expressed in all examined T-ALL cell lines (*data not shown*). These data show that it remains critically important to test potential mutant forms of kinases to discriminate between driver and passenger mutations, as was also shown recently in the context of *FLT3*.[9]

In the MOLT-4 and RPMI-8402 cell lines, we identified another transcript variant, which lacks exons 7 and 8 (nucleotides 648-1178). This results in a shift in the open reading frame with generation of a premature stop codon in exon 9. Consequently, a truncated form of *JAK1* is expressed in these cell lines, lacking the entire kinase, pseudokinase and SH2 domain as well as part of the FERM domain (Figure 1G, 1H). Due to the absence of a kinase domain, no further functional studies were performed on this variant.

To investigate if the 18 T-ALL cell lines were dependent on JAK1 signaling for their proliferation and survival, we examined the effect of treatment with a small molecule JAK inhibitor (JAK inhibitor I, Calbiochem, San Diego, CA, USA). This compound inhibited JAK1 autophosphorylation in control cells with an IC_{50} value of 100 nM (*data not shown*). The majority of T-ALL cell lines were completely insensitive to treatment with this inhibitor (IC_{50} values ≥10 μM), while other T-ALL cell lines (ALL-SIL, SUP-T1, DND-41, TALL-1) displayed an increased sensitivity with IC_{50} values below or around 1 μM. In none of the T-ALL cell lines was proliferation completely inhibited at a concentration of 10 μM of the JAK inhibitor (*data not shown*). Despite the presence of *JAK1* variants in the cell lines HPB-ALL, MOLT-4 and RPMI-8402, these cell lines were not more sensitive to JAK1 inhibition, confirming that the observed variants were unlikely to contribute to *JAK1* activation. As a final experiment, we knocked down JAK1 expression in the HPB-ALL and RPMI-8402 cell lines using a JAK1 siRNA (Validated Stealth siRNA, Invitrogen, Carlsbad, CA, USA), which again confirmed that these T-ALL cell lines were not dependent on *JAK1* expression for their survival and proliferation (*data not shown*).

Flex *et al*. reported association of hyperactive *JAK1* mutants with advanced age at diagnosis in ALL patients. Unfortunately, we were not able to identify a T-ALL cell line with an activating mutation in JAK1 that could be used as a T-cell model to study JAK1 signaling and study the effect of JAK1 inhibition. As most of the cell lines we tested correspond to samples from childhood/adolescent T-ALL, the results obtained in this study are in line with the data presented by Flex *et al*. Furthermore, our observation that concentrations in the range of 1 μM or more of a JAK kinase inhibitor are required to inhibit T-ALL cell lines indicate that T-ALL cell lines in general are not critically dependent on *JAK1* activity for their proliferation and survival.

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A father and his son with systemic AL amyloidosis

In systemic AL amyloidosis, fibrils are derived from a monoclonal immunoglobulin light chain produced by a plasma cell clone in the bone marrow.[1] AL amyloidosis may be associated with multiple myeloma but more commonly the plasma cell clone is not malignant. A monoclonal gammopathy of undetermined significance (MGUS) may have preceded the development of amyloidosis. AL amyloidosis is often recognized and diagnosed at a late stage thereby giving the patients an average 10-14 months survival if they do not respond to treatment.[2] Even though it was one of the first types of amyloidosis biochemically characterized, still little is known about how the light chains cause disease, why they are deposited in certain organs and why there is an enormous variation in patients' clinical symptoms and outcome.

There are several types of familial amyloidoses usually inherited dominantly. The most recognized one is familial amyloidotic polyneuropathy where a mutation in the transthyretin (TTR) gene leads to an amyloidogenic protein variant.[3] AL amyloidosis has not been considered to be hereditary[4-6] and to date no two identical amyloidogenic light chains have been identified.

Nevertheless, there are a few reports of cases of systemic AL amyloidosis in the same family.[5,6] In none of these cases was a biochemical characterization of the amyloid fibril protein performed. In this paper we describe a father and his son, both dying from systemic AL amyloidosis of λ type. The study was approved by the ethical committee of the Sahlgrenska University Hospital.

