Barts
Cancer Institute
Queen Mary, University of London

Barts and The London
School of Medicine and Dentistry

SVENSKA GRUPPEN AML

Familial Leukaemia – what genetics is teaching us

Jude Fitzgibbon

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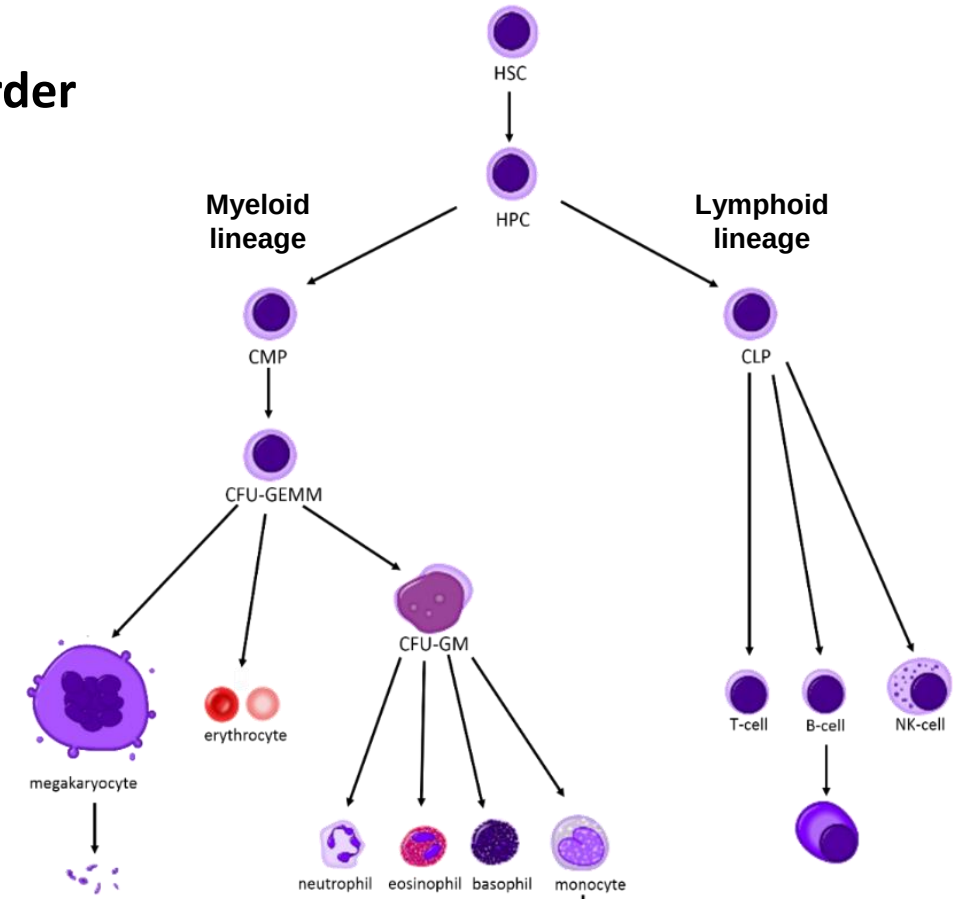
What's in Store

- Run through the current landscape of familial leukaemia.
- Genetic testing
- The transcription factor *CEBPA*
- Identify new loci
- New ideas emerging from the study of inherited leukaemia.

Acute Myeloid Leukaemia (AML)

- **Haematopoeitic Stem Cell Disorder**
- **Median age:** 68 years
- **Incidence:** 3/100,000 in Europe
- **5-year overall survival:** 42%



Familial AML/MDS

- > 95 % of all AML cases are sporadic.
- << 5% of the cases where two or more affected individuals are found within the same family.
- New WHO Entity 2016 provisional diagnostic category for heritable myeloid malignancies

Clinical challenges in Familial AML

1. Recognition of inherited forms of these diseases is difficult;
   - Patients may be unaware of their predisposition.
   - Wide variation in the age of onset and disease phenotype.
   - Absence of customized diagnostics.

2. Variable penetrance of the disease mutations: symptomatic and asymptomatic carriers.

3. How to manage patients with GL mutation? - Paucity of clinical guidelines.

Roadmap to improve diagnosis, treatment and management

Identification of new familial cases, Individual at risk

Molecular diagnostics, 13 predisposition loci

Tissue bank (Data collection)

New disease genes and functional validation

NOVEL GUIDELINES AND IMPROVED MANAGEMENT

Familial MDS/AML Genetic Discovery

Familial MDS/AML can be divided into three groups:

- Examples where approved testing exists.
- Emerging from basic research and requiring validation.
- Without an identified genetic basis.

