Genetic analyses of bone morphogenetic protein 2, 4 and 7 in congenital combined pituitary hormone deficiency

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Abstract

Background: The complex process of development of the pituitary gland is regulated by a number of signalling molecules and transcription factors. Mutations in these factors have been identified in rare cases of congenital hypopituitarism but for most subjects with combined pituitary hormone deficiency (CPHD) genetic causes are unknown. Bone morphogenetic proteins (BMPs) affect induction and growth of the pituitary primordium and thus represent plausible candidates for mutational screening of patients with CPHD.

Methods: We sequenced BMP2, 4 and 7 in 19 subjects with CPHD. For validation purposes, novel genetic variants were genotyped in 1046 healthy subjects. Additionally, potential functional relevance for most promising variants has been assessed by phylogenetic analyses and prediction of effects on protein structure.

Results: Sequencing revealed two novel variants and confirmed 30 previously known polymorphisms and mutations in BMP2, 4 and 7. Although phylogenetic analyses indicated that these variants map within strongly conserved gene regions, there was no direct support for their impact on protein structure when applying predictive bioinformatics tools.

Conclusions: A mutation in the BMP4 coding region resulting in an amino acid exchange (p.Arg300Pro) appeared most interesting among the identified variants. Further functional analyses are required to ultimately map the relevance of these novel variants in CPHD.

Keywords: Combined pituitary hormone deficiency, Bone morphogenetic proteins, BMP2, BMP4, BMP7

Background

The development of the pituitary gland is a highly complex process, involving many signalling molecules and transcription factors [1-3]. During embryogenesis cells from the oral ectoderm form the adenohypophysis, while the posterior part develops from neural tissue. With the help of animal models it has been shown that transcription factors like HesX1, Prop1, Pou1F1, Lhx3, Lhx4, Pitx1, Pitx2, Otx2, Sox2 and Sox3 play a crucial role in the development of the pituitary gland [4-6]. Several mutations in genes encoding these transcription factors have been reported in combined pituitary hormone deficiency (CPHD). However, for most of the patients the genetic cause of hypoplasia or at least functional insufficiency of the pituitary gland remains to be discovered.

Bone morphogenetic proteins (BMP) 2, 4 and 7 have a crucial role during the embryonic development of the pituitary gland [7]. In early development Bmp4 contributes to the formation of the rudimentary Rathke’s pouch in the mouse (reviewed in [4]). Later BMP 2, 4 and 7 secreted by surrounding tissues contribute to the polarisation of the pouch [7,8]. The development of the pituitary gland is completed within the first trimester of pregnancy in humans [9].

The BMPs are members of the transforming growth factor (TGF)-ß family and bind to type 1 and 2 serine-threonine kinase receptors (BMPR1A and BMPR2). Among different isoforms, three type 1 receptors (BMPR1A/ALK3,
BMP1B/ALK6, and ACVR1A/ALK2) and three type 2 receptors (BMPR2, ACTR2A, and ACTR2B) mediate most of the effects of BMPs [10-14]. Bmp2 null mice die between embryonic day E7.5 and E10.5, suffering from cardiac defects [15]. Selective inhibition of Bmp4 in mouse embryos results in a loss of nearly all pituitary cell lines except a few corticotrophs [8]. Bmp4 knock-out mice are characterized by pituitary aplasia, suffer from severe facial, kidney and skeletal abnormalities, and die early in embryogenesis [16]. Severe eye defects and skeletal and renal anomalies are found also in Bmp7 null mice [17], which die shortly after birth [18]. However, systematic search for mutations in BMP2, 4 and 7 in patients with combined pituitary insufficiency has not been performed yet. So, the aim of our study was to investigate whether genetic variants in BMP2, BMP4 and/or BMP7 are associated with congenital pituitary insufficiency.

Methods

Subjects
In the present study, we included 19 patients (13 males, 6 females) with congenital combined pituitary hormone insufficiency (Table 1). Prior to direct sequencing of BMP genes, screening for known mutations in PIT1 and PROP1 has been performed in 19 subjects and did not reveal any aberrant results. Screenings for mutations in further genes are specified in Table 1.

