Molecular and clinical analysis of haemoglobin Lepore in Campania, a region of Southern Italy

Paolo Ricchi, Massimiliano Ammirabile, Anna Spasiano, Silvia Costantini, Tiziana Di Matola, Patrizia Cinque, Caterina Saporito, Aldo Filosa and Leonilde Pagano

aUOSD Malattie rare del globulo rosso, AORN A. Cardarelli, Naples, Italy; bUOC Clinical Pathology, AORN Monaldi-Cotugno-CTO, Naples, Italy; cLaboratory of clinical chemistry and microbiology, Fondazione IRCCS Ca’Grande Ospedale Maggiore Policlinico, Milan, Italy

ABSTRACT
Objective: To date in Italy, there is paucity on data about the prevalence, clinical and haematological features of patients carrying the haemoglobin (Hb) Lepore variant in homozygous or in association with other haemoglobinopathies.

Methods: Here we report the results of a retrospective analysis on 33 patients from Campania, a region of Southern Italy, historically followed at ‘UOSD Malattie Rare del Globulo Rosso’ of Cardarelli hospital, Naples, Italy.

Results: We described 33 patients carrying the Hb Lepore variant: 21 compound heterozygotes with a common thalassaemia allele, six patients with homozygous state for Hb Lepore, five patients with Hb Lepore/Hb S and one patient with Hb Lepore/Hb Neapolis were identified. All individuals carried haplotype I or V.

Discussion: These thalassaemic patients showed different phenotypes ranging from severe disease with early blood transfusion dependency to moderate form of thalassaemia intermedia. In most cases, thalassaemia mutation type determined the severity of the disease.

Conclusion: A great variability of clinical phenotype among the same genotypes was also observed suggesting the presence of unknown genetic modifiers acting in combination with Hb Lepore.

KEYWORDS
Thalassaemia; haemoglobin; variant; haplotype; genotype; phenotype; haematopoiesis

Introduction

Haemoglobin (Hb) Lepore is a structurally abnormal Hb in which the abnormal globin results from an unequal crossover between the δ and β genes, because of a misalignment of homologous chromosomes during meiosis [1]. The mutant genes result from unequal recombination leading a 7.4Kb deletion [2]. Three structurally distinct types of Lepore Hbs have been described where the transition from δ to β sequences occurs at different positions along the polypeptide chain. Hb Lepore–Boston–Washington (δ87-β116) and Hb Lepore Baltimore (δ50-β86) are the two most common molecular types while the Hb Lepore Hollandia (δ22-β50) represents a very rare variant [3]. Hb Lepore–Boston [4] is virtually the only Hb Lepore found in Italy [5], being frequent in southern populations, chiefly in those of Campania region (Napoli and Caserta) [6] and in some well-defined areas of Calabria, Puglia and Sicilia region. For this reason, in several of these Italian regions, the combination of Hb Lepore with β-globin mutation may be a common event either in the homozygous or compound heterozygous condition. We describe main molecular and haematological features concerning 33 individuals with Hb Lepore at first diagnosis and during their follow up, if available, at our Centre.

Methods

This was a retrospective study and all medical records of our patients with Hb Lepore and followed routinely at our unit from first diagnosis to December 2015 were reviewed carefully. Thirty-three adult patients with Hb Lepore belonging to unrelated families from Campania were evaluated. While data at first diagnosis were available for all but one patient, eight were lost during the follow up period: one patient underwent bone marrow transplantation, two patients died and five patients referred to another thalassaemic centre and then data were not available for our evaluation.

The study was approved by the Ethic Committee of A.O.R.N. Cardarelli Hospital, and informed consent was obtained from all patients. Haematological parameters were measured by standard methods. The different Hb components were separated and quantitated by cation exchange high performance liquid chromatography using VARIANT-II system (Bio-Rad Laboratories, Richmond, CA, U.S.A.). Electrophoresis on cellulose acetate from Helena was performed in Tris-EDTA-Borate (pH 8.6); electrophoresis on agar gel from Beckman was performed at pH 6.

DNA was extracted from peripheral blood leukocytes by salting out extraction methods [7]. Thalassaemia mutations were analysed by amplification...
refractory mutation system [8], or reverse dot blot or by direct DNA sequencing as already described [9]. Specific primers were used to identify Hb Lepore–Boston–Washington according to Fioretti [10]. The RFPLs HindIII/Gy, HincII/Ay, HincII/µβ, HincII/ ψβ, AvaII/Band BamH133 analysis was carried out on PCR-amplified DNA fragments [11,12]. Haplotype associated with the mutations were determined through family-linkage analysis and classified according to Orkin [13].

