Identification of Two Novel Chromosome Regions Associated with Isolated Growth Hormone Deficiency

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ABSTRACT

The goal of this study was to identify novel candidate genes that may cause or predispose to growth hormone (GH) deficiency. DNA samples from 45 individuals with isolated GH deficiency were assessed using oligonucleotide microarray comparative genomic hybridization. Five individuals with previously unreported copy number variants were identified. Two of the five individuals were hemizygous for regions already known to cause GH deficiency (chromosomes 22q11.21 and 15q26.3). The remaining three individuals had copy number changes involving two novel chromosome regions. One individual had a homozygous deletion of a 2.2 Mb region of 13q33.1 that contains a single gene: integrin, beta-like 1 (ITGBL1). The remaining two individuals had duplications of 4.7 Mb on chromosome 20q13.13. This region includes eight genes not previously identified as copy number variants. These genes are ARFGEF2, CSE1L, DDX27, ZNFXI, C20orf199, SNORD12, KCNBL1, and PTGIS. Thus, further investigations into these potential candidate genes are necessary.

KEY WORDS

growth hormone, growth hormone deficiency, growth hormone genetics, gene identification, aCGH, chromosome copy number variation

INTRODUCTION

Growth is a fundamental aspect of childhood influenced by a multitude of factors, both genetic and environmental. One of the central causes of childhood growth failure is deficiency in the production and/or secretion of growth hormone (GH). However, to date, less than a dozen genes have been identified that impact this axis. Recently, Mullis¹ as well as Walenkamp and Wit² published comprehensive reviews of the genetics of GH deficiency.

We sought to identify novel genes associated with GH deficiency. The strategy was to identify genomic copy number changes in individuals who have isolated GH deficiency. These newly identified regions of copy number change will implicate specific candidate gene(s). These genes will then be targets for future experiments that attempt to identify mutations within the candidate genes in individuals with GH deficiency who do not have copy number changes.

METHODS

We used oligonucleotide microarray comparative genomic hybridization (aCGH) to assess DNA samples from 45 deidentified individuals enrolled in the GeNeSIS (Genetics and Neuroendocrinology of Short Stature International Study) research program sponsored by Eli Lilly and Company. GeNeSIS is a prospective, multi-center, multinational, observational study of children with growth disorders of various etiologies. As part of its modular structure, the DNA Analysis Sub-study has investigated DNA sequence alterations in genes associated with GH deficiency, including in relatives of affected participants. GeNeSIS is conducted in accordance with The Declaration of Helsinki and principles of Good Clinical Practice. Institutional review committee approval and written consent for data collection, electronic
processing and publication were obtained from subjects in accordance with national laws. Consent for participation in the DNA Analysis Sub-study was collected for all participating participants and relatives.

Enrollment criteria for the GeNeSIS study were a diagnosis of isolated GH deficiency or hypopituitarism. The following genes were sequenced prior to our acquisition of the samples: GH1, GHRHR, HESX1, LHX3, PROP1 and POU1F1. No mutations were identified, making these samples a rich source for the identification of novel genes that may play a role in GH deficiency and hypopituitarism.

Array Hybridization and Analysis

The arrays were constructed with 105,000 oligonucleotide probes assessing the entire genome. The hybridization was performed as described in the Agilent protocol. Comparative Genomic Hybridization (CGH) uses a two sample comparative method in which the test (or participant) sampled is assessed in comparison to a reference sample (Agilent Technologies, Santa Clara, CA). The two DNA samples are labeled with different fluorophores, then mixed together and allowed to competitively hybridize to the oligonucleotides on the array slide. The reference DNA samples were from Promega (Madison, WI) and consisted of pooled same sex DNA samples. The reference DNA sample was from the opposite sex as the test sample thus providing an internal copy number control with regard to the X and Y chromosomes. Each experiment was replicated using a dye swap.

Each array was scanned using the Agilent laser scanner. The scan data were extracted using Agilent Feature Extraction (version 8.1.1). Those data were then analyzed using the CGH Analytics 3.4.27 software. Data points were analyzed in continuous groups of 8 probes and log 2 ratios of sample DNA were compared to control DNA. Arrays were normalized to a median log 2 ratio of zero, except for the X and Y chromosomes. Features that were less than -1 or greater than +0.5 were identified by the grey bar (Figure 1). Breakpoints were determined to be between the ends of the array features on either side of the deletion breakpoint. Any copy number changes detected were checked against the Database of Genomic Variants (http://projects.tcag.ca/variation/) for their possible identification as a normal variant.

RESULTS

Using oligonucleotide microarray comparative genomic hybridization (aCGH), we analyzed the DNA of 45 individuals with isolated GH deficiency enrolled in the GeNeSIS research program sponsored by Eli Lilly and Company. Copy number changes in the 45 individuals that were identified as normal variants in the Database of Genomic Variants were eliminated from further consideration as pathogenic. We identified previously unreported copy number changes in 5 of the 45 samples analyzed (participants 40, 179, 210, 277 and 278). These are listed in Table I. The whole chromosome view of each copy number change is shown in Figure 1.