To determine the frequency of newly identified genetic variants in the general population, we included a set of 1046 healthy subjects (Germany) without any history of pituitary disorders [19]. The study was approved by the ethics committee of the University of Leipzig and all subjects provided written informed consent before taking part in the study.

DNA extraction and sequencing
Genomic DNA was extracted from lymphocytes using the Fujifilm (Düsseldorf, Germany) QuickGene DNA whole blood kit according to the manufacturer’s protocol. We sequenced all exons, exon-intron boundaries, 5′- and 3′-untranslated regions (UTR) of BMP2 (Ensembl ENSG00000125845), BMP4 (Ensembl ENSG00000125378) and BMP7 (Ensembl ENSG00000101144) in DNA samples from 19 non-related Caucasian subjects. Sequencing was performed using the Big Dye® Terminator (Applied Biosystems, Inc., Foster City, CA) on an automated DNA capillary sequencer (ABI PRISM® 3100 Avant; Applied Biosystems, Inc., Foster City, CA). Sequence information and PCR conditions for all oligonucleotide primers used for variant screening are available in Additional files 1 and 2. Known single nucleotide polymorphisms (SNPs) are designated according to dbSNP (http://www.ncbi.nlm.nih.gov/snp/) reference accession numbers.

Prediction of functional relevance
To predict the potential impact of an identified variant on protein structure and function we used several online tools and databases: SIFT (http://sift.bii.a-star.edu.sg/) [20], PolyPhen (http://genetics.bwh.harvard.edu/pph/) [21], Mutpred (http://mutpred.mutdb.org) [22], FATHMM (http://fathmm.biocompute.org.uk) [23], Mutation Taster (http://www.mutationtaster.org) [24], SNP and Go (http://snps-and-go.biocomp.unibo.it/snps-and-go) [25].

Genotyping of novel variants in control subjects
We genotyped all newly identified variants that predict an amino acid exchange in the cohort of healthy subjects by employing the TaqMan allelic discrimination assay (Applied Biosystems, Inc., Foster City, CA). The genotypes were detected on an ABI PRISM 7500 sequence detector (Applied Biosystems Inc.) according to the manufacturer’s protocol. Genotyping success rates for all analyzed SNPs were 99%.

Phylogenetic analysis of the newly identified BMP4 variant c.899G > C
For the coding region of BMP4, the conservation between species was determined by using Phylogenetic Analysis by Maximum Likelihood (PAML) [26]. Specifically, the aim of this analysis was to identify the ratio of non-synonymous to synonymous base substitutions (omega, \( \omega = dN/dS \)). The coding sequences of 37 BMP4 orthologues were downloaded from ENSEMBL (http://www.ensembl.org) and the NCBI (http://www.ncbi.nlm.nih.gov) databases.

Results

BMP2
Direct sequencing of BMP2 revealed 10 that have been previously reported. The non-synonymous SNP rs2273073, found to be heterozygous in one out of 19 analyzed subjects represents a T to G base pair exchange resulting in a serine to alanine amino acid (aa) substitution at protein position 37 (p.Ser37 Ala). A second non-synonymous variant (rs235768) also located within the coding region results in an arginine to serine exchange (p.Arg190Ser) and was found with a minor allele frequency (MAF) of 0.34 in our analyzed cohort. Detailed information of all identified SNPs in BMP2 is presented in Table 2.

