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Protein Identification and Haplotype Description of Homozygote Mutation Causing Congenital Plasminogen Deficiency

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Abstract

Severe type I Plasminogen (PLG) deficiency was clinically diagnosed after hyaline-positive periodic acid Schiff material was detected in the histologic study of superior tarsal conjunctiva and vulvar pseudomembrane of the patient. Direct immunofluorescence also confirmed multiple deposits of fibrinogen in the dermis. Plasma plasminogen activity was calculated in a <5% value (reference values, 75% to 150%) and sequencing of the PLG gene evidenced the homozygous mutation in c.2377T/A (p.Tyr793Asn), confirming the molecular diagnosis of congenital deficiency of plasminogen type 1. Genotype-Phenotype correlation among family members evidenced the recessive hereditary pattern of clinical manifestations of chronic inflammatory disease of the mucous membranes due to PLG deficiency, but co-dominance effect to present a decreased plasma plasminogen activity (46%) among heterozygous asymptomatic individuals. SNPs/CNVs whole genome array hybridization analysis in the patient, detected long Loss of Heterozygosity regions (LOH) and demonstrated the consanguinity in the family. Proteomic analysis identified impaired secretion of mutant PLG tissue specific proteins, as definitive molecular etiopathogenesis of the type I PLG deficiency in the patient.

Keywords: Fibrin; Proteomic; Loss of heterozygosity; Plasminogen deficiency

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Introduction

A 60-year-old woman was diagnosed of congenital plasminogen deficiency (OMIM #217090) for oral and genital mucosal lesions [1]. The patient had a vast clinical history with multiple consultations in different specialties, with a set of nonspecific signs and symptoms, as well as various symptomatic treatments that did not achieve relief. The most frequent symptoms had been recurrent pneumonia and recurrent cervicitis, and a history of infertility that led the patient to reject having biological offspring. We were also able to assess the medical history of her sister, who died at 42 years of age after serious complications from a respiratory process; the clinical diagnosis of her sister, also with an extensive clinical history due to having suggested various diagnostic suspicions, was Cowden syndrome.

In the last visit to the Dermatology Service, we observed lesions on both extensor surfaces of the arms that had not been previously described. Two brown warty patches were observed, which the patient reported having appeared in the recent months. A 4-millimeter punch biopsy was performed and the presence of eosinophilic bodies in the dermis was identified in the histological section, being the immunofluorescence of these bodies positive for fibrinogen (Figure 1). Plasminogen activity assessment in plasma, with chromogenic substrate, calculated an activity value of <5% (reference values, 75% to 150%) for the codified protein. Genetic study by complete Sanger sequencing of

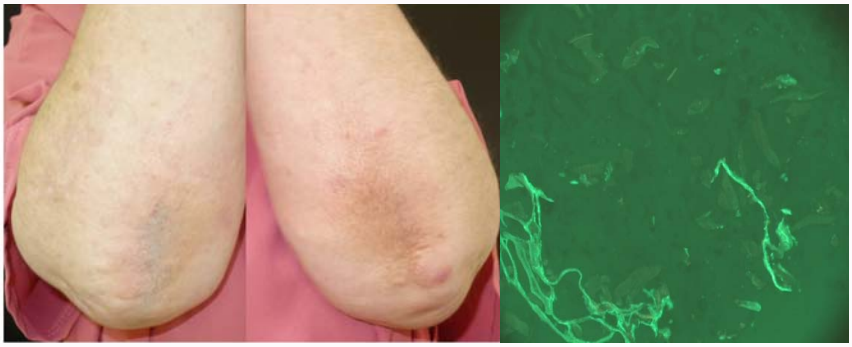

Figure 1: Warty brown patches in both elbows and immunofluorescence fibrinogen positive deposits.
We describe the presence of warty plaques on the extensor surface of the upper extremities that correspond to fibrinogen deposits in the dermis as a new clinical presentation of this disease, not yet reported.

the PLG gene, found the mutation c.2377T/A (p.Tyr793Asn) in homozygosis; the molecular diagnosis of congenital deficiency of plasminogen type 1 was confirmed.

