DATA REPORT

Report of an Italian family carrying a typical Indian variant of the Nilgiris tribal groups resulting from a de novo occurrence

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G6PD deficiency is quite common in Italy where it is characterized by extreme molecular and biochemical heterogeneity. We report a 15-year-old Italian boy with G6PD Nilgiri (c.593G>A, p.Arg198His), a typical Indian variant of the Nilgiris tribal groups. Further, this variant was biochemically characterized, and the molecular screening of the family highlighted a de novo mutational event. To date, this family is the first Caucasian family carrying the G6PD Nilgiri variant.

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Although it is difficult to detect G6PD-deficient patients given that affected people are asymptomatic until they are exposed to triggers, > 400 million individuals are thought to be G6PD-deficient, exhibiting high genetic heterogeneity and making this enzynamy the most common clinically significant enzyme defect.1

G6PD deficiency occurs most frequently in Africa, Asia, the Mediterranean and the Middle East, synchronizing with endemic malaria.2 In fact, the prevalence of G6PD deficiency among 1,125 male individuals from different tribal groups of the Nilgiri district in Southern India. A hitherto unreported G6PD variant was identified in four individuals. This new variant (c.593G>A, based on NM_001042351.2, rs137852332) causes a predicted amino acid change of arginine (Arg) to histidine (His) at codon 198 in exon 6. This variant was confirmed by a family study and was designated G6PD Nilgiri.

Chalvam indicates that further studies must be performed to determine the prevalence and distribution of this variant in different population groups. Moreover, Chalvam et al. was unable to classify this variant given that a sufficient blood sample was not available for biochemical characterization of the residual enzyme. To date, G6PD Nilgiri has never been reported in the literature again.

In Italy, G6PD deficiency is characterized by extreme molecular and biochemical heterogeneity; in addition to Sardinia and Sicily, where higher disease prevalence is present (from 2 to 15%), G6PD-deficient subjects are also found in other Italian regions, such as Campania, Basilicata, Puglia and Lazio.7 All these regions presented endemic malaria in the past.

At least 10 distinct G6PD point variants have been reported in Italy,8 and >94% of those (based on NM_001042351.2 reference) include c.563C>T (G6PD Mediterranean), c.844G>C (G6PD Seattle c.202G>A/c.376A>G (G6PD A+), c.1003G>A (G6PD Chatam) and c.1347G>C (G6PD Cassano). Thus, we are only able to perform preliminary mutational scanning of these variants.8 Moreover, novel and rare G6PD variants are also present.9

We report a case of an asymptomatic 15-year-old male born to parents of Italian descent (Campania region) with severe G6PD deficiency.

Table 1. G6PD activity, some hematological values and molecular results of proband and his relatives

|                      | Proband             | Mother             | Grandmother | Grandfather |
|----------------------|---------------------|--------------------|-------------|-------------|
| G6PD activitya       | 0.1                 | —                  | —           | —           |
| G6PD/6PGDe           | —                   | 0.4                | —           | —           |
| Hemoglobin           | 14.5                | 12.8               | —           | —           |
| RBCSC                | 4.25                | 4.46               | —           | 12.2–16.6 g/dl |
| G6PD Nilgiri         | Hemiogyote4         | Heterozygote4      | WT5         | WT5         |

9.2–13.8 U/gHb

> 0.85

12.2–16.6 g/dl

4.20–5.60 × 1012/l

4G6PD biochemical activity was performed using a commercial Kit (Sentinel diagnostics, Milano, Italia).

5G6PD/6PGD was evaluated using a commercial kit (NUREX diagnostics, Sassari, Italy) able to detect females G6PD variants carriers.

6RBCs: red blood cells.

7Molecular testing performed on DNA from peripheral blood.

8Molecular testing performed on DNA from buccal swab.

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deficiency discovered during military recruiting (Table 1). He had no relevant family history. After written informed consent was provided, direct sequencing of the entire G6PD gene was performed given that the patient was negative for the most common Italian variants. The patient was hemizygous for the G6PD Nilgiri variant.

The family study confirmed the maternal inheritance of the variant (Figure 1a). Conversely, the p.Arg198His variant was not identified in the patient’s grandparents, from whom DNA from buccal cell samples was analyzed (Figure 1b). The G6PD intragenic markers c.1365-13T>C (rs2071429) and c.1311C>T (rs2230037) were informative of the mother-grandparents relationships. These
findings suggested that the G6PD Nilgiri variant in this family was a result of a de novo mutational event.

G6PD Nilgiri involves the same codon 198 that is mutated in G6PD Coimbra (c.592C>T, p.Arg198Cys). However, in G6PD Coimbra, the mutation is at nt 592, which is the first base of the codon, whereas the G6PD Nilgiri variant is located at the second base of the same codon. G6PD Coimbra is very close to the G6PD Mediterranean variant within exon 6 and has similar kinetic properties, namely high affinity for G6P and NADP+ and a high rate of deamino-nicotinamide adenine dinucleotide phosphate (dNADP) and 2-deoxy glucose-6-phosphate utilization compared with the G6PD-B enzyme (considered the normal phenotype). It has been suggested that the region encompassing the Coimbra and Mediterranean variants is spatially close and involved in the enzyme’s interactions with its substrate. Both variants are classified as Class II.6

This study reports for the first time the G6PD Nilgiri in Italy, and we were able to definitively classify this variant as Class II based on both on the residual enzymatic activity and the clinical manifestations of the patient and his family members. In addition, this case provides further evidence on the prevalence and distribution of the G6PD Nilgiri in different population groups, confirming the high genetic heterogeneity of the G6PD deficiency in Italy,7 although owing to a de novo occurrence. In this context, we underscore c.592C>A (p.Arg198Ser)10 as an additional variant, indicating that the mutation at the same nucleotide site results from a de novo event; this consideration may suggest that this region is prone to such molecular events.

In conclusion, we highlight four main points: (a) a clinical picture of the patient and residual enzymatic activity are of primary importance for the definitive classification of each G6PD variant; (b) sequencing of the entire G6PD gene is mandatory especially in those patients from peculiar Italian regions; (c) the use of next-generation sequencing in G6PD-deficient subjects to diagnose common, rare and novel G6PD variants is now suggested,11 and finally, (d) G6PD is confirmed to be a gene prone to de novo events, as reported.12–17

HGV DATABASE
The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.1738.

COMPETING INTERESTS
The authors declare no conflict of interest.

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