BMP4
Sequencing of the BMP4 gene revealed four SNPs. Three of them have already been described by others (Table 2). The newly identified variant c.899G > C leads to an aa exchange from arginine to proline at position 300 (p.Arg300Pro) within the protein and was found as a heterozygous mutation in one of the 19 analyzed subjects (patient number 5; Table 1). Genotyping of this variant...
Table 1 Patient characteristics

| Patient | Sex | Genetic screening | Further genetic tests | Lack of Pituitary gland in MRI-scan | Special aspects | Symptoms leading to diagnosis | Age of diagnosis | Family history |
|---------|-----|-------------------|-----------------------|------------------------------------|----------------|-----------------------------|-----------------|--------------|
| 1       | m   | PIT1, PROP1, HESX1, LHX3 | /                     | + + + + hypoplastic               | /              | growth retardation and hypopituitarism | childhood/adolescence | no           |
| 2       | m   | PIT1, PROP1, HESX1, LHX3 | SOX2, OTX2            | + + + + hypoplastic               | midline defect, right anophthalmia, mental retardation | severe malformations | birth         | no           |
| 3       | f   | PIT1, PROP1, HESX1, LHX3 | /                     | + + + + hypoplastic               | brain atrophy  | growth retardation           | childhood/adolescence | no           |
| 4       | f   | PIT1, PROP1, HESX1, LHX3 | /                     | + + no no hypoplastic             | /              | growth retardation           | childhood/adolescence | no           |
| 5       | m   | PIT1, PROP1, HESX1, LHX3 | LHX4                  | + + + + hypoplastic               | sclerosed nodules at the hands, short metacarpalia IV, azoospermia, | growth retardation | childhood/adolescence | no           |
| 6       | f   | PIT1, PROP1, HESX1, LHX3 | LHX4                  | + + no + n.a.                     | /              | /                           | unknown          |              |
| 7       | f   | PIT1, PROP1, HESX1, LHX3 | /                     | + + no + small and ectopic neuropituitary gland | left optic atrophy | growth retardation, postpartal hypoglycaemia | childhood/adolescence | no           |
| 8       | m   | PIT1, PROP1, HESX1, LHX3 | GLI2                  | + + no + small pituitary and ectopic neuropituitary gland | /              | /                           | unknown          |              |
| 9       | m   | PIT1, PROP1, HESX1, LHX3 | /                     | no + no + ectopic adeno- and neuropituitary gland | /              | prolonged jaundice, hypothyroidism | early infancy     | no           |
| 10      | f   | PIT1, PROP1, HESX1, LHX3 | /                     | + + + + hypoplastic               | /              | hypoglycaemia, hypothyroidism | early infancy     | no           |
| 11      | m   | PIT1, PROP1, HESX1, LHX3 | LHX4, GLI2            | + no + + n.a.                     | Asperger syndrome | /                           | unknown          |              |
| 12      | m   | PIT1, PROP1, HESX1, LHX3 | /                     | + + + + n.a.                     | /              | growth retardation, puberty onset at the age of 18, hypogonadism | childhood/adolescence | yes          |
| 13      | m   | PIT1, PROP1, HESX1, LHX3 | /                     | + + no + n.a.                     | /              | /                           | unknown          |              |
| 14      | m   | PIT1, PROP1, HESX1, LHX3 | /                     | + + + + hypoplastic               | /              | pericardial effusion         | adulthood        | yes          |
| 15      | m   | PIT1, PROP1, HESX1, LHX3 | /                     | + + no + normal size, but ectopic neuropituitary gland | /              | prolonged jaundice, hypoglycaemia, microphallus, muscular hypotonia, hypothyroidism | early infancy     | no           |
| 16      | f   | PIT1, PROP1, HESX1, LHX3 | LHX4                  | + + + + hypoplastic               | /              | hypoglycaemia, hyponatremia, hepatopathy, muscular hypotonia | 3rd day of life   | n.a.         |
| 17      | m   | PIT1, PROP1, HESX1, LHX3 | GLI2, SHH             | + + + + hypoplastic               | /              | /                           | complex facial malformations | childhood/adolescence | no            |
| 18      | m   | PIT1, PROP1, HESX1, LHX3 | /                     | + + + + small and ectopic neuropituitary gland | arachnodactyly, pulmonalisectasia, cryptorchidism, scoliosis | /              | unknown          |              |
| 19      | f   | PIT1, PROP1, HESX1, LHX3 | /                     | + + no + hypoplastic              | /              | growth retardation           | childhood/adolescence | no           |

