The New Genomics: What Molecular Databases Can Tell Us About Human Population Variation and Endocrine Disease

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Major recent advances in genetics and genomics present unique opportunities for enhancing our understanding of human physiology and disease predisposition. Here I demonstrate how analysis of genomic information can provide new insights into endocrine systems, using the human growth hormone (GH) signaling pathway as an illustrative example. GH is essential for normal postnatal growth in children, and plays important roles in other biological processes throughout life. GH actions are mediated by the GH receptor, primarily via the JAK2 protein tyrosine kinase and the STAT5B transcription factor, and inactivating mutations in this pathway all lead to impaired somatic growth. Variation in GH signaling genes has been evaluated using DNA sequence data from the Exome Aggregation Consortium, a compendium of information from 60,000 individuals. Results reveal many potential missense and other alterations in the coding regions of GH1, GHR, JAK2, and STAT5B, with most changes being uncommon. The total number of different alleles per gene varied by ∼threefold, from 101 for GH1 to 338 for JAK2. Several known disease-linked mutations in GH1, GHR, and JAK2 were present but infrequent in the population; however, three amino acid changes in GHR were sufficiently prevalent (∼4% to 44% of chromosomes) to suggest that they are not disease causing. Collectively, these data provide new opportunities to understand how genetically driven variability in GH signaling and action may modify human physiology and disease. (Endocrinology 158: 2035–2042, 2017)
somatic growth during childhood through its induction of insulinlike growth factor-1 (IGF1), but additionally it is an important regulator of intermediary metabolism and tissue repair throughout life (5, 6). GH actions also have a dark side because they have been implicated in the development of certain cancers and in the pathophysiology associated with aging (7–9). Like related hormones and cytokines, GH acts after binding to a transmembrane receptor (10). Ligand binding to the growth hormone receptor (GHR) activates several signaling pathways (5, 10, 11). Among the most important is the just another kinase (JAK)–signal transducer and activator of transcription (STAT) cascade, in which induction of tyrosine kinase activity of JAK2 (5, 10, 11) causes phosphorylation of tyrosine residues on the intracellular part of the GHR (5, 11), leading to recruitment of several signaling molecules, including STATs. The seven members of the STAT family are inducible transcription factors that function as effectors of activated cytokine and growth factor receptors (12, 13). STAT5B in particular is a critical component of GH actions, being responsible for GH-stimulated production of IGF1 by promoting its gene transcription (14–16).

Decreased activity through the GH-IGF1 axis causes growth defects in humans that result in short stature (17–19). A variety of human genetic abnormalities have been shown to impair GH messenger RNA expression or protein biosynthesis [specifically the GH1 gene (20, 21)], and mutations in the GHR have been described that reduce its synthesis or biological actions (22). A few individuals also have been reported with growth deficiency and inactivating mutations in the IGF1 or IGF1 receptor genes (23, 24), or in STAT5B (25).

**Database Mining and Analysis**

DNA sequence information from the Exome Aggregation Consortium (ExAC) (26–30) comprises the primary data source for this mini-review (http://exac.broadinstitute.org/), and consists of results of exome sequencing from 60,706 different individuals. Other information was obtained from the following resources: human messenger RNAs and genes from the Ensemble Genome Browser, genome assembly GRCh38 (www.ensemble.org) (31); and human protein sequences from the Uniprot browser (http://www.uniprot.org/), and from the National Center for Biotechnology Information Consensus CDS Protein Set (https://www.ncbi.nlm.nih.gov/CCDS/). The mutations in human GH, GHR, JAK2, and STAT5B reported in Tables 2 and 3 are from Online Mendelian Inheritance in Man (https://www.omim.org/), which is a compendium of human genes and genetic conditions (and has an excellent online tutorial for those who wish to learn how to use it), and the Growth Genetics Consortium (http://www.growthgenetics.com/). Among other useful resources for studying genetic connections with endocrine and other diseases are the following: the GWAS catalog (https://www.ebi.ac.uk/gwas/) contains a manually curated collection of published genome-wide association studies, and is searchable by disease, gene name, author, single nucleotide polymorphism, or human chromosomal location. COSMIC (http://cancer.sanger.ac.uk/cosmic) consists of a compendium of cancer mutations, and can be searched by gene name, cancer type, cancer site, molecule, author, single nucleotide polymorphism, and other topics.

