As a further step, we focused on the clinical definition of these items and the grading of severity adopted in MDS-CI and ACE-27, and evaluated their goodness of fit in the MDS patient population. To this purpose, Akaike information criterion (AIC) was employed which allows the evaluation of a model by combining goodness of fit and complexity (among different models, a lower AIC indicates a better trade-off between fit and complexity, while models with an AIC difference of 4 or more with respect to the reference model have considerably less support).[10] For all comorbid conditions considered in the analysis, clinical definition and grading of severity by MDS-CI had greater goodness of fit for capturing the prognostic information of these comorbid conditions in MDS patients compared to ACE-27 (AIC for ACE 27 *vs.* MDS-CI cardiac disease 2929 *vs*. 2925, hepatic disease 2932 *vs.* 2930, pulmonary disease 2931 vs. 2928, renal disease 2930 *vs.* 2926, and solid tumor 2929 *vs.* 2926). Taken together, these data strengthen the importance of not basing definition of the prognostic value of comorbidity in the clinical setting of MDS patients exclusively on the severity of the comorbid condition *per se*, but also taking MDS-specific features into account.

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Low frequency of type-I and type-II aberrations in myeloid leukemia of Down syndrome, underscoring the unique entity of this disease

We recently published in this journal an overview of the currently known genetic events required for the development of pediatric acute myeloid leukemia (AML).[1] These aberrations can be subdivided into type-I aberrations that result in uncontrolled proliferation, and type-II aberrations that lead to the impaired differentiation of the leukemic cells.[1-2]

Recent advances in technology have allowed many novel genetic and molecular abnormalities to be detected, including cryptic translocations (such as *NUP98-NSD1*), and single gene mutations, occurring for instance in the *NPM1*, *CEBPA*, *WT1* and *MLL*-gene (MLL-PTD) which are predominantly found in patients with cytogenetically normal (CN)-AML.[1,3] Newly discovered mutations identified by whole genome sequencing include mutations in the genes encoding for *IDH1/ IDH2* and the DNA methyltransferase (*DNMT3A*) gene, which are rare in pediatric AML.[4-5]

Children with Down syndrome have an increased risk of developing acute myeloid leukemia (ML-DS).[6] ML-DS is a unique disease entity, and differs in clinical characteristics and biology from AML in non-DS children. It is characterized by somatic mutations in the *GATA-1* gene[7] which are unique for every patient. The role of the well-known and newly discovered type-I/II aberrations in myeloid leukemia of Down syndrome (ML-DS) has not yet been systematically investigated.

Therefore, we screened 34 newly diagnosed ML-DS patients for the presence of the above mentioned type-I and type-II aberrations. Samples were provided by the Dutch Childhood Oncology Group, the AML-'Berlin-Frankfurt-Munster' Study Group, and the Nordic Society for Pediatric Hematology and Oncology. Of the 34 patients, 12 ML-DS patients had a normal karyotype; this is important to note since some of the novel aberrations in non-DS AML are highly associated with a normal karyotype.

Screening of gene mutations was carried out according to availability of material. Mutation analysis was performed for the hotspot regions of the *NPM1*, *CEPBA*, *MLL* (i.e. partial tandem duplications, PTD), *WT1*, *FLT3* (i.e. internal tandem duplications, ITD) and tyrosine kinase domain mutations (TKD), *N-RAS* and *K-RAS*, *PTPN11*, *KIT*, *IDH1/ IDH2*, and the *DNMT3A* genes, as previously described.[1,5] In addition, we investigated the presence of the cryptic translocation *NUP98/NSD1* by reverse transcriptase-polymerase chain reaction (RT-PCR).[3] A complete list of investigated regions, primers and PCR condi-

Table 1. Characteristics of ML-DS patients.

