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Wiskott-Aldrich Syndrome: A retrospective study on 577 patients defines the genotype as a predictive biomarker for disease severity and survival

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- The type of genetic variant is a predictive biomarker for disease severity and survival in WAS.
- Patients with less severe variants experience a later onset of disease-related complications, but remain prone to morbidity and premature mortality.

Abstract

WAS is a multifaceted monogenic disorder with a broad disease spectrum and variable disease severity and a variety of treatment options including allogeneic hematopoietic stem cell transplantation (HSCT) and gene therapy (GT). No reliable biomarker exists to predict disease course and outcome for individual patients.

A total of 577 patients with a *WAS* variant from 26 countries and a median follow-up of 8.9 years (0.3-71.1), totaling 6118 patient-years, were included in this international retrospective study.

Overall survival (OS) of the cohort (censored at HSCT or GT) was 82% (95% CI 78-87) at 15 years and 70% (61-80) at 30 years of age. The type of variant was predictive of outcome: patients with a missense variant in exons 1 or 2 or with the intronic hotspot variant c.559+5G>A (class I variants) had a 15-year OS of 93% (89-98) and a 30-year OS of 91% (86-97), compared to 71% (62-81) and 48% (34-68) in patients with any other variant (class II; $p<0.0001$). The cumulative incidence rates of disease-related complications such as severe bleeding ($p=0.007$), life-threatening infection ($p<0.0001$), and autoimmunity ($p=0.004$) occurred significantly later in patients with a class I variant. The cumulative incidence of malignancy ($p=0.6$) was not different between classes I and II.

This study represents the largest cohort of WAS patients studied so far. It confirms the spectrum of disease severity and quantifies the risk for specific disease-related complications. The class of variant is a biomarker to predict the outcome for WAS patients.

Introduction

Wiskott-Aldrich syndrome (WAS) is an inborn error of immunity (IEI) with X-linked inheritance pattern characterized by immunodeficiency, thrombocytopenia, eczema, and a predisposition to autoimmunity and malignancy [1]. Due to its characteristic clinical presentation with thrombocytopenia and eczema it is relatively easy to distinguish clinically from other rare monogenic IEI [2]. This explains why WAS was recognized early as a distinct, inherited clinical entity [3,4] and its genetic cause identified in the 1990s [5]. It was one of the first IEI to be cured by allogeneic hematopoietic stem cell transplantation (HSCT) [6] and treated by hematopoietic stem and progenitor cell gene therapy (GT) [7]. Nevertheless, WAS remains a multifaceted disorder posing very complex challenges to physicians and families alike [2,8].

Patients affected by WAS carry hemizygous variants in the *WAS* gene and consequently express either no WAS protein (WASP) or reduced amounts of a defective protein [9]. The penetrance, severity and time of onset of clinical disease manifestations can be extremely variable between affected individuals and even within families[1,10-12]. Some patients may suffer from life-threatening opportunistic infections and bleeding in early infancy and are prone to premature death in childhood unless treated curatively, while others may have a normal life span with mostly asymptomatic thrombocytopenia as the only symptom [10,13]. A disease score ranging from 1 to 5 according to severity of symptoms has been developed [2], but is not well suited for therapeutic decision making as it only describes disease severity at a given time point, while progression from milder to a more severe score can occur [10,14].

Different therapeutic approaches are available for WAS, ranging from symptomatic treatment, prophylactic application of antimicrobial agents or immunoglobulin replacement, splenectomy, thrombopoietic agents, to definitive therapeutic options such as HSCT or GT [2,8,15-22]. Anti-infective drugs or immunoglobulin replacement may prevent many severe infections, but will not eliminate

the risk for bleeding, autoimmunity or malignancy. Splenectomy - and to a lesser degree thrombopoietic agents - can elevate the platelet count and reduce the risk of bleeding. Splenectomized patients are at significant and persistent risk of life-threatening bacterial infections, in particular sepsis [10,15,21]. HSCT has become increasingly successful and can completely cure the disease but carries a small but significant risk of mortality as well as short and long-term morbidity [17,18,22]. Lentiviral GT results in at least partial correction of WASP expression across all hematopoietic cell lineages and leads to reversion or improvement of disease manifestations. Long-term effectiveness and safety still require additional follow-up, but no events of insertional mutagenesis have been observed with lentiviral GT for WAS after follow-up of up to 13 years [16,20,23].

