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Case Report

Molecular basis of favism triggered by ingestion of frozen pumpkin cross-contaminated with fava beans

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1. Introduction

G6PD-deficient patients are difficult to detect, given that affected people are asymptomatic until they are exposed to triggers. More than 400 million individuals are thought to be G6PD-deficient, exhibiting high genetic heterogeneity, stating this enzymopathy the most common clinically significant enzyme defect [1]. To date, about 200 different *G6PD* pathogenic variants (PVs) have been identified worldwide and each ethnic population exhibits a peculiar mutational profile [2].

G6PD deficiency (G6PDd) commonly manifests as neonatal hyperbilirubinemia or acute hemolytic anemia (AHA), induced by oxidative stress. Common triggers include ingestion of fava beans, systemic infections or exposure to certain medications. Less commonly, G6PDd can manifest as a chronic non-spherocytic hemolytic anemia (CNSHA).

The degree of AHA, in patient's with favism, is largely dictated by the age of the red blood cells (RBC), the nature of the trigger and the severity of the G6PDd which, in turn, is determined by the specific PV. Only a close association between type of genetic defect, residual enzymatic activity and clinical manifestations allows a definitive phenotype stratification of these patients.

2. Case presentation

We focused our attention on an atypical case of favism described by Zuccotti et al. [3]. This study reported an 8-month-year-old Italian neonate presented at Luigi Sacco Hospital (Milan University) with AHA after frozen pumpkin (*Cucurbita maxima*) ingestion. Biochemical evaluation showed severe G6PDd. Zuccotti et al. demonstrated that AHA was caused by a cross-contamination between pumpkin and fava beans. To date, this is the first case of AHA triggered by ingestion of food cross-contaminated fava beans. It underlines the importance considering possible cross-contamination of food, growing on the same field or which is processed on the same food supply chain. Recently, this case was reported also by Luzzatto and Arese in their detailed review [4] as peculiar and unique case of favism due to foods other than classical fava beans. Although this case has been well described, the molecular basis of the G6PDd was missing. In the absence of the genetic test for G6PDd, Zuccotti et al. left the possibility open that AHA could depend on a peculiar *G6PD* variant, as previously reported [5–7]. We underline the importance to report information regarding the type of *G6PD* variant, in order to fully elucidate the phenotype-genotype relationship in this patient.

For this reason, the patient underwent in 2018, at the age of 5, *G6PD* gene screening, as reported [8], at our Laboratory at the "A. Gemelli" University hospital in Rome, to add important information to this medical case.

Results showed hemizygosity for the *c.563C > T* variant (*G6PD Mediterranean*), together with a C > T silent variant at nucleotide 1311 and a T > C polymorphism at nucleotide 93 of intron 11 in the *G6PD* gene. The compound variants (*c.563C > T*, *c.1311C > T*, *c.1365-*

Abbreviations: PVs, pathogenic variants; G6PDd, G6PD deficiency; AHA, acute hemolytic anemia; CNSHA, chronic non-spherocytic hemolytic anemia; RBC, red blood cells; ACP1, acid phosphatase