(Little is known regarding secondary genetic mutations)

Current Mutational Landscape

GENE	AUTHORS	YEAR
RUNX1	Song *et al*. Boston, Massachusetts, USA	1999
TERC	Vulliamy *et al*. London, UK	2001
CEBPA	Smith *et al.* London, UK	2004
TERT	Hiroki Yamaguchi *et al.* Atlanta, USA	2005
GATA2	Hahn *et al*. Adelaide, Australia	2011
ANKRD26	Noris *et al.* Pavia, Italy	2011
SRP72	Kirwan *et al.* London, UK	2012
ACD	Guo *et al.* Philadelphia, USA	2014
ETV6	Zhang *et al.* Seattle, Washington, USA and Leila Noetzli *et al*. Colorado USA	2014/2015
ATG2B/GSKIP	Saliba *et al.* Villejuif, France	2015
DDX41	Polprasert *et al*. Cleveland, USA	2015
SAMD9	Narumi *et al*. Tokyo, Japan	2016
SAMD9L	Tesi *et al.* Stockholm, Sweden	2017

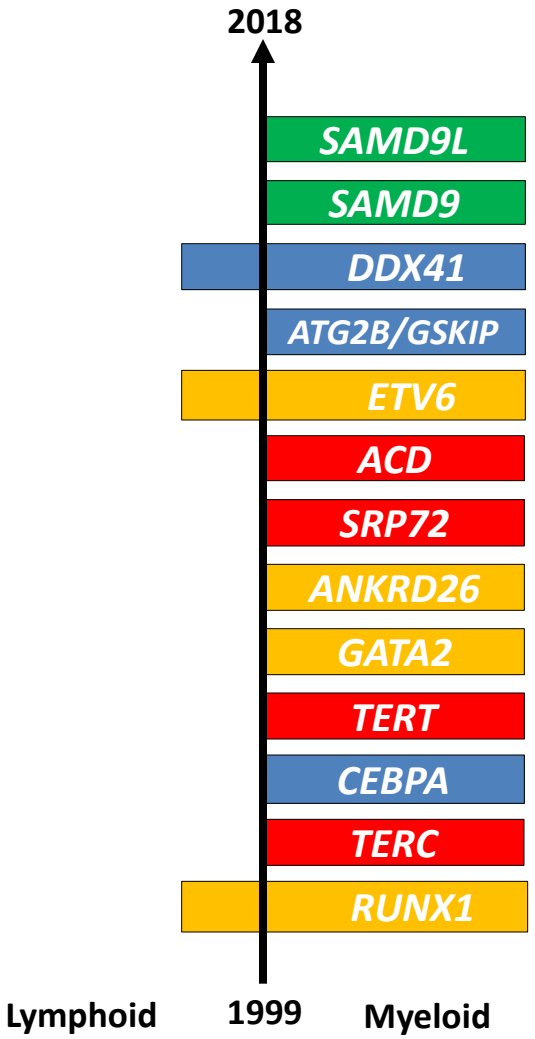
Current Mutational Landscape – 13 genes and counting

Myeloid malignancies only
AML: *CEBPA*
MDS/AML: *DDX41*
MPNs/AML: *ATG2B/GSKIP*

Cytopenias and/or platelet dysfunction
FPD/AML: *RUNX1*
GATA2 deficiency
Thrombocytopenia 2: *ANKRD26*
Thrombocytopenia 5: *ETV6*

Bone marrow failure syndromes
Telomere syndromes: *TERT*, *TERC, ACD*
Aplastic anemia/MDS: *SRP72*

Other syndromes
Cytopenia, immunodeficiency, MDS, and neurological symptoms: SAMD9L
MIRAGE syndrome: SAMD9