Previous studies have established a certain degree of genotype/phenotype correlation in WAS but demonstrated that even patients with milder variants are at significant risk of severe disease associated events [9-12]. For patients with a classic WAS phenotype in childhood with a suitable stem cell donor, HSCT is the best available option and clearly indicated [17,18,24]. On the other hand, it can be quite difficult to choose the optimal treatment strategy for patients with milder WAS phenotypes, considering risk and benefit since disease severity may aggravate during a patient's life, and even clinically mild symptoms may have a significant negative impact on quality of life [25]. Here we provide a comprehensive overview of the distribution of disease burden in WAS patients, assess their natural disease outcome, define the risk for specific disease-related events, and possibly describe genetic biomarkers that could be helpful in defining the most appropriate treatment strategy based on the risk profile of each WAS patient.

Materials and methods

Data accrual

Pseudonymized case report forms (CRFs) asking for retrospective data retrieved from patient records were sent out to members of the inborn errors working party (IEWP) of the European Society for Blood and Marrow Transplantation (EBMT) and the European Society for Immunodeficiencies (ESID), as well as to the mailing list of the International Union of Immunological Societies (IUIS). Additionally, centers known to treat WAS-patients and former collaboration partners were contacted directly. The cut-off data for data collection was 31st December 2014. This retrospective chart-based survey was given a waiver by the ethics committee of the Ludwig-Maximilians-University of Munich, Germany. Individual centers gained local ethics committee approval for data transfer where it was required.

Patients

All 581 submitted CRFs were evaluated and 577 patients could be enrolled in our study. Patients without genetic analysis of the *WAS* gene (two patients) or with a variant in a different gene (*WIP*, two patients) were excluded. Patients with X-linked neutropenia caused by *WAS* variants were not part of this study.

Definitions

Disease-related complications were defined as follows: Severe bleeding was defined as intracranial, gastrointestinal or other life-threatening bleeding or any bleeding episode requiring red blood cell transfusion. Severe infections were defined as sepsis, meningitis, pneumonia

requiring respiratory support, systemic viral (viremia), or invasive fungal infections. For classification of current disease severity we used the WAS score as previously published [2].

For the purpose of this manuscript we divided variants into two classes:

class I: missense variants in exons 1 and 2 as well as the intronic hotspot variant c.559+5G>A, all of which had previously been defined as hotspot variants often found in mildly affected patients and which are expected to allow for some WAS protein expression [9-12,26]

class II: All other *WAS* variants. WAS protein expression was not systematically recorded or experimentally assessed in this study. *WAS* variants were curated according to American College of Medical Genetics and Genomics (ACMG) criteria using the Franklin by Genoox tool[27,28].

Karnofsky/Lansky scores were translated to Eastern Cooperative Oncology Group (ECOG) scores as suggested by Oken et al: Karnofsky/Lansky 100% = ECOG 0, 80-90% = ECOG 1, and ≤70% = ECOG ≤2 [29].

Statistical analysis

Kaplan Meier survival estimates were compared using the log rank test. The survival analyses were censored at the time point of first HSCT or GT where indicated (all survival analyses except figure 2A). Cumulative incidences for different events factored in only the first event of the specified category and were adjusted for competing risks. The mean incidences per patient-year were calculated for different time intervals taking the patients at risk into account, counting each event of the specified category. Since those estimated mean incidences do not follow a normal distribution, the confidence intervals were calculated with bootstrap techniques (percentiles of 1000 samples). All incidence analyses were censored at the time point of first procedure (HSCT, GT or splenectomy). Only the first event of a category was counted for cumulative incidence,

while all events of a specific category were counted for incidence per patient-year. All statistical analyses were performed with R version 3.5.3 [30].

Results

Patients

We included 577 patients from 63 centers in 26 countries born between 1932 and 2014 into this study. The median age at diagnosis was 1.5 years (0-68). Of these patients, 464 (80.4%) were alive at last follow-up, and median age at last follow-up was 8.9 years (range 0.3-71.1). This resulted in a total of 6,118 reported patient-years.

The disease severity was variable across the cohort, with a WAS score at last follow-up or before the first procedure of 1 in 54 (9%), 2 in 144 (25%), 3 in 161 (28%), 4 in 109 (19%) or 5 in 86 (15%) patients, respectively. HSCT had been performed in 255 patients (44%), splenectomy in 79 (14%), gene therapy in 14 (2%), and 42 (7%) had more than one of these procedures. More details of the cohort are reported in table 1.

