1.9	2.2-8.2
FSH (IU/L)	1.0±1.2	2.2-7.4	2.5±2.1	2.4-7.9
IGF-1 (ng/mL)	251±105	198-397*	252±112	170-367*
Prolactin (ng/mL)	7.2±4.3	9-20	9.0±6.1	9.0-21
TSH (mIU/L)	2.2±1.7	0.8-4.3	1.9±1.1	1.1-3.9
FT4 (pmol/L)	15±3.4	12-22	14.6±2.4	12.5-22.4
Cortisol [8 am] (ng/mL)	192±76	94-214	173±71	89-225
DHEAS (ng/mL)	1766±929	643-3731	1323±725	416-3685

Data are reported as means ± standard deviations (SD). N: number of patients. BMI: body mass index, LH: luteinizing hormone, FSH: follicle stimulating hormone, IGF-1: insulin-like growth factor 1, TSH: thyroid stimulating hormone, FT4: free thyroxine, DHEAS: dehydroepiandrosterone sulfate. *Normative values for circulating IGF1 are from previous published work (ref. 15-17).

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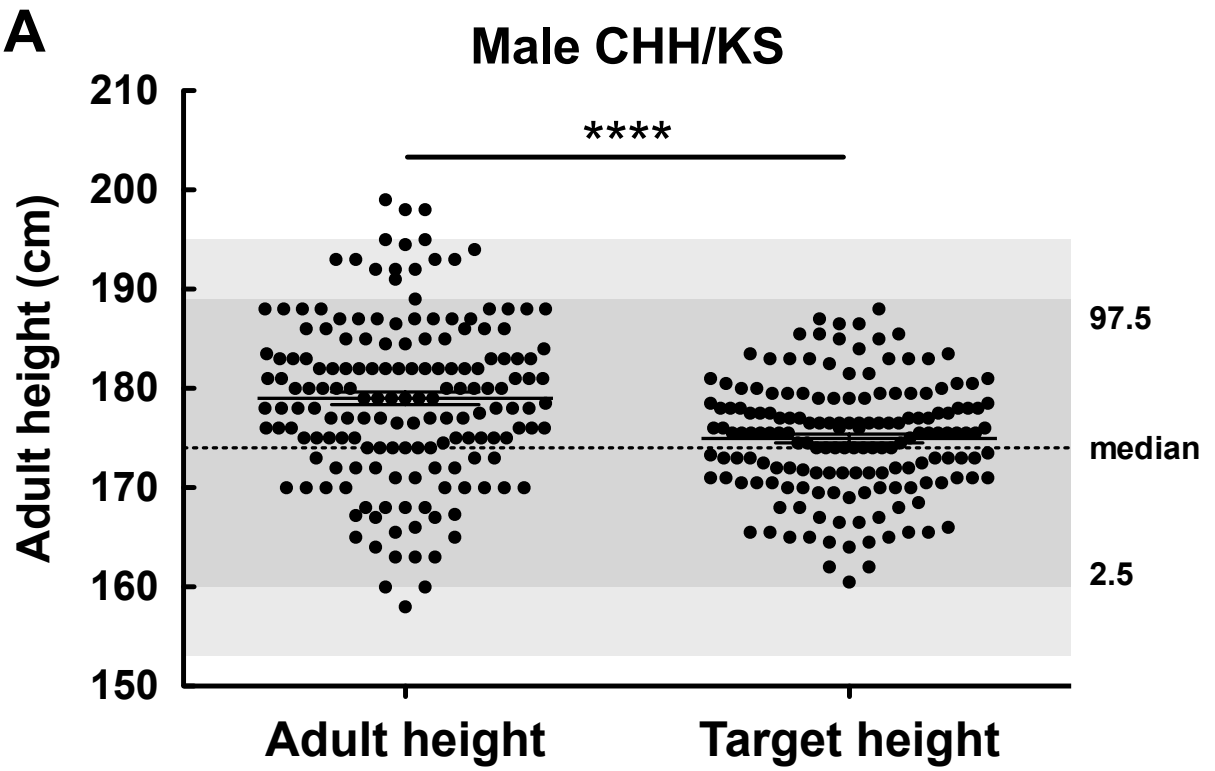
Fig. 1



