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SHORT REPORT

Insulin-Like Growth Factor Binding Protein, Acid-Labile Subunit Serum Level During the Acute and Convalescent Stage of SARS-CoV-2 Infection Depicted in a Longitudinal Study of 72 Patients During the First Wave of Pandemic

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Purpose: Recent discoveries have pointed to the role of IGFALS immunological response to viral challenges, potentially leading to the emergence of a cytokine storm. Here, we investigate if serum IGFALS during the acute response to SARS-CoV-2 will not be likely to accompany immune response during acute phase and convalescence.

Patients and Methods: We recruited patients hospitalized with PCR-confirmed SARS-CoV-2 infection in 2020 and have blood collected after securing consent (t_{adm}), at 48 hours (t_{48hrs}), 7 days (t_{7d}), and after discharge from hospital (t_{long}). IGFALS and IGF-1 in serum were measured to assess dynamics of the illness and compared against non-specific inflammatory (C-reactive protein, IL-6) markers. Serum titers of IgG against proteins S and N assessed specific viral responses. Serum HMGB-1 was measured to assess level of necrosis. Demographic and clinical data were collected using electronic health records (EHR). Survival was determined at six months from admission.

Results: No difference between serum IGFALS and IGF-1 levels was seen across the studied time points. $IGFALS_{adm}$ showed significant positive correlations with $IGFALS_{48hrs}$ (r^2=0.18; p<0.001), $IGFALS_{7d}$ (r^2=0.19; p=0.004), and $IGFALS_{long}$ (r^2 = 0.23; p=0.045). IGFALS correlated negatively with IGF-1 but positively with growth hormone. $IGFALS_{adm}$ showed significant inverse correlations with serum levels of $HMGB1_{adm}$ (r^2=0.26; p = <0.001) and t_{48hr} (r^2 = 0.27; p<0.001). A significant correlation in the case of $IGFALS_{48hrs}$ and CRP_{48hrs} (r^2 = 0.09, p = 0.021), $IGFALS_{long}$ and CRP_{long} (r^2 = 0.271, p=0.039) was seen. Similar correlations were seen at 48 hours of sampling time for IL-6 (r^2 = 0.14, p=0.006). In terms of specific antiviral response, we observed that serum $IGFALS_{adm}$ demonstrated correlation levels of serum IgG_{adm} (r^2 = 0.09, p=0.024). A positive correlation was found between length of stay in hospital or ICU and serum $IGFALS_{48hrs}$.

Conclusion: Though IGFALS serum levels did not change significantly during SARS-CoV-2 infection, we observed correlations with markers of tissue destruction, C-reactive protein, IL-6, and length of hospital stay.

Keywords: IGFALS, COVID-19, SARS-CoV-2, cytokine storm, IL-6, longitudinal study, infection

Introduction

Insulin-like growth factor binding protein, acid-labile subunit (IGFALS), forms complexes with insulin growth factor (IGF) and insulin growth factor binding proteins critical for glucose metabolism and growth hormone (GH) function.[1,2] However, more recent discoveries suggested the role of IGFALS in viral protection.[3] Overexpression of IGFALS

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potentiates interferon (IFNγ) production in cells exposed to viral particles.[3] IGFALS co-immunoprecipitates with TRAF and IRAK, which is critical in regulating the production of IL-6, T cell activation, and recovery from acute inflammation.[4] Furthermore, IGFALS can also interact with tumor growth factor β, integrins, and nuclear class II nuclear hormone receptors.[3,5] All of these components determine the natural trajectory of SARS-CoV-2 infection, where a significant number of patients suffer from overactivation of the immune system, leading to increased morbidity and mortality.[6] Interestingly, one study demonstrated a link between serum IGFALS and adverse outcomes in COVID-19 infection but the clinical data regarding studied population are very sparse.[7]

Most of what we know about the biologically important roles of IGFALS is linked to its mutations.[8–11] IGFAL's genetic deficit is related to delayed puberty, growth retardation, and some features of Down syndrome.[12,13] To date, no study has demonstrated any obvious immunological abnormalities associated with IGFALS deficits in individuals affected by mutations but IGFALS may be critical in dynamic immunological response as shown by interaction with IRAK6.[4]

Here, we compare the time course of IGFALS to markers of viral burden (specific immunoglobulins against S&N protein, tissue destruction markers (HMGB1), and non-specific immunological response (interleukin 6 (IL-6), C-reactive protein (CRP)). We hypothesize that SARS-CoV-2 will accompany an intense immune response paralleling immunological activation secondary tissue destruction markers, resulting in abnormalities and signatures in blood.[6]

Materials and Methods

IRB Approval

The Institutional Review Board of the Hospital of the University of Pennsylvania approved the study (IRB#813913).

