
**Adult-onset beta-thalassaemia intermedia caused by a 5-Mb somatic clonal segmental deletion in haemopoietic stem cells involving the β-globin locus**

Beta-thalassaemia major is amongst the most severe common monogenic diseases worldwide and, with few exceptions, is due to inheritance two defective beta-globin (HBB) genes in a Mendelian fashion. Heterozygous individuals, inheriting a single defective allele, are usually clinically asymptomatic. This study describes the molecular genetic investigation in a patient with an adult-onset and progressively worsening anaemia, culminating in a condition comparable with transfusion-dependent beta-thalassaemia. The female patient, born in 1962, self-reported as an asymptomatic beta-thalassaemia heterozygote in infancy, childhood and adolescence. In 1991 she reported having anaemia and moderate splenomegaly following the uncomplicated delivery of her second child. In 1996, she presented with severe anaemia [haemoglobin (Hb) 49 g/l, mean cell haemoglobin 23 pg, mean cell volume 76 fl], raised HbA2 (4.5%) and HbF (12%), with nucleated red blood cells (11/100 white cells) and reticulocytosis (5%). DNA analysis of peripheral leucocytes identified heterozygosity for a single beta-thalassaemia mutation HBBc.315+1G>A (IVSII-1G>A) in the patient and her father (the latter with haematology consistent with heterozygous beta-thalassaemia). Her mother had normal haematology and HBB genes. Variants in the alpha-globin genes and gene-cluster were excluded in all family members. All initial haematology and DNA studies were performed by standard methods (Traeger-Synodinos et al, 2015): ferritin, folic acid and vitamin B12 levels were within normal range, with mild indirect hyperbilirubinemia (30.8 µmol/l), and a negative Coombs test (direct and indirect). Bone marrow biopsy found marked erythroid hyperplasia, without myelodysplasia. Paroxysmal nocturnal haemoglobinuria was excluded (Ham test and flow cytometry). A 51Cr-labelled red blood cell (RBC) survival study demonstrated a RBC half-life of 11.5 days, without splenic sequestration. The patient began regular transfusions (3–4 units of packed RBCs/month) when aged 34 years; her transfusion requirements then remained unchanged, following a splenectomy in 1997, and a trial with weekly subcutaneous recombinant erythropoietin. In 2007 a portal vein thrombosis was treated with heparin, followed by long-term warfarin. Hypercoagulability evaluation was negative, and the thrombosis was attributed to haemolytic anaemia, along with a markedly elevated platelet count secondary to splenectomy. Anagrelide was initiated to reduce the number of platelets. The patient was managed with folic acid replacement and iron chelation therapy (Deferasirox), to maintain ferritin around 1000 µg/l. Magnetic resonance imaging excluded clinically significant liver and heart iron levels.

In 2015, repeat DNA analysis by Sanger sequencing was performed on leucocyte DNA, along with DNA from oral mucosal epithelia and hair follicles. Although the IVSII-1G>A mutation was confirmed in leucocyte DNA, the ratio of normal (G) versus variant (A) was skewed, with extremely low levels of normal allele. DNA from oral mucosal epithelium and hair follicles showed classic heterozygosity for IVSII-1G>A (Fig 1A, 1).
Following this result, peripheral blood DNA was analysed for genomic copy number variations (CNVs) using the Affymetrix CytoScan HD Array and Chromosome Analysis Suite (ChAS, version 3.2) software (Thermo Fisher Scientific, Santa Clara, CA, USA) (Gijsbers et al., 2009). A heterozygous deletion of 4.97 Mb (hg19/GRCh37: 1, 313, 792–6, 287, 277) was identified on chromosome (chr) 11 (11p15.5p15.4) showing a skewed ratio of 90% deletion vs. 10% normal (Fig 1). CNV analysis on peripheral blood DNA from the mother (the father is now deceased), excluded any deletions in chr11p15.5p15.4. The array results from the patient and her mother were evaluated to determine the parental origin of the deletion. The 4.97 Mb deletion region included 1608 single nucleotide polymorphisms (SNPs). Of the 1227 SNPs homozygous in the propositus (informative), 376 (30%) were absent in the mother, indicating that the 4.97 Mb deletion involves the maternally inherited allele, which harbours the normal HBB gene (the region flanking the 4.97 Mb deletion showed biallelic distribution of SNPs). Thus, the DNA in the patient’s peripheral blood is hemizygous for the maternally inherited beta-thalassaemia mutation.