ExAC contains DNA sequencing results from the exons of genes of 60,706 people, and has been available publicly for less than a year (26). These individuals live in various parts of the world, and have different ethnic backgrounds. Their overall health status is unknown (26). Among the general conclusions from the initial published analysis of these 121,412 alleles is that variation within the coding regions of human genes is extensive (26). It appears however that most of the detected modifications are uncommon, with over half being seen in a single allele, and >99% being found in <1% of the ExAC population (26). Also, most the observed differences among people studied appear to consist of synonymous nucleotide changes or amino acid substitutions (26), with only the latter altering protein sequence. However, it seems likely that the extent of variation may differ significantly for individual human genes, and more importantly, that there may be substantial variability among components of pathways in which certain of the encoded proteins act. Hence, the analysis of the human GH signaling cascade presented here, which serves as an illustrative example of how such publicly available databases can be assessed to gain new insights into human endocrine physiology.

**Allelic Variation in GH Signaling Molecules in Humans**

Examination of the genes for GH signaling molecules in ExAC reveals a wide range of coding variation, with most changes in GH1, GHR, JAK2, and STAT5B being missense mutations (94% to 97%) (Table 1). Second most common were alterations in the protein reading frame, including addition of truncating stop codons (~2% to 4%) (Table 1). The total number of different alleles per gene varied by more than threefold, from 101 for GH1 to 338 for JAK2. When corrected for protein length, protein modifications also varied by a factor of 3, from 0.14 nonsynonymous changes/codon for STAT5B, to 0.47 for GH1 (Table 1). In terms of prevalence in the
study population, 98% of missense alleles were found in <0.1% of individuals, and 99.6% were detected in <1.0%. These results show that overall variation in GH signaling proteins is low in this study group, and is consistent with general conclusions from the initial analysis of ExAC data (26).

Splicing changes at exon-intron and intron-exon junctions, reading frame alterations caused by insertions or deletions of DNA, and nucleotide changes that lead to the addition of stop codons each can contribute to loss of protein expression. The number of alleles showing these changes was very low among GH signaling genes, comprising a handful of different instances that were rare in the study population (0.007% to 0.11% allelic frequency). Copy number variation, in which a gene is absent or is present in more than one copy per chromosome, was similarly infrequent, and ranged from no instances for GH1 and GHR, to two for STAT5B and 42 for JAK2.

Table 1. Human Population Variation in GH1, GHR, JAK2, and STAT5B

| Protein | No. of Codons | Missense and In-Frame Insertions-Deletions | Frame Shifts, Stop Codons, Splicing Site Changes | Loss of Start Codon | Loss of Stop Codon | Total No. of Different Changes | Variants per Codon | Total Variant Alleles in Population (%) |
|---------|---------------|-------------------------------------------|-----------------------------------------------|----------------------|---------------------|--------------------------------|-------------------|----------------------------------------|
| GH      | 217           | 96                                        | 4                                             | 1                    | 0                   | 0                              | 0.47              | 1.4                                    |
| GHR     | 638           | 229                                       | 8                                             | 6                    | 1                   | 0                              | 0.38              | 53.9                                   |
| JAK2    | 1132          | 327                                       | 6                                             | 5                    | 0                   | 0                              | 0.29              | 2.6                                    |
| STAT5B  | 787           | 104                                       | 4                                             | 0                    | 0                   | 0                              | 0.14              | 0.4                                    |

*Based on transcripts used in ExAC database. One predicted variant does not correspond with the amino acid sequence of GHR and is not included in the compiled data.