m = male; f = female; MRI-scan = magnetic resonance imaging; MPHD = screening for PIT1, PROP1, HESX1, LHX3; PIT1 = POU domain, class 1, transcription factor 1; PROP1 = Homeobox protein prophet of Pit-1; HesX-1 = HESX homeobox 1; SOX2 = SRY (sex determining region Y)-box 2; OTX2 = SRY (sex determining region Y)-box 2; LHX4 = LIM/homeobox protein Lhx4; GLI2 = Zinc finger protein GLI2; SHH = Sonic hedgehog homolog; GH = growth hormone; TSH = thyroid stimulating hormone; LH = luteinizing hormone; FSH = follicle stimulating hormone; ACTH = adrenocorticotropic hormone; n.a. = not available.
in 1046 healthy subjects did not reveal any further heterozygous or homozygous c.899G > C carrier. Additionally, we have found the non-synonymous variant rs17563, resulting in a p.Val152Ala substitution. This SNP was found with a MAF of 0.45 within the cohort.

PAML analyses showed an overall strong conservation of the gene. Positional analyses further indicated that most positions are conserved or strongly conserved. Position number 300 is highly conserved. Regarding each species separately revealed that the human lineage seems

| Gene region | Exon/Intron | SNP | MAF according to NCBI | MM/mm in analyzed cohort | aa-exchange | MAF in analyzed cohort |
|-------------|-------------|-----|------------------------|--------------------------|-------------|------------------------|
| 5′-UTR      | Exon 1      | rs35123420 | C = 0.040              | G/C                      | C = 0.026   |
|             | Exon 2      | rs2273073  | G = 0.028              | T/G                      | p.Ser37Ala  |
|             | Exon 3      | rs235768   | A = 0.240              | T/A                      | p.Arg190Ser |
|             | Exon 3      | rs13037675 | T = 0.046              | C/T                      | synonymous  |
|             | Exon 3      | rs15705    | C = 0.280              | A/C                      |            |
| 3′-UTR      | Exon 3      | rs3178250  | C = 0.264              | T/C                      | C = 0.368   |
|             | Exon 3      | rs235769   | A = 0.234              | G/A                      | A = 0.368   |
|             | Exon 3      | rs170986   | A = 0.162              | C/A                      | A = 0.053   |

| Gene region | Exon/Intron | SNP | MAF according to NCBI | MM/mm in analyzed cohort | aa-exchange | MAF in analyzed cohort |
|-------------|-------------|-----|------------------------|--------------------------|-------------|------------------------|
| 5′-UTR      | Intron 2    | rs2855532 | T = 0.427              | C/T                      | T = 0.342   |
|             | Intron 2    | rs2761880 | T = 0.221              | C/T                      | T = 0.053   |
| coding      | Exon 4      | rs17563   | C = 0.373              | C/T                      | p.Val152Ala |
| region      | Exon 4      | c.899G > C | G/C                    | p.Arg300Pro             | C = 0.026   |

| Gene region | Exon/Intron | SNP | MAF according to NCBI | MM/mm in analyzed cohort | aa-exchange | MAF in analyzed cohort |
|-------------|-------------|-----|------------------------|--------------------------|-------------|------------------------|
| 3′-UTR      | Intron 7    | rs6025417 | C = 0.478              | G/C                      |              |
|             | Intron 7    | rs6025416 | C = 0.452              | T/C                      |              |
|             | Intron 7    | rs6025415 | G = 0.478              | G/C                      | C = 0.473   |
|             | Intron 7    | rs6025414 | G = 0.478              | G/C                      | C = 0.473   |

**SNP** = single nucleotide polymorphism, **BMP** = bone morphogenetic protein; **MAF** = minor allele frequency; **MM** = major allele, **mm** = minor allele in analyzed cohort; **aa** = amino acid; **UTR** = untranslated region; **n.a.** = not available; novel identified SNPs are presented in bold; *) only in ENST00000378827 but not in NM_001200 part of exon 1 (5′UTR); **) variants are located within an additional exon only present in isoform BMP7 ENST00000433911; $) for transcript variant ENST00000450594 the SNPs is located within the coding region.
to have no synonymous substitutions leading to an infinite omega. The absence of synonymous changes in the data leads to the infinite omega, as there is a positive number divided by zero. A likelihood ratio test (LRT) against the model with the average omega reveals that $P < 0.005$, underlining that we have a strongly conserved gene.

