Novel mechanism of pituitary hormone deficiency: genetic variants shift splicing to produce a dominant negative transcription factor isoform

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Abstract

Recent studies have shown a novel mechanism of combined pituitary hormone deficiency associated with mutations in *POU1F1*, altering the balance of alternative-splicing, which results in over-expression of the beta isoform of POU1F1. These studies underscore the need for biologists, in the context of routine molecular diagnosis of this condition, to investigate alternative splicing in *POU1F1* as well as in other genes.

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Two recent papers have shown a novel mechanism of combined pituitary hormone deficiency (CPHD) associated with mutations in *POU1F1*, the gene that encodes the pituitary transcription factor POU1F1 or PIT-1 (1, 2). These disease-causing mutations alter the balance of alternative-splicing resulting in over-expression of the beta isoform of POU1F1. The identification of this mechanism is of importance both for the understanding of the function of this major transcription factor and in terms of practical implications for genetic screening and molecular diagnosis for children with congenital CPHD or isolated growth hormone deficiency (IGHD). It is also relevant for other genetic diseases, as genetic variants that alter splicing are often overlooked, especially when they do not alter an amino acid.

POU1F1, a founding member of the POU family of homeodomain-containing transcription factors, is a key regulator of pituitary differentiation and function. Indeed, it directly regulates the transcription of growth hormone

(GH), prolactin (PRL), and both the α and β subunits of thyroid stimulating hormone (TSH). In addition, studies in mice reveal that POU1F1 is critical for developing the lineage of three specialized cell types that produce and secrete these hormones. POU1F1 was the first pituitary transcription factor to be identified in the mouse and in human, and it was initially described as growth hormone factor 1 (GHF-1) and Pit-1 (3, 4). A naturally occurring isoform of POU1F1, initially called GHF-2, was described in which an additional 26 amino acids are incorporated into the protein because of alternative splicing (5, 6). These extra amino acids disrupt the transactivation domain and introduce an ETS (E26 transformation-specific)-1 binding domain (E26 avian leukemia oncogene 1, 5' domain) that acts as a dominant and independent repressor (7, 8). This long isoform, now called POU1F1 β , is produced by utilization of an alternative 3' splice acceptor at the 3' end of the first intron (Fig. 1). A comparative genomics study analyzed POU1F1 expression data from over 100 species (9).

The expression of the β -variant is low ($\leq 12\%$ of total POU1F1 expression) relative to the major, activating α -isoform in all species examined. The function, if any, of this minor isoform is unknown.

In 1992, four independent groups described mutations in *POU1F1* in children with variable pituitary hypoplasia and deficiencies in GH, PRL, and TSH (10, 11, 12, 13). Many more mutations have been described subsequently (Fig. 1). Deficiencies of GH and PRL are generally complete, while TSH deficiency is more variable in terms of age of onset (14, 15). In a review covering 114 patients identified from 82 distinct pedigrees, GH, TSH, and PRL deficiencies were present in 100, 87.5, and 95.6% of the cases, respectively (16). Cohort studies have shown that *POU1F1* mutations are typically characterized by a pure endocrine phenotype, but in some cases, *POU1F1* mutations may also be observed in association with other malformations that include pituitary stalk interruption and eye and genital

abnormalities (17, 18). It is not clear whether these other manifestations are caused by variation in other genes, as oligogenic etiology is common in some endocrine disorders, such as hypogonadotropic hypogonadism (19). Based on the experience of the Genhypopit network, *POU1F1* gene defects were particularly prevalent in CPHD patients with normal corticotroph function during infancy (20).

Following the first descriptions of *POU1F1* mutations, a growing number of distinct genes, including those encoding hypothalamic and pituitary transcription factors and several signaling pathways, have been discovered in children with congenital pituitary hormone deficiencies (15, 21, 22). Thirty-four genes have been reported so far, and this number is expected to grow because high throughput screening approaches are just beginning to be applied to CPHD patients (23, 24, 25, 26). Until recently, most screening included the first genes identified: *POU1F1*,