All patients in this cohort had a clinical diagnosis of WAS and a presumably disease-causing genetic variant in the *WAS* gene. In 52 patients the *WAS* variant was confirmed yet not unambiguously reported. Of the 525 patients with clearly reported variants, 239 (46%) had a missense variant, 80 (15%) a nonsense variant, 90 (17%) an intronic variant, 76 (15%) a deletion and 40 (8%) an insertion (table 2). After curation according to ACMG criteria 290 (55.2%) variants were classified as “pathogenic”, 170 (32.4%) as “likely pathogenic”, and 60 (11.4%) as “variants of uncertain significance (VUS)” (figure 1, table 2). Two (0.4%) were curated as “likely benign”, and 3 (0.6%) as “benign”. Of these, 120 individual variants were at the time of writing not registered in ClinVar, 80 (66.7%) of which were found to have been previously published [9-12,31-47]. Details of these variants and their curation according to ACMG criteria are provided in supplementary table 1.

Overall survival

Given the paucity of natural outcome data for WAS patients in the current era, we performed a survival analysis of the entire cohort. The probability for overall survival (OS) was 78% (95% confidence interval [CI] 74-82) at 15 years, 65% (58-73) at 30 years, and 55% (44-69) at 45 years of age, respectively (figure 2A). Because potentially curative therapies such as HSCT or GT may significantly influence the natural course of disease either by curing the disease or by causing premature death, we performed an additional survival analysis where patients treated by either HSCT or GT were censored on the date of that procedure (meaning their last follow-up was recorded on that date as "alive"). This resulted in an OS of 82% (95% CI 78-87) at 15 years and 70% (61-80) at 30 years, and 62% (50-77) at 45 years of age, respectively (figure 2B). The approach of censoring at HSCT or GT was also used for all subsequent survival analyses.

Of the 113 patients who were reported deceased at last follow-up, 31 (27%) had died from infectious causes, 26 (23%) from bleeding, 8 (7%) from malignancy, 4 (4%) from autoimmunity, 18 (16%) from HSCT related events, and 26 (23%) from other or unknown causes (table 3).

Disease-related events

To better assess not only the mortality but also the morbidity caused by WAS, we analyzed the incidence of the most important disease-related events: severe bleeding, severe infection, autoimmunity and malignancy, regardless of whether their outcome was fatal or not. The cumulative incidences of a first severe event at 15, 30 and 45 years of age were 33% (96% CI 27-39), 49% (36-59) and 56% (36-70) for severe bleeding, 49% (43-55), 58% (49-66) and 74% (53-86) for severe infection, 30% (23-36), 40% (30-49) and 45% (31-56) for autoimmunity and 4% (1-7), 15% (5-24) and 15% (5-24) for malignancy, respectively (figure 3). For cumulative incidence

analyses of events, the follow-up was also censored at time of any first procedure (HSCT, GT or splenectomy).

We calculated per-patient-year incidences of disease-related events in order to assess morbidity during a specific age period. The overall incidence of any severe event was 0.19 (0.17-0.20) per patient-year (/yr), and 0.03/yr (0.03-0.04) for severe bleeding, 0.11/yr (0.10-0.13) for severe infection, 0.03/yr (0.03-0.04) for autoimmunity and 0.01/yr (0.01-0.01) for malignancy. Of note, bleeding, infection and autoimmunity had a higher observed incidence in the first five years of life and then returned to a steady level for the next 25 years of life, while malignancy was equally frequent during any age period (figure 3 B, D, F, H, K). This may at least partially be explained by the fact that many severely affected patients had undergone HSCT in early childhood and were therefore censored from the analysis at later time points. Nevertheless these data give an approximation of the true incidence of severe disease-related events during a specific age period in WAS patients in the absence of a potentially definitive procedure such as HSCT or GT.

Class of variant predicts outcome

Because the variability in disease severity in WAS can make it challenging to counsel families with respect to the most appropriate treatment choice, we intended to establish genotype as a predictive biomarker of disease outcome. For the purpose of this study, we grouped together all missense variants in exons 1 and 2 as well as the variant at the variant splice site c.559+5G>A, which generally allow for some (reduced) WAS protein expression, under the term “class I variants”. These types of variants had previously been defined as hotspot variants often found in mildly affected patients [9-12,26]. All other *WAS* variants were grouped as “class II variants” (table 2). Patients who had genetically confirmed WAS, but in whom the exact variants were not reported and those with “benign” or “likely benign” variants were excluded from this part of the analysis.