All identified genetic variants in the BMP4 gene are presented in Table 2.

**BMP7**

All protein-coding exons that constitute the various transcripts of BMP7 were sequenced. In total we found 18 genetic variants. One variant (c.611 + 3366C > T) has not been reported so far. The novel intronic SNP at position c.611 + 3366C > T showed a MAF of 0.026. This variation was found in patient number 17 (Table 1). This subject presented a phenotype including a hypoplastic pituitary gland and complex facial malformations. The previously known genetic variant rs61733438 results in the aa exchange p.Asn321Ser and was found in one out of the 19 subjects. Finally, two further previously known SNPs (rs192121279 and rs2148328) resulted in the aa exchange p.Asn321Ser and was found in one out of two known BMP7 isoforms (ENST00000433911 and ENST00000433911). The results for sequencing of BMP7 are summarized in Table 2.

**Prediction of functional relevance**

Potential impacts of all newly identified variants on protein structure and function was investigated by use of several online tools [20-25]. The only variant with consistent evidence for functional consequences was c.899G > C in BMP4. Results are summarized in Table 3.

**Discussion**

The development of the distinct cell lines of the pituitary gland is directed by nuclear mediators of cell type commitment, including the BMP pathway and a number of transcription factors (reviewed in [2]). The role of BMP2, BMP4 and BMP7 as signalling peptides in the programming of pituitary development makes them plausible candidates for pituitary disorders including congenital insufficiency as well as pituitary adenomas. In our study we systematically screened for genetic variation in these genes in a group of patients with CPHD.

Inhibition of Bmp2/Bmp4 in mice causes loss of the Pit-1 lineage and gonadotropes but not of POMC-expressing cells [8]. In detail, BMP2 is essential for the expression of ventral markers such as the insulin gene enhancer protein ISL-1 and human glycoprotein hormone α-subunit gene and necessary for terminal differentiation of pituitary cell types [8,27]. We could identify two known SNPs, rs2273073 and rs235768, in two different patients. To predict the potential impact of an identified variant on protein structure and function we used several online tools and databases [20-25]. However, we are aware of limitations of these tools and used comparative considerations and degree of conservation of amino acid residues do not provide functional evidence. The p.Ser37Ala substitution caused by rs2273073 is assumed to be tolerated according to SIFT and PolyPhen whereas it is predicted to be disease associated by Mutation taster and SNP&GO. The carrier of this variant in our study did not present any further phenotype other than CPHD. There is evidence in the literature that variation at rs2273073 affects bone mineral density [28] but we do not possess any clinical data on this phenotype in our study. Additionally, we identified rs235768 predicting the p.Arg190Ser exchange in one subject in our cohort of CPHD patients. An association of p.Arg190Ser substitution with the development of childhood IgA nephropathy has been described [29], the functional relevance cannot be predicted explicit based on SIFT and PolyPhen database search, it described as neutral or tolerated by MutPred and FATHMM but potentially disease associated by SNP&GO. Further functional studies are required to elucidate detailed effects of this variant.

