GENETIC ANALYSIS OF FAMILIAL MYELOID DISORDERS

PhD Thesis

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I. Introduction

Although familial clustering of haematological malignancies has long been known, the genetic background of these disorders has just started to be elucidated. The exact number of these disorders is still unknown, however they are likely to be more common than currently appreciated. This is supported by the fact that familial MDS/AML represents a new category in the recently revised 2016 WHO classification of myeloid neoplasms and acute leukemia. The first described syndrome was the familial platelet disorder with the propensity to myeloid malignancies (FPD-AML) with the mutations of *RUNX1*, followed by the discovery of *CEBPA* and *GATA2* mutations in the background of familial haematological malignancies. The currently recognized familial leukaemia predisposition syndromes are classified according to the clinical symptoms and predisposing germline mutations. AML or MDS caused by *CEBPA* or *DDX41* mutation occurs alone without other clinical symptoms or antecedent haematological condition In addition to the *RUNX1* mutations, *ANKRD26* and *ETV6.* mutations were recently found to be associated with FPD-AML. Familial MDS caused by *GATA2* mutations often

occurs in the setting of cytopaenias and rare immunological syndromes whereas mutations of *TERT* or *TERC* and *SRP72* emerge in bone marrow failure syndromes associated with elevated risk of MDS/AML. Familial myeloproliferative neoplasms are characterized by the internal tandem duplication of the long arm of chromosome, 14 affecting the *ATG2B* and *GSKIP* genes. Although familial clustering of lymphoid malignancies is also well known, the list of the culprit genes is shorter compared to the familial myeloid malignancies. Recently, germline mutations of the *PAX5* gene were reported to be associated with familial acute lymphoid leukaemias, and the recent discovery of the Shelterin complex mutations represented the first germline variants identified in familial chronic lymphocytic leukaemia

FPD-AML is characterized by a heterogeneous clinical course and variable chance of developing MDS/AML. Leukemic transformation has been associated with a heterogenous profile of mutations, including the mutation of the second *RUNX1* allele, however the exact mechanism of leukaemic evolution is not yet clear.

Identification and genetic characterization of the familial hematological malingnancies in Hungary has not been performed yet. Therefore, our aim was to collect families with

suspected familial myeloid malignancies in Hungary and to perform mutation screening of the currently known predisposing mutations in these families with a view to establish a genetic screening approach for hereditary myeloid malignancies in Hungary.

II. Aims

- To study the incidence of familial myeloid malignancies in Hungary,

- To perform genetic testing of the known predisposing mutations in the newly identified pedigrees,

- and using an FPD-AML family as a model, to screen the secondary co-operating genetic lesions driving the leukemic transformation in the cases with a full blown aggressive disease.

III. Materials and methods

We identified four families with apparent clustering of myeloid malignancies with nine affected individuals across these pedigrees.

Bone marrow or peripheral blood samples were collected from the affected members of these families. Following DNA isolation samples were tested for mutations in all currently known predisposition genes, including the full coding sequences of *CEBPA*, *GATA2*, *RUNX1*, *DDX41*, *TERT* and *TERC*, and for mutation hot-spots of *SRP62* and *ANKRD26* genes. In the case of an FPD-AML family with four affected siblings with MDS/AML, we performed whole exome sequencing (WES), to reveal the secondary cooperating mechanisms. The variants detected by WES were validated by Sanger sequencing. Ultra-deep sequencing (10000x) was performed to screen two candidate genes (*JAK2* and *SH2B3*).

To analyse the allele specific expression of RUNX1, an allele specific digital PCR assay for performed using specific probes for the wild type as well as the mutant RUNX1 allele. Standard bioinformatic pipelines were used to analyse the data.

IV. Results

We examined four families with apparent clustering of myeloid malignancies with nine affected individuals across these pedigrees.

In the case of the first family (**I**.), two siblings were affected with MDS (age of 10 and 14 years). We identified wild type *RUNX1*, *GATA2*, *CEBPA*, *TERC*/*TERT*, *SRP72* and *ANKRD26* genes.

The second family (**II**.), presented with three siblings affected with MDS (18, 20 and 12 years of age), mutational screening also identified wild type sequences of the above mentioned genes.

In the third family (**III**.), four individuals between age of 53 and 82 presented with AML. Mutation screening for the known predisposing genetic factors also revealed wild *RUNX1*, *GATA2*, *CEBPA*, *TERC*/*TERT*, *SRP72* and *ANKRD26* sequences.

In the case of the family **IV**, four children were affected with MDS/AML, including a dizygotic twin couple. We identified the *RUNX1* p.R201* stop gain mutation in case of

the four siblings and their asymptomatic carrier mother. The twin couple was diagnosed with AML at age of 5 years, and died within a year of the diagnosis. Ten years later, the youngest sibling who, was followed due to thrombocytopaenia, was diagnosed with MDS-AML at age of 5 years. The fourth sibling of the family was diagnosed with multilineage dysplasia (RCMD) at 14 years. Their mother (44 years) remains an asymptomatic carrier, with normal PB indices.