The typical heterozygosity (50%) for the beta-thalassaemia mutation in DNA from the patient’s hair follicles and oral mucosal cells suggests a somatic origin of the segmental deletion, affecting (at least) the haemopoietic cells. The late onset of beta-thalassaemia intermedia indicates a preferential survival of the haemopoietic cells containing the deleted region, which have become the main source of erythropoiesis.

Previous cases of late-onset beta-thalassaemia have been described (Badens et al., 2002; Galanello et al., 2004; Chang et al., 2008; Harteveld et al., 2013; Bento et al., 2013). Two molecular mechanisms are distinguishable: segmental duplication of the paternal chr 11p allele (mosaic segmental uniparental isodisomy) or mosaic deletion of the maternal allele.

Correspondence

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Table I. Summary of literature search of late onset beta-thalassaemia cases.

<table>
<thead>
<tr>
<th>Case</th>
<th>Clinical and haematological findings</th>
<th>Tissue from which DNA examined</th>
<th>HBB mutation (parental origin)</th>
<th>Genetic mechanism</th>
<th>Size of somatic deletion</th>
<th>% Mosaicism (mutant clone vs. wild-type)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female, 28 years: Hb 21 g/l (Hb 86 g/l at 20 years)</td>
<td>WBC, bone marrow, hair follicles, oral mucosal epithelium</td>
<td>c.52A&gt;T (Gd17 A&gt;T) (Paternal)</td>
<td>Mosaic segmental uniparental isodisomy of chromosome 11p</td>
<td>SNP array: 11p14.3 to 11p15.5 (approx. 2.73–22.39 Mb)</td>
<td>Not determined</td>
<td>Chang et al (2008)</td>
</tr>
<tr>
<td>2</td>
<td>Patient A: Female. At 8 years, phenotypic carrier; at 26 years Hb 80 g/l and splenomegaly. Transfusions after 30 years</td>
<td>WBC, oral mucosal epithelium</td>
<td>c.230delC (cd76(-C)) (Paternal)</td>
<td>Mosaic segmental uniparental isodisomy of chromosome 11p</td>
<td>SNP arrays: approx. 47-7 Mb</td>
<td>65%</td>
<td>Harteveld et al (2013), Patient A</td>
</tr>
<tr>
<td>3</td>
<td>Patient B: Male. At 38 years, severe beta-thalassaemia intermedia; at 43 years transfusion-dependent and splenectomised</td>
<td>WBC, oral mucosal epithelium</td>
<td>c.118C&gt;T (Paternal)</td>
<td>Mosaic segmental uniparental isodisomy of chromosome 11p</td>
<td>SNP arrays: approx. 47-2 Mb</td>
<td>70%</td>
<td>Harteveld et al (2013), Patient B</td>
</tr>
<tr>
<td>4</td>
<td>Patient C: Male. At 46 years, severe thalassaemia intermedia and splenomegaly; at 51 years transfusion-dependent and splenectomised</td>
<td>WBC, oral mucosal epithelium</td>
<td>c.118C&gt;T (Paternal)</td>
<td>Mosaic segmental uniparental isodisomy of chromosome 11p</td>
<td>SNP arrays: approx. 48-9 Mb</td>
<td>80%</td>
<td>Harteveld et al (2013), Patient C</td>
</tr>
<tr>
<td>5</td>
<td>Female. At 7 years, phenotypic carrier; from 21 years, regular transfusions (3–4 weeks)</td>
<td>WBC, oral mucosal epithelium, skin fibroblasts</td>
<td>c.48G&gt;A (Gd15G&gt;A) (Paternal)</td>
<td>Mosaic segmental uniparental isodisomy of chromosome 11p</td>
<td>Approx. 8.8 Mb</td>
<td>80%</td>
<td>Bento et al (2013)</td>
</tr>
<tr>
<td>6</td>
<td>Male: 10–18 years, no transfusion (Hb 70–80 g/l), but anaemia, hepatosplenomegaly and growth failure</td>
<td>WBC, hair root, oral mucosal epithelium</td>
<td>c.118C&gt;T (Paternal)</td>
<td>Mosaic deletion of chromosome 11p</td>
<td>ND</td>
<td>20%</td>
<td>Badens et al (2002)</td>
</tr>
</tbody>
</table>
Table 1. (Continued)