Case 1. The son. A 48-year old man who suffered from fatigue and impaired general condition for two years. He had signs of peripheral sensoric polyneuropathy. Cardiac biopsy revealed amyloid depositions, which were preliminarily believed to be of TTR origin. However, neither genetic nor protein analysis could confirm this. Instead, analysis of an abdominal fat tissue biopsy with Western blot showed reaction with antibodies against protein of AL λ type. Bone marrow analysis revealed no signs of myeloma but there was a small IgG M-component in the plasma. The patient died suddenly a month later, before start of treatment aimed at autologous stem cell transplantation. Autopsy showed systemic amyloidosis involving many organs, including pronounced cardiac deposits.

Case 2. The father. A 60-year old man who suffered from cardiac insufficiency. He had an IgA MGUS, but bone marrow examination revealed no overt myeloma. Two months before death he underwent surgery due to gastric retention and a suspicion of gastric rupture. Histological examination of the gastric wall and peritoneal fat tissue revealed heavy amyloid deposition. At autopsy, a pronounced systemic amyloidosis was found with deposits in the heart, kidneys, liver, pancreas, adrenal glands and stomach. From both patients, blocks of heart tissues were used for further studies (Figure 1 A and B).

Characterization of amyloid in subcutaneous fat tissue from the son was performed by Western blot analysis developed in our laboratory.[7,8]

From the autopsy material of the son, we obtained a formalin-fixed, paraffin-embedded heart block. Congo red staining revealed that about 50% of the material was amyloid. This material was used for the microextraction method described by Kaplan *et al*.[9] The N-terminus was deblocked with pyroaminoglutamate aminopeptidase (Takara; Otsu, Shiga, Japan) according to the manufacturer's description.

From the father, only formalin-fixed and paraffin-embedded heart material was available for extraction and characterization as described.[9,10] A Congo red stained section showed that approximately 75% of the material was amyloid. Sequence analyses were performed with a 477A Protein Sequence Analyzer connected to a 120A PTH-analyzer from from Applied Biosystems and with a Procise 494 protein sequenator (Applied Biosystems, Foster City, CA).

Western blot analysis of subcutaneous fat tissue revealed that the amyloid was of immunoglobulin lambda light chain origin (*data not shown*). Formalin-fixed and paraffin-embedded material was extracted and electrospray mass spectrometry of four tryptic peptides obtained by reversed phase-high performance liquid chromatography (RP-HPLC) indicated AL protein of lambda type, most likely subgroup λ 2, (*data not shown*). Further characterization of the material with Edman degradation was successful for 24 cycles and revealed a lambda 2a amino acid sequence. There were three substitutions compared to the germ line sequence (Figure 2).

The 21-year old formalin-fixed and paraffin-embedded material was extracted for amyloid proteins. After the RP-HPLC, material from the major protein peak was analyzed by Edman degradation. The reaction was success-