Statistics

Results for descriptive statistics were expressed as mean ± standard deviation (M ± SD). The Fisher’s exact test was used to compare the incidence of different parameters between the two groups of patients. Student’s t test was used to compare the difference in parametric data. A p-value below 0.05 was considered as significant. A statistical software package (MedCalc”, version 10.2.0.0) was used.

Results

Table 1 lists the laboratory findings at diagnosis of all but one patient with Hb Lepore; all patients were from main provinces of Campania region: 55% from the province of Caserta, 26% from the province of Naples and 10% from the province of Benevento (data not shown). There were several groups of patients with identical genetic defect: 11 compound heterozygotes for CD 39(C→T)/ Hb Lepore, six compound heterozygotes for IVS 1–110 (G→A)/Hb Lepore, six with homozygous state for Hb Lepore, two compound heterozygotes for IVS 1–6 (T→C)/Hb Lepore, two compound heterozygotes for −87/Hb Lepore and five compound heterozygotes for HbS/Hb Lepore. Hb Lepore–Boston was the unique type of Hb Lepore in all patients, associated with haplotype I in 22/27 (81%) chromosomes and with haplotype V in 5/27 (19%). As shown in Table 1, all patients presented at diagnosis increased level of HbF and a percentage of liver iron overload.

In Table 2 several clinical and haematological characteristics of all regularly transfused patients are detailed. All had a considerable degree of anaemia and transfusion dependency; however, even if with a wide spectrum among patients with identical genotype, compound heterozygosity CD39(C→T)/Hb Lepore and compound heterozygosity IVS 1–110 (G→A)/Hb Lepore, behaved more like thalassaemia major (TM) and thalassaemia intermedia (TI) transfusion-dependent patients, respectively. In fact, compound heterozygotes for CD39(C→T) had a tendency to have lower age at first transfusion, higher transfusion iron intake, number of nucleated red blood cells (NRBC), level of soluble transferrin receptor factor (sTFR) and lower presence of extramedullary haematopoiesis (EMH) as compared to those heterozygotes for IVS 1–110 (G→A); however, the sample size evaluated was inadequate to demonstrate statistical significance. Nevertheless, a case (n.11) of spinal cord compression with severe neurological abnormalities was detected among compound heterozygotes CD39 (C→T)/Hb Lepore, as previously described [14]. The same patient died of hepatocellular carcinoma in 2008. There was only one patient (n.9) among compound heterozygotes for CD39(C→T)/Hb Lepore, with coinherence of mutations in the gene encoding the alpha globin chain (3.7Kb) but its clinical picture was not particularly ameliorated.

Similar characteristic of variability was observed among five out of the six patients with homozygous state for Hb Lepore. Despite generally requiring regular transfusion from early in life, the blood consumption was slightly lower, but not statistically significant, as compared to those observed in compound heterozygosity IVS 1–110(G→A)/Hb Lepore. The remaining patient with Hb Lepore/Hb Lepore needed monthly blood transfusion in childhood, before splenectomy, but thereafter he was able to spontaneously maintain Hb around 8.5 g/dl, showing therefore a clinical picture of TI; subsequently, because of the presence of leg ulcer, he was again started on regular transfusion therapy. The patient died in 2009 of viral gastroenteritis.

Current iron-overload parameters were available for only 13 patients. All but two patients (one with liver cirrhosis, n.14; one with recent pregnancy, n.20) had normal cardiac T2* value (>20 ms) but broad spectrum of liver iron overload.

Table 3 shows several clinical characteristic of patients with Hb Lepore in combination with a β3 allele or with another Hb variant. The patients with IVS-1.6(T→C) or -87 showed a typical mild TI phenotype with haemoglobin level ranging from 8.8 to 11 g/dl; they had never been transfused or had only been transfused sporadically during infections, pregnancy or surgery. They had elevated levels of sTFR and NRBC and were frequently (75%) affected by EMH (50%) [15]. The two patients with the genetic compound IVS 1–6(T→C)/Lepore belonged to the same family and that one (n.27) carrying also alpha globin chain (3.7Kb) was in good clinical condition but had a very large spleen.

