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A high proportion of germline variants in pediatric chronic myeloid leukemia

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Abstract

Chronic myeloid leukemia (CML) typically occurs in late adulthood. Pediatric CML is a rare form of leukemia. In all age groups, the characteristic genetic driver of the disease is the *BCR::ABL1* fusion gene. However, additional genomic events contribute to leukemic transformation, which is not yet well-characterized in pediatric CML. We investigated the mutational landscape of pediatric CML to determine whether predisposing germline variants may play a role in early-age disease development. Whole exome sequencing and targeted sequencing were performed in pediatric and adult CML samples to identify age-related germline and somatic variants in addition to the *BCR::ABL1* translocation. Germline variants were detected in about 60% of pediatric patients with CML, with predominantly hematopoietic genes affected, most frequently *ASXL1*, *NOTCH1*, *KDM6B*, and *TET2*. The number of germline variants was significantly lower in adult patients with CML. If only confirmed pathogenic variants were regarded as cancer-predisposing variants, the occurrence was ~10% of pediatric CML, which is comparable to other hematological malignancies and most childhood cancer entities in general. We hypothesize that the interaction with the strong oncogene *BCR::ABL1* may also favor the development of leukemia by weaker variants in the same genes. In pediatric patients, the germline variants of genes associated with clonal hematopoiesis may increase the likelihood that an incidental *BCR::ABL1* translocation triggers the early manifestation of CML.

Keywords Chronic myeloid leukemia, Pediatric CML, Germline variants, Cancer predisposition

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Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disease with incidence markedly increasing with age. The typical age at diagnosis in Western populations is in the sixth decade of life. CML rarely occurs in children and adolescents, with an annual incidence of one per million in children aged < 15 years and 2.2 cases per million in adolescents aged 15–19 years, constituting 2% and 9% of all leukemias in these two age groups, respectively [1].

At all ages, CML is characterized by the underlying fusion of the *BCR* gene on chromosome 22 with the *ABL1* gene on chromosome 9 forming the chromosomal translocation t(9;22)(q34;q11.2). In most cases, the disease is diagnosed in the chronic phase (CP). If untreated, progression usually occurs with the expansion of clones with high-risk features and transition to the terminal blast phase (BP) [2].

While *BCR::ABL1* fusion is generally considered necessary for CML manifestation, model systems for *BCR::ABL1*-driven leukemia induction, detection of *BCR::ABL1* gene expression in healthy individuals, and detection of preexisting mutations in Ph-negative preleukemic hematopoietic clones demonstrate that translocation alone is not sufficient for leukemogenesis in all cells and additional mutations in cooperating genes play a role in CML development [3, 4]. The frequency of additional mutations and the number of mutations in a patient increase considerably as the disease progresses to BP [5]. Many of the alterations identified in CML-CP [6], mainly affecting *DNMT3*, *TET2*, and *ASXL1*, are also present with high frequencies in other hematologic malignancies and can also be detected in individuals with blood cell parameters in the normal range (clonal hematopoiesis of indeterminate potential [CHIP]) in an age-related manner. CHIP is rarely detected in people aged < 40 years but in about 10% of people aged > 70 years. Individuals with CHIP showed an increased risk of developing hematological neoplasms [7]. However, clonal hematopoiesis as a basis for leukemia development plays only a minor role at a young age.

In contrast, in pediatric cancers predisposing germline variants have been described in 10–25% of the affected individuals [8], and in 5–15% of patients with myeloid malignancies [9]. However, no data are currently available for CML, particularly with regard to age dependency. Therefore, we surveyed the genomic landscape in a German pediatric and adult CML cohort. A high proportion of young individuals had germline variants in genes that play a role in hematopoiesis and leukemia development and that are recurrently present in related myeloid malignancies.

Methods

Patients

The study included 62 pediatric patients with CML from the German pediatric CMLpaed II study ($n = 156$; EudraCT 2007-001339-69), all of whom were classified as European origin by SNP genotyping and had sufficient material and informed consent for genetic analysis. For whole-exome sequencing (WES) 12 of the youngest patients with CML (3–13 years; median, 7 years) diagnosed in CP were selected, and the cohort for our enrichment sequencing panel contained the remaining 50 pediatric cases. This investigated cohort is representative of the whole CMLpaed II study cohort. Patient characteristics are listed in Supplemental Table 1.