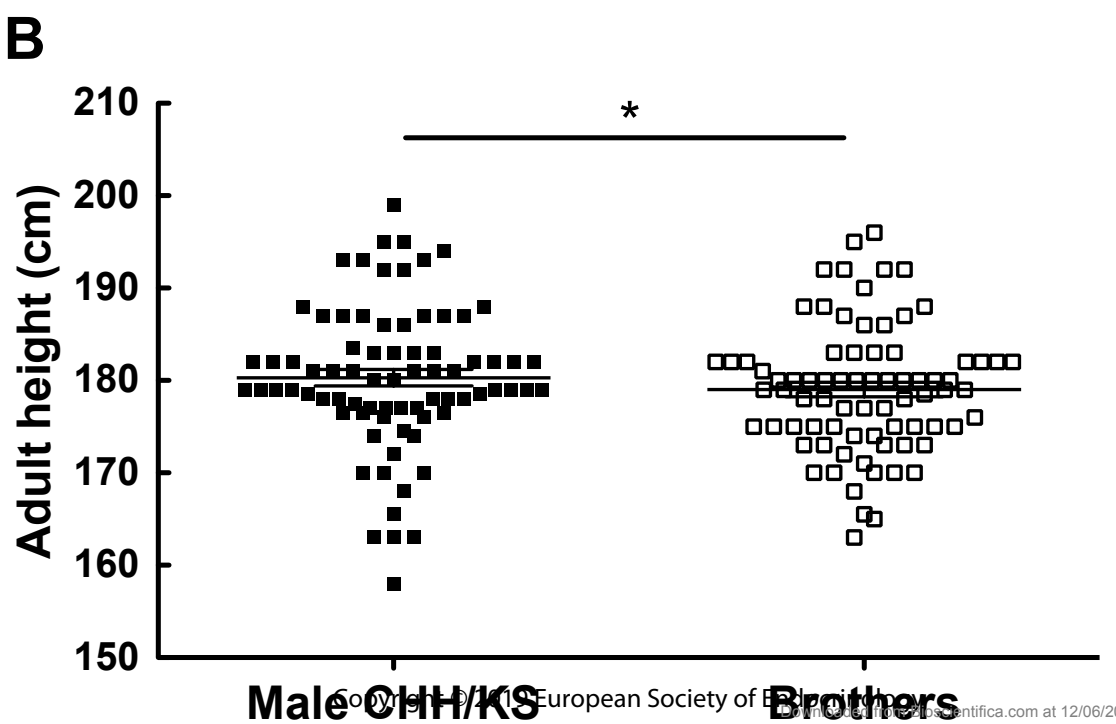
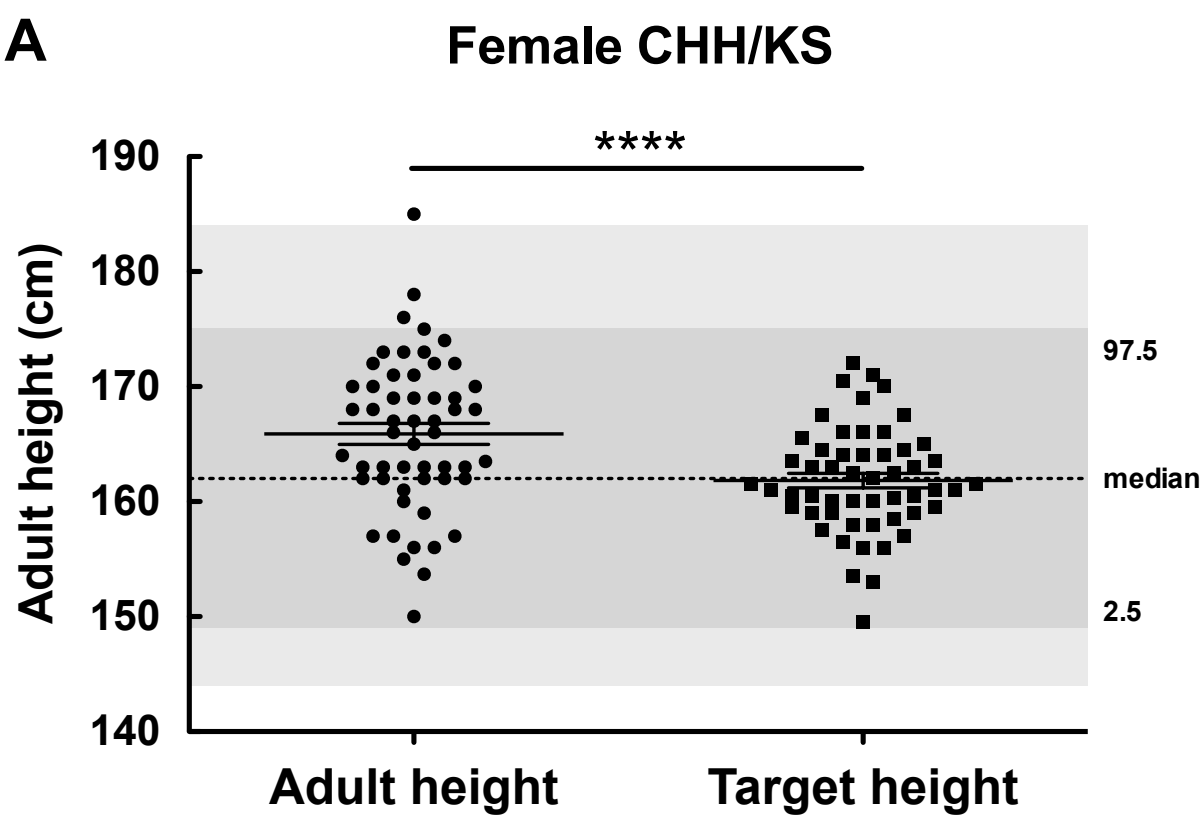