Targeted sequencing panel for familial MDS/AML

Index cases were tested for mutation in 11 **known disease genes**

West Midlands Regional Genetics Laboratories

Birmingham Women's NHS
NHS Foundation Trust

ACD, ANKRD26, ATG2B, CEBPA, DDX41, ETV6, GATA2, RUNX1, SRP72, TERC and *TERT*

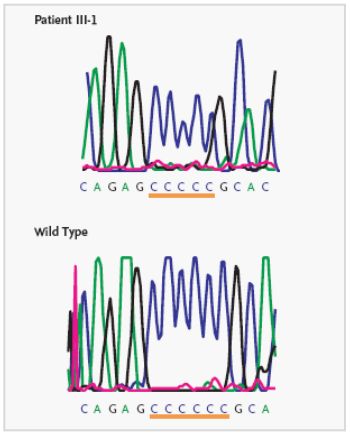
Our Interest in Familial *CEBPA*

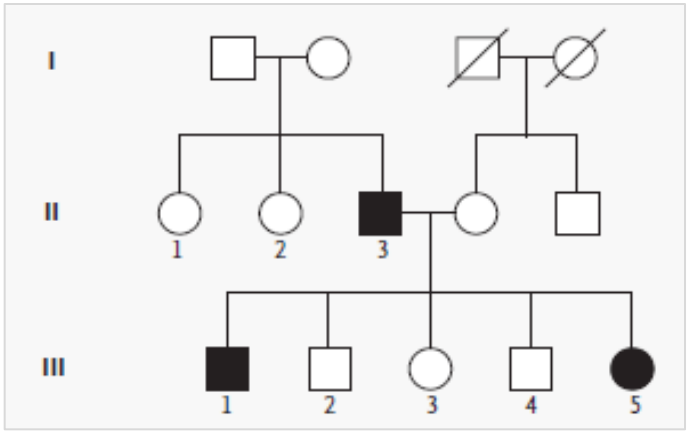
The NEW ENGLAND JOURNAL of MEDICINE

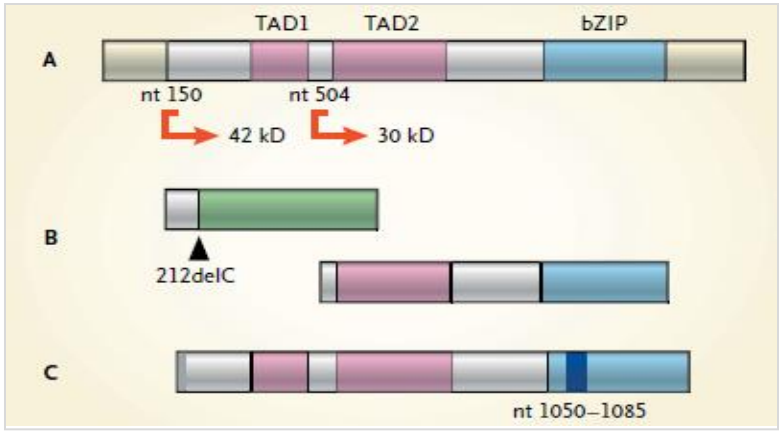
BRIEF REPORT

Mutation of *CEBPA* in Familial Acute Myeloid Leukemia

Matthew L. Smith, M.B., B.S., Jamie D. Cavenagh, M.D., T. Andrew Lister, M.D., and Jude Fitzgibbon, Ph.D.







Smith et al., NEJM 2004

International collaboration on Familial *CEBPA*-AML: 25 patients – 11 pedigrees

Smith *NEJM*, 2004 (UK)

Sellick *Leukemia*, 2005 (UK)

Pabst et al, *JCO*, 2008 (Switzerland)

Renneville *Leukemia,* 2009 (France)

Nanri *GCC*, 2010 (Japan)