62 samples from adult patients with CML were received from hematological centers in Ulm and Munich Leukemia Laboratory. The cohort undergoing WES comprised 11 patients (21–77 years; median, 58 years; one diagnosed in the accelerated phase (AP) and 10 in CP; one of the 12 initially selected samples did not pass quality control). The panel sequencing cohort included 50 individuals aged 20–77 years (median age, 46 years, evenly distributed over the age range with approx. 10 individuals per age decade) to investigate an age-dependent distribution of mutations.

To validate the germline variants, saliva samples or fingernails were utilized as reference biomaterials in addition to remission samples (*BCR::ABL1* < 5%), when available. To investigate the heritability of the identified germline variants in 15 pediatric patients, blood and saliva samples of the parents and, if available, of the siblings were also examined in trio analyses.

For genetic analyses, all participants or legal guardians provided informed consent in accordance with the Declaration of Helsinki (pediatric CML: EK282 122 006, EK 236_18 B; adult CML: EK05117, EK3456-05/12).

Whole exome and targeted enrichment sequencing

Whole exome sequencing (WES) was performed on diagnosis and matched remission samples from either 1000 ng DNA using the TruSeq DNA Sample Prep v2 kit (Illumina) with the TruSeq Exome Enrichment kit (Illumina) or from 50 ng DNA using the Nextera Rapid Capture Exome kit (Illumina) according to the manufacturer's instructions. The libraries were sequenced on HiSeq 2000 with 100 bp paired-end reads using a 200-cycle TruSeq SBS v3 kit (Supplemetal Methods).

For the targeted approach, a custom amplicon panel, HaloPlex HS (Agilent Technologies, CA, USA), was designed based on the usage of molecular barcodes. Panel design was performed based on findings from exome sequencing. In addition, genes commonly mutated in CML and other hematologic malignancies were included in the panel (Supplemental Table 2).

Sequencing data analysis

Specification of the mutation analysis pipeline, WES and amplicon-sequencing coverage statistics are shown in the Supplemental Methods, Supplemental Tables 3 and 4.

Mutational processes (*"Alexandrov" signatures)* were investigated with the sigProfilerExtractor.py script with 5000 replicates and corresponding reference genome GRCh37. Details are listed in the Supplemental Methods.

Classification and assessment of germline variants

The sequence variants that passed the filtering steps described in the sequence data analysis section were evaluated in addition to manual curation using several prediction tools to obtain a quantitative score for the potential effect of the variant sequence on the gene product. In addition to the widely used AlphaMissense and VIPUR algorithms, a more specialized Hematological Predictor of Pathogenicity (HePPy) score has been used. Details are listed in the Supplemental Methods. All scores obtained and the mean of the scores when several variants are present in an individual are listed in Supplemental Table 6.

Results

WES in pediatric CML reveals a high proportion of individuals with germline variants but few somatic variants

For the first exploratory cohort, 12 pediatric cases of CML with age at diagnosis below the median age of onset (13.5 years) in the German Childhood Cancer Registry population (Fig. 1A) were selected for WES, which identified 33 genetic variants in 32 genes. Of those, 14 variants found in 9/12 patients were somatic (range of variant allele frequency [VAF] 25–75%), and 19 variants in 11/12 cases were identified as constitutional. Most patients had 1–2 variants, except for a 6-year-old patient (UPN 295) who presented with five variants (Fig. 1B, Supplemental Table 6). One non-frameshift substitution in *SPRR3*, a polymorphism, was identified in two patients, and all other variants were private to the affected individual.

WES analysis revealed a list of 32 affected genes of which the predominant majority are known for their involvement in myeloid neoplasms, with chromatin modifiers *(ASXL1*, *KMT2D*, and *KMT2A)*, transcription factors (*NPAT*, and *NOTCH1)*, tyrosine kinase (co)receptors (*DYRK1A*, *KIT*, *JAK1*, *GIGYF2*, *PTPN11*, *CSF1R*, *EPHA5*, and *ROS1)*, cell cycle control (*CUX1*), tumor-suppressor (*EPOR*), and DNA damage repair genes (*FANCM*, and *NBN*).