Fig. 2



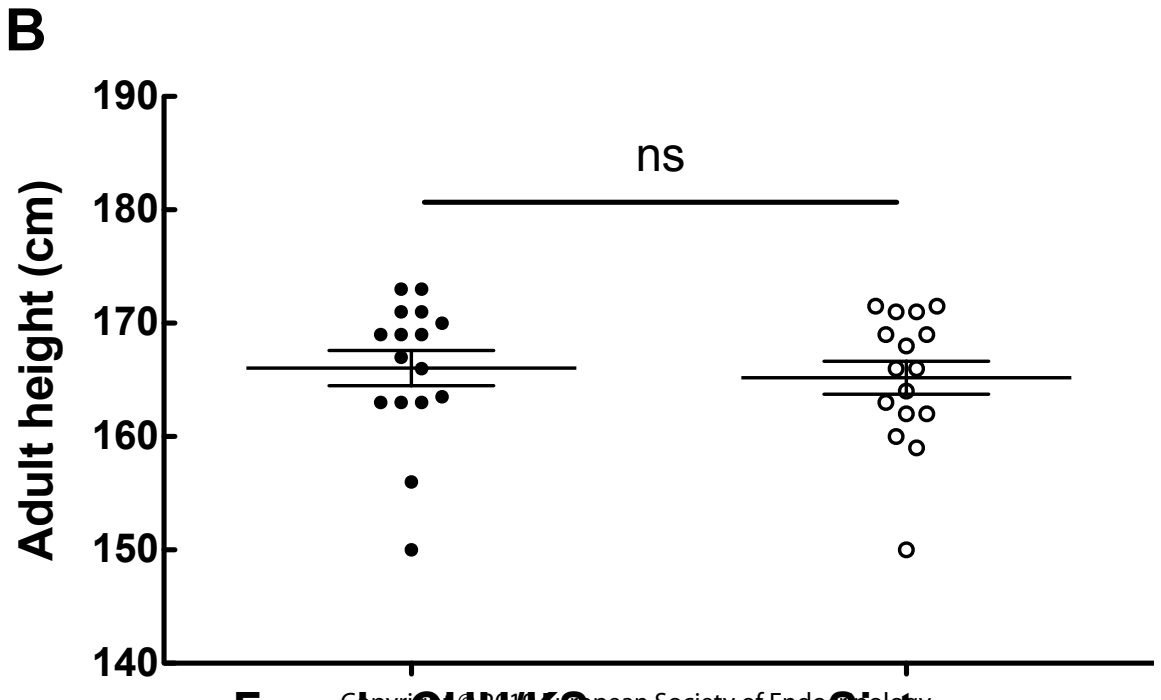


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