Two of the patients (n. 29 and n.32) with HbS disorders had quite a severe disease and painful events of vaso-occlusion. One (n.29) had relatively high haemoglobin level but, because of recurrent crisis of splenic sequestration underwent splenectomy; however, she received occasional blood transfusions during infection. Patient n.33 had a mild disease
without major complications but with a conspicuous spleen enlargement reduced level of Hb and transfusion dependency in young adult age too; she died in 2011 of brain stroke. The other two patients (n.30 and n.31) had a mild disease with spleen enlargement but without transfusion dependency and few painful events. The only patient (n.28) with Hb Lepore/Hb Neapolis behaves as a very mild TI and was already described [16,17].

**Discussion**

Epidemiological data in Italy are scarce: apart from previous reports from Sicily [18], even more inadequate is the knowledge about the presence, the type and the relevance of Hb Lepore in Southern Italy, a region with high prevalence of haemoglobinopathies. On the other hand, very little is known about clinical phenotype of Hb Lepore homozygotes and previous description of patients from our region were restricted to children or to young adult patients [6,19,20]. Small size studies have already described the clinical features of Hb Lepore interactions with β/α thalassaemia alleles and with other haemoglobinopathies [21–23]. In this study we reviewed, from diagnosis to current clinical manifestations, all data of our 33 patients with Hb Lepore, to identify their disease severity and phenotypes as adult patients.

Similarly to what was found among patients with HbE/β-thalassaemia, our data suggest that the type of thalassaemia mutation determined the degree of clinical severity in compound heterozygotes. To construct a type of disease severity score, we used several of the parameters previously validated for the HbE/β-thalassaemia disease such as the age at first transfusion, the requirement for transfusion and the level of sTfR as surrogate marker for erytron expansion as most patients had previously underwent splenectomy [24]. Our data confirmed that patients with the more severe phenotypes were compound heterozygotes for IVS 1–110(G→A) and CD39(C→T), being those for CD39(C→T) connected to a more TM-like phenotype [25]; on the other hand, those compound heterozygotes for a β+ allele such as IVS-1.6(T→C) and -87, showed typical characteristics of TI. All compound heterozygotes were characterised by the tendency to develop EMH, particularly evident in those for β+ and IVS 1–110(G→A) mutation and this may account for the observed pattern of iron overload [26].

Historical authors from our region previously discussed the relative mildness of some of cases with Hb Lepore/Hb Neapolis behaves as a very mild TI and was already described [16,17].

### Table 1. Laboratory findings at diagnosis.

| Age at first diagnosis | SEX | Second allele associated | Aplotype Beta/HbS | Aplotype Lepore | Hb F | Hb Lepore | Variant | Fenotype |
|------------------------|-----|--------------------------|-------------------|----------------|------|-----------|---------|----------|
| 1a 10m M               |     | CD39                     | II                | I              | 87   | 11.0      | TM      |
| 2 5m F                 |     | CD39                     | II                | N.A.           | n.a. | n.a.      | TM      |
| 3 12m F               |     | CD39                     | IX                | I              | 86   | 12.0      | TM      |
| 4a n.a. M             |     | CD39                     | II                | V              | 88   | 10.0      | TM      |
| 5 1y 5m F             |     | CD39                     | N.A.              | N.A.           | 89   | 9.0       | TM      |
| 6 6m F                |     | CD39                     | II                | I              | 90   | 8.0       | TM      |
| 7 1y 5m F             |     | CD39                     | II                | I              | 88   | 9.0       | TM      |
| 8 6m F               |     | CD39                     | N.A.              | N.A.           | 87   | 11.0      | TM      |
| 9 5y M               |     | CD39                     | II                | I              | 89   | 8.8       | TM      |
| 10+ n.a. M           |     | CD39                     | II                | I              | 89   | 9.0       | TM      |
| 11– 3y M             |     | CD39                     | II                | I              | 87   | 10.0      | TM      |
| 12 4y F              |     | IVSI-110                 | I                 | I              | 80   | 10.0      | TM      |
| 13 5y F              |     | IVSI-110                 | N.A.              | N.A.           | 88   | 9.8       | TM      |
| 14 7m F              |     | IVSI-110                 | N.A.              | N.A.           | 88   | 9.7       | TM      |
| 15 4y M              |     | IVSI-110                 | N.A.              | N.A.           | 65   | 8.0       | TM      |
| 16 2y 7m F           |     | IVSI-110                 | N.A.              | N.A.           | 85   | 7.8       | TM      |
| 17+ n.a. M           |     | IVSI-110                 | N.A.              | N.A.           | 87   | 11.0      | TM      |
| 18 1y 6m M           |     | Hb Lepore                | –                 | I/I            | 88   | 12.0      | TM      |
| 19 1y M              |     | Hb Lepore                | –                 | I/I            | 84   | 15.0      | TM      |
| 20 11m F             |     | Hb Lepore                | –                 | N.A.           | 85   | 14.0      | TM      |
| 21 1y 6m M           |     | Hb Lepore                | –                 | V/V            | 89   | 10.0      | TM      |
| 22+ n.a. M           |     | Hb Lepore                | –                 | V/V            | 87   | 13.0      | TM      |
| 23– 3y M             |     | Hb Lepore                | –                 | I/I            | 85   | 14.0      | TI      |
| 24 3y F              |     | –87                      | VIII              | I              | 73   | 10.3      | TI      |
| 25 10y F             |     | –87                      | VIII              | I              | 89   | 9.0       | TI      |
| 26 1y F              |     | IVSI-6                   | VI                | I              | 51   | 9.0       | –TI     |
| 27 5y M              |     | IVSI-6                   | VI                | I              | 48   | 12.0      | –TI     |
| 28+ – M              |     | Hb Neapolis              | V                 | I              | 18   | 9.1       | 63% Ti  |
| 29 3y F              |     | Hb S                     | Benin             | I              | 11   | 11.0      | 73% Hb S d |
| 30 50y M             |     | Hb S                     | Benin             | –               | 20.2 | 10.0      | 68% Hb S d |
| 31 47y M             |     | Hb S                     | Benin             | –               | 16.7 | 12.4      | 69% Hb S d |
| 32 11y F             |     | Hb S                     | Benin             | I              | 18   | 10.0      | 65% Hb S d |
| 33– 20y F            |     | Hb S                     | Benin             | I              | 13   | 10.0      | 72% Hb S d |