In comparison, 11 adult patients with CML (aged 21–77 years) presented with fewer germline variants ($n=8$), but significantly more somatic variants ($n=65$) (Fisher´s exact test: $^{****}p<0.0001$). Eight germline variants were detected in five patients. Interestingly, four of the 11 individuals (36%) with at least one germline variant were particularly young (21, 23, 29, and 43 years, respectively). 65 somatic variants were present in all adult patients, with 2–9 variants per patient (Fig. 1B, supplemental Table 6). All variations were unique to individual patients.

Germline and somatic variant allele frequencies were monitored during tyrosine kinase therapy in six pediatric patients with multiple variants and sampling time points. In comparison, *BCR::ABL1* transcript and *BCR::ABL1* genomic fusion gene copy numbers were quantified. Germline variants show a consistent presence over time, whereas a decrease in the number of CML cell clones can be demonstrated by a reduction in *BCR::ABL1* copy numbers. By contrast, somatic variants show a differential therapy-induced reduction, indicating the variable response of different CML subclones. Figure 1C presents the results from three representative cases.

To study the inheritance of the germline variants, saliva and blood samples from parents and siblings were analyzed in 9/12 families with pediatric CML patients when the complete set was available. Each germline variant could be detected in at least one parent (Fig. 1D; Table 2).

The genomic landscape of pediatric CML has similarities and differences to adult CML

To confirm the results of the WES cohort, 50 additional pediatric patients with CML were analyzed by targeted sequencing. Genes with germline variants identified in WES, genes previously identified to be involved in CML pathogenesis, or genes with crucial functions in myeloid differentiation and leukemogenesis were included in the panel and analyzed. The combined study cohort ($n=61$, of which WES $n=12$, and targeted sequencing $n=49$; note: one case was discarded due to insufficient data quality) is representative of the entire cohort of pediatric patients with CML in the CMLpaed II study cohort (Supplemental Table 1).

The identified variants were validated by Sanger sequencing and stratified to a somatic or germline status by VAF and analysis of matched remission samples. The germline variants were also sequenced in the parents if the material was available (Supplemental Table 5).

In addition to the *BCR::ABL1* translocation detected in all cases, 67 variants were identified in 43/61 (70%) individuals, including 52 germline variants in 38/61 (62%) patients (Fig. 2A, Supplemental Table 6). The most frequent variants were found in *ASXL1*, *NOTCH1*, *KDM6B*, *TET2*, *RUNX1*, *GIGYF2*, and *CUX1*.

To determine age-related effects, 61 adult patients with CML were analyzed in an identical pipeline (WES $n=11$, and targeted sequencing $n=50$). In total, 56 variants in 35/61 (57%) individuals could be detected. The pattern of the affected genes was comparable to the pattern of childhood CML (range of VAF 6–66%; Supplemental