Overall survival censored for HSCT and GT in patients with class I variants was significantly better than that of patients with class II variants: 93% (89-98) versus 71% (62-81) at 15 years, 91% (86-97) versus 48% (34-68) at 30 years and 84% (70-100) versus 41% (26-66) at 45 years, respectively ($p<0.0001$; figure 4A). When being more rigorous and also excluding patients with VUS, OS was also significantly better for patients with class I variants ($p<0.0001$; figure 4B).

The cumulative incidence of the first severe event was also significantly higher in patients with class II variants with 75% (67-81) versus 55% (45-63) at 15 years and 87% (76-93) versus 70% (56-79) at 30 years, respectively ($p<0.0001$; figure 5A). Accordingly, severe bleeding episodes, severe infections and autoimmunity, but not malignancy occurred significantly earlier in life in patients with class II variants (figure 5C, E, G, I). Excluding patients with VUS, severe bleeding episodes, severe infections and autoimmunity, but not malignancy also occurred significantly earlier in class II patients (figure 5B, D, F, H, K). Patients with class II variants were diagnosed earlier in life, had a higher WAS score, and received their first procedure at a younger age ($p\leq0.0001$; table 1). Yearly incidence rates for severe events are provided in supplementary figure 2.

In summary, severe events occurred in both variant groups at comparable overall cumulative incidences, but significantly later in life in those patients with class I variants.

Discussion

This study describes the largest cohort of WAS patients ever collected. In many previous studies either patients with a distinct disease phenotype ("mild", "classic" or "early severe") [10,13], or just one therapeutic approach (usually HSCT or GT) were analyzed[16-18,22]. In contrast, this cohort includes patients with all grades of disease severity and thus enabled us to give a comprehensive overview over the distribution of disease burden in WAS patients and assess their natural disease outcome.

A certain degree of genotype/phenotype correlation in WAS has been known for a while [9,11,12]. In this study we identified a group of *WAS* genetic variants, which we termed class I, as predictive biomarkers for a less severe disease phenotype. Nevertheless, even these patients have a high probability of severe disease-related complications, which - on average - occur a few years later in life compared to patients with class II variants, but both patient groups have a significant risk of premature death. Our data advocate not to call class I variants "mild", because that would imply a perpetuous benign course of disease, which is not the case for many patients. This seemingly stands in contrast to a cohort of X-linked thrombocytopenia (XLT) patients with normal life expectancy that we previously reported [10]. However, in the latter study only patients with an already known mild disease course were included and retrospectively assessed, and even those had a significant incidence of disease-related events, but much less frequent than the class I cohort described here [10]. About 73% of patients in that study had class I variants [10].

Our findings are significant because they potentially allow treating physicians to counsel families on the expected disease severity based on the type of variant which is usually known at the time of diagnosis or shortly after. Given the excellent overall survival after HSCT or GT [16-18,23], this knowledge opens the discussion towards offering curative treatments even to patients carrying a class I variant who do not – or maybe not yet – exhibit the full clinical spectrum of WAS disease

manifestations. Currently, patients with a WAS score of 1 or 2 are labelled as XLT and often not considered for immediate curative therapy, which can be problematic if they develop life-threatening complications at an older age when permanent organ damage makes HSCT a risky procedure [48]. On the other hand, the decision to transplant a young child with "mild" WAS has to be carefully weighed against the long-term risks of the procedure and discussed with the family, especially if a well-matched donor is not available. Our data suggest that the label "XLT" should be used with caution, or even abandoned in favor of calling all patients with presumably disease-causing *WAS* variants "WAS patients" in order to encourage a genetics-based and more pragmatic therapeutic approach. Along the same lines these data emphasize that the established "WAS score" should only be used to describe current disease severity, but not as a basis for treatment decisions, because the score does not properly reflect the possibility of progression in disease severity. We also propose that the most suitable therapeutic approach should be reevaluated and set in context with the available scientific evidence for every WAS patient at regular time intervals.

The categorization of *WAS* variants as class I or II was not arbitrary. We grouped missense variants located in exon 1 or 2, which have been reported to result in a reduced amount of WASP expression, into class I. One additional intronic variant which results in transcription of multiple splicing products, including normal WASP, previously identified as a hotspot for a less severe disease course, was also added to class I [9-12]. Nevertheless, we cannot exclude that other *WAS* variants exist which are less detrimental than those labelled class II, but have not been identified as such because they are rare.