The deletion identified on 22q is known to be associated with velocardiofacial syndrome. The region identified on 15q contains the Insulin-Like Growth Factor-1 Receptor gene (IGF1R) known to be involved in growth disorders. Thus, only three of the five individuals had copy number changes in chromosome regions not known to be associated with GH deficiency. Two of these three had identical duplications of 4.7 Mb on chromosome 20q13. However, these two samples were from a child enrolled in the GeNeSIS program and the child’s mother. The fifth individual had a homozygous deletion on 13q. This has not been reported in previous studies. Thus, in total, two new chromosomal regions were identified which are likely to include novel genes involved in the GH/IGF-1 axis: 13q33.1 and 20q13.13.

All 5 participants have the diagnosis of “classic” GH deficiency, which was reported as “isolated” for participants 210, 40, 277 and 278. However, participant 210 was also reported as having micropenis. Participant 179 was reported to have short stature with some dysmorphic features, such as bifid nose and micropenis, suggesting a possible midline defect; this participant was also reported to have multifocal...
areas of cortical dysplasia and attention deficit hyperactivity disorder, for which he was receiving treatment with methylphenidate. For participant 210 measurements of thyroid function tests, follicle stimulating hormone and testosterone were within normal ranges at baseline. For participant 179 measurements of free thyroxine and cortisol were within normal ranges at baseline. No hormone measurements other than GH were available for participant 40 or participant 210. However, participant 40 was reported to have entered puberty spontaneously. Clinical data on these participants, with genomic copy number changes, is provided in Table 2.

TABLE 1
Location and type of copy number change and the genes in those regions

<table>
<thead>
<tr>
<th>ID #</th>
<th>Chr.</th>
<th>Duplication /deletion</th>
<th>Locus of copy number change</th>
<th>Genes in the region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>From To</td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>13q33.1</td>
<td>homozygous del</td>
<td>100,966,310-101,166,684-</td>
<td>ITGBLI</td>
</tr>
<tr>
<td>40</td>
<td>15q26.3</td>
<td>del</td>
<td>96,783,964-98,788,607-</td>
<td>IGF1R, et al.</td>
</tr>
<tr>
<td>277</td>
<td>20q13.13</td>
<td>dup</td>
<td>47,083,596-47,582,293-</td>
<td>CSEIL, STAUI,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>47,083,537-47,597,647</td>
<td>DDX27, ZNFX1,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>KCNB1</td>
</tr>
<tr>
<td>278</td>
<td>20q13.13</td>
<td>dup</td>
<td>47,083,537-47,553,878-</td>
<td>Same as above</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>47,098,987-47,582,234</td>
<td></td>
</tr>
<tr>
<td>179</td>
<td>22q11.21</td>
<td>del</td>
<td>17,080,599-19,829,971-</td>
<td>55 RefSeq Genes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17,269,389-19,886,024</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: The whole chromosome view of the oligonucleotide array comparative genomic hybridization microarray (aCGH) data for each of the chromosomes found to have copy number changes. The study ID number of the participant is shown above their data. The grey bars indicate the location of features that are significantly different from 0 on the log2 scale. This indicates the location of a deletion when there is a shift to less than 0 and duplication when there is a shift to greater than 0. Features exactly on the 0 vertical axis have a 1:1 red green color ratio between the test and reference DNA samples indicating 2 copies.
DISCUSSION

This study was able to identify five of 45 individuals with isolated GH deficiency and genomic copy number changes not previously identified as a normal genomic variant. Two of these individuals had genomic deletions that created regions of hemizygosity previously known to result in growth disorders (15q26.3; 22q11.21). The other 3 individuals identified 2 new genomic regions which potentially contain novel genes not previously associated with growth disorders (13q33.1; 20q13.13).

The two previously identified growth related regions involve the DiGeorge/Velo cardiofacial syndrome region of chromosome 22 and the Insulin Growth Factor 1 Receptor (IGF1R) gene on chromosome 15q. Participant 179 had a hemizygous region on chromosome 22q that was 2.8 Mb in size and included 55 RefSeq genes in the DiGeorge/Velo cardiofacial region. Ryan and coworkers described a large cohort of 558 individuals with 22q hemizygosity and found that 36% were below the 3rd percentile for height. Subsequently, Weinzimer and coworkers reported four individuals with 22q11.2 deletions and GH deficiency. Participant 40 was hemizygous for a region on 15q that includes the insulin-like growth factor 1 receptor (IGF1R) gene. Hemizygosity of this gene is a known cause of intrauterine growth retardation as well as postnatal growth failure.