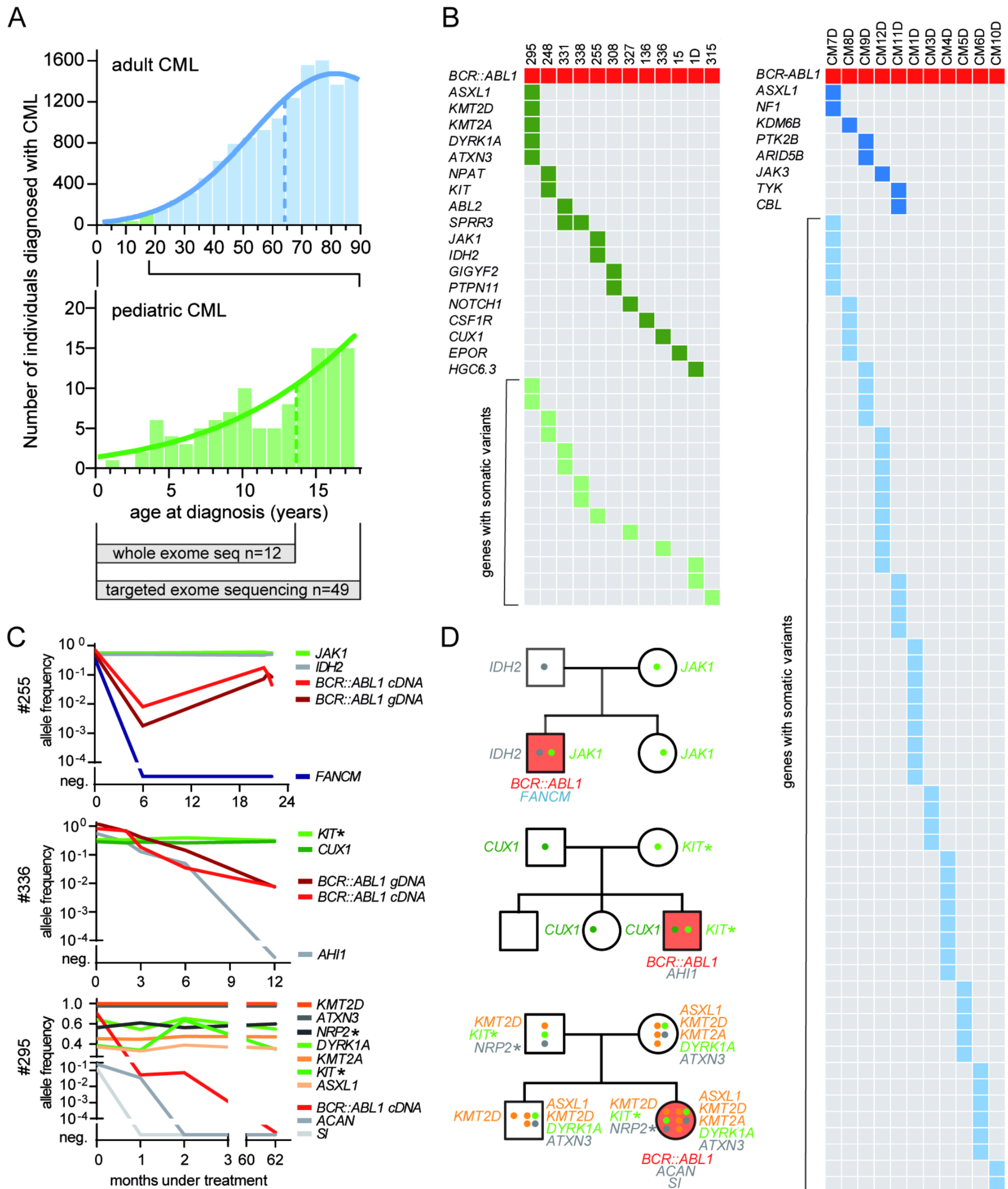

Fig. 1 Whole exome sequencing in pediatric CML reveals a high proportion of individuals with germline variants but few somatic variants. (**A**) Age distribution of CML in children based on data from the German CMLpaed II study (2004–2015), and adults based on cancer registry data from the Robert Koch Institute over 11 years (2004–2014). The median age at diagnosis in the respective cohort is indicated by dashed lines. (**B**) Oncoplot showing germline (dark colors) and somatic (light colors) variants in 12 pediatric (green) and 11 adult (blue) patients with CML. Each column represents one patient. Every patient has a detectable *BCR::ABL1* fusion gene (red). (**C**) Quantification of germline and somatic variants in parallel to the *BCR::ABL1* fusion gene and *BCR::ABL1* fusion transcript at initial diagnosis and under tyrosine kinase inhibitor treatment in three pediatric patients with CML. Corresponding pedigree charts (**D**) show the inheritance of germline variants. An asterisk indicates SNP with > 1% allele frequency