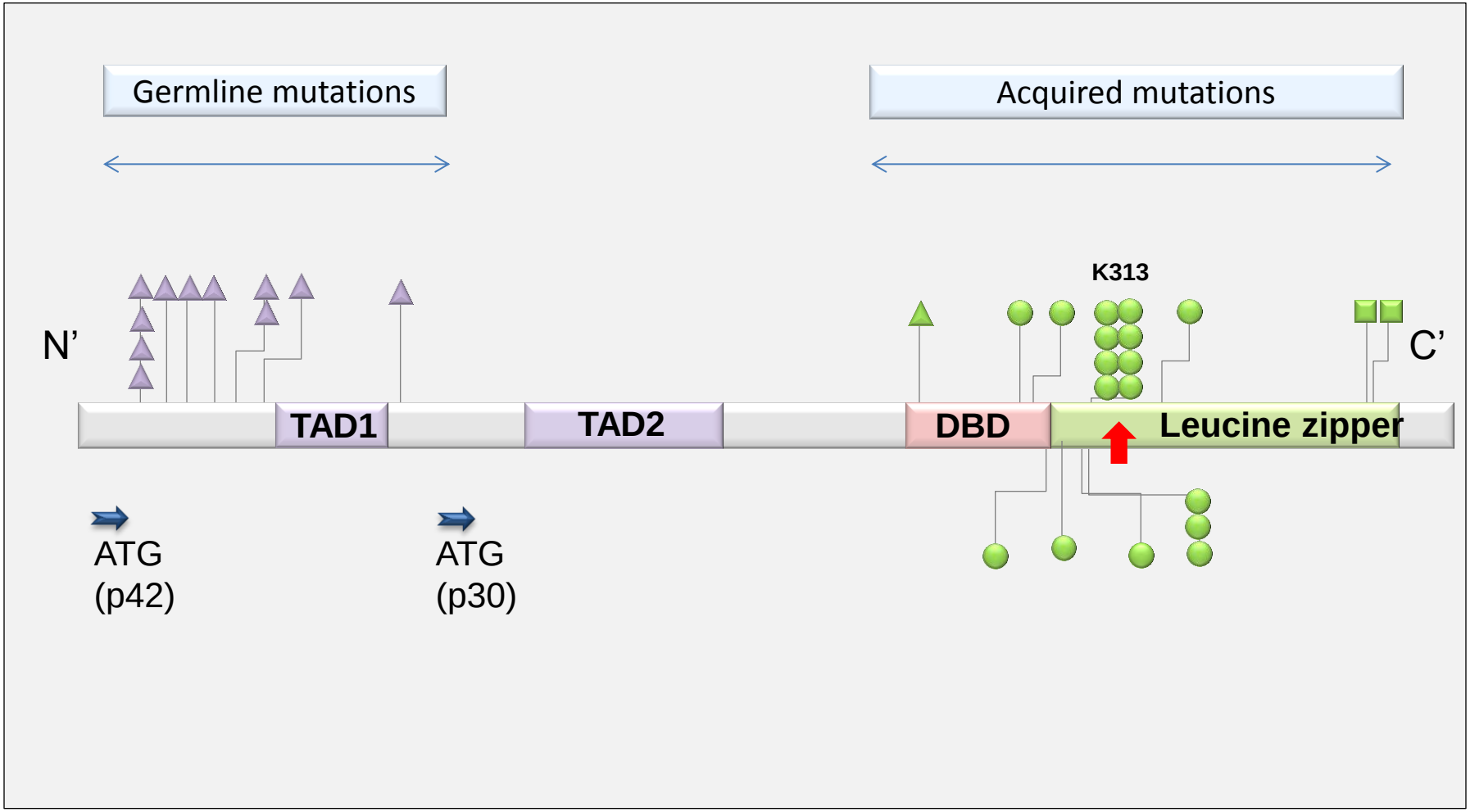
Stelljes *Leukemia,* 2011 (Germany)

Taskesen *Leukemia*, 2011 (Germany)

Savic A, unpublished (Serbia)

Debeljak *Haematologica* 2013 (Slovenia)