*Lost at observation; +Bone marrow transplantation; – deceased (n.11, in 2008; n.23, in 2009; n.33, in 2011) N.A.: not available; HbS d: HbS disorders
Table 2. Main clinical and laboratory parameters of transfused patients.

|   | Age (y) | SEX | Age at first transfusion | Hb pre (gr/dl) | sTfR (mg/ml) | HCV RNA | NRBC (x10^9/l) | L.I.C. | T2* (ms) | DEXA | Hypot | Hypog | Cholel | DM |
|---|---------|-----|--------------------------|---------------|-------------|---------|----------------|-------|----------|------|-------|-------|--------|----|
| 1*| 31.2    | M   | 10 m                     | N.A.          | N.A.        | +       | N.A.           | N.A.  | 380      | 14   | 35    |       |        |    |
| 2 | 44.6    | F   | 5 m                      | CD39          | 1972        | 10.5    | 0.26           | 1.6   | +        | -    | 380   | 14    | 35     |    |
| 3 | 39.7    | F   | 2y                       | CD39          | 1976        | 9.6     | 0.33           | 3.1   | +        | +    | 2800  | 1.8   | 42     |    |
| 4*| 42.3    | M   | N.A.                     | CD39          | 1972        | 10.5    | 0.26           | 2.8   | +        | -    | 1500  | 6.3   | 20     |    |
| 5 | 33.9    | F   | 1y 5m                    | CD39          | 1985        | 9.5     | 0.23           | 3.1   | +        | -    | 150   | 1.8   | 42     |    |
| 6 | 35.7    | F   | 6 m                      | CD39          | 1990        | 9.6     | 0.53           | 2.9   | +        | -    | 100   | 7     | 45     |    |
| 7 | 37.6    | F   | 1y 5m                    | CD39          | 1991        | 9.6     | 0.30           | 2.6   | +        | -    | 500   | 2.5   | 44     |    |
| 8 | 37.4    | F   | 8 m                      | CD39          | 1992        | 9.6     | 0.32           | 4.6   | -        | -    | 1000  | N.A.  | N.A.   |    |
| 9 | 23.4    | F   | 5 y                      | CD39          | 2002        | 9.6     | 0.32           | 4.6   | -        | -    | 4000  | N.A.  | N.A.   |    |
| 10*| N.A.    | M   | N.A.                     | CD39          | 2002        | 9.6     | 0.32           | 4.6   | -        | -    | 4000  | N.A.  | N.A.   |    |
| 11| 39.8    | M   | 3 y                      | CD39          | 2003        | 10.0    | N.A.           | 6.6   | +        | +    | 4000  | N.A.  | N.A.   |    |
| 12| 45.2    | F   | 4 y                      | IVSI-110      | 2005        | 9.6     | 0.14           | 5.6   | -        | +    | 1500  | 1.4   | 32     |    |
| 13| 48.1    | F   | 5 y                      | IVSI-110      | 1971        | 8.7     | 0.24           | 6.7   | -        | -    | 800   | N.A.  | N.A.   |    |
| 14| 35.5    | F   | 7 m                      | IVSI-110      | 1992        | 8.9     | 0.20           | 2.4   | +        | -    | 1500  | 13    | 5      |    |
| 15| 34.7    | M   | 4 y                      | IVSI-110      | 2002        | 9.4     | 0.19           | 5.0   | +        | -    | 5000  | 1.7   | 39     |    |
| 16| 32.8    | M   | 2y 7m                    | IVSI-110      | 1996        | 9.6     | 0.20           | 7.5   | +        | +    | 150   | 1.4   | 42     |    |
| 17*| N.A.    | M   | N.A.                     | IVSI-110      | 2003        | N.A.    | N.A.           | N.A.  | N.A.     | N.A. | N.A.  | N.A.  | N.A.   |    |
| 18| 35.6    | M   | 1y 6m                    | Hb Lepore      | 1994        | 9.9     | 0.28           | 6.6   | +        | -    | 3000  | 7.7   | 37     |    |
| 19| 36.7    | M   | 1y 6m                    | Hb Lepore      | 1990        | 9.6     | 0.20           | 6.0   | -        | -    | 12000 | 1.2   | 42     |    |
| 20| 30.2    | F   | 11 m                     | Hb Lepore      | 2001        | 9.5     | 0.31           | 3.2   | -        | -    | 1500  | 7.6   | 15     |    |
| 21| 38.8    | M   | 1y 6m                    | Hb Lepore      | 1987        | 9.7     | 0.18           | 5.5   | -        | -    | 320   | 1.6   | 49     |    |
| 22*| 35.6    | M   | N.A.                     | Hb Lepore      | 1981        | N.A.    | N.A.           | N.A.  | N.A.     | N.A. | N.A.  | N.A.  | N.A.   |    |
| 23| 74.2    | F   | 20 y                     | Hb S           | 2000        | 9.0     | 0.30           | 2.5   | +        | -    | 250   | N.A.  | N.A.   |    |

Hb pre: pre-transfusion haemoglobin level; sTfR: serum transferrin receptor; EMH: extramedullary haematopoiesis; NRBC: nucleated red blood cell; L.I.C.: liver iron concentration; DEXA: dual-energy X-ray absorptiometry; Hypot: hypothyroidism; Hypog: hypogonadism; Cholel: cholelithiasis; DM: diabetes mellitus; N.A.: not available.

*Lost at observation; +Bone marrow transplantation; – deceased (n.11, in 2008; n.33, in 2011)
regimen, they maintain an increased level of soluble transferrin receptor and this is in line with the major expansion of the erythroid bone marrow, the high level of soluble transferrin receptor and of erythropoietin production in response to the anaemia, as previously reported [28].

Data on patients with HbS disorders partially agree with those reported in previous studies in Sicilian patients and again sustain previous observation that, a marked difference in clinical severity may be found also in these syndromes [29,30]. Data on haplotype analyses showed that haplotype I was the most frequent in our population, followed by haplotype V; this remark is in line with previous studies from Campagna [31,32], supporting the general observation that, in regional populations, a few mutations are prevalent.

Currently, there is growing emphasis to accurately score and predict the haematological severity of thalassaemic patients using genetic markers as it could be helpful in patient management and in providing accurate genetic counselling [33]. Our data, although highlighting useful information for patients with such Hb variant and indicating that they seemed not to have specific characteristics and management peculiarities with respect to patients with TM or TI, reinforce previous findings that they may have an unpredictable phenotypic severity. Further studies are needed to assess the presence of unknown genetic modifiers responsible for such a phenotypic variability among individuals carrying Hb Lepore alone and in combination with other beta globin defects.