Two predicted alterations in mature GH, A39>V and V136>I, account for over half of all population changes among ExAC alleles [Fig. 1(a); Table 1]. Remarkably A39>V maps to site 1, one of two three-dimensional recognition domains that mediate binding of one molecule of GH to two molecules of the GHR (36) [site 1 comprises amino acids F36 to M40, F80 to Q94, and D195 to V206, and site 2 comprises amino acids E27 to R34 and D142 to E145 (36, 37)]. Experiments now may be performed to address whether the valine side chain at position 39 could alter hormone-receptor recognition, and thus influence GH-mediated receptor activation. There are other predicted amino acid substitutions in the GH molecule, such as those associated with growth defects as previously noted (Table 2), but these occur with allelic frequencies that seem too low (from 1 in 2500 to 1 in 120,000) to have an appreciable population impact on human physiology.

Population Variation in GH

GH1 resides on chromosome 17q23.3 in a cluster of five related genes that include two genes coding for placental lactogen (chorionic somatomammotropin), one encoding a placental lactogen analog, and the other encoding the GH gene variant, GH2 (32), which unlike GH1, is minimally expressed in the pituitary gland (32). The first characterized mutations in GH1 were gene deletions in individuals with familial GH deficiency (33), and multiple instances of gene and partial locus deletions have been described subsequently (34, 35). Later studies identified individuals with short stature, who had presumptive inactivating amino acid changes in GH1 exons, or who had frame shift mutations or stop codons that prevented full-length GH from being synthesized in pituitary somatotrophs (34, 35). Listed in Table 2 are eight such previously characterized mutations, and their prevalence in the ExAC population. Alterations at four of these sites are present in ExAC, but are rare, because only one substitution, F205>M, is detected as frequently as ~0.04% of the population (56 alleles), and the other three are found in <1 in 10,000 chromosomes (Table 2).

Variability in the GHR

The GHR was the first member of the cytokine receptor family to be characterized, and was among the first receptors to be shown to engage the JAK-STAT signaling cascade (5, 10, 11, 38). Many different mutations in the GHR gene have been associated with human growth deficiency syndromes (17, 22, 39). Even though the first subject to be characterized molecularly had a complex rearrangement within the GHR locus on chromosome 5p13-p12 (40), most mutations identified to date are splicing alterations, protein truncating stop codons, or amino acid substitutions (17, 22, 39) (Table 2). Of the 23 different disease-associated frame shifts, stop codons, and splicing mutations listed in Table 2, only four are found in the ExAC database, with all being present at very low frequencies (C56stop, one allele; R61stop, two alleles; R235stop, one allele; I293> K-frame shift, one allele). In addition, amino acid substitutions at the same locations as the frame shifts and stop codons in the GHR gene also are rare, being detected at allelic frequencies of <0.01% in the population (Table 2).

Of 22 different disease-associated amino acid substitution locations in the GHR, only eight are present in
the ExAC database (Table 2). Most changes that are connected to GHR dysfunction and growth defects map to the extracellular domain of the receptor (17, 22, 39), and five of these disease-linked alterations are very uncommon in the general population, being found in only one or three alleles of >121,000 surveyed (Table 2). However, two changes are present in ~0.1% of chromosomes (V162I and R229H, 166 and 149 alleles, respectively) and one in ~0.4% (R179C, 496 alleles). The high prevalence of these three substitutions suggests either that the modifications may not be disease causing or that potentially subtle differences in the ability of the GHR to bind GH may be polymorphic in the human population. Further, one presumptive amino acid change in the intracellular region of the GHR that has been associated with growth impairment, L544I, is actually the most common allele in ExAC, being found in 56% of all chromosomes sequenced (67,843 alleles) (Table 2). The other polymorphic allele accounts for the remaining 44% of chromosomes in the population [Fig. 1(b)]. Similarly, two other amino acid substitutions in the intracellular part of the GHR that were each first identified in an individual with a Laron-type syndrome of short stature and lack of response to GH treatment, C440T and P579T (41), are found in 4% of the population [Fig. 1(b)]. Thus, it seems likely that these latter modifications are also polymorphic variants. Of note, none appears to map to segments of the GHR that have been found previously to be critical for signal transduction (42–44). Experiments now may be designed to assess their effects on receptor function.