ID	Sex	Age	WBC	Karyotype
1	M	2.5	6	48,XY,t(9;14)(p21;q24),+11,der(16)t(1;16)(q22;p13),+21c[21]/47,XY,+21c[14]
2	M	1.4	6	48,X,ins(Y;5)(q11;?),der(3)t(3;6)(q2?8;?) or ins(3;6)(q2?8;?),-5,del(6)(q1?4q2?4),+21c,+21,+mar[24]
3	F	2.3	49	47,XX,der(9)inv(9)(p24q2?1)del(9)(q2q3),+21c[14]
4	F	1.9	40	47,XX,der(1)t(1;1)(p36;q21),t(5;6)(p15;p23),+21c[23]
5	F	2.4	3	47,XX,r(7)(p22q22).ish r(7)(WCP7+,D7Z1+,D7S486-,164D18-,3K23-),+21c
6	F	1.2	53	47,XX,?del(3)(q?26),add(17)(q?21),add(20)(q11),+21c[10]
7	F	1.4	10	46,XX,dic(15;21)(p11;p11),+21c[10]/46,idem,t(3;5)(q24-q25;p15)[4]/46,idem,t(3;5)(q24-q25;p15),add(10)(p31~32)[6]
8	M	1.8	21	47,XY,del(13)(q13q21),+21c[3]/47,XY,+21c[3]
9	F	1.9	11	47,XX,del(7)(p11.1),+21c[3]/47,XX,+21c[4]
10	M	1.3	6.4	47,XY,t(4;15)(q?21;q?21),del(7)(q?31q?33),+21c/47,XY,+21c
11	F	2.2	7	45-46,XX,der(1)t(1;6)(q31;q?),ins(4;1)(q12;q25q44),-6,-7,der(7)t(6;7)(p21;p22),der(7)t(7;8)(q2?2;q2?3),der(11)t(7;11)(p14;p15),+21c,der(22)t(1;22)(q25;p11)[cp13]/47,XX,+21c[28]
12	F	1.5	7.2	48,XX,+8,+21[5]/47,XX,+21c[18]
13	M	1.0	3.5	47~49,XY,+?19,+21c,+21[cp7]/47,XY,+21c[5]
14	F	2.0	6	48,XX,der(9)t(1;9)(q23;p22),+11,?del(13)(q14q21),+21c[18]
15	M	2.7	4.9	48, XY, +21, +21c
16	F	0.9	3.2	47,XX+21c[13]
17	M	2.3	12	47,XY,+21c [11]
18	F	2.7	4.8	47,XX+21c[6]
19	M	1.1	20	47,XY+21c[15]
20	M	1.3	18	47,XY+21c[1]
21	M	0.9	47	47,XX+21c
22	M	1.8	7	47,XY,+21c [13]
23	M	0.9	4.7	47,XX+21c
24	M	2.0	26	47,XY+21c[25]
25	F	0.8	4.2	47,XX+21c
26	F	1.2	17	47,XX+21c
27	F	1.8	5.9	47,XX+21c
28	M	2.0	26	NA
29	F	2.2	9	NA
30	F	0.9	19	NA
31	F	1.1	26	NA
32	F	2.0	168	NA
33	M	1.3	6	NA
34	F	0.7	4	NA

Age in years; WBC: white blood cell count (x10⁹/L); NA: not available.

tions is provided in the *Online Supplementary Table S1*. Purified PCR products were bi-directionally sequenced on an ABI Prism 3100 genetic analyzer (Applied Biosystems Inc., Foster City, CA, USA). The sequence data were assembled and analyzed for mutations using CLC Workbench version 3.5.1 (CLC Bio, Aarhus, Denmark).

Median age of the ML-DS patients was 1.8 years (range 0.7-2.7 years). Median WBC was 7.1×10⁹/L (range 3-168×10⁹/L) and 42% were male. Patients' characteristics are described in detail in Table 1.

