IGF-I Deficiency in the Era of Genomics: Lessons Learned

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In this issue, Professor Joachim Woelfle and his colleagues report the results of whole exome sequencing in 22 patients from 17 families with severe primary IGF-I deficiency. These patients were recruited as part of a national effort termed the German Growth Hormone Insensitivity study group. The patients met a standard definition of severe primary IGF-I deficiency with heights below -3 SDS, IGF-I concentrations below the 2.5th percentile, and normal growth hormone secretion. While they performed whole exome sequencing, the current manuscript only focuses on the analysis of a list of 26 candidate genes, a number of which have no current known relation to the growth hormone/IGF-I axis. They found a definitive etiology for the patients' growth disorders in 3 out of 17 families, a diagnostic yield of 18%. One of these was a homozygous nonsense mutation in the growth hormone receptor, which is clearly an expected outcome. The other 2 were de novo mutations in genes not known to be directly associated with the growth hormone/IGF-I axis, *HRAS* and *FAM111A*, which cause Costello syndrome and Kenny-Caffey syndrome, respectively. In addition, they identified another 9 rare or novel nonsynonymous variants, all of which were inherited from an unaffected parent except for 1 missense variant in *IGF2*

which was inherited from a father who was at the 3rd percentile for height.

This manuscript raises a number of interesting questions and observations as our field rapidly moves into the genomic era. First, what is the role of genomic testing in patients with severe growth disorders, and what type of results can we expect from exome sequencing in these patients? Second, why was the diagnostic yield in this study only 18%? Third, what does it mean when we do large-scale sequencing and find rare or novel genetic variants in a candidate gene?

It is well known amongst pediatric endocrinologists that the standard biochemical and hormonal workup for patients presenting with short stature has a low diagnostic yield [1]. The vast majority of patients are left with the nondiagnosis of idiopathic short stature. Do these patients have genetic etiologies for their short stature, and will wide-scale genomic testing lead to an increased diagnostic yield in these patients? The truth is that no one really knows. To date, there have been no large-scale exome sequencing studies published in patients with idiopathic short stature. However, there is some evidence to suggest that exome sequencing will identify a monogenic cause in patients with more severe short stature or those with syn-

dromic features. Our group, for example, previously found a genetic etiology in 5 out of 14 patients with severe syndromic short stature who underwent exome sequencing [2]. The current study focused on patients with severe primary IGF-I deficiency, a subgroup of growth disorders which one would posit to have a higher likelihood of monogenic causes, especially since human mutations have been found in multiple genes in the growth hormone/IGF-I axis [3]. Notably though, 2 of the 3 genetic causes identified in this article were in genes outside of this axis. The authors present some potential links between *HRAS* and IGF-I, but we currently do not understand why this patient or the one with the *FAM111A* mutation presented with apparent primary IGF-I deficiency. As the authors note, Storr et al. [4] previously performed a similar study investigating 69 patients with growth hormone insensitivity. They found that 19% of the families had mutations in the growth hormone/IGF-I axis (the majority of which were mutations in the growth hormone receptor), while another 2 patients had homozygous mutations in *OBSL1*, a gene responsible for 3-M syndrome, and 2 others had genetically proven Silver-Russell syndrome, giving a total yield of 25%. The higher yield seen in the Storr article is almost certainly due to patient demographics, with a much higher percentage of patients coming from consanguineous families and an increase in the number of referrals for suspected Laron syndrome.

Looking at these studies together, it is clear though that a significant number of patients who present with biochemical findings suggestive of “primary IGF-I deficiency” will end up having genetic defects outside of the canonical growth hormone/IGF-I axis. We do not know whether the low IGF-I levels are truly part of the causal pathway in these patients or whether they are chance associations. Recently, a group of growth researchers led by Jeffrey Baron have suggested a new conceptual framework focused on the biology of the growth plate as opposed to the growth hormone/IGF-I axis when evaluating growth disorders [5]. Another clear lesson is that as we perform more exome sequencing, we will identify patients with clinical presentations that differ from the classical descriptions of a known syndrome. For example, the patient reported in Woelfle’s article with Kenny-Caffey syndrome lacked the key clinical feature of hypocalcemia. These patients will be difficult to diagnose on clinical grounds alone.

Given the severity of the patients included in the current study, why was the diagnostic yield only 18%? First and foremost, this study only examined a very limited number of candidate genes which can cause growth dis-

orders. There are hundreds of genes listed in OMIM which are known to be associated with short stature. As noted earlier, the fact that these patients presented with primary IGF-I deficiency does not exclude the possibility of a genetic etiology outside of the classical growth hormone/IGF-I axis. Once a more complete analysis of the exome data is concluded, it is possible that the diagnostic yield will increase. Second, as the authors correctly note, a routine exome analysis does not detect genomic deletions or duplications or methylation abnormalities. Numerous groups have shown that genomic copy number variants (deletions and duplications) underlie a significant portion of patients with severe growth disorders [6, 7], and the article by Storr et al. [4] suggests that Silver-Russell syndrome may be a cause for a number of these patient’s short stature. Silver-Russell syndrome is most commonly caused by methylation abnormalities which will not be identified by exome sequencing. Even with these caveats, the diagnostic yield of genetic testing in these patients will never be 100% as some of them may have nongenetic causes for growth failure or others may have highly polygenic etiologies which will be impossible to definitively identify.

Finally, what does it mean when exome sequencing is done and a novel variant is identified in a candidate gene? The answer is quite complex as it depends on the type of variant found, the inheritance pattern in the family, the similarity of the phenotype to that expected to be caused by defects in that genes, the penetrance of the variant, and many other factors. A complete exploration of this topic is beyond the scope of this editorial, but interested readers are directed to the variant classification criteria established by the American College of Medical Genetics [8]. When these criteria were applied to the novel or rare variants identified by Woelfle and his colleagues, all of the variants outside of the 3 mentioned above were categorized as either variants of uncertain significance or likely benign. One must be very careful in ascribing any potential pathogenicity to these variants. Wide-scale sequencing will almost always identify rare variants in candidate genes when the list of candidate genes is sufficiently long. As an example, there are 46 individuals in the Exome Aggregation Consortium data set who have nonsynonymous variants in *IGF2* that are not found in anyone else in this data set, which includes over 60,000 individuals. Do we think that these 46 individuals are likely to have growth disorders or is it more likely that these represent rare benign genetic variants? In their report, Woelfle and his colleagues describe a similar variant and raise the possibility that this variant could be causing the patient’s

growth failure. This patient had a height of -3.3 SD and her father, who also carried the variant, had a height at the 3rd percentile. *IGF2* is an imprinted gene which further complicates matters, but both of them inherited the variants from their fathers and thus the variant should be expressed. Is the difference in clinical presentation due to a difference in clinical expressivity of this variant or in other genetic modifiers? If this is a pathogenic variant, why does a variant in *IGF2* cause a low IGF-I level and why was the patient not born small for gestational age as one would expect in *IGF2* deficiency? Is it possible that this variant is playing a role in this patient's growth or is more likely to be a benign incidental finding? While I favor the

latter explanation, the truth is that our current state of knowledge does not allow us to definitively answer this question.

While exome sequencing has great potential to improve diagnostics in pediatric endocrinology, many more questions than answers will be raised. This does not mean that we should shy away from genomics as it will inevitably play a larger role in the future. Rather, as a field, we must work together to answer these questions. This will require partnerships between clinicians and researchers as well as large collaborative efforts across the continents. Thankfully, our field has a wonderful tradition of collaboration and will no doubt rise to the occasion.

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