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Individual Genome Sequence

Clinical Report

Ordering Physician	George Besser MD Palo Alto Medical Foundation 4050 Dublin Blvd Dublin, CA 94568

Patient Name	K. Thomas Pickard
Patient Sex	Male
Patient Record Number	PG0001189-BLD
Patient Date of Birth	10/8/1963
Indications for Testing	Risk Assessment
Date Reported	2/1/2014

Sample Type	Sample Collection Date	Sample Receipt Date
Blood	11/20/2013	11/21/2013

Test - Individual Genome Sequencing - Wellness

Genome level sequencing was performed and calls made across greater than 90% of the genome. Clinical interpretation was performed for all single nucleotide variants in 1,600 genes associated with 1,221 conditions for predisposition and carrier assessment. Only variants considered clinically significant are discussed within this report, however all variants that were interpreted can be found in the Clinical Variant Interpretation appendix. References for all interpreted variants are included here. The complete list of 1,600 genes and 1,221 conditions along with the callability of these genes can be found in the Gene-Disease appendix. Finally, the Annotation appendix lists variants found in the sample that were identified in genome wide association studies, along with position, associated condition, and interpretation with associated phenotype.

Individual Genome Sequence Results

PG0001189-BLD
K. Thomas Pickard

Results

A total of **5375** variants were detected in the subset of genes for this patient. Each variant was evaluated for clinical significance and placed into one of six possible categories for classification, which are described at the end of this report.

Summary of Clinically Significant Findings		
Category	**# Variants**	**Condition**
Pathogenic	0	
Likely Pathogenic	0	
VUS - Suspicious	0	

Summary of Findings Regarding Carrier Status		
Category	**# Variants**	**Condition**
Pathogenic	2	Hereditary Hemochromatosis Glucocorticoid Deficiency
Likely Pathogenic	2	Spastic Paraplegia, Recessive Dihydropyrimidine Dehydrogenase Deficiency
VUS - Suspicious	0	

Clinically Significant Findings

Variants that are clinically significant increase the individual's risk for a specific disease/disorder that is typically inherited as a dominant condition. Clinical correlation is recommended. The patient's first-degree relatives each have a 50% chance to carry the same variant as the patient. Testing for these at-risk family members should be considered, although the interpretation may be limited by the current understanding of this variant in the case of likely pathogenic variants.

No pathogenic, likely pathogenic or VUS-Suspicious variants were found in the 1600 genes evaluated that are expected to be clinically significant for the patient. However, this screen only detects nucleotide substitutions. Other types of genetic variants, including but not limited to insertions, deletions, copy number variants and trinucleotide repeats are not reported in this screening test. Further, the coverage of each gene is less than 100%. Therefore, clinically significant variants could exist in this genome that are not detected with this test. The coverage for each gene is provided in the Gene-Disease appendix.

Findings Regarding Carrier Status

Variants affecting carrier status indicate that an individual does not have the associated disease/disorder but that they may pass the variant to their offspring. These are typically disorders that are inherited in a recessive manner. For some disorders, carriers can manifest symptoms that are typically milder than for affected individuals and they are then referred to as symptomatic carriers. If two carriers of pathogenic variants in the same gene have a child, each child has a 25% chance to be affected when the disease/disorder is inherited in an autosomal recessive fashion. The patient's first-degree relatives each have a 50% chance to be carriers of this same variant. Testing for these at-risk family members should be considered.

Findings Regarding Carrier Status			
Variant	**Interpretation**	**Associated Condition**	**Mode of Inheritance**
HFE c.845G>A (p.Cys282Tyr)	Pathogenic	Hereditary Hemochromatosis	Autosomal Recessive
MC2R c.221G>T (p.Ser74Ile)	Pathogenic	Glucocorticoid Deficiency	Autosomal Recessive
SPG7 c.1529C>T (p.Ala510Val)	Likely Pathogenic	Spastic Paraplegia, Recessive	Autosomal Recessive
DPYD c.496A>G (p.Met166Val)	Likely Pathogenic	Dihydropyrimidine Dehydrogenase Deficiency	Autosomal Recessive

Disease and Variant Detail

Hereditary Hemochromatosis

Hereditary hemochromatosis (HH) is an autosomal recessive disorder characterized by abnormally high iron absorption. Excess iron is stored in tissues and organs, which can lead to tissue and organ damage. Clinical symptoms of HH include fatigue, joint pain, abdominal pain, arthritis, liver disease, heart abnormalities, skin discoloration, and diabetes. Onset of symptoms of HH can occur during adolescence or adulthood. Hereditary hemochromatosis is caused by mutations in several genes, including the genes HFE, SLC40A1, and TRF2. Hereditary hemochromatosis is one of the most common genetic disorders in the United States, with prevalence estimates ranging between 1/200 and 1/400. Significantly reduced penetrance has been demonstrated in HH.