Distribution of *CEBPA* mutations



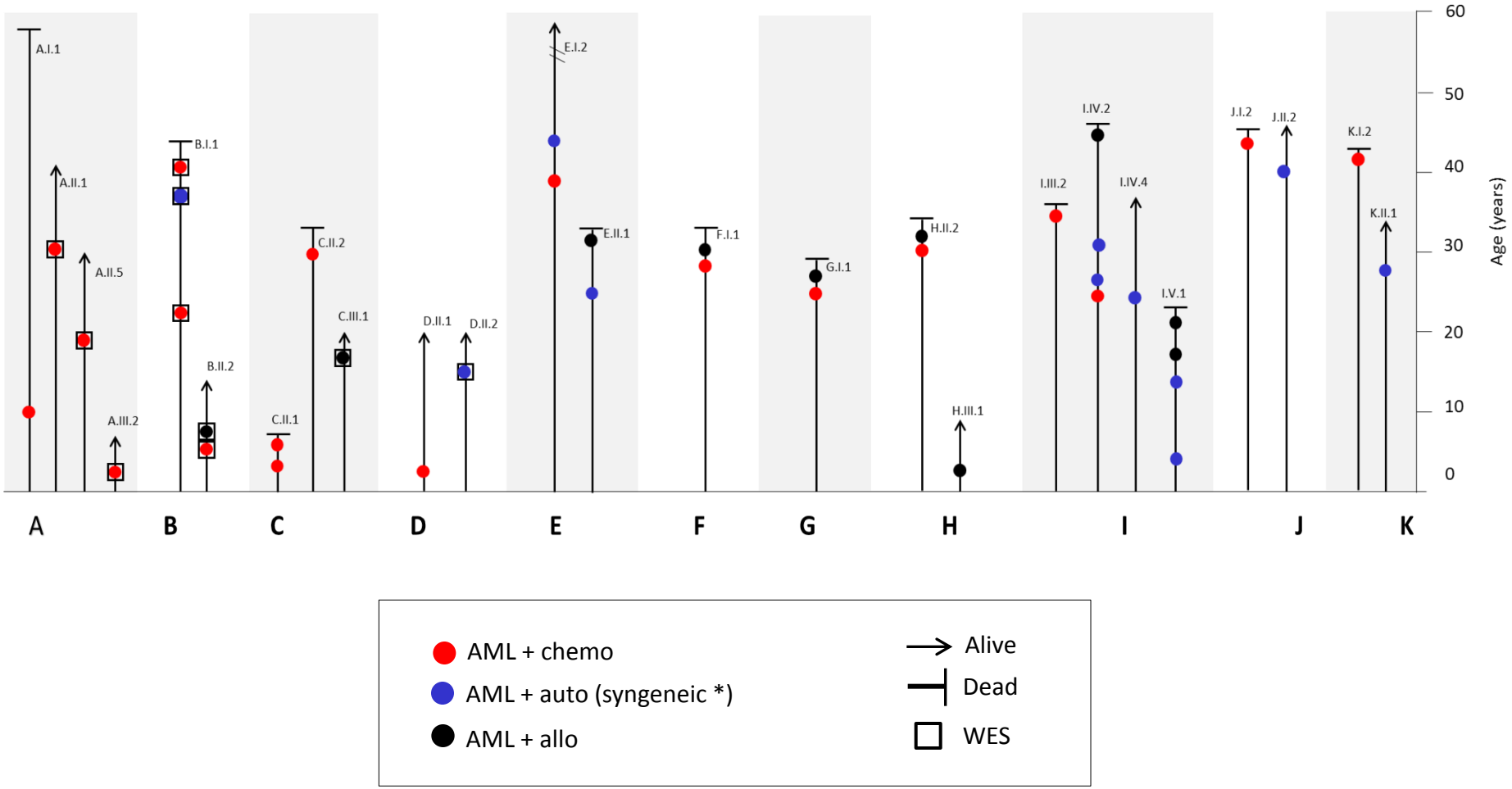
Key

○ In-frame insertion or deletion

△ Frameshift mutation

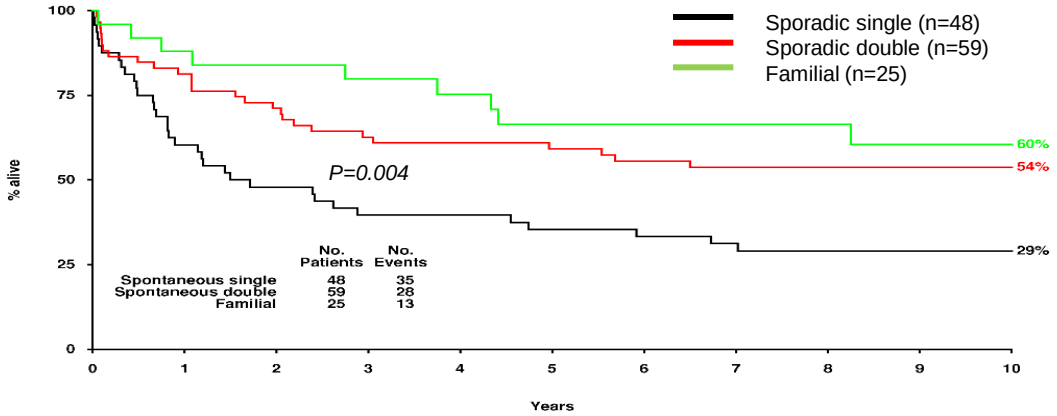
□ Missense mutation

Timeline of clinical events in all 25 Familial *CEBPA* AMLs

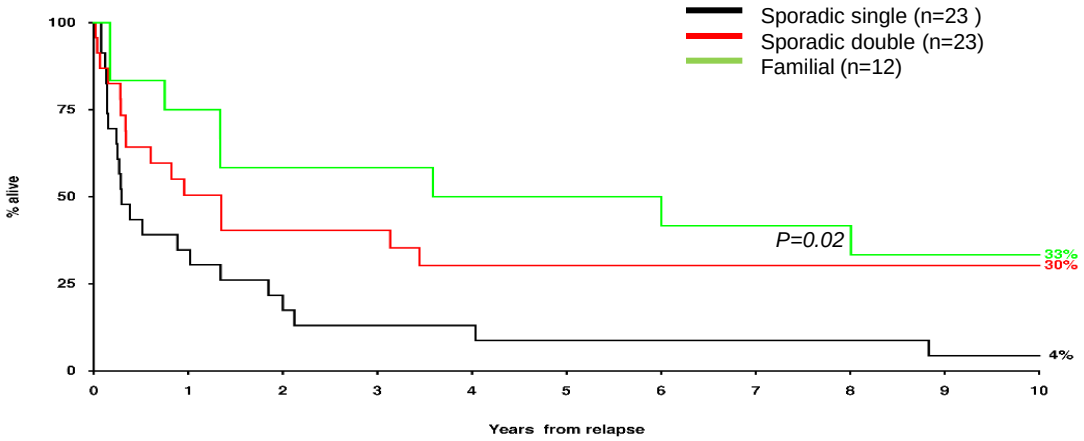


Comparison Familial versus MRC *CEBPA* series

A) Overall survival



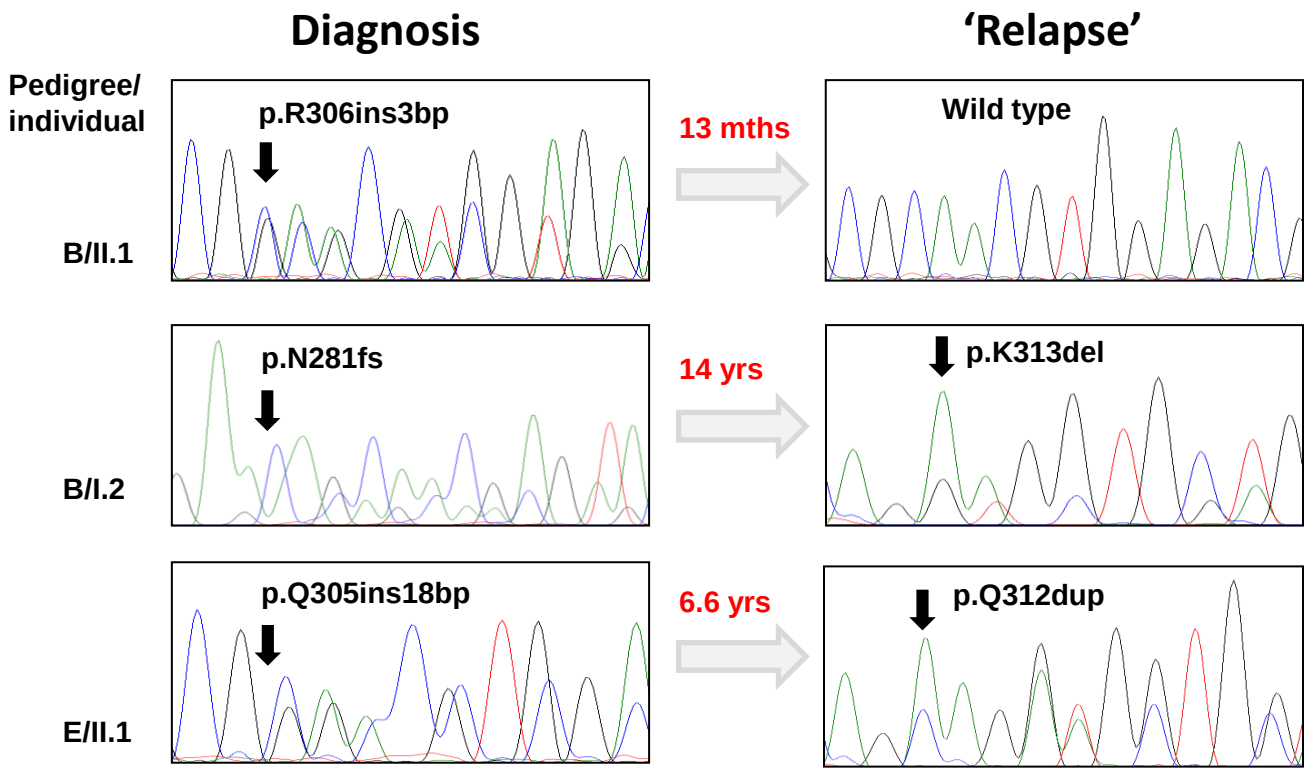
B) Post-relapse survival



Tawana *et al.,* Blood 2015

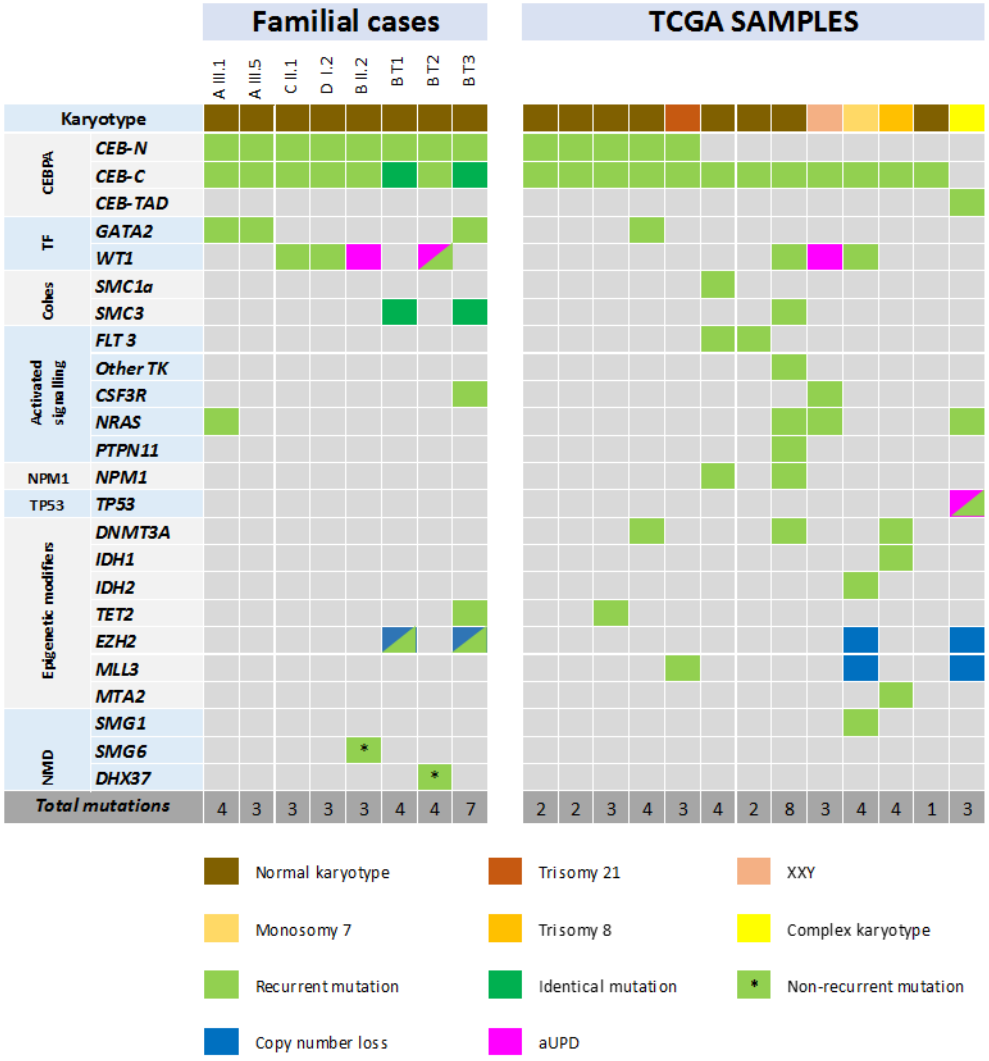