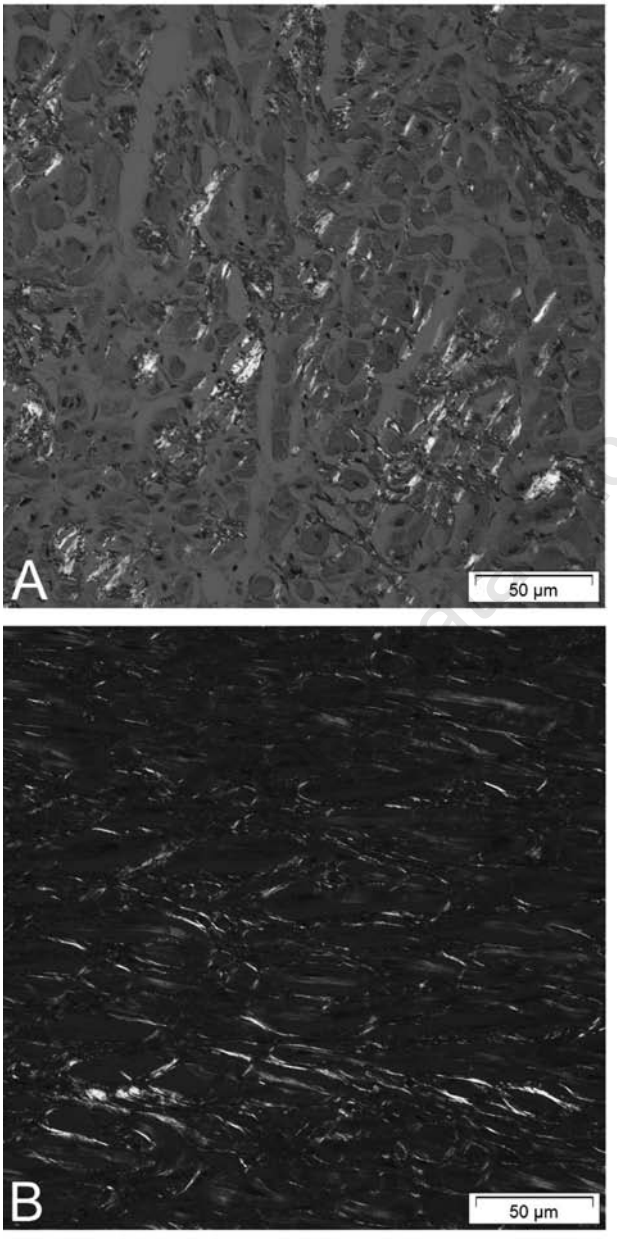

Figure 1. Section of myocardium from the father (A) and the son (B). There is pronounced amyloid infiltration in both specimens. Congo red, polarized light with partially crossed polars.

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          1         10         20
λ2a       QSALTQPASV SGSPGQSITI SCTGT
AL-son    XSALTQPDSE SGSPGQSITI SCSGT

          1         10         20         30
λ3a       SYVLTQPPSV SVAPGKTARI TCGGNNIGSK
AL-fath    YVLAESPSV SVAPGQTARI SCGGNDIGSY
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Figure 2. N-terminal amino acid sequences of the AL proteins of the son and the father compared with the germ line sequences.

ful for 32 cycles and revealed a lambda 3a sequence. There were 6 amino acid substitutions compared with the germ line sequence (Figure 2).

We report systemic AL amyloidosis affecting a father and his son. A possible genetic predisposition for AL amyloidosis would be complex, since all light chains have different amino acid sequences, depending both on the existence of about 50 different light chain variable domain genes and on somatic mutations. The light chain is composed of three segments, the variable (V) domain which is attached to the constant (C) chain via the joining (J) segment. Most of the variation is found in the variable region and in the area around the joining of this domain to the constant part. The literature has documented that the light chain involved in amyloidosis usually consists of the N-terminal part of a monoclonal protein. On the other hand, there have been three cases described where the amyloidogenic protein was from the C-terminal part.[10-12] A missense mutation leading to AL amyloidosis is theoretically possible if it occurs in the gene for the constant or joining segment. This could lead to an amyloidogenic constant or joining region and affect the aggregation propensity of the variable domains. In this scenario, our material should consist of several different variable domains combined with one specific pair of lambda J segment and constant domain. Instead, we found two different monoclonal variable regions. Therefore, other factors are probably involved in the pathogenesis of the disease and may be responsible for the inheritance.

In summary, we report a patient who originally was believed to suffer from familial TTR amyloidosis since his father had died from systemic amyloidosis two decades earlier. However, biochemical analysis of the fibril protein of both individuals showed AL amyloidosis. These patients underline the importance of a direct determination of the amyloid type in all individuals with systemic amyloidosis.

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Donor lymphocyte infusions in amyloid light chain amyloidosis: induction of a "graft-versus-plasma cell-dyscrasia effect"

Systemic amyloid light chain (AL) amyloidosis is a rare protein folding disorder with poor prognosis due to multiple organ impairment (mainly heart and kidney). Underlying disease is a monoclonal gammopathy in most patients, only a small portion has symptomatic multiple myeloma. Treatment results in AL amyloidosis have improved in recent years. Apart from high-dose chemotherapy with melphalan supported by autologous stem cells (HDM)[1] and melphalan-dexamethasone combination therapy[2] new drugs active in multiple myeloma (MM) have become available.[3-5] The main goal of treatment is to improve or at least preserve the function of the affected organs by elimination or control of the monoclonal plasma cell disorder.[1] Long-term survival (>10 years) in about 20% of patients with complete remission (CR) after HDM could be shown recently.[6] However, more than 50% of the patients do not achieve CR or relapse after initial therapy. Treatment of these patients has not been well investigated. It was recently shown that allogeneic stem cell transplantation (allo-SCT) could be an option for eligible patients leading to sustained CR.[7] However, allo-SCT is a clinical challenge in this fragile patient group. Donor lymphocyte infusion (DLI) might be an approach to further increase CR rate after allo-SCT using reduced-intensity conditioning (RIC) as shown in MM.[8] However, there are no data about application of DLI in this rare and fatal disorder.

Two patients with systemic AL amyloidosis were treated with allo-SCT in both centers until 2007. We administered DLI in both patients because they did not reach CR after allo-SCT. Patient characteristics and