### Population Aspects of JAK2 and STAT5B

JAK2 is one of four members of a nonreceptor tyrosine kinase family that also includes JAK1, JAK3, and Tyk2 (45). All these molecules function as obligate protein kinases for multiple cytokine receptors (46, 47). Seven different amino acid substitution mutations in JAK2 have been described in individuals with blood dyscrasias, including polycythemia vera, thrombocytopenia, and myelofibrosis, and several classes of leukemia (48–52), but no abnormalities in JAK2 have been associated with growth disorders in humans. Of these disease-linked alterations, only V617F is detected in ExAC, and surprisingly is present in 0.07% of chromosomes (82 alleles) (Table 2), perhaps indicating that a predisposition to one of these precancerous diseases is prevalent among different human populations.

Overall, variant alleles are found in JAK2 in 2.6% of the population (Table 1), with three substitutions, G127D, L393A, and R1063H, accounting for half of the changes [Fig. 1(c)]. The biological significance of these three modifications is unknown. Although they are found in different domains of the protein [Fig. 1(c)], none map to segments of the molecule involved in

| Mutation | Population Variant | ExAC Prevalence |
|----------|--------------------|-----------------|
| GH1      |                    |                 |
| W20stop  | None               |                 |
| E58K     | None               |                 |
| C79S     | C79fs              | 1 Allele        |
| R103C    | R103H              | 1 Allele        |
| D138G    | None               |                 |
| G157fs   | G157D              | 13 Alleles      |
| I205M    | I205M              | 56 Alleles      |
| R209H    | None               |                 |
| GHR      |                    |                 |
| W4stop   | None               |                 |
| W34stop  | None               |                 |
| C56stop  | C56stop            | 1 Allele        |
| R61stop  | R61stop, R61G, R61L, R61Q | 2, 1, 10 Alleles |
| Q83stop  | Q83L               | 1 Allele        |
| W98stop  | None               |                 |
| C101stop | None               |                 |
| Y113stop | None               |                 |
| V143Lfs  | V143I              | 2 Alleles       |
| L159stop | None               |                 |
| W175stop | None               |                 |
| E198splice | None            |                 |
| V199-M206del | V199A, V199I | 6, 1 Alleles |
| E201stop | E201G              | 2 Alleles       |
| M206-M207ins | None        |                 |
| M207fs   | M207V              | 2 Alleles       |
| R235stop | R235stop           | 1 Allele        |
| E242stop | E242D, E242K       | 3, 7 Alleles    |
| I293Kfs  | I293Kfs            | 1 Allele        |
| I297Kfs  | None               |                 |
| V301fs   | V301I              | 1 Allele        |
| I328Pfs  | I328T              | 2 Alleles       |
| A442fs   | None               |                 |
| C56S     | None               |                 |
| S58L     | None               |                 |
| E60K     | E60K               | 1 Allele        |
| E62K     | E62K               | 3 Alleles       |
| W68R     | None               |                 |
| R89K     | None               |                 |
| Y104D    | None               |                 |
| C112S    | None               |                 |
| F118S    | None               |                 |
| Q148P    | None               |                 |
| V162I    | V162I, V162F       | 166, 17 Alleles |
| H168Q    | H168Q, H168P       | 1, 2 Alleles    |
| D170H    | None               |                 |
| I171T    | None               |                 |
| Q172P    | None               |                 |
| V173G    | None               |                 |
| R179C    | R179C, R179H       | 496, 36 Alleles |
| Y226C    | Y226F              | 1 Allele        |
| R229H, G | R229H, R229C, R229L | 159, 3 Alleles |
| S244I    | None               |                 |
| D262N    | None               |                 |
| L544I    | L544I              | 67,843 Alleles  |
signaling with either the GHR or other cytokine receptors (11, 53).