Patients are cured of initial disease but are predisposed to new leukemic episodes

New *CEBPA* C-terminal mutations at relapse



Tawana *et al.,* Blood 2015

Familial *CEBPA* reminiscent of dm*CEBPA*



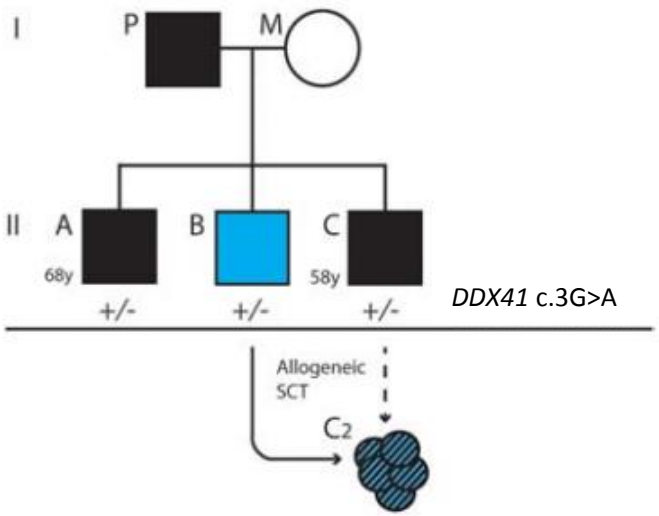
Tawana *et al.,* Blood 2015

Collective experience

- Chemotherapy alone can be used to treat patients without adverse disease features.
- High frequency of late 'chemosensitive' relapses, where intensive consolidation, preferably with allogeneic transplantation, may well prove beneficial in preventing further disease events.
- Perform germ-line testing in sporadic *CEBPA* AML of <40 yrs at diagnosis.
- Counselling of affected individuals and asymptomatic carriers requires full knowledge and understanding of the implications of inherited *CEBPA* mutations and, above all, recognition and consideration for an individual's choice.
- Where possible, we advocate screening of all potential sibling donors, preparing for both treatment escalation in affected cases and identifying asymptomatic mutation carriers for counselling and surveillance.