Accrual of Study Subjects

We recruited patients hospitalized with PCR-confirmed SARS-CoV-2 infection in 2020. Study cohort of patients represent sample of convenience. All patients admitted to the hospital, or their surrogates, were screened for consent by research staff during period of 4.2020 and 9.2020. If consent could not be secured within first 24 hours after hospitalization, patient's consent was not pursued any further. If the consent could be secured patients was approach by study personnel. After securing consent, enrollment progressed immediately.

All participants were informed about the purpose of the study, in accordance with the Declaration of Helsinki.

Demographic and clinical data were collected using electronic health records (EHR). The clinical condition was assessed with the Acute Physiology and Chronic Health Evaluation II (APACHE II) Score calculated within 1 hour ($APACHE_{1hr}$) and 24 hours after admission ($APACHE_{24hr}$). Charlson's Comorbidity Index (CCI) was used to assess the burden of pre-existing illnesses. The application of remdesivir, convalescent plasma, and steroids was extracted from EHR. Survival was determined at six months from admission. Characteristics of the enrolled individuals (n = 72) are described in Table 1.

Table 1 Demographic and Clinical Characteristics of Studied Cohort

Demographics	
Age [X ± SD]	62.13±17.00
Over 60 (%)	63.90%
BMI [X ± SD]	31.06±7.33
Gender	
Male (%)	62.50%
Female (%)	37.50%
Not Reported (%)	0%

(*Continued*)

Table 1 (Continued).

Race	
Caucasian/Hispanic Latino [%]	23.60%
Black [%]	65.30%
Other/Asian/unknown [%]	11.20%
Admitted to the ICU [%]	63.90%
Intubated [%]	43.10%
Extra corporeal membrane oxygenation [%]	12.50%
Length of stay in the Hospital [X ± SD]	23.49±29.72
Length of stay in the ICU [X ± SD]	15.5±28.31
APACHE SCORE 1 hR [X ± SD]	12.76±7.85
APAHE SCORE 24 hR [X ± SD]	12.4±7.04
Pre-existing conditions	
Myocardial Infarction [%]	5.60%
Congestive Heart Failure [%]	18.10%
Peripheral Vascular Disease [%]	9.70%
Cerebrovascular Stroke/Transient Ischemic Attacks [%]	15.30%
Dementia [%]	5.60%
Chronic obstructive pulmonary disease [%]	19.40%
Connective Tissue Disease [%]	4.20%
Peptic Ulcer Disease [%]	2.80%
Liver Disease [%]	1.40%
Diabetes Mellitus [%]	41.70%
Hemiplegia [%]	5.60%
Chronic Kidney Disease [%]	30.60%
End Stage Renal Disease [%]	2.80%
Solid Tumor [%]	8.30%
Leukemia [%]	1.40%
Lymphoma [%]	0%
Acquired immunodeficiency syndrome [%]	1.40%
Smoking status	
Smoker [%]	9.43%
Former smoker [%]	32.08%
Non-smoker [%]	58.49%
Outcome at 28 days	
LOS ICU (Mean ± SD) [Days]	15.5 ± 28.31
LOS ICU Hospital (Mean ± SD) [Days]	23.7 ± 30.04
Discharged Home / Deceased [%]	82% / 18%
Outcome at 6 months	
Discharged Home / Deceased [%]	72% / 28%

Study Procedure and Sample Collection

The first blood samples were collected after securing consent (t_{adm}), with subsequent at 48 hours (t_{48hrs}), 7 days (t_{7d}), if the patient was in the hospital. Finally, we obtained one long-term sample (t_{long}) between 3–6 months since admission. Blood was collected in BD Vacutainer tubes and centrifuged at 2,000×g for 10 minutes at 4°C to separate serum. The serum was subsequently aliquoted and stored at −80°C.