All reported *WAS* variants in this cohort were classified according to ACMG criteria. The variants of five patients were classified as "likely benign" or "benign". Three of these variants were previously reported in other WAS patients [10,34,35]. All five had classical WAS features with

thrombocytopenia, eczema and WAS scores between 2 and 5, but they were nevertheless excluded from the analysis of genotype/phenotype correlation. Without access to original patient material we are unable to decipher whether these variants are in fact pathogenic or whether these patients may have had additional deleterious variants in *WAS* which could not be detected, or had a variant in another gene resulting in a phenocopy of WAS, also considering that the genetic analysis of some of these patients was performed in the early 2000s or before.

While “benign” or “likely benign” variants have a sufficient probability to be not disease-causing, this is not necessarily the case for VUS. They have to be carefully interpreted in conjunction with clinical evidence and adequate variant curation is important. We therefore performed two separate analyses of genotype/phenotype correlation: one including patients with VUS and one more rigorous one excluding them. The results were very similar with regards to the diffrence between class I and II with a slightly more severe phenotype for patients with known pathogenic and likely pathogenic variants. To make the variant curation that we performed more helpful for clinicians, those variants that are currently not described in ClinVar were provided with their respective curation criteria as a reference in supplemental table 1. Many of these were reported for multiple patients in our cohort and/or had been previously reported in WAS [9-12,31-47].

It is important to note that other factors besides the underlying variant can influence the individual disease course of a WAS patient, such as somatic reversions, possibly disease-modifying genes, or administration of anti-infectives or thrombopoietic agents [19,21,49]. Also, the absolute numeric values for incidences of events in this study need to be interpreted cautiously, not only because of possible reporting bias, but also due to the fact that we censored analysis after HSCT or GT - often carried out at a young age. The numbers of patients at risk tend to get very low at ages >15 years and the incidences of disease complications at higher ages may therefore be underestimated because patients with more severe phenotypes were transplanted early and

excluded from the analysis after their HSCT or GT date. The same caveat applies to all Kaplan-Meier graphs in this manuscript where the number of patients beyond 30 years of follow-up are small, thus resulting in wide confidence intervals. It is important to note that this survey contains historic data, which precludes generalizing the findings to the current era with presumably improved supportive care and a lower threshold to proceed to HSCT in current times. There are other limitations to an international, retrospective chart-based analysis like this, where it is impossible to control for reporting bias or to monitor source data. The detail of HSCT-related data collected in this survey was very limited by design and not a focus of this study, and therefore not reported here. There was no significant difference in overall survival of patients who underwent HSCT and those who did not (supp. figure 1A), but this comparison using the Kaplan-Meier method is hampered by significant bias, because severely affected patients are more likely to undergo HSCT early and the transplant-related mortality will be higher in older patients. Survival after HSCT was 80% at two years and plateaued after that (supp. figure 1B). Two large, more recent cohorts from the US and Europe which evaluated the outcomes of HSCT in WAS were published in 2020 and 2022, respectively, showing improved survival after HSCT regardless of donor source [17,18].

In conclusion, this study demonstrates that the type of *WAS* variant is a valid biomarker predictive of the expected disease severity of WAS patients. Patients with the less severe class I variants can on average expect a later onset of severe WAS related complications, but remain prone to morbidity and premature mortality, justifying the evaluation for early definitive therapy in this group of WAS patients. These findings shall help to improve the counselling of families with WAS patients.

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Author contributions

MA, TV and JG designed the research, collected the data. MA, TV, JG and HB analyzed the data. All authors except HB provided original patient data. All authors reviewed the final manuscript and consented to its submission.

Conflict of interest statements

IM reports research support from CSL Behring, and being on a scientific advisory board for Boehringer-Ingelheim. MA reports research support by GSK and Orchard, and being on a scientific advisory board for CSL.