STAT5B, a 787-amino acid protein (54), is a critical transcription factor for GH-activated IGF1 gene expression (14–16), and mutations in human STAT5B phenotype GH and GHR deficiencies regarding growth deficits; however, most of the handful of individuals found to have inactivating STAT5B gene alterations also presented to medical attention with evidence of immune system defects (25). Compared with the other genes studied here, STAT5B is an outlier because its extent of variation in the population (0.4%, 0.14 changes per codon) was much lower than was detected for GH1, GHR, or JAK2 (Table 1). Additionally, no amino acid substitution or frame shift mutation was present in 0.08% of chromosomes (Table 3). Although speculative, perhaps the minimal level of variability in the STAT5B gene reflects its multifactorial role as a signal transducer for GH, prolactin, erythropoietin, and multiple other cytokines (13, 46, 54).

As noted, a small number of humans have been identified with STAT5B gene mutations (19, 25). In all cases, these individuals have been ascertained because of severe growth failure associated with evidence of immune deficiency (19, 25), and of these, two are single amino acid changes and five are frame shift mutations or stop codons (Table 3). Alterations at two of these sites are present in the ExAC database, with predicted amino acid substitutions at the Q368 frame shift location being present in ~0.1% of chromosomes in the population (132 alleles) (Table 3). The other mutations are either very rare or absent in ExAC (Table 3).
on variation in insulinlike growth factor signaling and action, and its roles in human physiology and disease, has recently been published (56).

The extensive variability captured by ExAC should be traceable to our ancestors, including extinct populations (57, 58). Modern humans contain marks in their DNA of past contacts with these populations, including both Denisovans and Neanderthals, and the legacy of these interactions still influences some traits and probably some disease susceptibilities (58). New hypotheses inspired by the data from ExAC and other genome sequencing projects could lead to novel insights about how the complex biology of GH actions has been shaped over millennia. Similar opportunities to develop new research questions and to define new physiologic paradigms exist for other areas of endocrinology, and should incentivize investigators to extract, critically evaluate, and interpret these data.

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### Table 3. Disease-Associated Mutations in JAK2 and STAT5B

| Mutation | Population Variant | ExAC Prevalence |
|----------|--------------------|-----------------|
| JAK2     |                    |                 |
| K539L    | None               | —               |
| K607N    | None               | —               |
| V617F    | V617F              | 82 Alleles      |
| R683G, K, S | None              | —               |
| STAT5B   |                    |                 |
| L142Rfs  | None               | —               |
| R152Stop | R152P, R152Q       | 1, 6 Alleles    |
| Q368Fs   | Q368K, Q368Pfs, Q368Rfs | 2, 91, 41 Alleles |
| N398fs   | None               | —               |
| E561Rfs  | None               | —               |
| A630P    | None               | —               |
| F646S    | None               | —               |

**Limitations and Strengths of Population-Based Genome Sequence Data in Understanding GH Actions in Humans**

As potentially expected with any large-scale DNA sequencing project, the ExAC database contains extensive material for novel biological insights, and both ambiguities and errors. From the perspective of GH signaling and actions, potential problems include the presence of at least one presumptive polymorphic coding variant that cannot be mapped to the GHR gene or protein. Other limitations of the data include the potential skewing of the study population. Although several different groups are represented, >60% of samples derive from Europeans, ~20% are East or South Asian, and only ~8% are either of Hispanic or African origin (26). Thus, the true rate and extent of variation among proteins in humans may not be established yet. In addition, there is a likely error rate associated with the many nucleotide changes that appear only once in 121,412 chromosomes evaluated.

Despite these limitations, these data provide a multitude of new opportunities to understand and reconsider what is normal GH physiology and to redefine the extent of its pathophysiology in humans. GH actions are critical for normal somatic growth in children, with growth rates functioning as a possible readout for dynamic interactions between genetic and environmental factors (55). These interactions, and the range of outcomes labeled normal, now may be reassessed in the context of many versions of GH and the GHR in the population. GH actions also have been postulated to be involved in aging (9) and in cancer pathogenesis (7, 8), and the hypothesis now may be considered that some variants of either GH or the GHR, or specific combinations with different types of JAK2 or STAT5B, may enhance disease susceptibility, whereas others may be protective. A similar perspective...
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