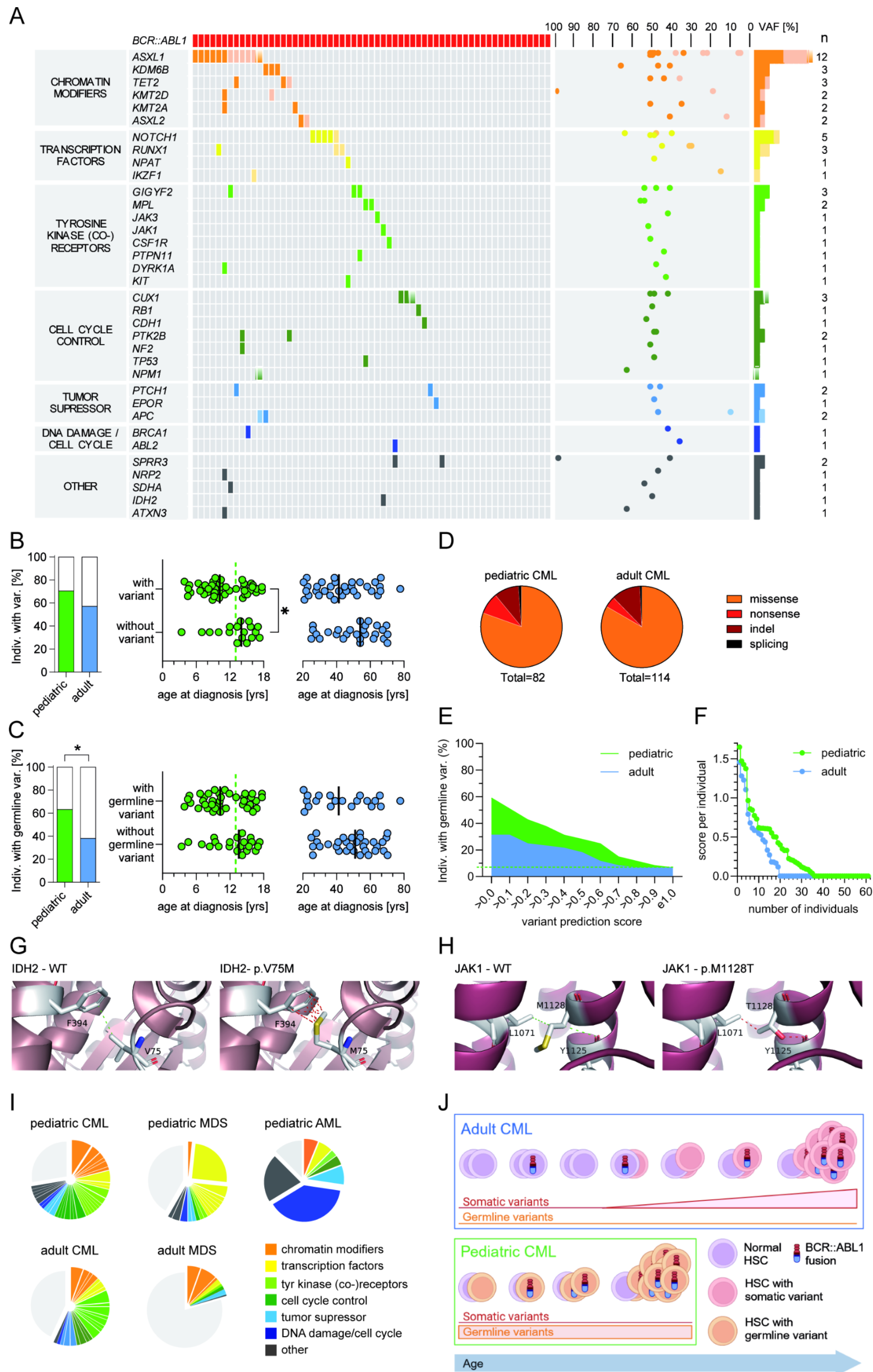

Fig. 2 (See legend on next page.)

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Fig. 2 Targeted exome sequencing in pediatric CML exhibits both similarities and differences to adult CML. (**A**) Germline (dark colors) and somatic variants (light colors) in 61 pediatric patients with CML are displayed, with variant allele frequency (VAF). Variants with unknown mutation status are presented using gradient colors. Each column represents one patient, and genes are functionally grouped. (**B, C**) The bar plots demonstrate the proportion of pediatric and adult patients with CML affected by any variation (somatic and/or germline) (B), and in germline variations only (C). Differences were tested for significance using Fisher´s exact test (*, $p<0.05$). The scatter plots show the age at diagnosis plotted for both age cohorts according to the group with all variants (top) and according to the group with germline variants (bottom). The Kolmogorov-Smirnov test was used to test for significant differences (*, $p<0.05$). The vertical black lines indicate the median in the respective group, and the dashed green line the median age of the analyzed cohort. (**D**) Pie charts showing the distribution of mutation types for pediatric and adult patients with CML. (**E**) Proportion of individuals with CML with germline variants, subdivided according to the variant prediction score. (**F**) Presentation of the number of patients with the sum score for all individual germline variants. (**G, H**) Visualization of the modeled structural change by the IDH2 V75M (G) and the JAK1 M1128T (H) variant in the study patient #255. (**I**) Pie charts illustrating the distribution of functionally grouped genes with germline variants in pediatric CML, adult CML, pediatric MDS, adult MDS, and pediatric AML. For pediatric AML, functional groups rather than individual genes are presented, as over half of the patients (52%) display multiple germline variants (3–8 germline variants). (**J**) Graphic illustration of the hypothesis regarding the contribution of predisposing germline variants or acquired somatic mutations with the crucial *BCR::ABL1* fusion in the progression of CML in children and adults