Tables

Table 1: Characteristics of the cohort

	entire cohort	class I variant	class II variant	variant not classified	p=*
number of patients	577 (100%)	209 (100%)	316 (100%)	52 (100%)	
region					
Europe	278 (48.2%)	106 (50.7%)	139 (44.0%)	33 (63.5%)	0.1545
USA	113 (19.6%)	67 (32.1%)	42 (13.3%)	4 (7.7%)	
rest of world:	186 (32.2%)	36 (17.2%)	135 (42.7%)	15 (28.8%)	
Russia	54 (9.4%)	12 (5.7%)	41 (13.0%)	1 (1.9%)	
China	103 (17.9%)	22 (10.5%)	79 (25.0%)	2 (3.8%)	
Brazil	15 (2.6%)	0 (0.0%)	6 (1.9)	9 (17.3%)	
other	14 (2.4%)	2 (1.0%)	9 (2.8)	3 (5.8%)	
age at diagnosis (years)					
≤2	309 (57.0%)	76 (36.4%)	199 (63.0%)	34 (65.4%)	<0.0001
>2-5	118 (20.5%)	46 (22.0%)	59 (18.7%)	13 (25.0%)	
>5-11	57 (9.9%)	23 (11.0%)	31 (9.8%)	3 (5.8%)	
>11-18	35 (6.1%)	24 (11.5%)	10 (3.2%)	1 (1.9%)	
>18	20 (3.5%)	14 (6.7%)	6 (1.9%)	0 (0.0%)	
not reported	38 (6.6%)	26 (12.4%)	11 (3.5%)	1 (1.9%)	
median (range)	1.5 (0.0-68.0)	2.4 (0.0-68.0)	1.3 (0.0-33.0)	1.2 (0.0-11.3)	
age at last follow-up (years)					
≤2	41 (7.1%)	12 (5.7%)	27 (8.5%)	2 (3.8%)	0.1343
>2-5	89 (15.4%)	27 (12.9%)	55 (17.4%)	7 (13.5%)	
>5-11	121 (21.0%)	42 (20.1%)	69 (21.8%)	10 (19.2%)	
>11-18	86 (14.9%)	40 (19.1%)	37 (11.7%)	9 (17.3%)	
>18	82 (14.2%)	44 (21.1%)	28 (8.9%)	10 (19.2%)	
not reported/dead (n,%)	158 (27.4%)	44 (21.1%)	100 (31.6%)	14 (26.9%)	
median (range)	8.9 (0.3-71.1)	11.0 (0.3-68.0)	6.7 (0.5-71.1)	11.0 (1.5-42.0)	
survival status at last follow-up					
alive	464 (80.4%)	190 (90.9%)	235 (74.4%)	39 (75.0%)	<0.0001
deceased	113 (19.6%)	19 (9.1%)	81 (25.6%)	13 (25.0%)	
WAS score at last follow-up or before first procedure					
1	54 (9.4%)	47 (22.5%)	7 (2.2%)	0 (0.0%)	<0.0001
2	144 (25.0%)	84 (40.2%)	53 (16.8%)	7 (13.5%)	
3	161 (27.9%)	40 (19.1%)	98 (31.0%)	23 (44.2%)	
4	109 (18.9%)	10 (4.8%)	86 (27.2%)	13 (25.0%)	
5	86 (14.9%)	19 (9.1%)	60 (19.0%)	7 (13.5%)	
not reported	23 (4.0%)	9 (4.3%)	12 (3.8%)	2 (3.8%)	
procedures					
splenectomy	79 (13.7%)	31 (14.8%)	40 (12.7%)	8 (15.4%)	0.5456
HSCT	255 (44.2%)	44 (21.1%)	177 (56%)	34 (65.4%)	
gene therapy	14 (2.4%)	4 (1.9%)	10 (3.2%)	0 (0%)	
more than one procedure	30 (5.2%)	4 (1.9%)	21 (6.6%)	5 (9.6%)	
no procedure	263 (45.6%)	134 (64.1%)	114 (36.1%)	15 (28.8%)	
age at first procedure (years)					
≤2	115 (19.9%)	13 (6.2%)	88 (27.8%)	14 (26.9%)	0.0001
>2-5	103 (17.9%)	28 (13.4%)	63 (19.9%)	12 (23.1%)	
>5-11	40 (6.9%)	15 (7.2%)	17 (5.4%)	8 (15.4%)	
>11-18	20 (3.5%)	6 (2.9%)	12 (3.8%)	2 (3.8%)	
>18	9 (1.6%)	4 (1.9%)	5 (1.6%)	0 (0.0%)	

no procedure or not reported (n, %)	290 (50.3%)	143 (68.4%)	131 (41.5%)	16 (30.8%)	
median (range)	2.3 (0.4-33.7)	3.5 (0.5-33.7)	2.1 (0.4-29.0)	2.2 (0.4-13.8)	

*: chi-square test for the Null hypothesis that the proportions of subjects in category x are equal in class I variant and class II variant subjects. x is the respective category in the same row. Subjects with missing values are not included in the analysis. All p-values are unadjusted.