The realization of the genealogical three of the family confirmed the consanguinity in the patient´s progenitors, four generations before them. Segregation analysis of the deleterious mutation in family members, identified four asymptomatic heterozygote's individuals who showed a decreased plasminogen activity also (similar level of 46%); suggesting a co-dominance biochemical effect of the mutation in heterozygosis. The evaluation of the clinical data of her deceased sister, suggested that she was most likely also a carrier of the homozygous familial mutation; that deceased sister of the patient did not have children either. The patient was thoroughly evaluated by the Allergology Service, to rule out any symptoms related to possible angioedema, given the susceptibility that certain mutations in the PLG gene have shown to its appearance after triggering agents [2]. Such suspicion was absolutely ruled out, and no signs, symptoms, lesions, or biochemical changes typical of a disorder related to allergic phenomena were found. The complete sequencing of the coding zones of the PLG gene, as well as its flanking intronic zones, showed a complete Loss of Heterozygosity (LOH) of the gene, suggesting that it was included in an inherited zone with identical molecular information from both parents. Sequencing defined the haplotype which was in linkage disequilibrium with the deleterious p.Tyr793Asn mutation (Table 1). The genetic analysis identified four variants in homozygous status, c.330C>T (p.=), c.1083A>G (p.=), c.1414G>A (p.Asp472Asn) and c.2377T>A (p.Tyr793Asn), located in exons 4, 9, 11 and 19, respectively. The c.2377T>A variant has not been described in other patient [1]. Bioinformatics tools (Mutation Taster, Polyphen-2 and SIFT) for in silico functional analysis, coincided with respect to the deleterious capability of this variant (Table 1).

Due to family history and the homozygous state of all detected SNPs in PLG, it was essential to verify that there was no heterozygous deletion of one PLG allele; a SNPs/CNVs microarray analysis was performed to evaluate homozygosity of genomics regions. arr[GRCh37]6q25.3q26(1600300007_163624883)hmz LOH region was identified, including the PLG gene (chromosomal location 161,123,224-161,175,085) (Figure 2). From the sum of all validated detected LOH regions, it was able to calculate the percentage of consanguinity that existed in the patient; the global percentage of consanguinity calculated for the genome of the patient was less than 3%, an accepted limit in the general population for offspring of unrelated parents. However, the LOH regions of size greater than the average found in the general population were detected. The region containing the PLG gene was one of them (Figure 2). To complete the pathological diagnosis, a histologic study of the white-yellowish pseudo membranes in the vaginal introitus and in the superior tarsal conjunctiva was carried out, identifying hyaline-positive periodic acide Schiff material in the stroma. Direct immunofluorescence showed multiple deposits of fibrinogen in the dermis [1]. The plaques in both elbows were histologically studied, finding the presence of eosinophilic bodies in the dermis and positivity in the immunofluorescence for fibrinogen.

From the conjunctiva and bronchial sputum sample of the patient, a proteomic study was carried out. The samples were digested with trypsin [3] and the resulting peptides analyzed by liquid chromatography coupled to mass spectrometry using a 5600 Triple TOF (SCIEX). Protein identification was performed with Protein Pilot v5.0. Search engine (SCIEX) software. We identified over 350 protein groups in each sample. Protein abundance, estimated by Top3 label-free quantitation [4], indicated that fibrinogens are the most abundant proteins. We estimated that fibrinogen alpha; beta and gamma chains represented about 26 and 14% of the total protein amount in conjunctiva and sputum samples, respectively. The most abundant proteins were common in both sample types. The functional analysis, performed with the bioinformatics tool DAVID [5], indicated that most proteins were secreted proteins found in plasma.

Discussion

The integration of several “omics” techniques, in combination

Table 1: Homozygous Variants in the *PLG* Gene. Four variants in the *PLG* gene were identified, all of them in homozygous status.