Fig. 1, Supplemental Table 6). However, the number of patients with germline variants was significantly lower in the adult group ($p=0.0157$; Fig. 2B).

The median age at diagnosis was younger in the group with variants (10 vs. 14 and 41 vs. 54 years, respectively) (Fig. 2B) and germline variants only (10 vs. 13 and 41 vs. 51 years, respectively) in both cohorts (Fig. 2C). The distribution of variant types (missense, nonsense, indel, and splicing) was identical in both age cohorts (Fig. 2D). In general, the median number of all mutations per megabase in children was 4.5-fold lower than that in adults (6.2 versus 27.8 mutations per megabase). To gain further insight into the potential mechanisms underlying the mutational pathogenesis in children and adults, the available collection of single base substitution (SBS) and insertions deletions (ID) signatures were analyzed (Supplemental Fig. 2) based on the COSMIC catalog of somatic mutations in cancer. The enrichment of the SBS3 mutational signature in the pediatric CML samples indicates the involvement of defective homologous recombination DNA damage repair in these young individuals. Additionally, the presence of SBS3-related events was correlated with an increase in ID1-associated events. The ID1 signature is found in most cancers and is thought to correlate with the age at diagnosis. This signature is particularly strong in cancer samples with defective DNA mismatch repair and microsatellite instability. As the ID1 signature is based on 1 bp insertion mainly in T homopolymers of length greater than 4 and given the multitude of contexts of single base substitutions in the SBS3 signature, it would be unlikely that either SBS3-related events be misclassified as ID1 events and vice versa. The presence of variants, neither germline nor somatic, had no prognostic effect on the achievement of treatment milestones such as the time to major molecular response (*BCR::ABL1* ≤ 0.1%) or deep molecular response (*BCR::ABL1* ≤ 0.01%) in our cohort (Supplemental Fig. 3).

When the total number of germline variants is plotted against the scores given by different prediction tools for the effect of the variant on protein structure or the likelihood of the presence of a pathological variant, the proportion of individuals with a germline variant is again higher in children and adolescents across the spectrum. However, both age groups show a similar proportion of highly probable or confirmed predisposing variants of 15% in the pediatric and 13% in the adult group with scores > 0.9, and the same proportion of 7% at scores ≥ 1 (Fig. 2E). Similar characteristics are found in the presentation of the sum of all scores per person (Fig. 2F). Four children and four adults have a sum score > 1. While about half of the children with CML have medium to low scores, this accounts for only 1/3 of the older patients. As an indirect validation of the prediction scores, the top ranks of all patients include #255, #336, and #295, which were independently identified as informative cases based on the pedigrees (Fig. 1D, Supplemental Table 6). The modeled structural effects of the IDH2 V75M and JAK1 M1128T mutations in #255 are exemplified in Fig. 2G and H. Of the eight individuals with the highest mean per variant of the scores analyzed, six were diagnosed with pediatric CML and two had CML at the age of 62 or 65 years. The affected genes were *SDHA*, *ABL2*, *JAK1*, *PTK2B*, *APC*, and *PTPN11* in pediatric CML, and *NF1* and *RB1* in adult CML, all of which are associated with cancer predisposition. However, seven of these germline variants have so far been classified as VUS or have not yet been described as variants (Supplemental Table 6). This is in line with our hypothesis that these variants only assume their predisposing role through the combination with the *BCR::ABL1* oncogene (Fig. 2J).