Serum Testing

IGFALS and IGF-1 [ng/mL] in serum were measured using a commercially available kit (Biolegend, San Diego, CA) at 1:4 and 1:50 dilution respectively. Inflammatory (C-reactive protein, IL-6) markers [pg/mL] were measured using a multiplex kit (ThermoFisher, Waltham, MA, USA) on a MagPix machine (Luminex; Austin, TX, USA) at 1:4 dilution. Serum titers of IgG against proteins S and N were measured using a commercially available kit (RayBiotech, Stanford, CA, USA). Serum HMGB-1 [pg/mL] was measured using (Aviva Systems Biology; San Diego, CA, USA) at 1:4 dilution. Growth hormone [pg/mL] was measured with immuno-enzymatic assay (Sino Biological Inc; Wayne, PA, USA) at 1:1 dilution. All samples were inactivated with (5%) Triton X-100 (ChemCruz, Dallas, TX, USA). All serum dilutions were standardized for optimal sample curve.

Statistical Analysis

Serum IGFALS was our primary variable. CRP, HMGB-1, serum level of S protein, titer of antibodies against S&N immunoglobulins were considered covariables.

The Shapiro–Wilk *W*-test and distribution plots were used to test the normality of distribution variables. Parametric variables will be expressed as mean ± SD and compared using t-Student. For non-parametric variables, median (M_e) and interquartile ranges (IR) will be shown, and the U-Mann–Whitney statistic will be employed to compare such variables. The data will be analyzed as dependent, paired samples. A double-sided p-value less than 0.05 will be considered statistically significant for all tests. Statistical analyses will be performed with the Statistica 11.0 (StatSoft Inc., Tulsa, OK).

Results

Characteristics of the Studied Cohort and Effect at Baseline Serum IGFALS Levels

Table 1 presents the demographic and clinical characteristics of the studied individuals, as well as the clinical outcome at 28 days and 6 months. $IGFALS_{adm}$ did not correlate with sex, BMI, CCI, age, or dichotomized age groups (over 60 years old).

Evolution of Serum IGFALS Level During SARS-CoV-2 Infection and Convalescence

No difference between IGFALS levels was seen across studied samples and time points (Figure 1A). $IGFALS_{adm}$ showed significant positive correlations with $IGFALS_{48hrs}$ (r^2 = 0.18; $p < 0.001$), $IGFALS_{7d}$ (r^2 = 0.19; p=0.004), and $IGFALS_{long}$ (r^2 = 0.23; p = 0.045).

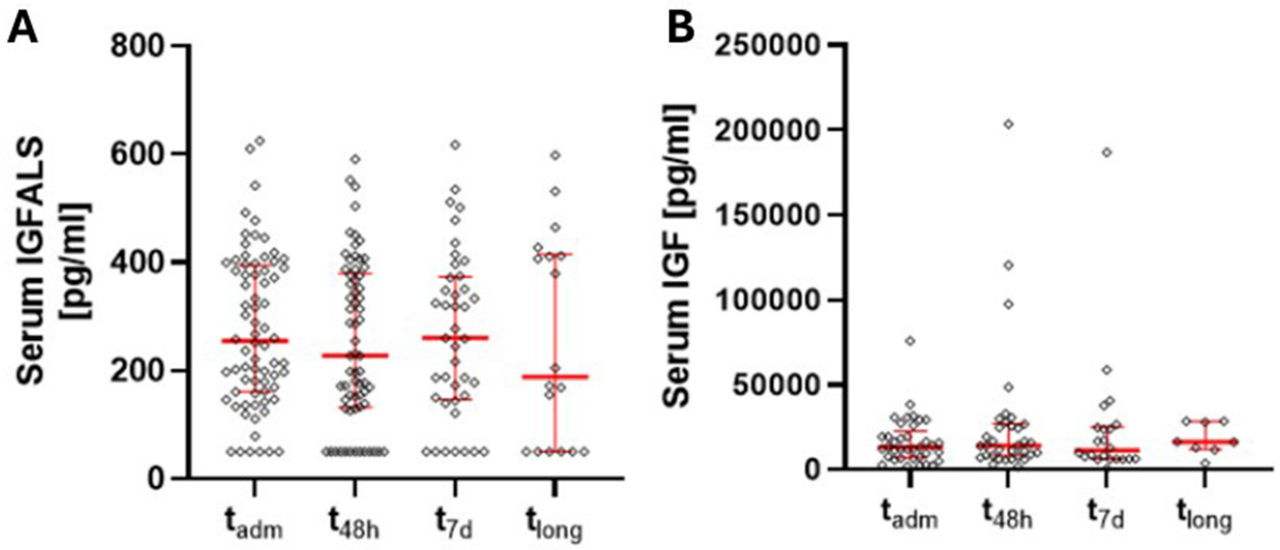

Figure 1 Variability in IGFALS (**A**) and IGF-1 (**B**) serum levels varied little during evolution of COVID-19 infection.