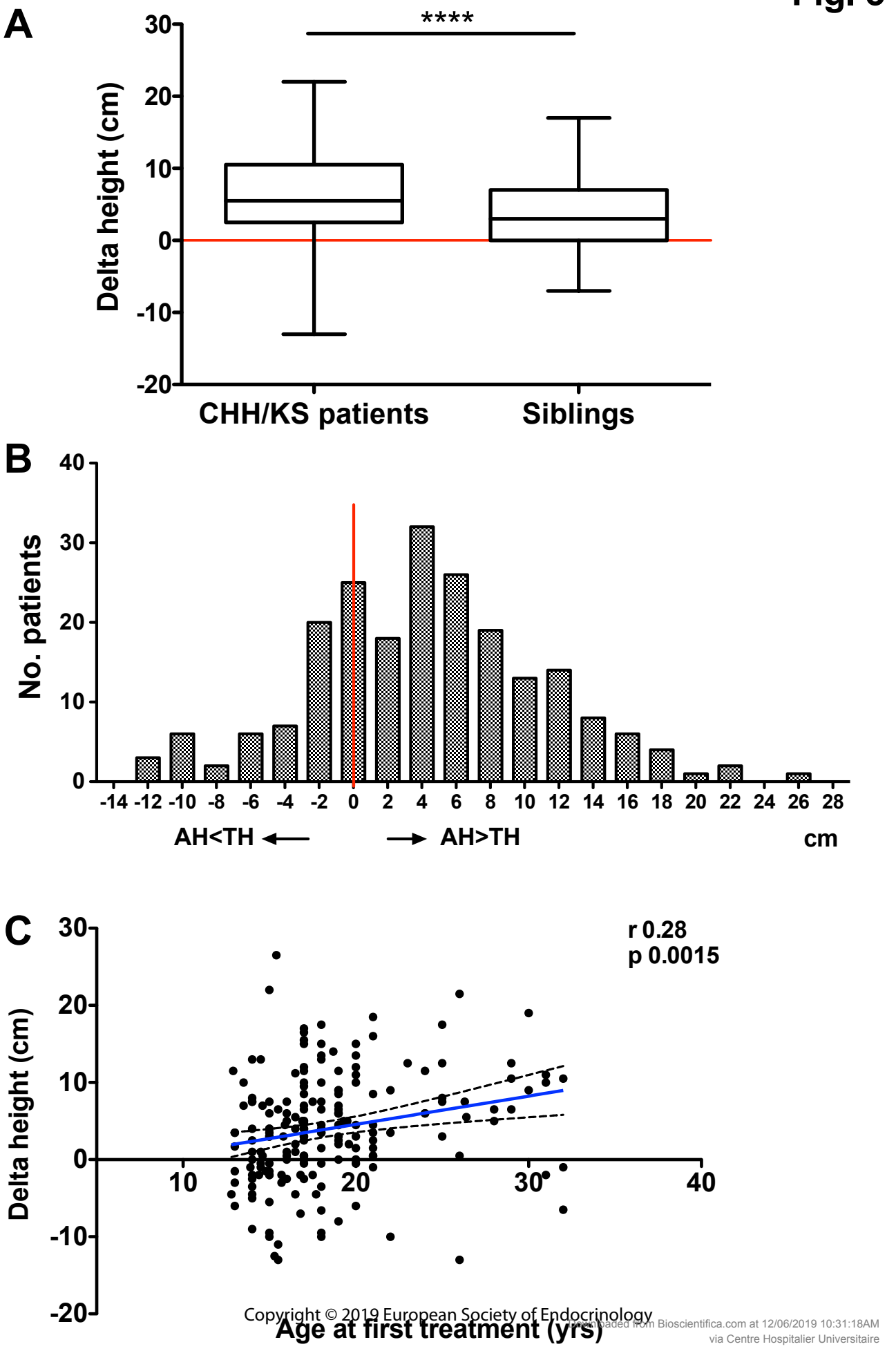
Fig. 3



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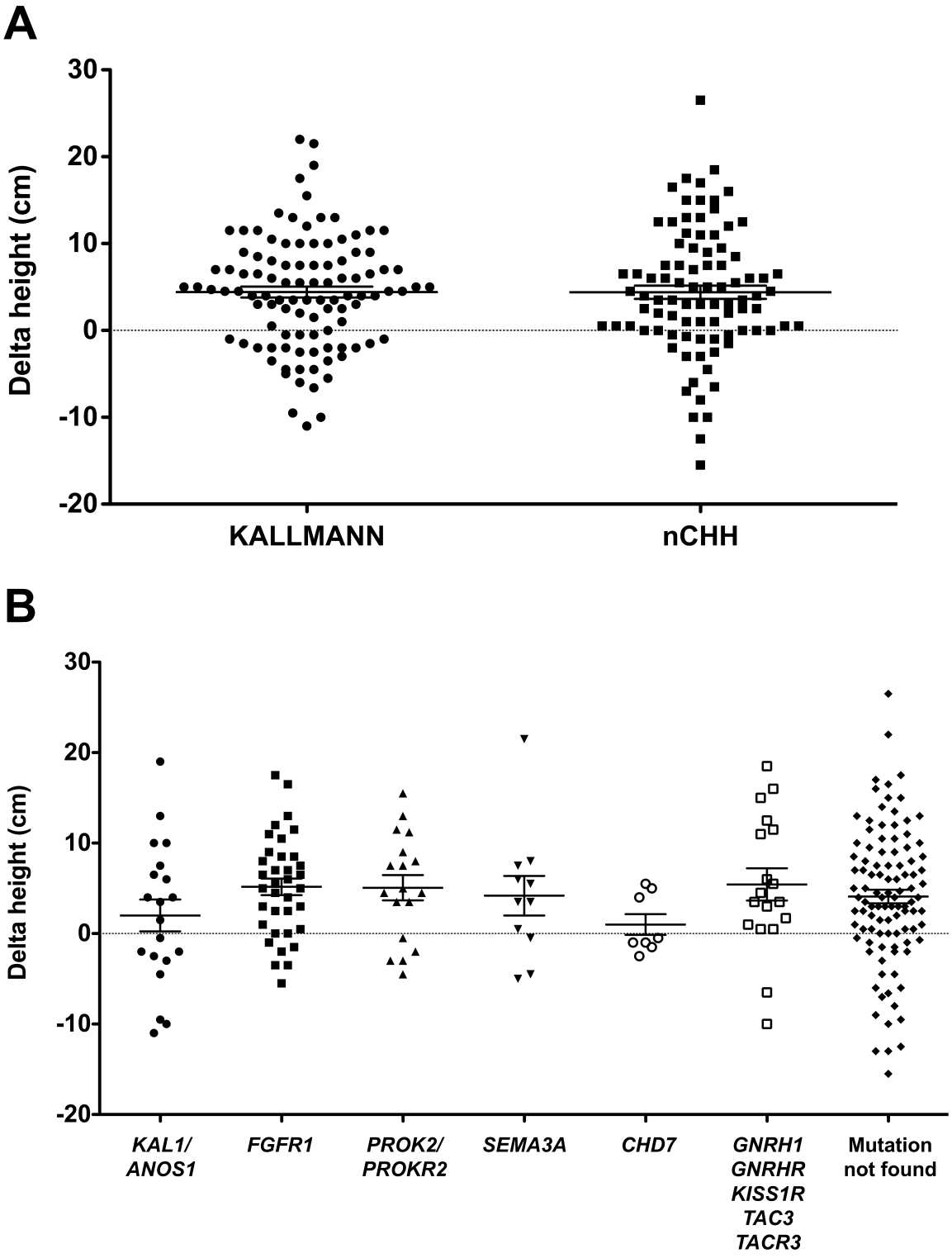