POU1F1 protein variants

- ▼ missense
- ▼ premature stop
- ▼ frameshift

POU1F1 splicing variants

Figure 1

Mutations previously described in *POU1F1*. Premature stop, frameshift, and missense mutations in *POU1F1* have been described in recessive cases of CPHD. These variants affect the transactivation domain (TAD), POU-specific domain (POU), and homeodomain (HOMEO) (shown above protein diagram). Alternate splicing produces the longer, normally rare, *POU1F1* β isoform, with extra amino acids disrupting the TAD. This isoform acts as a transcriptional repressor in some contexts, in contrast to the transactivating *POU1F1* β isoform. Three dominant negative missense variants have been reported (27, 28, 29) which alter protein-protein interactions (shown below the protein diagram). Splice disruptive variants are illustrated relative to the gene diagram. Seven reported splice disruptive mutations in human I are recessive. Recently, a homozygous intron 4 variant, c.605-3C>A, was found in canine *POU1F1* in Karelian Bear dogs with pituitary (43) (not shown). This variant is expected to cause skipping of exon 5. The only previously reported dominant splicing mutation in human *POU1F1* is c.143-5A>G, which results in production of *POU1F1* β only.

HESX1, *LHX3*, *LHX4*, *SOX3*, and *PROP1*. Although *PROP1* mutations are common in some populations, these genes account for less than 20% of CH cases worldwide. The ever-increasing list of candidate genes associated with hypothalamic-pituitary development and the phenotypic heterogeneity among patients has emphasized the growing need for fast, inexpensive methods to uncover the genetic etiology. The laborious and outdated Sanger sequencing methods have been exchanged for targeted gene panels, which can screen known causative genes over a 100 patients simultaneously. However, recent data suggest that screening for all the known genes still leaves most cases unsolved (26). Thus, next generation sequencing (NGS) techniques, including whole exome and genome sequencing, need to be applied broadly to identify pathogenic variants for most patients.

Many mutations have been reported in *POU1F1*, including dominant and recessive inheritance, splicing defects, and protein alterations including missense, frameshift, and premature stop codons (Fig. 1). Loss of function mutations are recessive, and dominant mutations are thought to act by interfering with the function of POU1F1 as a dimer or in a protein complex (27, 28, 29). Lesions occur throughout the α -isoform, including the transactivation domain, POU-specific domain, and the homeodomain. Splicing mutations have been reported near the splice donor and acceptor sites in introns 1 and 2, the acceptor site in intron 4, and the donor site in intron 5 (14, 16, 30, 31, 32, 33). Most of these are recessive, result in exon skipping, and are predicted to produce nonfunctional proteins (Fig. 1). However, the c.144-5A>G variant in intron 1 is dominant and results in production of only the POU1F1 β isoform (32).

Suzuki and colleagues reported a novel cause of dominant CPHD in a three generation Japanese pedigree (1). They identified a missense variant in POU1F1 β , c.152T>G, and p.Ile51Ser (NM_001122757). They used a standard minigene splicing assay to establish that a shift in splicing occurred that favored the production of the POU1F1 β isoform over the α -isoform. Transfection studies in rat pituitary GH₃ cells, which produce endogenous GH and PRL, revealed that the POU1F1 β , p.Ile51Ser variant interferes with basal and ras-mediated activation of a rat PRL reporter gene by co-transfected POU1F1 α expression vector. This effect was more pronounced when endogenous POU1F1 expression was suppressed with a siRNA. While *POU1F1* is highly expressed only in the pituitary gland, trace amounts of transcripts can be amplified from lymphocytes. Epstein-Barr virus-transformed lymphocytes from two affected family members and a normal control

revealed elevated production of the transcript encoding the β -isoform in the affected individuals. This is a clever approach that could be employed to detect altered splicing for other genes suspected to cause disease, or potentially, to implicate regulatory variants that could ablate expression entirely.

In a collaborative study involving patients from Brazil, Germany, France, and Argentina, four missense variants and two silent nucleotide substitutions were found in the POU1F1 β coding region that cause CPHD or IGHD (2). This team found another example of p.Ile51Ser reported by Suzuki and colleagues, as well as the novel variants p.Ser50Ala, p.Ser50=, p.Ile51=, p.Leu52Trp, and p.Ser53Ala. Minigene splicing assays in both, heterologous Cos7 cells and pituitary GH₃ cells indicate that the variants shift splicing to favor the POU1F1 β -isoform almost exclusively. This suggests that the splice site selection is not cell-type specific. They showed that the missense variants suppressed autoactivation of the *Pou1f1* auto-regulatory enhancer by the POU1F1 α -isoform. Using a high throughput splicing assay, Kitzman and Smith tested 1070 single-nucleotide variants in POU1F1 and identified 96 splice-disruptive variants, including 14 synonymous variants that prompted the team to identify the affected individuals with p.Ser50= and p.Ile51= variants. The collection of splice disruptive variants cause exon skipping, isoform switching, utilization of cryptic splice sites, or a combination of these. The team also evaluated several software programs to predict splice utilization and found the best predictor of their experimentally determined sites was SpliceAI, although some discrepancies existed. This new catalog of *POU1F1* variants tested in parallel provides a useful resource for medical geneticists and endocrinologists for interpretation of variants of uncertain significance in *POU1F1*.

