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Mutations in the gene encoding the PML nuclear body protein Sp110 are associated with immunodeficiency and hepatic veno-occlusive disease

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We describe mutations in the PML nuclear body protein Sp110 in the syndrome veno-occlusive disease with immunodeficiency, an autosomal recessive disorder of severe hypogammaglobulinemia, combined T and B cell immunodeficiency, absent lymph node germinal centers, absent tissue plasma cells and hepatic veno-occlusive disease. This is the first report of the involvement of a nuclear body protein in a human primary immunodeficiency and of high-penetrance genetic mutations in hepatic veno-occlusive disease.

Hepatic veno-occlusive disease with immunodeficiency syndrome (VODI, OMIM235550) is an autosomal recessive primary immunodeficiency associated with hepatic vascular occlusion and fibrosis[1]. VODI has an estimated frequency of 1:2,500 live births in the Sydney Lebanese population, with 19 cases identified over a period of 30 years. VODI is associated with an 85% mortality if unrecognized and untreated with intravenous immunoglobulin and *Pneumocystis jerovici* prophylaxis. The association of an immunodeficiency with hepatic veno-occlusive disease (hVOD) has not been recognized in other classes of immunodeficiency, suggesting that hVOD is not a secondary event but is a primary feature of VODI.

We identified six children from five families of Lebanese ethnicity who presented between 3–7 months of age with either a combined T and B cell immunodeficiency and/or a personal or family history of hVOD (**Table 1**) who met the clinical criteria for VODI (see **Supplementary Methods** online). The clinical correlates of immunodeficiency included *Pneumocystis jerovici* infection, enteroviral infection or mucocutaneous candidiasis, but there was no evidence of mycobacterial infection. Hepatic VOD was verified by biopsy in at least one individual in each sibship and was indistinguishable clinically and pathologically from the sinusoidal obstruction syndrome described after hematopoietic stem cell transplantation.

The B cell immunodeficiency was characterized by evolving severe hypogammaglobulinemia, absent memory (CD19+ CD27+ IgD–) B cells and tonsillar lymph nodes, and circulating CD19+ B cell numbers and percentages within the normal range. Absence of lymph node germinal centers and tissue plasma cells have been noted as a consistent finding in autopsies that have been reported on 12 affected individuals, consistent with a block in B cell differentiation (M.W., personal communication). The T cell immunodeficiency was characterized by reduced numbers of memory (CD4+ CD45RO+ CD27–) T cells (1–2%; normal is >40%) and low or reduced intracellular T cell cytokine expression (IFNγ, 1−4% (reference range, 25−30%); IL2, 1–3% (12–32%); IL4 1–2% (4–7%) and IL10 1–2% (1–6%)) after stimulation with phorbol myristate acetate (PMA) and ionomycin. CD4 and CD8 T cell numbers and percentages and T cell proliferation assays were normal.

We performed homozygosity mapping in four affected individuals and their parents from families A, B and C after obtaining approval from the relevant institutional ethics committees and informed written consent. We mapped VODI to chromosome 2q36.3-37.1. Fine-mapping studies identified a conserved haplotype of three short tandem repeat (STR) markers spanning a genomic interval of 1.422 Mb (**Fig. 1a**,**b**) that contained the gene *SP110* (encoding 110-kDa Speckled). *SP110* is an immunoregulatory gene expressed in T and B lymphocytes, lymph nodes, spleen and liver[2,3]; therefore, we screened its coding exons for mutations by DNA sequencing. Mutation screening (**Supplementary Table 1** and **Supplementary Methods**) identified a homozygous single-base deletion, 642delC (P214PfsX15), in exon 5 in affected individuals from families A–C and subsequently in family D (**Fig. 1c**). No living affected individuals were available from family E, but the consanguineous parents and unaffected children were shown to share a heterozygous single-base deletion, 40delC (Q14SfsX25) in exon 2 on a different haplotype background from families A–D. We confirmed that this mutation was