The HFE gene is the most commonly contributing gene to hereditary hemochromatosis (HH). The vast majority of HH type 1 patients are homozygous for the HFE c.845G>A (p.Cys282Tyr) variant (Feder et al., 1996). The p.Cys282Tyr variant affects HFE protein activity by preventing the formation of a disulfide bridge in the alpha-3 domain, which impairs the beta-2-microglobulin interaction and prevents the protein from reaching the cell surface (Feder et al., 1997). The highest reported allele frequency in the 1000 Genomes database was 0.056, identified in the Great Britain cohort.

Glucocorticoid Deficiency

Glucocorticoid Deficiency (GCCD) is an autosomal recessive condition characterized by progressive adrenal insufficiency. Individuals with GCCD are unable to synthesize glucocorticoids due to an insensitivity to the hormone adrenocorticotropin. Clinical features of GCCD are noticeable during early infancy and include low levels of cortisol, recurrent hypoglycemic episodes, hyperpigmentation, seizures, and immunodeficiency resulting in frequent infections. GCCD is caused by mutations in several genes involved in the glucocorticoid synthesis pathway, including the genes MC2R and MRAP.

Variants in MC2R have been shown to cause glucocorticoid deficiency. The c.221G>T (p.Ser74Ile) variant was first described by Clark et al. (1993) in two affected siblings who were homozygous for the variant. The authors report that the variant segregated with disease throughout the family. Subsequent studies have reported the p.Ser74Ile variant in at least 43 additional affected individuals in either the homozygous or compound heterozygous state (Chan et al., 2009; Chung et al., 2010; Clark and Weber, 1994; Lin et al., 2007). Fluck et al. (2002) tested the enzyme activity in a male who was compound heterozygous for the p.Ser74Ile variant, and reported the variant elicited

virtually no measurable enzyme activity. Elias et al. (1999) demonstrated that the p.Ser74Ile variant was associated with an impaired maximal cAMP response when compared to the wild-type. Additionally, Chung et al. (2008) postulated that because the p.Ser74Ile gene product is retained within the cell, it may cause a misfolding or trafficking defect in the receptor. This variant is considered to be highly conserved. Although there was limited control data, the highest allele frequency is 0.008 in the Americans of African Ancestry in SW USA population, according to the 1000 Genomes database so this variant appears to be rare in the general population.

Spastic Paraplegia, Recessive

Spastic paraplegia (SPG) refers to a group of disorders characterized by progressive lower extremity spasticity, which may occur as an isolated finding or in association with other neurologic abnormalities such as optic neuropathy, retinopathy, extrapyramidal disturbance, dementia, ataxia, ichthyosis, mental retardation, and deafness. The main symptoms are weakness and spasticity of the leg and hip muscles, which can lead to progressive difficulties with movement. Genetic heterogeneity is seen, with mutations in multiple genes causing different forms of the disorder. Depending upon which gene is implicated, the onset of the disease may begin in childhood or adulthood with variable associated features. Autosomal recessive SPG may be characterized by severe lower limb spasticity, variable lower limb weakness, hyperreflexia, posterior column sensory impairment, and bladder dysfunction.

Multiple genes, including the SPG7 gene, have been reported to cause a recessive form of spastic paraplegia. Brugman et al. (2008) reported on four individuals affected by spastic paraplegia who were compound heterozygotes for the c.1529C>T (p.Ala510Val) variant and another variant. Elleuch et al. (2007) reported the p.Ala510Val variant in 13 cases and no control subjects. Affected individuals were homozygous or compound heterozygous for the p.Ala510Val variant. Affected individuals in four additional families were compound heterozygotes for the p.Ala510Val variant and two different polymorphisms in the SPG7 gene. One unaffected family member in this study carried the p.Ala510Val variant and a presumed pathogenic mutation, however, age-related penetrance may account for her unaffected status. The highest allele frequency is 0.008 in the Americans of African Ancestry in SW USA population, according to the 1000 Genomes database, so this variant is rare enough to be consistent with disease prevalence.