### Notes on contributors

**Paolo Ricchi** is an assistant in the Department of Oncology in the "UOSD Malattie rare del globulo rosso" Unit at Antonio Cardarelli Hospital in Naples since 2005. He has a specialization in oncology and in haematology and a PhD in molecular and cellular biology and pathology. His research interests includes combined modality therapy, thalassaemia and haemoglobinopathies. He is first author and co-author of more than 45 papers published in international journals, and he is sub-investigator in several clinical trials. He is also a fellow in the "UOSD Malattie rare del globulo rosso" Unit at A.O.U. Cardarelli Hospital in Naples. His main research fields include haematology, drug resistance and genetic and biological factors. He is a member of several national and international associations.

**Massimiliano Ammirabile** is a biologist director of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (laboratory of clinical chemistry and microbiology) in Milan. He was a fellow in the "UOSD Malattie rare del globulo rosso" Unit at Antonio Cardarelli Hospital in Naples. His research interests includes combined modality therapy, thalassaemia and haemoglobinopathies. He is first author and co-author of more than 45 papers published in international journals, and he is sub-investigator in several clinical trials. He is also a fellow in the "UOSD Malattie rare del globulo rosso" Unit at A.O.U. Cardarelli Hospital in Naples. His main research fields include haematology, drug resistance and genetic and biological factors. He is a member of several national and international associations.

**Anna Spasiano** is an assistant in the Department of Oncology in the "UOSD Malattie rare del globulo rosso" Unit at Antonio Cardarelli Hospital in Naples since 2005. She has a specialization in oncology and in haematology and a PhD in molecular and cellular biology and pathology. Her research interests includes combined modality therapy, thalassaemia and haemoglobinopathies. She is first author and co-author of more than 45 papers published in international journals, and she is sub-investigator in several clinical trials. She is also a fellow in the "UOSD Malattie rare del globulo rosso" Unit at A.O.U. Cardarelli Hospital in Naples. Her main research fields include haematology, drug resistance and genetic and biological factors. She is a member of several national and international associations.

### Disclosure statement

No potential conflict of interest was reported by the authors.

### Table 3. Main clinical and laboratory parameters of non-transfused patients.

| Age | SEX | Age at first transfusion | Second allele associated | If splenectomy or date | Hb (g/dl) | sTfR (mg/ml) | HCV RNA | EMH+ | NRBC (>10^11/l) | DEXA | Hypot | Hypog | Cholel | DM | CHELATION |
|-----|-----|-------------------------|--------------------------|-----------------------|----------|-------------|--------|------|----------------|------|-------|-------|--------|----|----------|
| 23- | 41,9 | M | 3y | Hb Lepore | 1974 | 8.3 | 17.0 | + | + | + | + | 75000 | Osteoporosis | + | + | + | DFX |
| 24  | 43,7 | F | – | – | 1986 | 11.0 | 11.4 | + | + | 29540 | Osteopenia | – | + | – | DFO |
| 25  | 66,3 | F | – | – | 225 mm | 10.0 | 10.0 | + | + | 25 | Osteopenia | – | – | + | – |
| 26  | 33,0 | F | 23y | IVS1-6 | 2002 + A.S. | 9.8 | 10.5 | – | – | 4685 | Normal | + | – | – | DFO |
| 27  | 36,5 | M | – | – | 220 mm | 9.9 | 10.9 | 150 | Osteopenia | – | – | + | – |
| 28  | 21,4 | M | N.A. | Hb Neapolis | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. |
| 29  | 27,3 | F | 13y | Hb S | 2010 | 10 | 2.8 | – | – | 1000 | Osteopenia | – | – | – | – |
| 30  | 59,9 | F | – | – | 188 mm + A.S. | 12 | 4.5 | – | – | 150 | N.A. | – | – | – | – |
| 31  | 53,0 | M | – | – | 195 mm | 9.9 | 5.0 | – | – | 130 | Normal | – | – | – | – |
| 32  | 41,5 | F | 11y | Hb S | 130 mm | 3.7 | 2 | – | – | 150 | Normal | – | – | – | – |

sTfR: serum transferrin receptor; EMH: extramedullary haematopoiesis; NRBC: Nucleated Red Blood Cell; L.I.C.: Liver Iron Concentration; DEXA: dual-energy X-ray absorptiometry; Hypot.: hypothyroidism; Hypog.: hypogonadism; Cholel.: cholelithiasis; DM: Diabetes Mellitus; A.S. = Accessory spleen; DFX: Deferasirox; DFO: desferrioxamine; N.A.: Not Available

*Lost at observation; deceased (n.23, in 2009)
a specialization in haematology and she is a principal investigator in Myocardial Iron Overload in Thalassaemia (MIOT) project. She is co-author of several papers published in international journals.