In our cohort, only mutations were found in the RAS-pathway, i.e. 2 of 34 (7%) of the patients (ID5 and ID30) carried the G12D mutation in the *K-RAS* gene, and in one patient (ID 32) (3%) the G12D mutation in *N-RAS* was found. These *RAS*-mutations have been described in approximately 20% of non-DS pediatric AML patients.[1] In a previous report by Chen *et al.*, N-RAS mutations were reported in one of 9 ML-DS and in none of 11 TMD patients.[8]

No *WT1* mutations were found, but 4 of 34 (17%) ML-DS patients carried the rs16754 single nucleotide polymorphism (SNP) in the *WT1* gene. *WT1* expression is used as a marker for minimal residual disease (MRD) detection in TMD[9] and non-DS pediatric AML.[10] To date, no *WT1* SNPs have been described in ML-DS patients. There is still some controversy about the prognostic impact of this SNP in non-DS pediatric AML; Hollink *et al.* did not find any prognostic significance[11] whereas Ho *et al.* identified this *WT1* SNP as an independent predictor of favorable outcome.[12] None of our patients with the *WT1* SNP had an event; however, one patient died due to resistant disease. Two patients with the *WT1* rs16754 SNP simultaneously had an RAS-mutation.

Mutations in *FLT3*, *KIT*, *CEBPA*, *NPM1*, *MLL-PTD*, *DNMT3A* and *IDH1/2* were not found. Two patients (7%) carried the *IDH1* rs11554137 SNP. In non-DS pediatric AML, this *IDH1* SNP was found in 47 of 460 cases (10%).[4] The *NUP98/NSD1* transcript was not detected in any of

Table 2. Frequency of aberrations in ML-DS patients compared to non-DS AML patients.

Genes	AML (n)	Aberrant (n)	Frequency (%)	95% CI interval	Genes	ML-DS (n)	Aberrant (n)	Frequency (%)	Expected frequency (%)	*P* value
NPM1	337	26	7.7	0.05-0.11	*NPM1*	34	0	0	2.6	0.066
CEBPA	282	17	6.0	0.04- 0.09	*CEBPA*	34	0	0	2.1	0.122
MLLPTD	309	7	2.3	0.01-0.05	*MLLPTD*	27	0	0	0.8	0.538
WT1	330	29	8.8	0.06-0.12	*WT1*	34	0	0	3.0	*0.044**
FLT3_ITD	372	67	18.0	0.14- 0.22	*FLT3_ITD*	34	0	0	6.1	*0.001**
FLT3_TKD	330	9	2.7	0.01- 0.05	*FLT3_TKD*	34	0	0	0.9	0.39
nRAS	353	57	16.1	0.13- 0.20	*nRAS*	34	1	2.9	5.5	*0.003**
kRAS	353	13	3.7	0.02- 0.06	*kRAS*	34	2	5.9	1.3	0.279
PTPN11	350	7	2.0	0.01- 0.04	*PTPN11*	34	0	0	0.7	0.503
KIT	368	31	8.4	0.06- 0.12	*KIT*	34	0	0	2.9	0.051
DNMT3A	140	3	2.1	0.01- 0.06	*DNMT3A*	27	0	0	0.7	0.479
IDH1	281	7	2.5	0.01- 0.05	*IDH1*	27	0	0	0.8	0.424
IDH2	281	9	3.2	0.02- 0.06	*IDH2*	27	0	0	1.1	0.331
NUP98/NSD1	293	13	4.4	0.03- 0.07	*NUP98/NSD1*	27	0	0	1.5	0.297

**Indicates a significant difference*

our samples.

When we examined the expected frequency of the aberrations in our ML-DS cohort compared to the observed frequency in non-DS AML pediatric AML patients as a reference cohort (calculated from a binomial distribution), only the frequency of *WT1*, *FLT3-ITD*, and *nRAS*-mutations appeared to be significantly lower. The lack of a statistically significant result in the other aberrations may be due to the low frequencies in non-DS AML. An overview of the frequencies and aberrations is provided in Table 2.

We conclude that the (molecular) type-I/type-II aberrations, relevant in pediatric non-DS AML, are absent or rare in ML-DS patients. Except for mutations in the *RAS*-gene (and SNPs in the *WT1* and *IDH1* genes), we did not detect any aberrations. Our study underscores the unique signature of ML-DS, and stresses the fact that further research is needed to unravel the molecular abnormalities involved in the leukemogenesis of this specific disease.

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