Dihydropyrimidine Dehydrogenase Deficiency

Dihydropyrimidine dehydrogenase deficiency (DPD deficiency) is an inherited metabolic disorder in which there is absent or significantly decreased activity of dihydropyrimidine dehydrogenase, an enzyme involved in the metabolism of uracil and thymine. Affected individuals present in infancy with seizures, microcephaly, hypertonia, growth and motor delays, and later, cognitive disabilities and autism-like features. This condition is highly variable, with some affected individuals showing very few signs of the disease phenotype. Regardless of the severity of symptoms, all individuals with the condition are at risk for severe, toxic reactions to fluoropyrimidines, which are used to treat cancer. It is reported that 2 -8 percent of the general population may be vulnerable to toxic reactions to fluoropyrimidines due to the high frequency of carrier status. The condition has an autosomal recessive mode of inheritance.

The DPYD gene is the only gene currently associated with dihydropyrimidine dehydrogenase deficiency. The DPYD c.496A>G (p.Met166Val) variant was identified in one compound heterozygous patient in the van Kuilenburg (2000) study. This study did not include a control population. A second study by Gross et al. (2003) identified the p.Met166Val variant as compound heterozygous with other DPYD variants in two out of four patients, while 24 out of 157 controls carried one allele of the p.Met166Val variant alone (with no second allele identified). A third study by Gross et al. (2008) found the p.Met166Val variant in 22/92 cases and 49/607 controls. Together these studies show a significant over representation of the p.Met166Val allele in cases compared to controls. The highest allele frequency is 0.188 in the Finnish population according to 1000 Genomes. This is consistent with the high prevalence and autosomal recessive nature of this condition.

Variants of Unknown Clinical Significance

Of the 5375 variants detected in this subset of genes, 1088 variants have little or nothing reported about them in the scientific literature, and therefore, are considered Variants of Unknown Significance. This includes variants in genes that could be clinically significant or confer carrier status. At this time, the evidence is too weak or contradictory to assess whether the variant is pathogenic or benign. The interpretations of these variants are likely to change as more individuals are sequenced and the community understanding of the effects of the variant improves. A complete list of these variants, the genes in which they were found, and annotation characteristics can be found in the Clinical Variant Interpretation appendix.

Benign/Likely Benign Variants

Finally, 2775 variants categorized as benign and 1508 variants categorized as likely benign polymorphisms were also found. A complete list of these variants, the genes in which they were found, and annotation characteristics can be found in the Clinical Variant Interpretation appendix.

Test Information

Background

All calls within the 1600 genes were evaluated for evidence of clinical importance including: allele frequency in population studies (dbSNP, 1000 Genomes, etc.), evidence in the scientific literature for likely causation of the condition, and consideration of the likely biological implications of the variant based on its expected characteristics. Interpretation was performed for single nucleotide variants only. This assessment represents our current best understanding of the clinical implications of the variants reported. No other variants beyond those contained within the listed genes and conditions were evaluated for possible clinical significance. Therefore, other variants of possible clinical significance may exist within this genome.

Recommendations

- As knowledge increases, periodic re-evaluation of the clinical implications of variants is appropriate.
- Genetic counseling is recommended to assess the specific implications of this variant relative to an individual's clinical context and concerns.
- Clinical correlation is appropriate.
- Additional verification of variants that are deemed medically actionable may be appropriate.
- Additional testing may be appropriate to evaluate for other types of variants not detected in this test.

Methodology

Sequence was generated from DNA that was extracted from peripheral whole blood. The regions of the genome not reported here include regions where the human reference genome has not been completely resolved, or where duplications of genetic regions make it impossible to align the fragments accurately. The official reference build 37.1 was used to align the Personal Genome Sequence reported here. (http://www.ncbi.nlm.nih.gov) The analytical accuracy of these calls is at least 97%. This test was developed and its performance characteristics determined by Illumina Clinical Services Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. Pursuant to the requirements of CLIA '88, this laboratory test has established and verified the test's accuracy and precision.

Criteria for classification

- Pathogenic: Reported in multiple unrelated cases, with control data. Functional or expression evidence suggests deleterious effect on gene function.
- Likely Pathogenic: Reported in limited cases, or in a single family cohort, with or without control data. Limited or no functional evidence available, but overall biological expectations suggestive of deleterious effect.
- VUS-Suspicious: There is some evidence that the variant could be causative of disease. However, the information available is insufficient to categorize the variant as likely pathogenic. This category was added to bring attention to variants that are on the border between unknown significance and likely pathogenic.

- Unknown Significance: Little or nothing has been reported on this variant or its effects.
- Likely Benign: This variant has been seen in cases, but also in controls. Variant may be present in a high percentage of the population, and may be present in a non-conserved region.
- Benign: Established in the literature as a variant that is not associated with Mendelian (single-gene inherited) disease, or known to have an allele frequency that is far too high to be compatible with the prevalence of disease, mode of inheritance and penetrance patterns known for that condition.