Silvia Costantini is an assistant in the Department of Oncohaematology in the “UOSD Malattie rare del globulo rosso” Unit at Antonio Cardarelli Hospital in Naples since 2005. She has a specialization in haematology.

Tiziana Di Matola is an assistant at UOC Patologia Clinica bUOC Clinical Pathology, AORN Monaldi-Cotugno-CTO, Naples, Italy since 2006. She has a specialization in Clinical pathology and a PhD in molecular oncology. He is first author and co-author of more than 35 papers in international journals.

Patrizia Cinque is an assistant in the Department of Oncohaematology in the “UOSD Malattie rare del globulo rosso” Unit at Antonio Cardarelli Hospital in Naples since 1995.

Caterina Saporito is a biologist and she has a fellow in the Department of Oncohaematology, Genetics and pathological anatomy of Antonio Cardarelli Hospital in Naples.

Aldo Filosa is Director of “UOSD Malattie rare del globulo rosso” Unit at Antonio Cardarelli Hospital in Naples from 2011. He has specialization in pediatrics, neonatology and haematology. He was Principal Investigator in several International Trials. He is first author and co-author of more than 50 papers in international journals.

Leonilde Pagano is a biologist specialized in medical genetics; she directed the genetics laboratory of haemoglobinopathies in the department of oncohaematology of Cardarelli hospital in Naples. She identified two new haemoglobin (Hb Neapolis and Hb Cardarelli), and is first author and co-author of 50 publications in international journals.