In accordance with previous studies [27] our phylogenetic analyses of the BMP4 gene revealed a highly conserved sequence of the BMP4 region which would suggest a potential functional relevance of variation at this locus. We identified a novel variant resulting in a c.899G > C substitution predicting a missense mutation within the protein sequence (p.Arg300Pro). Bioinformatic prediction tools provide substantial evidence to functional consequences and the fact that we have not found a second heterozygous or homozygous c.899G > C carrier in a set of 1046 healthy subjects and the high conservation at this locus furthermore support a potential association with the phenotype. An X-ray of the patient at the age of 17 presents skeletal abnormalities described as vertebral platyspondyly, sclerosis of the metaphyses and a short metacarpalia IV, which would be in line with the diagnosis of spondyloepiphyseal dysplasia tarda. Since BMP4 is known to increase osteoblast differentiation the affection of the skeletal system would be consistent with a functional relevance of the newly identified c.899G > C substitution. Furthermore, fibro-dysplasia ossificans progressiva is characterized by an overexpression of BMP4 in lymphocytes [30], so detailed functional analyses are required to assess effects on BMP4 expression and interaction with BMPR1A receptor pathway. A detailed family history or genetic material of the patient’s family are unfortunately not available which is a clear limitation of the study. According to the self reported family history all other relatives do not
| Table 3 Assessment of potential functional relevance of identified variants |
|---------------------------------------------------------------|
| **BMP2**                                                      |
| rs2273073 Ser37Ala  | - tolerated          | - tolerated          | - aas changed                          | - aas changed                          | - tolerated                          | - score 0.123                       |
|                  |                     |                     | - heterozygous in TGP                  | - disease associated variation         | - score 0.61                         | - neutral                           |
|                  |                     |                     | - known disease mutation at this position (HGMD CM034611) | - protein features (might be) affected | - disease associated variation (probability – 0.527) |                     |
|                  |                     |                     | - splice site changes                  | - splice site changes                  | (probability – 0.974)                |                     |
| rs235768 Arg190Ser | - functional relevance cannot be predicted explicit | - functional relevance cannot be predicted explicit | - aas changed                          | - aas changed                          | - tolerated                          | - score 0.293                       |
|                  |                     |                     | - homoyzous in TGP                     | - disease associated variation         | - score –0.045                       | - neutral                           |
|                  |                     |                     | - protein features (might be) affected | - disease associated variation         | (probability – 0.755)                |                     |
|                  |                     |                     | - splice site changes                  | - splice site changes                  | (probability – 0.906)                |                     |
| **BMP4**                                                     |
| rs17563 Val152Ala  | - tolerated          | - tolerated          | - aas changed                          | - aas changed                          | - tolerated                          | - score 0.145                       |
|                  |                     |                     | - homoyzous in TGP                     | - disease associated variation         | - score –0.08                        | - neutral                           |
|                  |                     |                     | - protein features (might be) affected | - disease associated variation         | (probability – 0.775)                |                     |
|                  |                     |                     | - splice site changes                  | - splice site changes                  | (probability – 0.906)                |                     |
| rs192121279 Thr105Met | - deleterious        | - unknown           | - not found                            | n.a.                                   | - No dbSNP mapping(s)               | - score 0.466                       |
|                  |                     |                     | - disease causing                      | n.a.                                   | - neutral                           |                     |
| c.899G > C Arg300Pro | - substantial evidence for functional consequences | - substantial evidence for functional consequences | - disease causing                      | - aas changed                          | - scored –0.78                      | - neutral                           |
|                  |                     |                     | - aas changed                          | - disease associated variation         | - score –0.78                        |                     |
|                  |                     |                     | - protein features (might be) affected | - disease associated variation         | (probability – 0.906)                |                     |
|                  |                     |                     | - splice site changes                  | - splice site changes                  | (probability – 0.906)                |                     |
| **BMP7**                                                     |
| rs192121279 Thr105Met | - deleterious        | - unknown           | - not found                            | n.a.                                   | - No dbSNP mapping(s)               | - score 0.466                       |
| c.611 + 3366C > T % intronic | n.a.              | n.a.               | n.a.                                   | n.a.                                   | - neutral                           |                     |
| rs61733438 Asn321Ser | - tolerated          | - tolerated          | - aas changed                          | - aas changed                          | - tolerated                          | - score 0.278                       |
|                  |                     |                     | - homoyzous in TGP                     | - disease associated variation         | - score –0.92                       | - neutral                           |
|                  |                     |                     | - protein features (might be) affected | - disease associated variation         | (probability – 0.848)                |                     |
|                  |                     |                     | - splice site changes                  | - splice site changes                  | (probability – 0.906)                |                     |
| rs2148328 Ala399Gly | - tolerated          | - unknown           | - aas changed                          | - aas changed                          | - No dbSNP mapping(s)               | - score 0.540                       |
|                  |                     |                     | - protein features (might be) affected | - disease associated variation         | - neutral                           |                     |