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Table 1 Summary of clinical and laboratory data in the VODI families

Individual	Sex	Age (months)	Presentation	Serum Ig (g l^{-1})			Percentage circulating cells		Memory T & B cells	T cell subsets	PHA/ConA Response	T cell cytokine production	Current clinical state
				IgM	IgG	IgA	B	T					
A II.1	F	5	Immunodeficiency, thrombocytopenia and hVOD	0.16	0.5	<0.07	N	N	–	N	N	–	Deceased; recurrent hVOD post HSCT
B II.1	M	7	Immunodeficiency	0.1	0.2	0.07	N	N	Decreased	N	N	Impaired	Well
B II.2	F	6	Hepatosplenomegaly and ascites	0.09	<0.33	<0.07	17	58	Decreased	N	N	Impaired	Mild aminotransferase elevation
C II.1	F	4	Hepatosplenomegaly and thrombocytopenia	0.2	0.55	<0.07	8	79	Decreased	N	N	Impaired	Chronic liver disease and portal hypertension
D II.1	M	3	hVOD	0.05	<0.33	<0.07	23	79	Decreased	N	N	Impaired	Deceased; hemophagocytic syndrome post hepatic transplant for hVOD
E I.1	M	3	Immunodeficiency & thrombocytopenia	0.05	0.8	<0.06	N	N	–	N	N	–	Deceased; enteroviral and *Pneumocystis jerovici* infection

PHA: phytohemagglutinin; ConA: concanavalin A; HSCT: hematopoietic stem cell transplantation.

homozygous in DNA extracted from archival tissue from EII.1. We did not detect either mutation in 50 unrelated Lebanese controls. In addition, we did not detect any mutations in the coding regions of *SP110* in 89 isolated cases of common variable immunodeficiency of European or Middle Eastern origin[4].

Sp110 nuclear staining was absent in EBV-transformed B lymphocytes from an affected individual with the homozygous 642delC mutation in exon 5. Sp100 staining was consistent with normal PML nuclear body numbers (**Fig. 2a–h**). Protein blot analysis using a rat anti-Sp110 antiserum confirmed the absence of the two major isoforms, Sp110b and Sp110c, in affected cell lines (**Fig. 2i**). A quantitative TaqMan real-time PCR assay demonstrated significantly reduced *SP110* mRNA levels in B lymphoblastoid cell lines established from 642delC homozygous patients compared with their heterozygous siblings and familial controls (**Fig. 2j**). We did not see any corresponding changes in mRNA levels in the evolutionarily related, physically adjacent genes *SP100* and *SP140*. Long-range PCR performed on random hexamer-primed cDNA generated from individuals with VODI and heterozygotes showed no evidence of exon skipping (data not shown). These data are consistent with absence of Sp110 without disruption of the PML nuclear body in individuals with VODI.

Given the association between mutations in *SP110* and hVOD, we were interested to determine if there was an association between Sp110 alleles and the risk of hVOD after hematopoietic stem cell transplantation. We performed a targeted *SP110* SNP association study using 69 SNPs with an average inter-SNP distance of 0.826 kb in a cohort of 47 affected individuals and 62 controls who had received standardized myeloablative conditioning therapy[5]. Measures of association between hVOD and alleles of *SP110* did not reach statistical significance.