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Signed electronically by Philip D. Cotter, PhD, FACMG on Saturday, February 1, 2014 8:08 AM

References:

Brugman F, Scheffer H, Wokke JH, Nillesen WM, de Visser M, Aronica E, Veldink JH, van den Berg LH. 2008 Nov 4. Paraplegin mutations in sporadic adult-onset upper motor neuron syndromes. Neurology. 71(19):1500-5.

Chan LF, Metherell LA, Krude H, Ball C, O'Riordan SM, Costigan C, Lynch SA, Savage MO, Cavarzere P, Clark AJ. 2009 Aug. Homozygous nonsense and frameshift mutations of the ACTH receptor in children with familial glucocorticoid deficiency (FGD) are not associated with long-term mineralocorticoid deficiency. Clin Endocrinol (Oxf). 71(2):171-5.

Chung TT, Chan LF, Metherell LA, Clark AJ. 2010 May. Phenotypic characteristics of familial glucocorticoid deficiency (FGD) type 1 and 2. Clin Endocrinol (Oxf). 72(5):589-94.

Chung TT, Webb TR, Chan LF, Cooray SN, Metherell LA, King PJ, Chapple JP, Clark AJ. 2008 Dec. The majority of adrenocorticotropin receptor (melanocortin 2 receptor) mutations found in familial glucocorticoid deficiency type 1 lead to defective trafficking of the receptor to the cell surface. J Clin Endocrinol Metab. 93(12):4948-54.

Clark AJ, McLoughlin L, Grossman A. 1993 Feb 20. Familial glucocorticoid deficiency associated with point mutation in the adrenocorticotropin receptor. Lancet. 341(8843):461-2.

Clark AJ, Weber A. 1994 Jul. Molecular insights into inherited ACTH resistance syndromes. Trends Endocrinol Metab. 5(5):209-14.

Elias LL, Huebner A, Pullinger GD, Mirtella A, Clark AJ. 1999 Aug. Functional characterization of naturally occurring mutations of the human adrenocorticotropin receptor: poor correlation of phenotype and genotype. J Clin Endocrinol Metab. 84(8):2766-70.

Elleuch N, Bouslam N, Hanein S, Lossos A, Hamri A, Klebe S, Meiner V, Birouk N, Lerer I, Grid D, Bacq D, Tazir M, Zelenika D, Argov Z, Durr A, Yahyaoui M, Benomar A, Brice A, Stevanin G. 2007 Nov. Refinement of the SPG15 candidate interval and phenotypic heterogeneity in three large Arab families. Neurogenetics. 8(4):307-15.

Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R Jr, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, Mintier GA, Moeller N, Moore T, Morikang E, Prass CE, Quintana L, Starnes SM, Schatzman RC, Brunke KJ, Drayna DT, Risch NJ, Bacon BR, Wolff RK. 1996 Aug. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet. 13(4):399-408.

Feder JN, Tsuchihashi Z, Irrinki A, Lee VK, Mapa FA, Morikang E, Prass CE, Starnes SM, Wolff RK, Parkkila S, Sly WS, Schatzman RC. 1997 May 30. The hemochromatosis founder mutation in HLA-H disrupts beta2-microglobulin interaction and cell surface expression. J Biol Chem. 272(22):14025-8.

Flück CE, Martens JW, Conte FA, Miller WL. 2002 Sep. Clinical, genetic, and functional characterization of adrenocorticotropin receptor mutations using a novel receptor assay. J Clin Endocrinol Metab. 87(9):4318-23.

Gross E, Busse B, Riemenschneider M, Neubauer S, Seck K, Klein HG, Kiechle M, Lordick F, Meindl A. 2008. Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. PLoS One. 3(12):e4003.

Gross E, Ullrich T, Seck K, Mueller V, de Wit M, von Schilling C, Meindl A, Schmitt M, Kiechle M. 2003 Dec.

Detailed analysis of five mutations in dihydropyrimidine dehydrogenase detected in cancer patients with 5-fluorouracil-related side effects. Hum Mutat. 22(6):498.

Lin L, Hindmarsh PC, Metherell LA, Alzyoud M, Al-Ali M, Brain CE, Clark AJ, Dattani MT, Achermann JC. 2007 Feb. Severe loss-of-function mutations in the adrenocorticotropin receptor (ACTHR, MC2R) can be found in patients diagnosed with salt-losing adrenal hypoplasia. Clin Endocrinol (Oxf). 66(2):205-10.

van Kuilenburg AB, Haasjes J, Richel DJ, Zoetekouw L, Van Lenthe H, De Abreu RA, Maring JG, Vreken P, van Gennip AH. 2000 Dec. Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. Clin Cancer Res. 6(12):4705-12.