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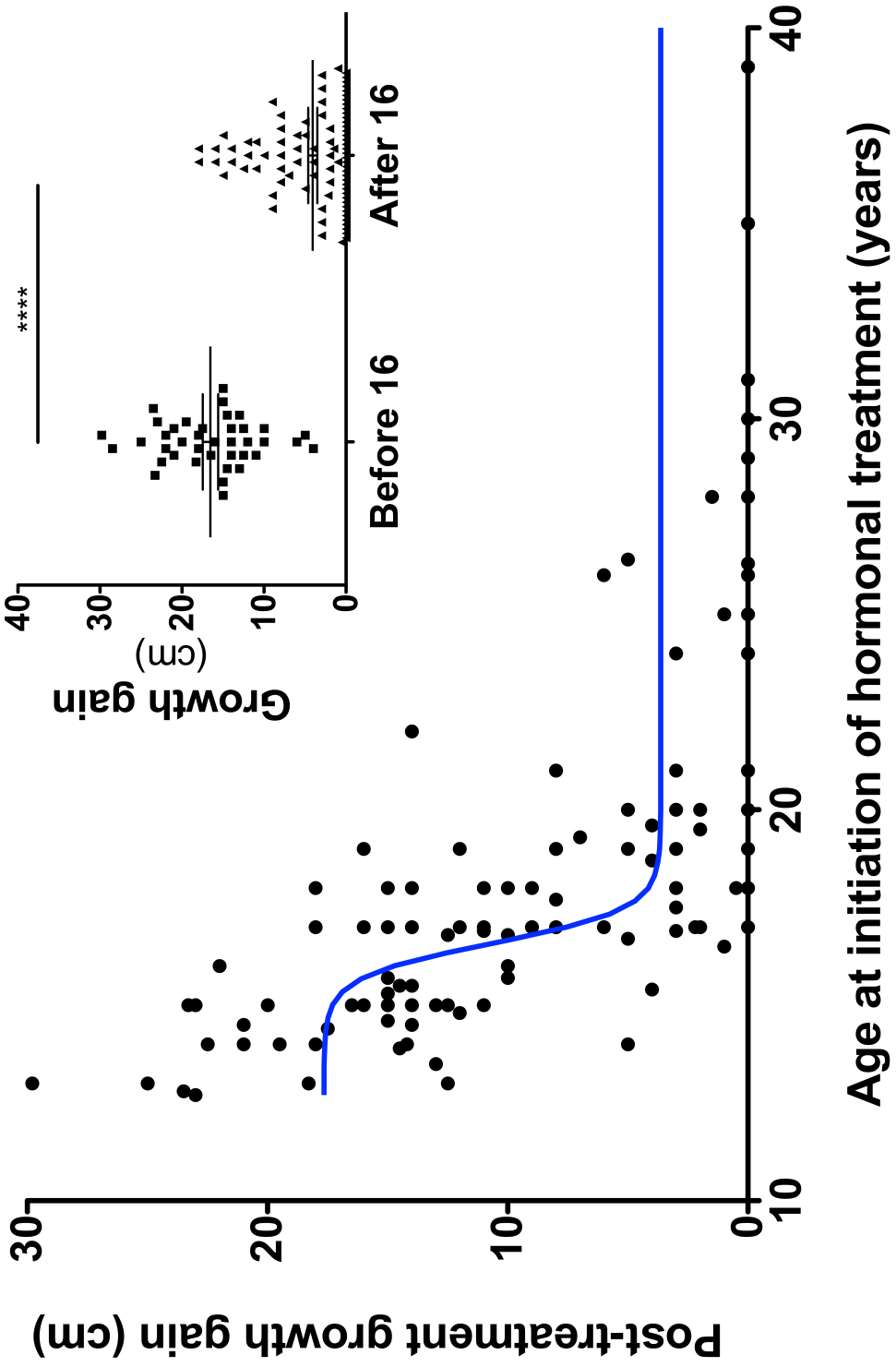