Serum IGF-1 levels were not altered over the trajectory of SARS-CoV-2 infection (Figure 1B). $IGFALS_{adm}$ exhibited a moderate negative correlation with $IGF\text{-}1_{adm}$ (r^2 = 0.14; p = 0.026). $IGFALS_{adm}$ also showed a significant positive correlation with serum GH_{adm} (r^2= 0.14, p = 0.002) as well as later points, including 48 hours (r^2 = 0.22; p = <0.001), 7 days (r^2 = 0.27; p = 0.002), and long term follow up (r^2 = 0.52; p = 0.008).

$IGFALS_{adm}$ showed significant inverse correlations with serum levels of $HMGB1_{adm}$ (r^2 = 0.26; p = <0.001) and t_{48hr} (r^2 = 0.27; p < 0.001). Also, 48-hour serum IGFALS correlated with HMGB1 at the same time (r^2 = 0.13, p = 0.024).

In terms of non-specific inflammation, we observed a significant correlation in the case of $IGFALS_{48hrs}$ and CRP_{48hrs} (r^2 = 0.09, p = 0.021), $IGFALS_{long}$ and CRP_{long} (r^2 = 0.271, p=0.039). Similar correlations were seen at 48 hours of sampling time for IL-6 (r^2 = 0.14, p=0.006). In terms of specific antiviral response, we observed that serum $IGFALS_{adm}$ demonstrated correlation levels of $sIgG_{adm}$ (r^2 = 0.09, p = 0.024). Serum IGFALS levels did not vary between patients who received therapeutic agents, including remdesivir, hydroxychloroquine, corticosteroids, or convalescent plasma, and those who did not.

IGFALS and Clinical Outcomes

A positive correlation was found between length of stay and serum $IGFALS_{48hrs}$ (r^2 = 0.06; p = 0.047) and t_{7d} (r^2 = 0.1; p = 0.04). $IGFALS_{adm}$ demonstrated a significant correlation with the Intensive Care Unit length of stay (r^2 = 0.05, p = 0.044). No important or strong correlation between APACHE at 1 hour and APACHE at 24 hours at the corresponding sampling time of IGFALS was seen. IGFALS levels demonstrated no significant association with mortality at one month or six months. Similarly, there were no differences in IGFALS levels between patients requiring ICU admission and those who did not or among those who underwent interventions such as mechanical ventilation or extracorporeal membrane oxygenation (ECMO).

Discussion

We observed that IGFALS serum levels did not change significantly during the course of SARS-CoV-2 infection. However, IGFALS levels are correlated with markers of tissue destruction, C-reactive protein, IL-6, and length of hospital stay. These findings suggest that IGFALS may not play a dynamic role in modulating the immune response during SARS-CoV-2 infection, as prior researchers suggested it.[1,2,4,7] Prior study suggested that IGFALS is negative prognostic for patients with ongoing infection but study had very limited clinical data set to support this statement.[7] Small sample study, lack of data when samples were collected and "speculative" finding in the words of authors of the manuscript may indicate accidental finding.[7] Alternatively, serum levels might not accurately reflect intracellular changes in the activation of IGFALS.[3] It is also unknown if serum IGFALS needs to undergo similar conformational changes as seen for its metabolic function.[2,9,13] Although there have been proposals suggesting IGFALS as an influencer of immunological responses, the supporting data remain insufficient.[1,3] Notably, the publication describing the role of IGFALS in the TRAF6 and IRAK signaling pathways requires replication.[3,4] Furthermore, while they study focused on the intracellular functions of IGFALS, our investigation measured its serum levels. Similarly, the data suggesting a connection between IGFALS and interferon-gamma are limited to in vitro studies and are not yet robust.[3] It is worth noting that IGFALS plays a critical role in carbohydrate metabolism, which is often disrupted during acute illness. Alternatively, IGFALS may be a constitutively secreted molecule influenced by factors not affected by acute viral infection. It is also unclear how the serum levels are affected by differential expression of the gene and its polymorphism.[1,2,8,11]