1) Loss of relative solvent accessibility (P = 0.0071); Gain of loop (P = 0.0166); Loss of helix (P = 0.0376); Loss of solvent accessibility (P = 0.0442); aas = amino acid sequence, TGP = 1000 Genome Project.
show any affection of the pituitary function. However, it is of note that there is a substantial variability in the clinical presentation of patients with combined pituitary hormone deficiency even if the same gene is affected and even in subjects with identical mutations. Intrafamilial penetrance can range from high to incomplete and it is not possible to draw direct conclusions form the clinical manifestation to the potential genotype. This indicates the remarkable influence of the genetic background, incomplete penetrance, highly variable expressivity, environmental factors and possibly stochastic events. Also co-occuring mutations in interacting genes have to be taken into account.

Additionally to this new variation we found the SNP rs17563 in the coding region of *BMP4*. This variation has been suggested to be involved in the development of otosclerosis [31]. According to the high prevalence in healthy subjects an association with pituitary disorders is rather unlikely.

*BMP7*, also called Osteogenic Protein 1 has an important function during the embryonic development of the eye, brain and ear [17,18]. In mice, Bmp7 is responsible for the expression of *Pax6* and *Sox2* [32] that are both known to be involved in the development of the pituitary gland [3]. We have identified rs61733438, resulting in p.Asn321Ser substitution. So far, rs61733438 has been described in patients with several eye defects [33]. The male patient identified in our CPHD cohort who is carrier of the heterozygous rs61733438 variant has an ectopic neurohypophysis but no other specific symptoms. Furthermore, there is no family history of CPHD.

Taken together, we identified several genetic variants in *BMP2*, *BMP4* and *BMP7* in a group of patients with CPHD. However, genotyping of further patients and mainly functional analyses are required to clarify the exact role in pituitary insufficiency. Clear limitation of our study is the missing genetic information for family members. These data would significantly support phenotype-genotype associations and would strengthen potential functional relevance of the identified variants. Furthermore, the group of CPHD patients included in our study presents a heterogeneous phenotype and most likely also diverse genetic source. We are also aware that by including only a few genes the data remain inconclusive. However, we believe that even by extending the list of studied genes by further candidates there would be no guarantee that further patients will be identified. Thus, a systematic approach including whole genome/exome sequencing strategies would be desirable here.

**Conclusions**

Our study provides a systematic analysis of *BMP* genes in patients with CPHD. We identified novel variants in *BMP2*, *BMP4* and *BMP7*. Of particular interest is a novel variant in *BMP4* (p.Arg300Pro) found in one patient with skeletal malformation in addition to CPHD. Further functional characterization of the newly identified variant is desirable not only to ultimately pinpoint their biological and clinical consequences but also to better understand the role of bone morphogenetic proteins in the pathophysiology of congenital combined pituitary insufficiency.

**Additional files**

Additional file 1: PCR conditions.  
Additional file 2: Primers.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

JB and SM sequenced the genes, analyzed the results, contributed to discussion and drafted the manuscript. JK and RP participated in the design of the study, provided samples and contributed to discussion. KW carried out the evolutionary analyses. DF and MS contributed to the study design and discussion of the results. PK and DS analyzed the results, contributed to discussion and edited the manuscript. AT designed the study, provided samples and contributed to discussion and manuscript writing. All authors read and approved the final manuscript.

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