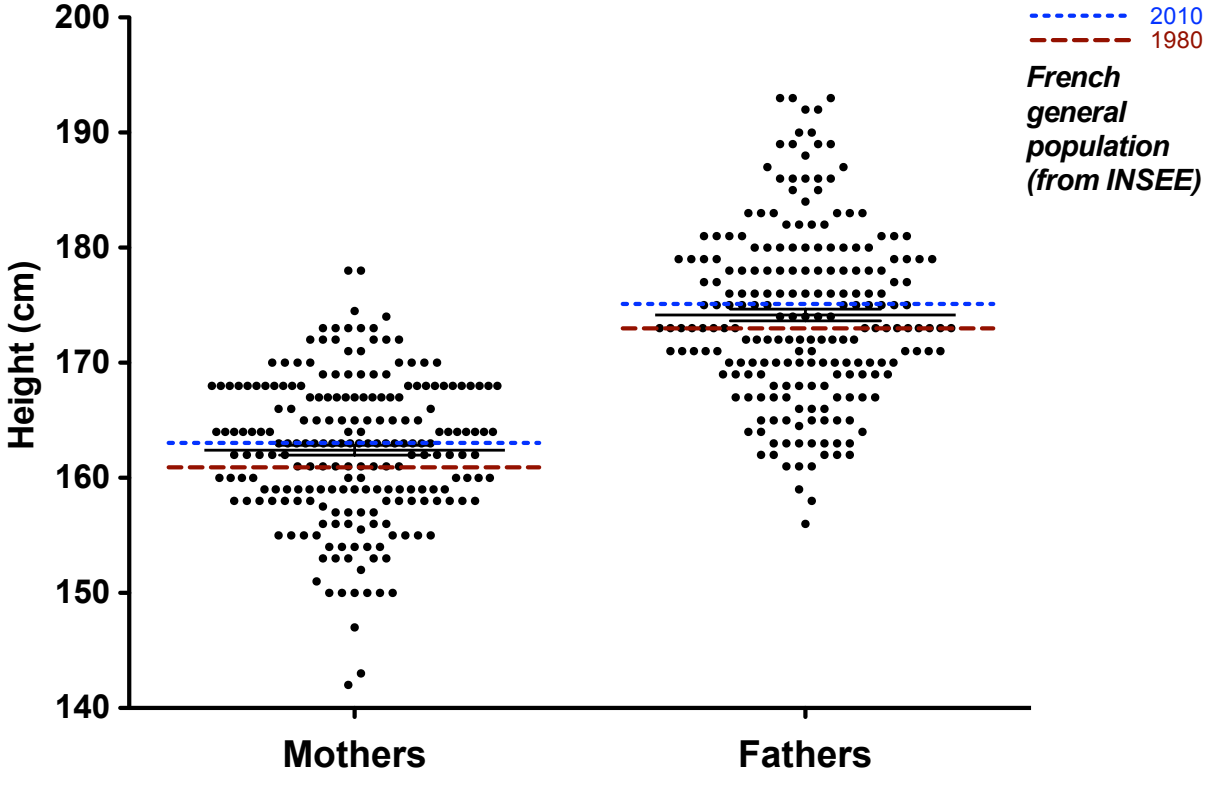
Fig. 5