We performed whole exome profiling to determine the genetic alterations responsible for the clinical heterogeneity observed within this pedigree and the stepwise evolution of MDS/AML across the 4 female siblings. WES identified the strong molecular addiction to the JAK-STAT signalling (mutated *JAK2* and *SH2B3* genes) in case of children with the more aggressive disease phenotype.

V. Conclusions

The main statements of the dissertation:

- We identified the first 4 families in Hungary with clustering of familial myeloid malignancies,

- In case of three families all individuals were tested for *CEBPA*, *GATA2*, *RUNX1*, *ANKRD26*, *ETV6*, *DDX41*, *TERC* or *TERT* and *SRP72* mutations and were found to be negative for the mutations of this target genes.

- In case of the first Hungarian FPD-AML family, we identified the *RUNX1* p.R201* stop gain mutation,

- We identified a novel cooperating mechanism in the background of the clinical heterogeneity of family IV. WES revealed the convergent evolution of mutations of the JAK-STAT pathway (*JAK2* and *SH2B3*).

- We found the concurrent disruption of *JAK2* and *RUNX1* rare in a sporadic AML cohort of 59 patients.

- We established a diagnostic algorithm for the screening of the known germ-line predisposing genes of MDS/AML.

VI. List of publications

VI.1 Publications in connection with the thesis

1. **Király P,** Kállay K, Gángó A, Kellner Á, Egyed M, Szőke A, Kiss R, Vályi-Nagy I, Csomor J, Matolcsy A, Bödör C. (2017). Familial Acute Myeloid Leukemia and Myelodysplasia in Hungary. *Pathology oncology research : POR*;10.1007/s12253-017-0216-4. doi: 10.1007/s12253-017-0216-4. **IF: 1.940**

2. Tawana K, Wang J, **Király P,** Kállay K, Benyó G, Zombori M, Csomor J, Al Seraihi A, Rio-Machin A, Matolcsy A, Chelala C, Cavenagh J, Fitzgibbon J, Bödör C (2017). Recurrent somatic JAK-STAT pathway variants within a runx1-mutated pedigree. *European Journal of Human Genetics*. doi:10.1038/ejhg.2017.80. **IF: 4.580**

3. **Király P,** Kállay K, Marosvári D, Benyó G, Szőke A, Csomor J, Bödör C. (2016). [Clinical and genetic background of familial myelodysplasia and acute myeloid leukemia] (Review article). *Orvosi hetilap*, 157(8), 283-9. **IF: 0.291**

VI.2 Other publications

1. **Király PA,** Alpar D, Fesus V, Marosvari D, Matolcsy A, Bödör C. Introduction to the molecular diagnostic methods of oncohematology. Hungarian Oncology. 2016;60(2):88-98.

2. Fesus V, Marosvari D, Kajtar B, **Király PA,** Demeter J, Gurbity Palfi T, Egyed M, Plander M, Farkas P, Matrai Z, Matolcsy A, Bödör C. (2017). TP53 mutation analysis in chronic lymphocytic leukaemia. *Orvosi hetilap*, 158(6), 220-8. doi: 10.1556/650.2017.30656.

3. Kiss R, **Király PA,** Gaal-Weisinger J, Marosvari D, Gango PA, Demeter J, Bödör C. (2017). Molecular monitoring of myeloid leukemia. Hungarian Oncology. 61(1), 57-66.

4. Marosvari D, Alpar D, **Király PA**, Rajnai H, Reiniger L, Bödör C. (2016). A krónikus limfocitás leukémia genetikai háttere az újgenerációs szekvenálás korszakában. [The genetic landscape of chronic lymphocytic leukemia]. *Magyar onkologia*, 60(2), 118-25.

5. Nagy N, Hajdu M, Mark A, **Király PA**, Toth M, Danko T, Csoka M, Sebestyen A. (2016). Growth inhibitory effect of rapamycin in Hodgkin-lymphoma cell lines characterized by constitutive NOTCH1 activation. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology*

and Medicine, 37(10), 13695-704. doi: 10.1007/s13277-016-5272-y. **IF: 2.926**

6. Posfai E, Marton I, **Király PA**, Kotosz B, Kiss-Laszlo Z, Szell M, Borbenyi Z. (2015). JAK2 V617F, MPL, and CALR mutations in essential thrombocythaemia and major thrombotic complications: a single-institute retrospective analysis. *Pathology oncology research : POR*, 21(3), 751-8. doi: 10.1007/s12253-014-9885-4. **IF: IF: 1.940.**

7. Rajnai H, **Király PA.** (2017) Pathogenesis and genetic landscape of acute myeloid leukemia. Hungarian Oncology. 61(1), 21-8.

8. Rajnics P, Kellner A, Karadi E, Moizs M, Bödör C, **Király PA**, Marosvari D, Andrikovics H, Egyed M. (2016). Increased Lipocalin 2 level may have important role in thrombotic events in patients with polycythemia vera and essential thrombocythemia. *Leukemia research*, 48101-6. doi: 10.1016/j.leukres.2016.04.016. **IF: 2.606.**