Gene	Variant	State	Access Number NCBI/HGMD	Population Allelic Frequency (*)
PLG	c.330C>T (p.=)	Homozygous	rs4757	24,9%
PLG	c.1083A>G (p.=)	Homozygous	rs13231	13,6%
PLG	c.1414G>A (p.Asp472Asn)	Homozygous	rs4252125 / CM043559	14,0%
PLG	c.2377T>A (p.Tyr793Asn)	Homozygous	-	-

(*)Source: 1000 Genomes Project

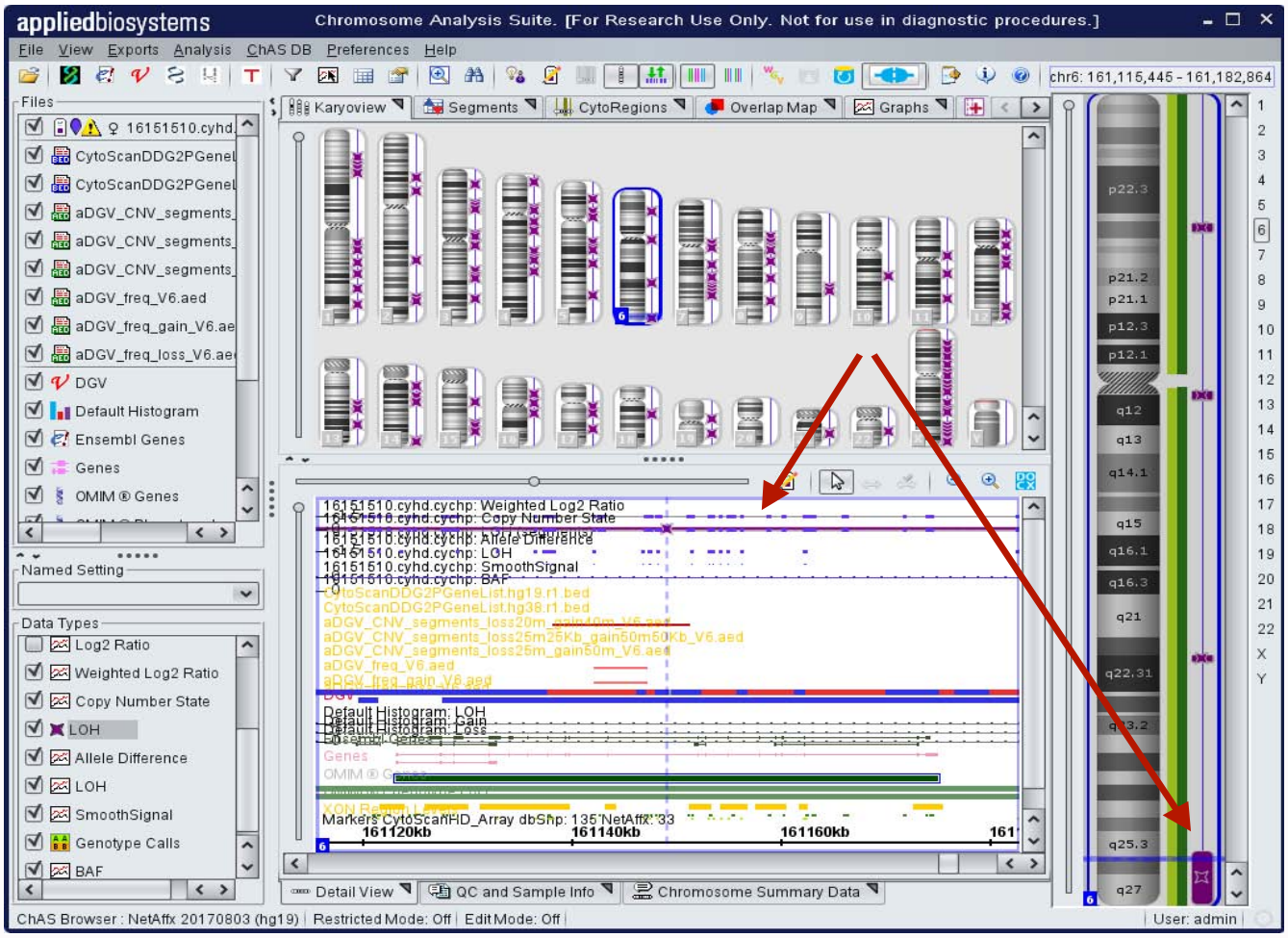

Figure 2: PLG mutation coincides in a LOH region, suggesting common ancestor.
Image from Chromosome Analysis suite software, analyzing SNPs/CNVs whole genome data, evidencing that the patient carried a percentage of LOH regions under the accepted consanguinity ratios, but existing some with size which led to suspect a common ancestor of both her progenitors. PLG gene location coincides in a LOH region (red arrows).

with family pedigree, medical symptoms interpretation, and hematology and biochemistry determinations, allowed us a better molecular diagnosis and precise medical advisement for the patient and her family. The proteomic analysis confirmed the high abundance of undigested fibrinogen chains in the pseudo membranes obtained from both tissues. Although there were some minor differences in the protein content, the majority and most abundant proteins were the same in both samples. Incorporation of proteomics into the clinical laboratory procedures greatly enhances its diagnostic capacity and opens the way to precision medicine. The genetic diagnosis could be definitive in our patient/family, and it was possible to carry out a individualized clinical follow up in the homozygous and even heterozygous individual carriers. The precise characterization of the patients' phenotype (Human Phenotype Ontology: https://hpo.jax.org/app/) allowed a very precise correlation with the identified genotype; this facilitated the inclusion of affected individuals in new clinical trials. Plasminogen deficiency is a rare entity that affects 1.6 of every 1 million individuals and is inherited in an autosomal recessive manner by mutations in homozygosis or compound heterozygosis in the PLG gene, which codes for plasminogen [6]. Despite this inheritance pattern, it is estimated that 0.13% to 0.42% of the population are asymptomatic heterozygous individuals. Plasminogen is the proenzyme of plasmin and is synthesized primarily in the liver. Although its role in intravascular and extravascular