<table>
<thead>
<tr>
<th>Case</th>
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<tbody>
<tr>
<td>7</td>
<td>Patient A: Male, 42 years; splenectomised at 40 years. Hb 70–90 g/l but no transfusions</td>
<td>WBC, oral mucosal epithelium, skin fibroblasts</td>
<td>c.118C&gt;T (Paternal)</td>
<td>Mosaic deletion of chromosome 11p (or Mosaic segmental uniparental isodisomy of chromosome 11p)</td>
<td>8 Mb (11p15.4)</td>
<td>Galanello et al (2004), Patient A</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Patient B: Female 23 years, splenomegaly. Hb 70–90 g/l but no transfusions</td>
<td>WBC, oral mucosal epithelium, skin fibroblasts</td>
<td>c.118C&gt;T (Paternal)</td>
<td>Mosaic deletion of chromosome 11p (or Mosaic segmental uniparental isodisomy of chromosome 11p)</td>
<td>8 Mb or larger (11p15.4)</td>
<td>10%</td>
<td>Galanello et al (2004), Patient B</td>
</tr>
<tr>
<td>9</td>
<td>Female, 29 years. At 34 years Hb 49 g/L, transfusion scheme initiated 3–4 units monthly</td>
<td>WBC, hair root, oral mucosal epithelium</td>
<td>c.315+1G&gt;A (IVS2-1G&gt;A) (Paternal)</td>
<td>Mosaic deletion of chromosome 11p</td>
<td>SNP array: 4.97 Mb (11p15.4–11p15.5) maternal allele</td>
<td>90%</td>
<td>Present paper</td>
</tr>
</tbody>
</table>

Hb, haemoglobin; ND, not done; SNP, single nucleotide polymorphism; WBC, white blood cells.
(Table I), with respective homozygosity or hemizygosity for the mutated paternal HBB allele in haemopoietic cells. Although a meaningful comparison the data between cases is hampered by the heterogeneity of methods, CNV nomenclature and stage of disease at analysis, the date indicates that the CNV in our case was amongst the smallest (~5Mb) and the level of deleted allele relative to normal amongst the highest (>90%).

Deteriorating clinical expression in all cases implies that cells with maternal segmental allelic loss or paternal homozygosity have a selective advantage over cells biallelic for chr 11p15. However, underlying mechanisms are not apparent. In our patient the deleted region contains 124 Ensembl coding genes, including 14 Online Mendelian Inheritance in Man® Morbid genes (CTSD, TNN12, TNI13, IGF2, INS, TH, CD81, KCNQ1, CDKN1C, SLC22A18, PGAP2 and STIM1), the β-globin gene cluster (HBB, HBD, HBG1, HBG2 and HBE) and imprinted genes, including H19 and IGF2 (exclusively transcribed from the maternal allele or paternal allele, respectively). It could be postulated that if the maternally-active growth suppressor gene H19 is lost, the paternally-active growth-enhancer gene, IGF2, is left unregulated. This mechanism is observed in 20% of Beckwith-Wiedemann patients with uniparental isodisomy of 11p, characterized by cellular over-growth and a predisposition to tumour development. A single case has been reported of a congenital Beckwith-Wiedemann Syndrome with a beta-thalassemia major phenotype, despite inheriting only a single beta-thalassemia mutation, carried by his father (Beldjord et al., 1992).

Overall cases of late-onset of beta-thalassaemia major or sickle cell disease are extremely rare, but based on the handful of cases described to date, molecular investigation should primarily consider somatic paternal uniparental isodisomy or maternal loss of chr 11p15, and analysis of the HBB gene mutations should not be restricted to the blood, but also be extended to the investigation of DNA from other tissues.

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Conflict of Interest

The authors declare no conflict of interest.

Author contributions

J. Traeger-Synodinos: wrote the paper and co-ordinated all stages of the study. C. A. J. Bosch, C. Vrettou, E. Maragoudaki, S.G.J. Arkesteijn: performed the research. J. Apostolidis: performed the clinical evaluation and currently manages the patient. E. Kanavakis: performed the initial clinical evaluation. C. Kattamis: performed the initial and current clinical evaluation. M. J. V Hoffer, C. A. L. Ruivenkamp: analysed the data. C.L. Harteveld: designed the research study.

Joanne Traeger-Synodinos
Cathy A. J. Bosch
Christina Vrettou
Lena Maragoudaki
Janos Apostolidis
Emmanuel Kanavakis
Christos Kattamis
Sandra G. J. Arkesteijn
Mariette J. V. Hoffer
Claudia A. L. Ruivenkamp
Cornelis L. Harteveld

1Department of Medical Genetics, National & Kapodistrian University of Athens, St. Sophia’s Children’s Hospital, Athens, Greece, 2Department of Clinical Genetics/LDGA, Leiden University Medical Centre, Leiden, the Netherlands, 3Department of Haematology and Bone Marrow Transplantation, Evangelismos Hospital, 4Genesis Genoma Laboratory and 5National & Kapodistrian University of Athens, St. Sophia’s Children’s Hospital, Athens, Greece.
E-mail: c.l.harteveld@lumc.nl