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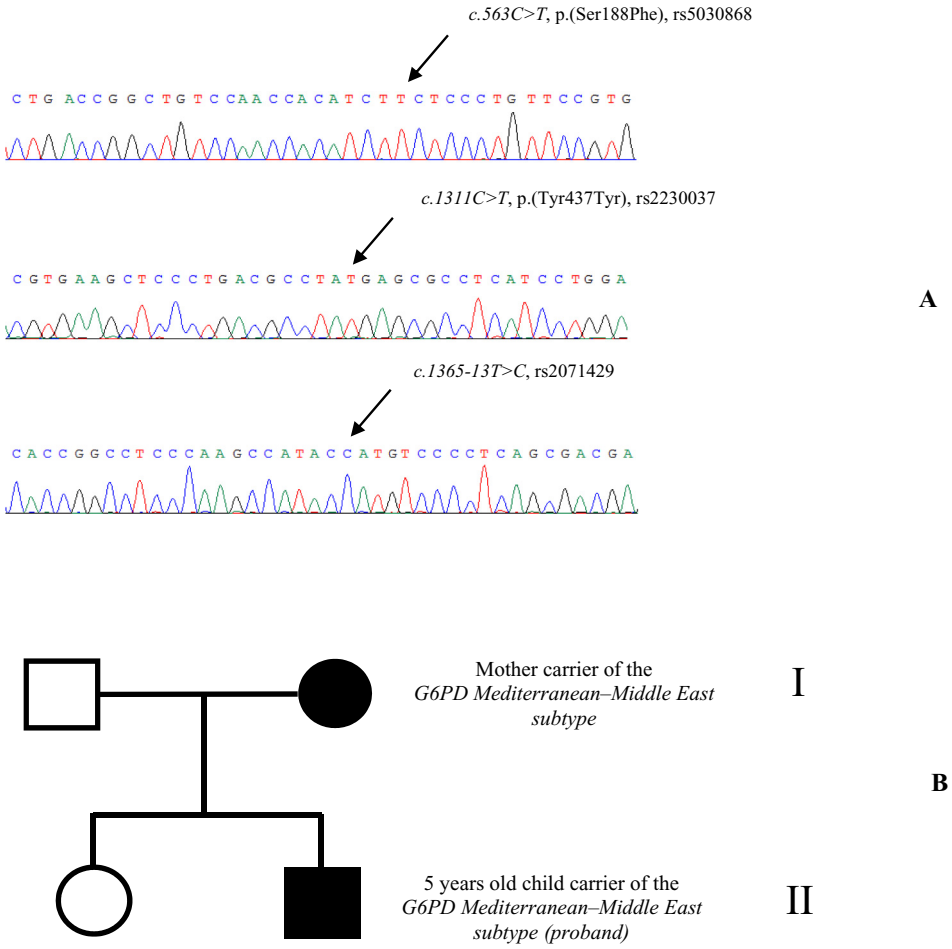

Fig. 1. Sequencing results and pedigree of proband's family.
(A) Sequence chromatogram showing the *G6PD Mediterranean–Middle East subtype* of *G6PD* gene in the proband. The variants are numbered according to GenBank NM_001042351.2; (B) The pedigree of the patient shows maternal inheritance of the *G6PD* haplotype.

13T > C, according to the reference sequence: NM_001042351.2) are responsible for the *G6PD Mediterranean–Middle East* subtype (Fig. 1). The family history confirmed the maternal inheritance of the variant and highlighted a wild-type genotype for the proband's sister (Fig. 1).

We also performed the molecular screening of the Acid phosphatases 1 (*ACP1)* gene, with the aim to identify other genetic modifiers of the G6PDd phenotype, since some *ACP1* genotypes give a predisposition to favism. The sequencing showed the wild type *ACP1* genotype (BB), which is not associated to favism predisposition [9]. In addition, a complete blood re-valuation showed the following results: haemoglobin, 12.1 g/dL, white cell cells (WBC), 6500/μL, platelet, 385,000/μL, RBC 4.03 million/μL and hematocrit 35.8%.

3. Discussion

The most common variant in the Italian population and Mediterranean area is the *G6PD Mediterranean,* which is characterized by very low activity (< 5% of normal activity) often causing favism in the G6PD deficient patients [1]. Fava beans, which are grown throughout the world and are a very popular foodstuff in Italy, are probably the most common trigger of hemolysis in patients with G6PDd [4]. Yet, for unknown reasons, favism does not affect all people with G6PDd and some patients can eat fava beans without any symptoms. Controversy exists as to whether typical symptoms may also be caused by simply inhaling pollen from the plants, or in babies who have been breastfed by mothers who have eaten fava beans. The combination of some factors, such as residual enzymatic activity, metabolic characteristics of the individual, general health status or the amount of fava beans ingested, may have a relevant role to determine the severity of symptoms. Children with G6PDd seem to be more prone to hemolytic crisis triggered by ingesting fava beans compared to adults. This is possibly due to overexposure related to a low body surface area [4].

Herein, the child's clinical picture [3] has been completed with molecular testing. This case underlines that the myth, other beans or foods can cause an attack of favism must be considered carefully. In fact, this study highlights the importance considering possible cross-contamination of food growing on the same field or which are treated along the same food supply chain. In particular, in G6PD deficient pediatric patients, cross-contamination of food with fava beans may represent a risk factor for AHA even if a small quantity of contaminated food is ingested, as reported by Zuccotti et al.

Finally, correlation between genotype and clinical phenotype of G6PD deficient individual's gives insight on the clinical expression of G6PDd and the complex interaction of molecular variants, environmental factors (such as infection and medications), food and residual enzymatic activity and is therefore mandatory.

Declaration of competing interests

The authors declare they have no competing interests.

Consent

Written informed consent was obtained from the patient's parents

for the publication of this report.

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