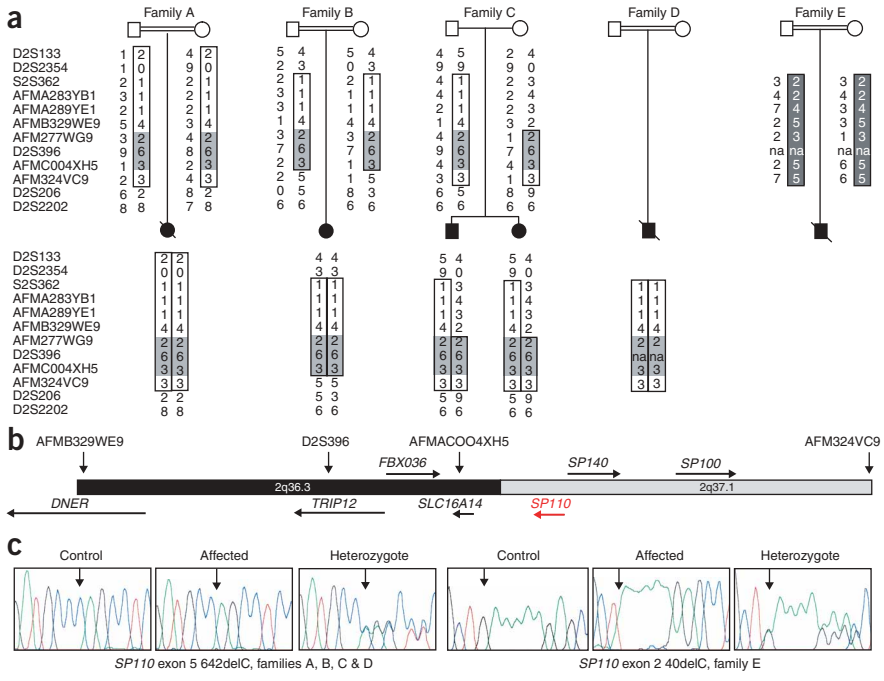

Figure 1 *SP110* mapping and mutation analysis. (**a**) Pedigree structure and haplotypes in the region 2q37.1 for five families with VODI. The shaded haplotype shared by affected family members is oriented with centromere towards the top of the page. Individuals II.1 and II.2 in family C and individual II.1 family B show the position of the centromeric and telomeric crossover events. 'na' in families D and E indicates an STR that was not analyzed. (**b**) Gene and STR localizations within the minimal critical region. (**c**) Partial *SP110* DNA sequences for exons 5 (forward) and 2 (reverse) in normal controls, affected individuals and heterozygotes. The reference sequence used is GenBank accession number NM_080424, with base 1 assigned to the initiator ATG in exon 2.