fibrinolysis is well defined, it also plays an important role as a proteolytic enzyme, degrading the proteins of the extracellular matrix, activating metalloproteases, and repairing wounds. Its deficit leads to an inability to lysis of fibrin, producing an accumulation of this protein, which is the cause of the clinically observed manifestations. Two types of plasminogen deficiency have been described: type 1 (hypoplasminogenemia), in which plasminogen levels and activity are reduced, and type 2 (dysplasminogenemia), in which plasminogen levels are normal but their specific activity is reduced. The clinical symptoms of type 1 plasminogen deficiency are derived by the accumulation of fibrinoid material in different systems of the organism, giving rise to mucocutaneous, otorhinolaryngologic, respiratory, or gynecologic manifestations. The most frequent manifestation is the formation of pseudo membranes of woody consistency in the ocular conjunctiva and less frequently in the upper and lower respiratory tract, vagina, and gastrointestinal tract. It must be confirmed by the diminished plasminogen activity in plasma and the genetic study of the PLG gene by complete sequencing (next-generation sequencing or Sanger), for the detection and description of the genetic alterations responsible for the appearance of this disease in each patient and their

family in the face of subsequent genetic counseling.

HPO describes in July 2021 (https://hpo.jax.org/app/browse/gene/5340) severe Hypoplasminogenemia (HPG) or type 1 Plasminogen deficiency (Plg), as a systemic disease characterized by markedly altered extracellular fibrinolysis that leads to the formation of woody pseudo membranes (rich in fibrin) that heal on the mucosa during injury. We show that the formation of pseudo membranes occurs continuously throughout life and independent of scarring processes. Furthermore, we have shown that fibrinogen deposits also occur in the dermis, which had not been previously described as a clinical manifestation of this disease. The patient is about to start treatment with humanized plasminogen replacement therapy, which represents a new therapeutic option specifically directed to these patients [7]. The data shows increasing levels of plasminogen in the treated individuals.

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