Less than half of the information needed for proper splicing exists in the canonical splice site motifs (34). Short motifs of 6–10 base pairs in the primary transcript are bound by RNA-binding proteins that can enhance or suppress splicing at specific sites (35). The splice acceptor site used to produce POU1F1 α is predicted to be much weaker than the one used to produce the POU1F1 β isoform. This suggests that there must be splice enhancers and/or splice repressors that allow splicing to favor production of POU1F1 α under normal conditions. The catalog of splice disruptive variants shows that the splice disruptive sequences are in and around the POU1F1 β splice acceptor, suggesting that they are splice repressors (Fig. 2). Future studies are necessary to test this idea. The results of Gergics and Suzuki confirm and extend the findings of Takagi and colleagues, who reported CPHD in two children with

Figure 2

Novel coding variants that alter splicing of POU1F1. Two teams of investigators recently reported dominant negative variants in the coding region of POU1F1 β that alter splicing to favor production of the POU1F1 β isoform. These expand on the previous description of a dominant splice disruptive variant near the splice acceptor site in intron 1. Interpretation of high-throughput splicing assays suggest the location of splice regulatory information in intron 1 and the coding region of the POU1F1 β isoform (red hatched lines) that may suppress utilization of the splice acceptor that produces the α -isoform. Future studies will be necessary to clarify the function of these sequences and identify the RNA binding proteins.

a c.143-5>G variant in intron 1 that produces only the POU1F1 β -isoform (32).

Ironically, the GH gene (*GH1*) is one of the first genes shown to have intronic and exonic splice enhancers (36, 37) (Fig. 3). This five-exon gene has weak splice donor and acceptor sites in intervening sequence 2, a cryptic splice site in exon 3, and a weak donor site in intervening sequence 3. Thus, exon 3 is skipped at a low but significant rate, and most healthy individuals have detectable, circulating levels of the dominant negative 17.5 kDa form of GH as well as the bioactive 22 kDa GH1. The normal use of weak splice sites is assisted by the presence two splice enhancer sequences in exon 3 and another splice enhancer in intron 3. Individuals with

IGHD type II typically have genetic variants that disrupt one of the three splice enhancer sequences, resulting in an increase in exon skipping and more predominant production of the 17.5 kDa dominant negative form of GH1. The phenotype of individuals with *GH1* splicing defects is highly variable, and the severity of the disorder may relate to the degree to which splicing is affected, either due to the nature of the mutation or to variation in genes that affect splicing efficiency in general (38). Studies in mice suggest that the progression of IGHD type II to multiple hormone deficiencies is due to endoplasmic reticulum stress associated with poor secretion of the 17.5 kDa variant and collateral damage to surrounding cells as GH producing cells undergo apoptosis (39).

Figure 3

Regulation of GH1 alternate splicing and variants that cause IGHD type II. Normal splicing to produce bioactive 22 kDa GH1 is shown at the top of the gene diagram. Exonic and intronic splice enhancers (green hatched lines) are required to overcome the naturally weak splice donor and acceptor sequences in intron 1 and the weak donor site in intron 3. Variants that disrupt these splice enhancer sequences can cause exon skipping to produce the 17.5 kDa GH1 or utilization of a cryptic splice within exon 3 that produces a 20 kDa form of GH1. These variants cause variable, dominant IGHD type II (adapted from (44)).

Conclusion

These studies underscore the importance of evaluating splicing defects as a disease mechanism, and the need, in routine molecular diagnosis, for using adequate tools to investigate alternative splicing in *POU1F1* as well as in other genes. In practice, such findings should be considered by molecular biologists, clinical geneticists, and clinicians when addressing the molecular diagnosis of CPHD. Moreover, the identification of such mechanisms may pave the way to antisense oligonucleotide therapies as such an approach has shown promise for treating diseases caused by abnormal splicing, including IGHD (40, 41) or acromegaly (42).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this commentary.

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