References

[1] Ribeiro ML, Cunha E, Gonçalves P, et al. Hb Lepore-Baltimore (G6Leu, G84Thr) and Hb Lepore-Washington-Boston (G72Cys, N87I–8) in Central Portugal and Spanish Alta Extremadura. Hum Genet. 1997;99:669–673.
[2] Flavell RA, Kooter JM, De Boer E, et al. Analysis of the beta-gamma-globin gene loci in normal and Hb Lepore DNA: direct determination of gene linkage and inter-gene distance. Cell. 1978;15:25–41.
[3] Baglioni C. The fusion of two peptide chains in hemoglobin Lepore and its interpretation as a genetic deletion. Proc Natl Acad Sci USA. 1962;48:1880–1886.
[4] Baglioni C. Abnormal human hemoglobins. X. a study of hemoglobin Lepore Boston. Biochim Biophys Acta. 1965;97:37–46.
[5] Marinucci M, Mavilio F, Massa A, et al. Haemoglobin Lepore trait: haematological and structural studies on the Italian population. Br J Haematol. 1979;42:557–565.
[6] Quattrin N, Bianchi P, Cimino R, et al. Study on nine families with haemoglobin Lepore in Campania: Hb Lepore trait, heterozygosity for Hb Lepore and beta-thalassaemia, homozygosity for Hb Lepore. Acta Haematol. 1967;37:266–275.
[7] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1213.
[8] Old JM, Varawalla NY, Weatherall DJ. Rapid detection and prenatal diagnosis of beta-thalassaemia: studies in Indian and Cypriot populations in the UK. Lancet. 1990;336:834–837.
[9] Ricchi P, Ammirabile M, Spasiano A, et al. Hypocholesterolemia in adult patients with thalassemia: a link with the severity of genotype in thalassemia intermedia patients. Eur J Haematol. 2009;82:219–222.
[10] Fioretti G, De Angioletti M, Masciiangelo F, et al. Origin heterogeneity of Hb Lepore-Boston gene in Italy. Am J Hum Genet. 1992;50:781–786.
[11] Kulozik AE, Lyons J, Kohne E, et al. Rapid and non-radioactive prenatal diagnosis of beta thalassaemia and sickle cell disease: application of the Polymerase Chain Reaction (PCR). Br J Haematol. 1988;70:455–458.
[12] Fullerton SM, Clegg JB. Hp, HindII, and BamHI polymorphisms of the human beta-globin gene could be detected by a single polymerase chain reaction amplification product. Am J Hematol. 1994;47:256.
[13] Orkin SH, Kazazian, HH Jr, Antonarakis SE, et al. Linkage of beta-thalassaemia mutations and beta-globin gene polymorphisms with DNA polymorphisms in human beta-globin gene cluster. Nature. 1982;296:627–631.
[14] Ricchi P, Ammirabile M, Spasiano A, et al. Extramedullary haematopoiesis correlates with genotype and absence of cardiac iron overload in polytransfused adults with thalassaemia. Blood Transfus. 2014;1:124–130.
[15] Ricchi P, Ammirabile M, Costantini S, et al. A useful relationship between the presence of extramedullary erythropoiesis and the level of the soluble form of the transferrin receptor in a large cohort of adult patients with thalassaemia intermedia: a prospective study. Ann Hematol. 2012;91:905–909.
[16] Pagano L, Carbone V, Fioretti G, et al. Compound heterozygosity for Hb Lepore-Boston and Hb Neapolis (Dhonburi) [beta 126(H4)Val-->Gly] in a patient from Naples, Italy. Hemoglobin. 1997;21:1–15.