Table 2: Genetics

patients with exact genetic information (n, %):	525 (100%)
missense	239 (45.5%)
nonsense	80 (15.2%)
intronic	90 (17.1%)
deletion	76 (14.5%)
insertion	40 (7.6%)
classes of variants:	525 (100%)
class I: missense in exon 1+2 or c.559+5G>A	209 (39.8%)
class II: other exact variant	316 (60.2%)
classification of variants according to ACMG criteria:	525 (100%)
class I: missense in exon 1+2 or c.559+5G>A	209 (39.8%)
pathogenic	160 (76.6%)
likely pathogenic	37 (17.7%)
VUS	11 (5.3%)
likely benign	1 (0.5%)
benign	-
class II: other exact variant	316 (60.2%)
pathogenic	130 (41.1%)
likely pathogenic	133 (42.1%)
VUS	49 (15.5%)
likely benign	1 (0.3%)
benign	3 (0.9%)
hotspots* (n):	
class I	
c.C134T; p.Thr45Met	15
p.Val75Met/Gly/Leu	60
p.Arg86His/Leu/Gly/Cys	59
c.559 +5 G>A	22
class II	
c.C121T ; p.Arg41X	10
c.C631T; p.Arg211X	21
c.777+1 G>A	10

*: defined as ten or more patients with this variant

Table 3: Causes of death

all deceased patients	113 (100%)
bleeding	26 (23.0%)
intracranial	16
pulmonary	3
other/unknown	7
infection	31 (27.4%)
bacterial	13
sepsis	8*
pneumonia	4
meningitis	2
fungal	2**
viral	8***
other/unknown	8
malignancy	8 (7.0%)
lymphoma	6****
leukemia	2*****
autoimmunity	4 (3.5%)
AIHA	3
colitis	1
directly HSCT related	18 (15.9%)
GVHD	5
VOD	3
infection	9
organ toxicity	3
other/unknown	2
other/ unknown******	26 (23.0%)

In the first category, the leading cause of death is given. Numbers in the subcategories may not add up because some patients had multiple reported causes of death.

*: 2 pneumococcus, other organisms not reported

**: 1 aspergillus, 1 organism not reported

***: 3 CMV, 5 organism not reported

****: 1 B-NHL, 1 DLBCL, others not specified

*****: 2 T-ALL, both relapsed after HSCT

******: contains deaths which occurred more than 6 months after HSCT and were not clearly attributable to HSCT.

Figure legends

Figure 1: Classification of *WAS* variants

Distribution of class I variants (A) and class II variants (B and C) across the *WAS* gene. Variants are displayed as classified according to ACMG criteria as either “pathogenic” (red), “likely pathogenic” (orange), “uncertain significance (VUS)” (grey). Variants classified as “likely benign” or “benign” not shown.

Figure 2: Overall survival.

Overall survival (OS) of the entire cohort (A), and of the entire cohort with patients censored at the time of HSCT or GT (B).

Figure 3: Severe disease-related events.

Cumulative incidence and incidence per patient year for severe disease-related events: any severe event (A, B), severe bleeding (C, D), severe infection (E, F), autoimmunity (G, H) and malignancy (I, K). Follow-up censored at the time of first procedure. Only the first event of a category was counted for cumulative incidence, while all events of a specific category were counted for incidence per year.

Figure 4: Overall survival by class of variant.

(A) Overall survival, comparing those with either a missense variant in exons 1+2 or the c.559+5G>A intronic variant (class I) to all others (class II). (B) Same analysis excluding patients with VUS according to ACMG criteria. Variants classified as “likely benign” or “benign” were excluded from this analysis.

Figure 5: Incidence of severe events by class of variant.

Cumulative incidence of the first severe disease-related event comparing those with class I variants (missense variant in exons 1+2 or the c.559+5G>A intronic variant) to class II variants (all other variants) including all patients (left panel) or excluding patients with VUS (right panel): any severe event (A, B), severe bleeding (C, D), severe infection (E, F), autoimmunity (G, H) and malignancy (I, K). Variants classified as “likely benign” or “benign” were excluded from this analysis.

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