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References

Clinical outcomes of hepatitis B or C virus infections in patients with malignant lymphoma receiving autologous stem cell transplantation: on behalf of the Adult Lymphoma Working Group of the Japan Society for Haematopoietic Cell Transplantation (JSHCT)

Hepatitis virus infection is a major health problem leading to significant morbidity and mortality worldwide. Post-transplantation liver complications are well recognized in patients with allogeneic transplantation (Ramos et al, 2009; Nakasone et al, 2013). However, no consensus has yet been reached concerning the actual benefit of autologous stem cell transplantation (ASCT) in hepatitis B virus (HBV)- and/or hepatitis C virus (HCV)-positive patients, and data on patients with HBV and/or HCV infection following ASCT are very limited. This is the first large retrospective analysis to show the influence of HBV and/or HCV infection on outcomes in lymphoma patients who underwent ASCT.

Subjects were 4639 malignant lymphoma patients undergoing ASCT between 1989 and 2010 (Table I). HBV- and HCV-positivity were defined by each transplant centre; detailed data on hepatitis virus antigen, antibody and viral loads were not available. HBV and HCV infection were present in 152 (3.3%) and 93 (2.0%) patients, respectively. Ten patients (0.2%) had HBV and HCV coinfection (Table I).

With a median follow-up of 2.8 years, the 2-year overall survival (OS) rates were 74% (95% confidence interval [CI]: 65–80), 77% (95% CI: 66–84), 60% (95% CI: 25–83) and 75% (95% CI: 73–76) in patients positive for HBV, HCV, HBV and HCV, and negative for HBV and HCV, respectively (P = 0.83, log-rank) (Fig 1A). Cumulative incidence of therapy-related mortality (TRM) at 1 year was 8.8% (95% CI: 4.8–14.2), 5.6% (95% CI: 2.1–11.7), and 5.5% (95% CI: 4.8–6.2) in patients positive for HBV, HCV, and negative for HBV and HCV, respectively (P = 0.97, log-rank) (Fig 1B).

In multivariable analysis of HBV-infected patients, factors associated with worse OS were male sex [hazard ratio (HR): 3.49, 95% CI: 1.35–9.03], performance status >1 (HR: 2.68, 95% CI: 1.05–6.82), and non-remission/relapse status at ASCT (HR: 2.30, 95% CI: 1.05–5.08). In patients with HCV infection, factors associated with worse OS were age ≥50 years at ASCT (HR: 11.9, 95% CI: 2.25–62.8), performance status >1 (HR: 27.6, 95% CI: 7.38–103.2), and non-remission/relapse (HR: 6.23, 95% CI: 2.18–17.8). Patient who received ASCT in 2005 or later showed better OS in both HBV- and HCV-positive groups (Tables S1 and S2). Performance status >1 (HR: 16.1, 95% CI: 2.06–126.2) and non-remission/relapse status at ASCT (HR: 5.80, 95% CI: 1.20–28.0) were associated with higher TRM in HCV-infected patients (Table S2). In patients negative for HBV or HCV infection, mature T-cell subtypes were associated with worse OS (data not shown); on the other hand, difference in histology could not be identified as adverse indicator in both HBV- and HCV-positive groups (Tables S1 and S2, Fig S1).

Forty-seven patients had liver dysfunction prior to ASCT. Hepatic veno-occlusive disease (VOD) occurred in 19 patients after ASCT, of which 13 patients were transplant-related complications. The median time to VOD diagnosis was 10 days (range: 1–158 days). Details of the patients diagnosed or died with VOD are shown in Table S3. Liver dysfunction at ASCT was not a risk factor in OS and TRM in both HBV/HCV-negative and -positive groups.

The current study showed comparable OS and TRM rates in patients with and without hepatitis virus infections. Our results were similar to that of a previous study (Varma et al, 2016).

Recent developments in the understanding of hepatitis B virus infection lead the successful treatment of HBV-positive lymphoma patients (Liang, 2009; Kusumoto et al, 2019). All patients who are candidates for ASCT should be screened for HBV [hepatitis B surface antigen (HBsAg), and antibodies against hepatitis B core antigen (anti-HBc) and HBsAg (anti-HBs)] and HCV [HCV antibody (anti-HCV)]. Japanese guidelines recommend that transplant physicians should confirm the information of screening tests before first-line therapy, because of decreased level of anti-hepatitis virus antibody following immunosuppressive therapy and existence of passive immunity against hepatitis viruses through blood products. To prevent reactivation of HBV, patients with resolved HBV infection (HBsAg negative, anti-HBc and/or