[17] Pagano L, Viola A, Fioretti G, et al. Neapolis (CD 126 beta+ GGT->GGG): a result of a screening in Campania, a region in Southern Italy. Haematologica. 2007;92:990–999.
[18] Schiliro G, Di Gregorio F, Samperi P, et al. Genetic heterogeneity of beta-thalassaemia in southeast Sicily. Am J Hematol. 1995;48:5–11.
[19] Ramirez F, Mears JG, Nudel U, et al. Defects in DNA and globin messenger RNA in homozygotes for hemoglobin Lepore. J Clin Invest. 1979;63:736–742.
[20] Quattrin N, Venturto V. Hemoglobin Lepore: its significance for thalassaemia and clinical manifestations. Blut. 1974;28:326–336.
[21] Chaibunruang A, Srivorakun H, Fucharoen S, et al. Complex interaction of hemoglobin Lepore (deltabeta hybrid hemoglobin) with various hemoglobinopathies: a molecular and hematological characteristics and differential diagnosis. Blood Cells Mol Dis. 2010;44:140–145.
[22] Viprikastra V, Pung-Ambritt P, Suwanthan L. Complex interactions of delta beta hybrid hemoglobin with various hemoglobinopathies: a molecular and hematological characteristics and differential diagnosis. Blood Cells Mol Dis. 2010;44:140–145.
[23] Sharma V, Choudhry VP, Saxena R. Association of HbE with Hb Lepore and a triplication in a Bengali family. Clin Chim Acta. 2006;359:175–177.
[24] Sririchai O, Makarasara W, Munkongdee T, et al. A scoring system for the classification of beta-thalassaemia/Hb E disease severity. Am J Hematol. 2008;83:482–484.
[25] Efremov DG, Efremov GD, Zisovski N, et al. Variation in the level of the soluble form of the transferrin receptor in a large cohort of adult patients with thalassaemia intermedia: a prospective study. Ann Hematol. 2012;91:905–909.
[26] Ricchi P, Meloni A, Spasiano A, et al. Extramedullary haematopoiesis is associated with lower cardiac iron
loading in chronically transfused thalassemia patients. Am J Hematol. 2015;90:1008–1012.

[27] Quattrin N, Luzzatto L, Quattrin S Jr. New clinical and biochemical findings from 235 patients with hemoglobin Lepore. Ann N Y Acad Sci. 1980;344:364–374.

[28] Olivieri NF, Rees DC, Ginder GD, et al. Treatment of thalassaemia major with phenylbutyrate and hydroxyurea. Lancet. 1997;350:491–492.

[29] Mirabile E, Testa R, Consalvo C, et al. Association of Hb S/Hb lepore and delta beta-thalassemia/Hb lepore in Sicilian patients: review of the presence of Hb lepore in Sicily. Eur J Haematol. 1995;55:126–130.

[30] Seward DP, Ware RE, Kinney TR. Hemoglobin sickle–Lepore: report of two siblings and review of the literature. Am J Hematol. 1993;44:192–195.

[31] Ferrari M, Matarese SM, Francese M, et al. Hematological and molecular analysis of beta-thalassemia and Hb Lepore in Campania, Italy. Hemoglobin. 2001;25:29–34.

[32] Camaschella C, Serra A, Bertero MT, et al. Molecular characterization of Italian chromosomes carrying the Lepore Boston gene. Acta Haematol. 1989;81:136–139.

[33] Denjou F, Francavilla M, Anni F, et al. A genetic score for the prediction of beta-thalassemia severity. Haematologica. 2015;100:452–457.