Case Report

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Next-generation sequencing: Application of a novel platform to analyze atypical iron disorders

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Abstract

The development of targeted next-generation sequencing (NGS) applications now promises to be a clinically viable option for the diagnosis of rare disorders. This approach is proving to have significant utility where standardized testing has failed to identify the underlying molecular basis of disease. We have developed a unique targeted NGS panel for the systematic sequence-based analysis of atypical iron disorders. We report the analysis of 39 genes associated with iron regulation in eight cases of atypical iron dysregulation, in which five cases we identified the definitive causative mutation, and a possible causative mutation in a sixth. We further provide a molecular and cellular characterization study of one of these mutations (TFR2, p.I529N) in a familial case as proof of principle. Cellular analysis of the mutant protein indicates that this amino acid substitution affects the localization of the protein, which results in its retention in the endoplasmic reticulum and thus failure to function at the cell surface. Our unique NGS panel presents a rapid and cost-efficient approach to identify the underlying genetic cause in cases of atypical iron homeostasis disorders.

Keywords: Next-generation sequencing; Hemochromatosis; Iron overload; Anemia; Transferrin receptor 2.
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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BMP, bone morphogenetic protein; GGT, gamma-glutamyl transferase; HIF1A, hypoxia inducible factor 1; HMOX1, heme oxygenase (decycling) 1; IRIDA, iron-refractory iron-deficiency anemia; NGS, Next-Generation Sequencing; TFR2, transferrin receptor 2; TMPRSS6, transmembrane protease, serine 6; HG19, human genome version 19; TS, transferrin saturation; MAF, minor allele frequency; SMAD, mothers against decapentaplegic homolog; SNP, single nucleotide polymorphism.

Introduction

The advent of next-generation sequencing (NGS) offers the ability to sequence large amounts of DNA rapidly and has promised to have the ability to overcome the restriction of single gene analysis; its practicality for clinical use has been limited until recently by the cost and complex post-sequencing interpretation. A more recent and strategic approach, however, has been the development of targeted gene panels and scalable NGS systems, which leverage the power and economics of NGS [1].

Atypical iron disorders represent a spectrum of iron dysregulation ranging from juvenile onset iron overload through to iron-refractory iron-deficiency anemia (IRIDA). The most common form of primary iron overload disease, HFE-related hereditary hemochromatosis (HFE-HH), accounts for anywhere between 60% and 95% of cases of iron overload in European populations [2]. However, a significant and growing number of atypical iron disorders are being identified, particularly within non-European populations [2]. These cases have historically proven difficult to genetically diagnose, as beyond genotyping for the common *HFE* mutations, there is no standardized process for the further investigation of the underlying genetic cause.

Iron homeostasis is regulated through the hepcidin/ferroportin axis. Hepcidin, a liver-expressed peptide hormone, regulates the protein levels of the iron exporter ferroportin. The regulation of hepcidin itself is significantly more complex. The primary iron sensing and iron responsive regulation of hepcidin occurs through the bone morphogenetic protein (BMP)/mothers against decapentaplegic homolog (SMAD) signaling pathway. This pathway can be augmented by innate immune responses to infection and inflammation, hypoxia and oxidative stress leading to changes in hepcidin expression, along with sex hormones that also affect hepcidin regulation [for review see 3]. Mutations in the genes involved in any of these systems thus have the potential to influence iron homeostasis, and ultimately result in iron dysregulation.

While the treatment of the more common forms of iron overload are similar, specific diagnosis remains important in atypical cases in which alteration in treatment and/or monitoring are recommended. An example of this is classical ferroportin disease where cellular iron export capacity is reduced, inhibiting both iron recycling through the reticuloendothelial system and the

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Table 1. Genes and promoter regions sequenced.