Although we did not observe significant changes in IGFALS serum levels, we cannot exclude its potential involvement in metabolic disturbances during acute infection despite unchanged levels over time.[1,2] Specifically, IGFALS may form non-functional complexes with other molecules required for optimal insulin action.[2,13] Post-translational modifications could induce conformational changes, impairing carbohydrate metabolism under certain conditions. Gene-controlling IGFALS transcriptome is also reported to be metabolically regulated with differential responses.[14] It is likely that the relationship between IGFALS and length of stay results from alterations in carbohydrate metabolism typical for critical illness. However, such finding clinical relationships will require another study.

Our study has several limitations. It was conducted on a relatively small cohort of patients. However, the sample size exceeds that of a typical pilot study, and the statistical features of the studied group provide sufficient statistical power to

support our conclusions. This lends relative confidence in discounting the hypothesis that IGFALS exhibits dynamic changes during COVID-19 infection.[3,4] There remains a risk of recruitment and drop-out biases, as patients were enrolled through a larger study conducted at an academic center, and their participation required engagement with the project throughout the study. Future validation studies should aim for similar recruitment in alternative settings to mitigate this limitation. Additionally, we did not investigate the potential effects of IGFALS complex formation on immunological reactivity during measurements.[13] However, these alterations are theoretical and are beyond the scope of the current study. Finally, the IGFALS gene demonstrates remarkable heterogeneity and the ability to mutate with differential effects on its function. However, our longitudinal design accounts for this particular variability and mutations are rare, with clearly apparent phenotypes associated with them.[1,8–11] Also, the expression of the gene is very variable but relatively stable in an individual.[14] Likely, this manifests in correlations we saw between IGFALS levels across different time points.

Our study also has several unique advantages. The study cohort is representative of the general population, ensuring broader applicability of the findings. All subjects were recruited during the initial wave of COVID-19, which eliminates the potential confounding effects of immunological memory from prior exposures. Additionally, we employed robust and reliable assays to measure IGFALS levels, enhancing the accuracy of our findings. The longitudinal design of the study further strengthens the analysis by allowing for dynamic observations over time. We found that IGFALS correlates baseline levels and growth hormone levels, as demonstrated in prior work, which validated our findings.[2,10,13]

In summary, our study demonstrated that serum IGFALS correlates with markers of tissue destruction and non-specific and specific immune responses at 48 hours after admission. This likely represents a non-specific response during maximal intensity of the viral immune system response when tissue destruction, concomitant immunological response, and buildup of specific immunoglobulins potentiate at the same time.[6]

Conclusion

In conclusion, our study provides valuable insights into the role of IGFALS in the context of SARS-CoV-2 infection. While serum IGFALS levels did not exhibit significant temporal changes during the course of infection, they demonstrated notable correlations with markers of tissue destruction, systemic inflammation, and specific immune responses. These findings suggest that IGFALS may function as a non-specific marker of immune system activation and tissue damage during acute viral infection, rather than playing a dynamic regulatory role as previously hypothesized. Although prior studies have proposed potential immunological functions for IGFALS, the current data do not support its involvement in modulating immune responses during COVID-19 infection. The observed correlations with growth hormone and markers of inflammation underscore the complexity of IGFALS's interactions in critical illness, potentially linked to metabolic disturbances and tissue injury. However, the lack of significant changes in serum IGFALS levels over time calls into question its utility as a dynamic biomarker for immune modulation during viral infections. Further investigations with larger cohorts, more detailed molecular analyses, and exploration of IGFALS's intracellular roles are warranted to clarify its precise functions in the context of SARS-CoV-2 and other viral diseases. Despite the limitations of the study, our findings contribute to the growing body of literature on IGFALS, providing a foundation for future research into its potential involvement in acute viral infections and critical illness.