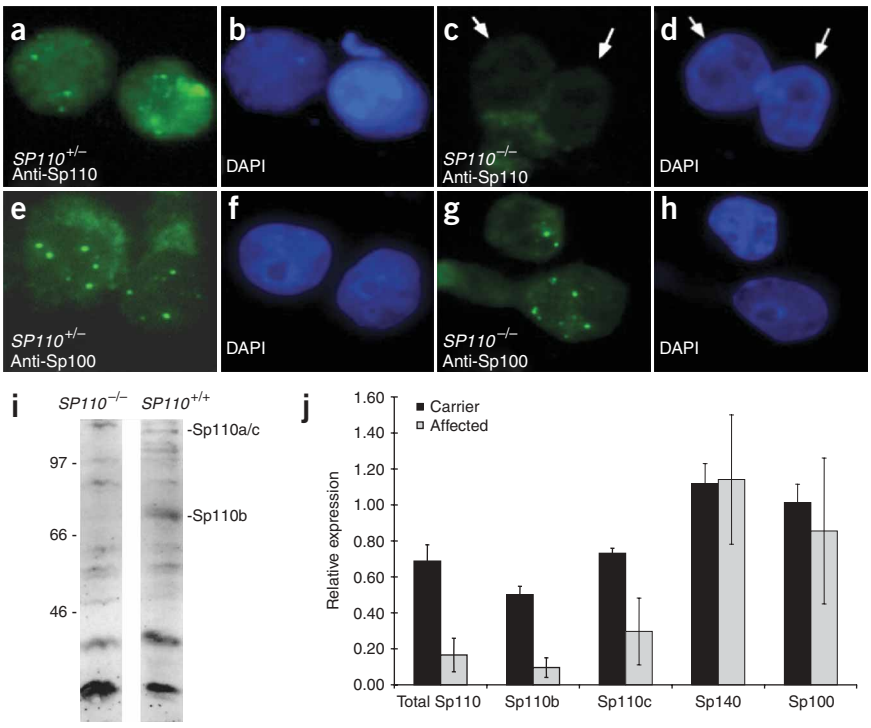

Figure 2 Functional consequences of *SP110* mutations. (**a**–**h**) Indirect immunofluorescence analysis of Sp110 expression with a rat anti-human Sp110 polyclonal antiserum showing absent Sp110 nuclear staining and the presence of normal Sp100 nuclear body staining in an affected individual. Also shown are images of DAPI staining, demonstrating nuclear localization. (**i**) Protein blot showing absence of Sp110 expression in EBV-transformed B cells from a homozygous affected individual versus a normal control. (**j**) Relative mRNA levels of total *SP110*, *SP110b*, *SP110c*, *SP140* and *SP100* in affected individuals ($n = 4$) and a carrier (normalized to expression from a control individual ± 1 s.d.).

SP110, an *SP100* gene family member with three isoforms, encodes a 713-residue protein that has structural features consistent with a role in transcriptional regulation, including a nuclear localization signal; *SP100*-like dimerization, plant homeobox, bromo and LXXLL-type nuclear hormone domains and Sp100/AIRE-1/NucP41/75 domains (SAND)[6,7]. Sp110 can also increase transcriptional activity from retinoic acid response elements by binding to the retinoic acid receptor α (RARα), whereas the Sp110b isoform binds RARα and acts as a transcriptional corepressor[7]. Sp110 is associated with the PML nuclear body, a macromolecular complex within the nucleus that is deployed to areas of active host or viral DNA replication, transcription and repair[8–10]. In addition to the regulation of gene transcription, the PML nuclear body is involved in other cellular processes such as apoptosis, cell cycle control and the immune response. Our data are consistent with Sp110 having an important role in the immune response without being essential for PML nuclear body formation.

This study also extends previous findings on the role of the mouse *SP110* orthologue, *Ifi75*, in the increased susceptibility to mycobacterial and listerial infections in C3HeB/FeJ inbred strain of laboratory mice[11]. Full-length transcripts of *Ifi75* are absent in C3HeB/FeJ. No abnormalities of T cell function, alveolar B cell numbers or expression of CD19, B220 or CD69 have been reported in C3HeB/FeJ. There is also no evidence of hVOD in this strain; however, this was assessed in the context of mycobacterial infection rather than bone marrow ablative therapy (I. Kramnik, personal communication). Notably, the full characterization of the C3HeB/FeJ *Ifi75* genomic region, including the genomic basis of the lack of full-length *Ifi75* expression, has yet to be defined. The discordance in the phenotypes observed in humans and mice may be related to different mutational bases of disruption of *SP110* and *Ifi75* in the two species. Phenotypic variation due to allelic heterogeneity has numerous precedents[12,13]. Alternatively, the variation in immune phenotype may reflect species-specific differences, as has been previously documented for the *ZAP70* and *ICOS* genes[14,15].

In summary, this study indicates that *SP110* has an important role in immunoprotective mechanisms against infectious organisms in humans. VODI presents with a T cell abnormality and a defect in B cell differentiation between the stages of CD19 sIg-positive mature B cells and antibody secreting plasma cells, with resulting severe hypogammaglobulinemia. The failure of T cells from affected individuals to express IFNγ, IL2 and IL4 and the expression of low levels of IL10 in response to PMA and ionomycin stimulation suggest either the abolition of transcriptional coactivation of these genes by *SP110* or an effect secondary to a block in B cell maturation. The mechanisms by which mutations in *SP110* lead to decreased T cell cytokine production, the failure of B cell differentiation and sinusoidal injury have yet to be elucidated. We hypothesize that *SP110* not only has a role in interferon-dependent transcription of genes central to cytokine production and lymphocyte differentiation but that it is also involved in cytoprotection. This represents the first report of a human immunodeficiency syndrome associated with mutations in a nuclear body protein and the identification of the molecular basis of a mendelian form of hVOD.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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