Supplementary Figure 1. CHH patients' parental height.
Individual values (n=219) and mean ± SE of CHH patients' mothers and fathers. Red line indicates general French population median in 1980. Blue line indicates general French population median in 2010. Data of French general population in the last thirty-year period are from the public database of the French National Institute of Statistics, INSEE, http://www.insee.fr/).

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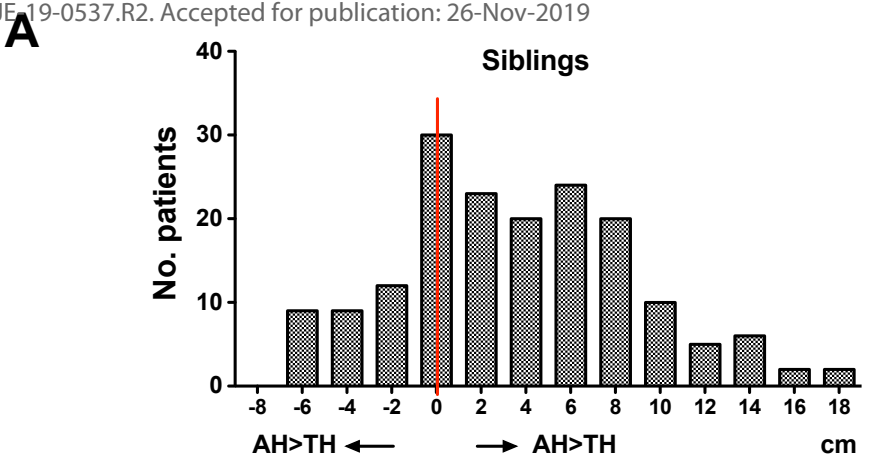
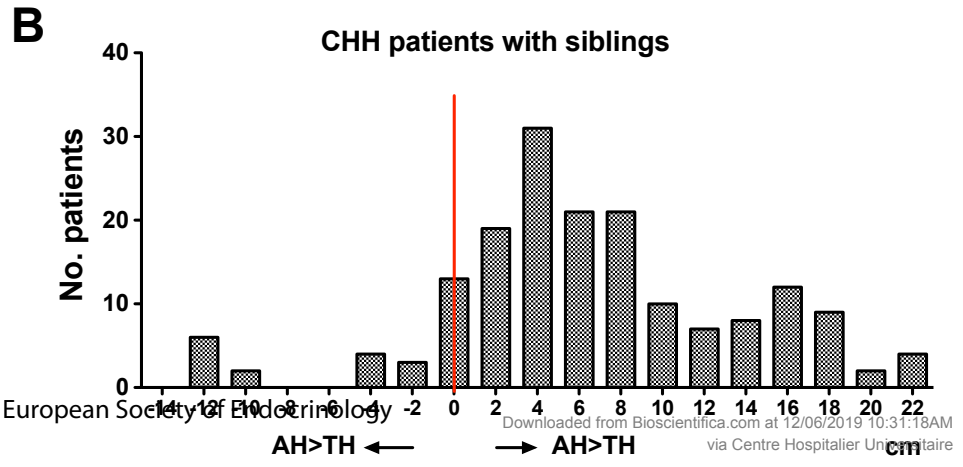
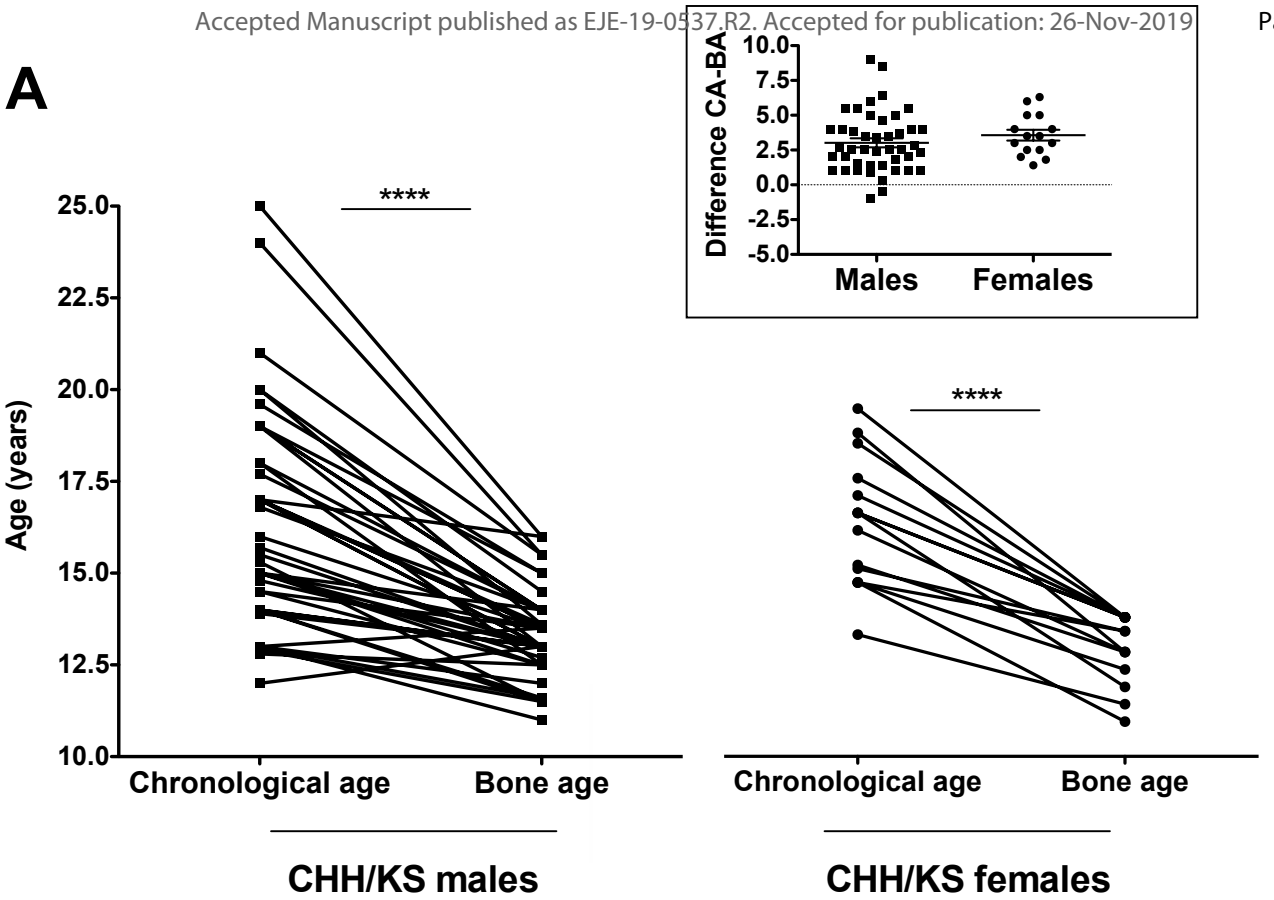
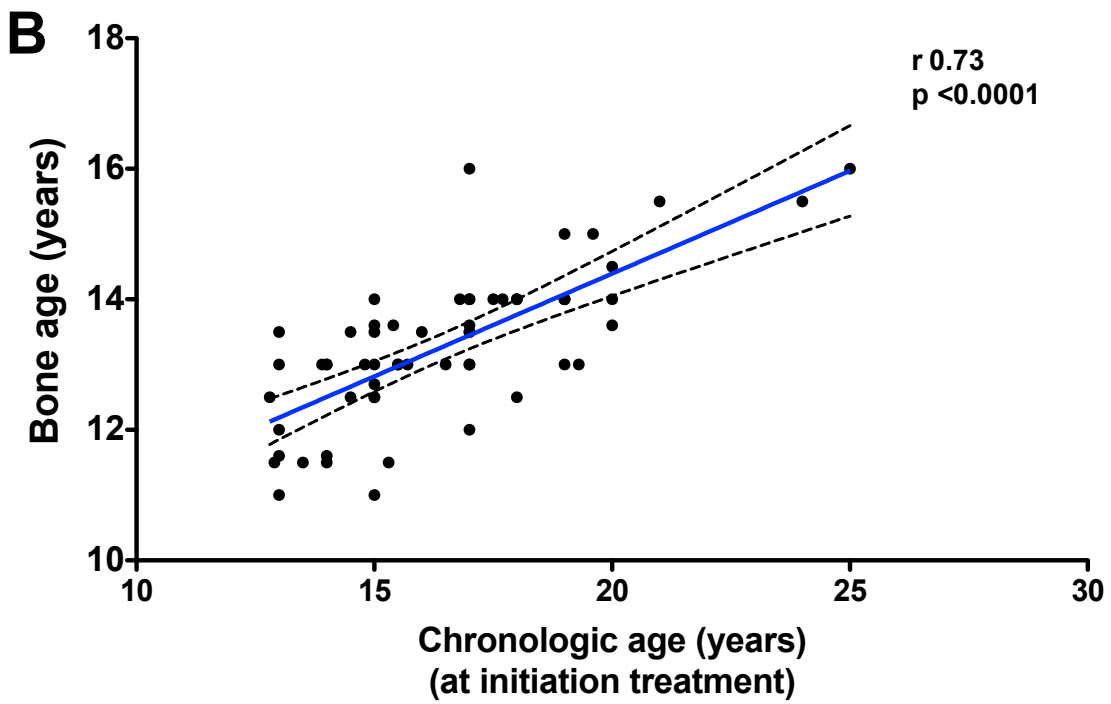




Supplementary Figure 2.
Distribution of Delta Heights in HHC/KS patients and in their siblings.
Panel A. Distribution of positive (>0 (cm)) and negative (<0 (cm)) delta heights in siblings of CHH/ KS patients. Each vertical bar represents number of patients. On x-axis numbers represent the delta (observed - target height, in cm). Red line indicates zero (no difference between final and predicted height).
Panel B. Distribution of of positive (>0 (cm)) and negative (<0 (cm)) delta heights in CHH/KS patients having siblings. Each vertical bar represents number of patients. On x-axis numbers represent the delta (observed - target height, in cm). Red line indicates zero (no difference between final and predicted height).





Supplemental Figure 3

A. Chronological and bone age comparisons in CHH/KS males and females patients. Data are showed as "before-after" graphic representation of individual chronological and bone age values in male (left) and in female (right) CHH/KS patients (n=59). ****p<0.0001. Inset: Difference between chronological and bone age (CA-BA) between males and females CHH/KS patients.

B. Correlation between chronologic and bone age in CHH/KS patients. Individual values of chronologic and bone age in male and female CHH/KS (n=59). R-square 0.53, P<0.0001, slope 0.31±0.01, 95%CI 0.23-0.39.

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