The spectrum of variants in CML in comparison with other myeloid neoplasms

We compared the proportion of individuals with germline mutations and the spectrum of affected genes with data from similar studies in children and adults with myeloid neoplasms, particularly pediatric MDS [10], pediatric AML [11], and adult MDS [12] (Fig. 2I). Published data for germline variants in adult patients with AML have not been available. Although childhood MDS and AML also have a high proportion of germline mutations, significant differences were found in the

distribution pattern of the affected genes. While chromatin modifiers, tyrosine kinase (co)receptors, or cell cycle genes are frequently affected in pediatric CML, these genes are less prevalent in pediatric MDS and pediatric AML. Instead, mutations are found mainly in transcription factors (MDS) and DNA damage repair proteins (AML). Adult MDS also has numerous variants in genes encoding chromatin modifiers; overall, far fewer genetic variants are found than pediatric CML. The most shared genes are present in pediatric and adult CML (Supplemental Fig. 4A).

In addition, we compared the proportion of somatic variants in childhood CML with published data from adult patients with CML-CP or in advanced disease stages (AP/BP), as well as with published CHIP variants (Supplemental Fig. 4B). Somatic variants showed a remarkable similarity between pediatric CML and adult CML in CP.

Discussion

Genetic predisposition plays a crucial role in cancer development. Recently, in pediatric oncology, expanded genetic studies have identified a previously unanticipated high prevalence of cancer predisposition syndromes and have unraveled numerous novel risk variants [8]. As CML has been reported only in a few familial cases and is rarely part of a syndromic disease, it has been assumed a malignancy without a clear monogenic predisposition. However, the role of germline variants and the age dependency of the prevalence of somatic mutation patterns, i.e., the genetic landscape of pediatric CML, has not been comprehensively studied to date.

Data in adult CML show that somatic mutations leading to clonal hematopoiesis are associated with an unfavorable disease course and inferior treatment response [13]. In patients who develop tyrosine kinase inhibitor (TKI) resistance or CML-BP, these somatic mutations most frequently involve *RUNX1*, *IKZF1*, *ASXL1*, *GATA2*, and *TET2* [14]. Only one study in a small cohort of children and adolescents used myeloid gene panel sequencing and revealed somatic *ASXL1* mutations in 6/21 included individuals, suggesting a different genetic landscape in young patients compared to older adults [15].

In our study, we wanted to address whether, conversely, preexisting mutations could constitute a genetic setting favoring the effectiveness of *BCR::ABL1* translocation and the manifestation of CML similar to the setting in older patients where it is linked to the aging hematopoietic stem cell compartment. While the *BCR::ABL1* translocation is considered causative for CML development and represents the hallmark of the disease, model systems, and in-depth analyzed cases have shown that cooperating mutations might be predisposing for the translocation [4]. Persistence of *TET2* or *DNMT3A* mutations with minimal variance in allelic load have been demonstrated in diagnostic and follow-up samples from adult patients with CML originated from a preleukemic clone [4], indicating that the presence of CHIP mutations in some patients may induce a favorable milieu for the development of transforming mutations, such as *BCR::ABL1*. The presence of preexisting germline mutations have not yet been explored.

For our analyses, we selected pediatric patients as uniformly as possible and compared them with older patients with the same genetic background. The median age of the adult cohort (46 years) was significantly lower than the median age at diagnosis of CML in Germany (65 years). Samples from the adult CML cohort represent each age decade equally, deviating from the natural distribution to account for age-related factors.

Our study shows that 70% of the investigated pediatric patients with CML have gene variants in addition to the *BCR::ABL1* translocation and highlights for the first time a high proportion of germline variants detectable in 62% of this young patient cohort. The distinction between germline and somatic variants was made based on the VAF at diagnosis versus remission and detectability in non-hematological tissues (saliva or fingernails) as well as transmission of the variant in the family pedigree. Patients with a germline variant are younger on average than patients without a variant. In practically all pedigrees the respective variants accumulate in the children affected by CML.