The identification of these copy number changes involving known genes is not surprising. The techniques used to assess these DNA samples in the GeneSIS laboratory before they were examined in the current study involved the sequencing of a series of specific genes. However, large deletions are not detected using sequencing technology. Based on sequencing alone, homozygosity and hemizygosity can not be distinguished. Until the aCGH data were available, homozygosity was presumed.

The other three individuals identified two new genomic regions of interest. The anomaly identified on 13q33.1, was a 2.2 Mb homozygous deletion that included most of the integrin, beta-like 1 gene (ITGBL1). The homozygosity of this rare deletion suggests consanguinity. However, family history information is not available to confirm this suspicion. Little is known about the function of this gene product. Berg et al. cloned the cDNA from an osteoblast cell line. It was found to be expressed in many tissues, especially the aorta. The GNF Expression Atlas 2 Data from 133A and GNF1H Chips shows highest expression in adipocytes and the pituitary gland.

A second novel region was identified on 20q13.13. Two related individuals (ID#288 and 278) had identical duplications of this region. In this study, this is the only region identified in which the genomic copy number change was a duplication instead of a deletion. The duplicated region was almost 0.5 Mb in size and included all or part of 9 Reference Sequence (RefSeq) genes (listed below), none of which are obvious candidate genes for GH deficiency. A portion of one of those genes, STAU1, was hemizygous in a control sample, making it less likely to be a dosage-sensitive locus. The other genes in this region are: ARFGEF2, CSE1L, DDX27, ZNF1, C20orf199, AK055386, SNORD12, SNORD12B, SNORD12C, KCNBI and PTGIS. The potential roles of the proteins encoded by these genes are described below.

Mutations in the gene encoding the ADP-ribosylation factor guanine 2 (ARFGEF2) are the cause of autosomal recessive periventricular nodular heterotopia type 2, which to date has no associated endocrine abnormalities. The chromosome segregation I-like protein (CSE1L) is involved in nuclear transport. DEAD (Asp-Glu-Ala-Asp) box polypeptide 27 (DDX27) is a putative RNA helicase and postulated to be involved in cell growth and division. Zinc-finger, NFX-1 type containing 1 (ZNF1) is anticipated to be a zinc-finger DNA binding protein and, as such, involved in the regulation of transcription and thus important in cellular growth and differentiation. There is no information available regarding the potential role in disease or any of these three genes. C20orf199 and AK055386 are defined by cDNA sequences for which there are no functional inferences or information. Small nuclear RNAs, (SNORD12, SNORD12B and SNORD12C) are non-coding RNAs with no known function. Potassium voltage-gated channel (KCNBI) does not have a known function;
however, these types of voltage-gated ion channels are involved in diverse functions including insulin secretion and therefore could possibly be involved in the secretion of other secretory hormones like GH. Prostaglandin 12 synthase (PTGIS) belongs to the cytochrome P450 family and loss of function is implicated in myocardial infarction, stroke, and atherosclerosis. Although none of these genes are obvious candidates for the pathogenicity of GH deficiency, most of them have little, if any, biological information available. Thus, there is fertile territory for further investigation. It is also interesting to note that three recent genome wide association studies investigating loci that influence adult height did not find association with any of the novel loci identified in this study.

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### TABLE 2
Clinical data from GeNeSIS database on participants with genomic copy number changes

<table>
<thead>
<tr>
<th>ID #</th>
<th>Sex</th>
<th>Country</th>
<th>Gestation (weeks)</th>
<th>Birth Weight (gm)</th>
<th>Age at Dx (yr)</th>
<th>Peak GH (µg/L)</th>
<th>IGF-I SDS</th>
<th>Baseline height (cm)</th>
<th>Baseline height SDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>210</td>
<td>M</td>
<td>Spain</td>
<td>36</td>
<td>3000</td>
<td>2.6</td>
<td>0.4</td>
<td>NA</td>
<td>68.2</td>
<td>-7.67</td>
</tr>
<tr>
<td>40</td>
<td>M</td>
<td>Lithuania</td>
<td>32</td>
<td>1700</td>
<td>6.3</td>
<td>6.9</td>
<td>NA</td>
<td>87.0</td>
<td>-3.93</td>
</tr>
<tr>
<td>277</td>
<td>M</td>
<td>Italy</td>
<td>42</td>
<td>1950</td>
<td>2.0</td>
<td>5.4</td>
<td>-0.09</td>
<td>77.0</td>
<td>-3.07</td>
</tr>
<tr>
<td>278</td>
<td>F</td>
<td>Italy</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>145</td>
<td>NA</td>
</tr>
<tr>
<td>179</td>
<td>M</td>
<td>Thailand</td>
<td>40</td>
<td>2850</td>
<td>10.6</td>
<td>5.8*</td>
<td>-0.37</td>
<td>125.3</td>
<td>-2.75</td>
</tr>
</tbody>
</table>

NA = not available; *sex steroid-primed. Participant 278 is the mother of participant 277.

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