Gene name	Short name (AKA)	Function in iron homeostasis
Hepcidin	HAMP	Master regulator
HFE	HFE	Mutated in HH / Iron sensing?
Transferrin receptor 2	TFR2	Mutated in HH / Iron sensing?
Aconitase 1, soluble	ACO1 (IRP1)	Iron sensing
Iron-responsive element binding protein 2	IREB2 (IRP2)	Iron sensing
Hemochromatosis type 2 (juvenile)	HFE2 (HJV)	BMP/SMAD hepcidin regulation
Transmembrane protease, serine 6	TMPRSS6 (MT2)	BMP/SMAD hepcidin regulation
Bone morphogenetic protein 6	BMP6	BMP/SMAD hepcidin regulation
Activin A receptor, type IIA	ACVR2A	BMP/SMAD hepcidin regulation
Activin A receptor, type I	ACVR1 (ALK2)	BMP/SMAD hepcidin regulation
Bone morphogenetic protein receptor, type IA	BMPR1A (ALK3)	BMP/SMAD hepcidin regulation
SMAD family member 1	SMAD1	BMP/SMAD hepcidin regulation
SMAD family member 4	SMAD4	BMP/SMAD hepcidin regulation
SMAD family member 5	SMAD5	BMP/SMAD hepcidin regulation
SMAD family member 6	SMAD6	BMP/SMAD hepcidin regulation
SMAD family member 7	SMAD7	BMP/SMAD hepcidin regulation
SMAD family member 9	SMAD9	BMP/SMAD hepcidin regulation
Growth differentiation factor 15	GDF15 (MIC1)	Hepcidin regulation
Twisted gastrulation homolog 1 (Drosophila)	TWSG1	Hepcidin regulation
Nuclear factor (erythroid-derived 2)-like 2	NFE2L2	Transcription factor
Beta-2-microglobulin	B2M	Chaperone/HFE interaction
Ferritin, light polypeptide	FTL	Iron storage
Ferritin, heavy polypeptide 1	FTH1	Iron storage
Transferrin	TF	Iron transport
Solute carrier family 40 (iron-regulated transporter), member 1	SLC40A1 (FPN)	Iron export
Transferrin receptor (p90, CD71)	TFRC (TFR1)	Iron import
Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	SLC11A2 (DMT-1)	Iron import/export
Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	SLC11A1 (NRAMP)	Metal ion transporter
Solute carrier family 39 (zinc transporter), member 14	SLC39A14 (ZIP14)	Zinc/iron transporter
Ceruloplasmin (ferroxidase)	CP	Ferroxidase
Amyloid beta (A4) precursor protein	APP	Ferroxidase
Hephaestin	HEPH	Ferroxidase
Hephaestin-like 1	HEPHL1	Ferroxidase
Cytochrome b reductase 1	CYBRD1 (DCYTB)	Ferric reductase
Nedd4 family interacting protein 1	NDFIP1	Trafficking
Nedd4 family interacting protein 2	NDFIP2	Trafficking
Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	HIF1α	Hypoxia/oxidative stress
Endothelial PAS domain protein 1	EPAS1 (HIF2α)	Hypoxia/oxidative stress
Heme oxygenase (decycling) 1	HMOX1	Hypoxia/oxidative stress
	Promoter size	Genomic region
Promoter region of HFE	-1485	chr6:26086024-26087509
Promoter region of hepcidin	-2255	chr19:35771155-35773410
Promoter region of hemochromatosis type 2 (juvenile)	-1000	chr1:145412191-145413191
Promoter region of solute carrier family 40 (iron-regulated transporter), member 1	-1681	chr2:190445537-190447218
Promoter region of ferritin, light polypeptide	-1350	chr19:49467216-49468566
Promoter region of ferritin, heavy polypeptide 1	-1000	chr11:61735132-61736132
Promoter region of bone morphogenetic protein 6	-1000	chr6:7726011-7727011
Promoter region of transmembrane protease, serine 6	-1000	chr22:37499693-37500693
Promoter region of transferrin receptor (p90, CD71)	-1000	chr3:195809032-195810032
Promoter region of transferrin receptor 2	-1000	chr7:100239173-100240173
Promoter region of transferrin	-1700	chr3:133463277-133464977

release of iron from tissues. In this pathophysiological setting, phlebotomy can be poorly tolerated by patients due to the reduced iron availability for erythropoiesis and often results in anemia. Consequently, the treatment regimen for classical ferroportin disease differs from other forms of iron overload, and increased monitoring of hemoglobin levels is required