Only a fraction of the identified germline variants has been recognized as high-penetrance cancer predisposing variants according to the American College of Medical Genetics guidelines, which per se can promote the development of neoplasia. We hypothesize that gene variants that are not considered pathogenic alone in particular may become functionally relevant under the pressure of the strong *BCR::ABL1* oncogene, thus accelerating leukemogenesis. Pathogenic variants, which are detected as secondary somatic alterations in CML, particularly at an advanced age, cannot exist as germline variants due to their embryonic lethality.

The genes with germline variants found here show a high degree of overlap with those associated with somatic variants in the course of CML progression. The most frequent variants were found in *ASXL1*, *NOTCH1*, *KDM6B*, *TET2*, *RUNX1*, *GIGYF2*, and *CUX1*. Most of the affected genes are shared between pediatric and adult CML. We do not know what the mechanism might be in the 40% patients without germline aberrations. Since we only performed WES, the germline aberrations in these cases could be in regions not covered by WES, e.g., enhancer/promoter regions, or they could be multifactorial predisposing variants that we cannot adequately identify with the number of cases in this rare disease.

Based on the results of our study, we hypothesize that *BCR::ABL1* translocation occurs frequently but additional genetic alterations, enhancing cell susceptibility to *BCR::ABL1*, are required for inducing transformation and rapid clonal expansion. With germline risk variants, translocation encounters a greater number of cells that already support the effect of *BCR::ABL1* (Fig. 2J). Thus, the likelihood of a compatible combination is higher, and the age of onset of disease shifts to younger ages. Further experimental studies examining cell clones with different combinations of genetic variants are needed to test this hypothesis. As a direct clinical consequence, the results justify the inclusion of CML among malignancies in childhood and adolescence for which human genetic testing for cancer-predisposing germline variants should be implemented.

Conclusion

We detected predisposing germline variants in addition to the *BCR::ABL1* fusion gene in over half of pediatric patients with CML. The identified predisposing germline variants mainly affect genes involved in myeloid neoplasms. The presence of germline variants in genes associated with hematopoiesis or leukemogenesis increases the likelihood that a random *BCR::ABL1* fusion event will result in the manifestation of CML at a young age.

Abbreviations

AML	Acute myeloid leukemia
AP	Accelerated phase
BP	Blast phase
CHIP	Clonal hematopoiesis of indeterminate potential
CML	Chronic myeloid leukemia
CP	Chronic phase
HePPy	Hematological Predictor of Pathogenicity
JMML	Juvenile myelomonocytic leukemia
MDS	Myelodysplastic syndrome
SBS	Single base substitution
SNP	Single nucleotide polymorphism
VAF	Variant allele frequency
WES	Whole-exome sequencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12943-024-02109-5.

Supplementary Material 1

Supplementary Material 2

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Author contributions

M. Krumbholz: conception and design, performed research, data analysis, writing-original draft, and writing-review and editing. A. Dolnik: conception and design, research, data analysis, and writing-review and editing. E. Sträng: data analysis and editing. T. Ghete: data analysis and editing, writing-review and editing. S. Skambraks: research and editing. S. Hutter: data analysis and editing. A. Simonis: research and writing-review and editing. F. Stegelmann: sample collection, data collection, and writing-review, and editing. M. Suttorp: sample collection, data collection, and writing-review and editing. A.H.C. Horn: data analysis and editing. H. Sticht: data analysis and editing. T. Haferlach: sample collection, data collection, and writing-review, and editing. L. Bullinger: conception and design, computational analysis, writing-review, and editing. M. Metzler: conception and design, computational analysis, writing-original draft, and writing-review and editing.

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Data availability

The datasets used and analyzed in this study are available from the corresponding author on request.

Declarations

Consent for publication

Not applicable.

Competing interests

LB: honoraria from AbbVie, Amgen, Astellas, Bristol-Myers Squibb, Celgene, Daiichi Sankyo, Gilead, Hexal, Janssen, Jazz Pharmaceuticals, Menarini, Novartis, Pfizer, Roche, and Sanofi, as well as research support from Bayer and Jazz Pharmaceuticals. MS: honoraria from Novartis, Pfizer, Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

Ethics approval and consent to participate

For genetic analyses, all participants or legal guardians provided informed consent in accordance with the Declaration of Helsinki (pediatric CML: EK282 122 006, EK 236_18 B; adult CML: EK05117, EK3456-05/12).

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