Abbreviations

IGFALS, Insulin-like growth factor binding protein, acid-labile subunit; IGF-1, Insulin-like growth factor 1; PCR, Polymerase Chain Reaction; CRP, C-reactive protein; IL-6, Interleukin 6; HMGB-1, High-mobility group box protein 1; sIgG, Serum Immunoglobulin G; HER, Electronic Health Records; APACHE II, Acute Physiology and Chronic Health Evaluation II; CCI, Charlson's Comorbidity Index; ICU, Intensive Care Unit; ECMO, Extracorporeal Membrane Oxygenation; TRAF, Tumor necrosis factor receptor-associated factor; IRAK, Interleukin-1 receptor-associated kinase; IFNγ, Interferon gamma; BMI, Body Mass Index; LOS, Length of Stay; GH, Growth hormone.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Domené HM, Hwa V, Argente J, et al. Human acid-labile subunit deficiency: clinical, endocrine and metabolic consequences. *Horm Res*. 2009;72(3):129–141. doi:10.1159/000232486
2. Baxter RC. Signaling pathways of the insulin-like growth factor binding proteins. *Endocrine Rev*. 2023;44(5):753–778. doi:10.1210/endrev/bnad008
3. Xu G, Deng F, Zuo Q, et al. Virus-inducible IGFALS facilitates innate immune responses by mediating IRAK1 and TRAF6 activation. *Cell Mol Immunol*. 2021;18(6):1587–1589. doi:10.1038/s41423-021-00649-0
4. King CG, Kobayashi T, Cejas PJ, et al. TRAF6 is a T cell–intrinsic negative regulator required for the maintenance of immune homeostasis. *Nature Med*. 2006;12(9):1088–1092. doi:10.1038/nm1449
5. Xu L, Xiong L, Chen Y, et al. IGFALS suppresses hepatocellular carcinoma progression by stabilizing PPAR-γ. *Int Immunopharmacol*. 2024;143:113414. doi:10.1016/j.intimp.2024.113414
6. Vardhana SA, Wolchok JD. The many faces of the anti-COVID immune response. *J Exp Med*. 2020;217(6). doi:10.1084/jem.20200678
7. Kimura Y, Nakai Y, Shin J, et al. Identification of serum prognostic biomarkers of severe COVID-19 using a quantitative proteomic approach. *Sci Rep*. 2021;11(1):20638. doi:10.1038/s41598-021-98253-9
8. Heath KE, Argente J, Barrios V, et al. Primary acid-labile subunit deficiency due to recessive IGFALS mutations results in postnatal growth deficit associated with low circulating Insulin Growth Factor (IGF)-I, IGF binding protein-3 levels, and hyperinsulinemia. *J Clin Endocrinol Metab*. 2008;93(5):1616–1624. doi:10.1210/jc.2007-2678
9. Poyrazoğlu Ş, Hwa V, Baş F, et al. A novel homozygous mutation of the acid-labile subunit *(IGFALS)* gene in a male adolescent. *J Clin Res Pediatr Endocrinol*. 2019;11(4):432–438. doi:10.4274/jcrpe.galenos.2019.2018.0301
10. Işık E, Haliloglu B, van Doorn J, et al. Clinical and biochemical characteristics and bone mineral density of homozygous, compound heterozygous and heterozygous carriers of three novel IGFALS mutations. *Eur J Endocrinol*. 2017;176(6):657–667. doi:10.1530/eje-16-0999
11. Domené HM, Scaglia PA, Lteif A, et al. Phenotypic effects of null and haploinsufficiency of acid-labile subunit in a family with two novel IGFALS gene mutations. *J Clin Endocrinol Metab*. 2007;92(11):4444–4450. doi:10.1210/jc.2007-1152
12. Ueki I, Ooi GT, Tremblay ML, et al. Inactivation of the acid labile subunit gene in mice results in mild retardation of postnatal growth despite profound disruptions in the circulating insulin-like growth factor system. *Proc Natl Acad Sci USA*. 2000;97(12):6868–6873. doi:10.1073/pnas.120172697
13. Sneppen SB, Lange M, Pedersen LM, et al. Total and free insulin-like growth factor I, insulin-like growth factor binding protein 3 and acid-labile subunit reflect clinical activity in acromegaly. *Growth Horm IGF Res*. 2001;11(6):384–391. doi:10.1054/ghir.2001.0254
14. Högler W, Martin DD, Crabtree N, et al. IGFALS gene dosage effects on serum IGF-I and glucose metabolism, body composition, bone growth in length and width, and the pharmacokinetics of recombinant human IGF-I administration. *J Clin Endocrinol Metab*. 2014;99(4):E703–E712. doi:10.1210/jc.2013-3718