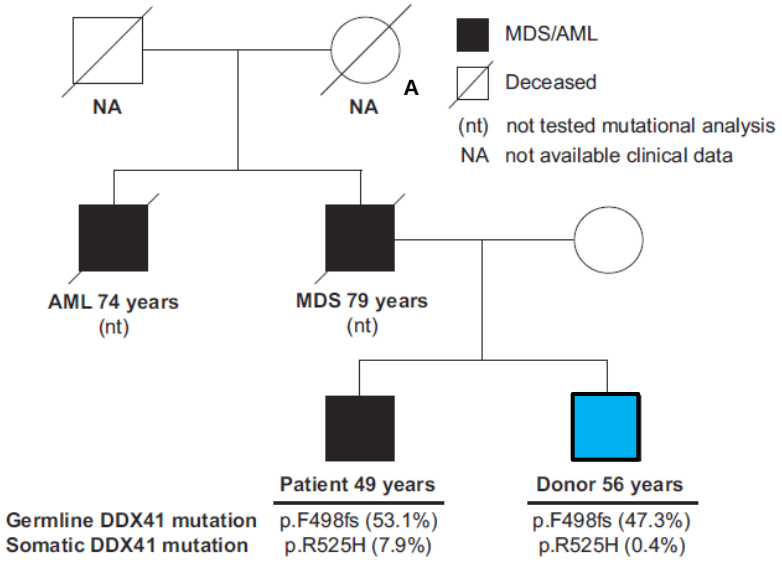
Donor cell leukaemia in *DDX41* families

FAMILY 1



Berger *et al,* 2017

FAMILY 2

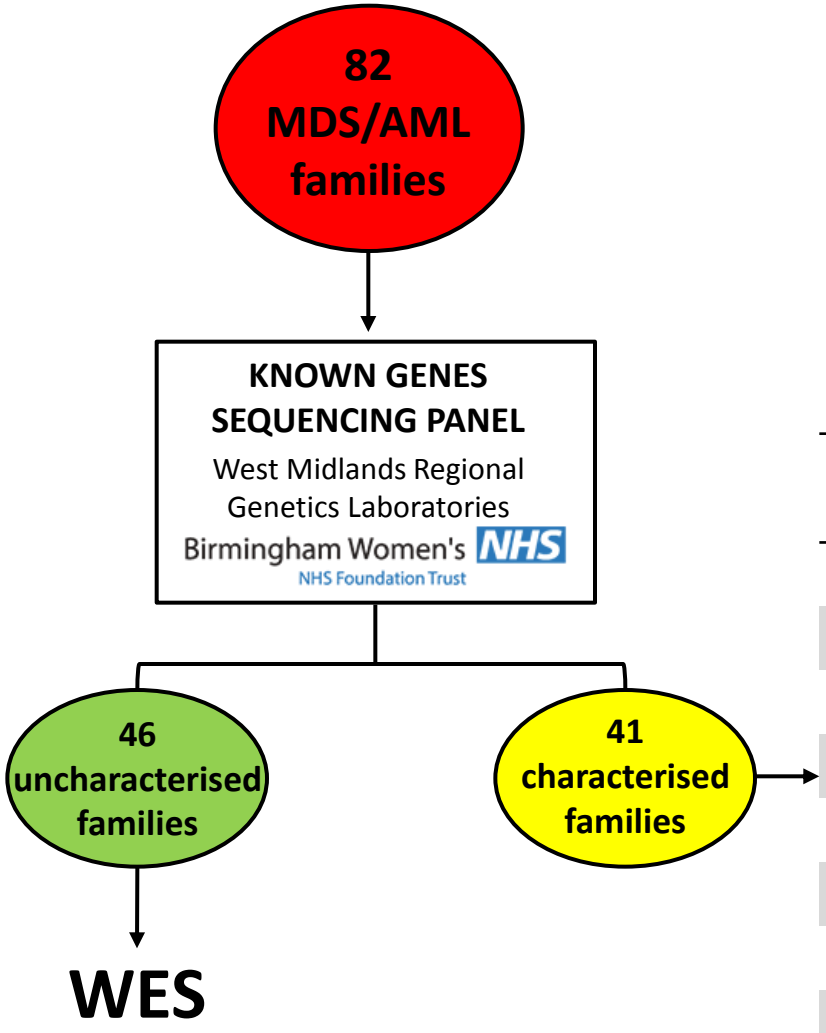


Kobayashi *et al,* 2017

Lesson from familial leukaemia

- Genes not mutated in sporadic AML - Novel mechanism
- Under appreciated mechanisms of mutation
- Clustering of mutations within pedigrees - Host genetics
- A clever way of making a monosomy
- Disease Latency and penetrance - Protection factor

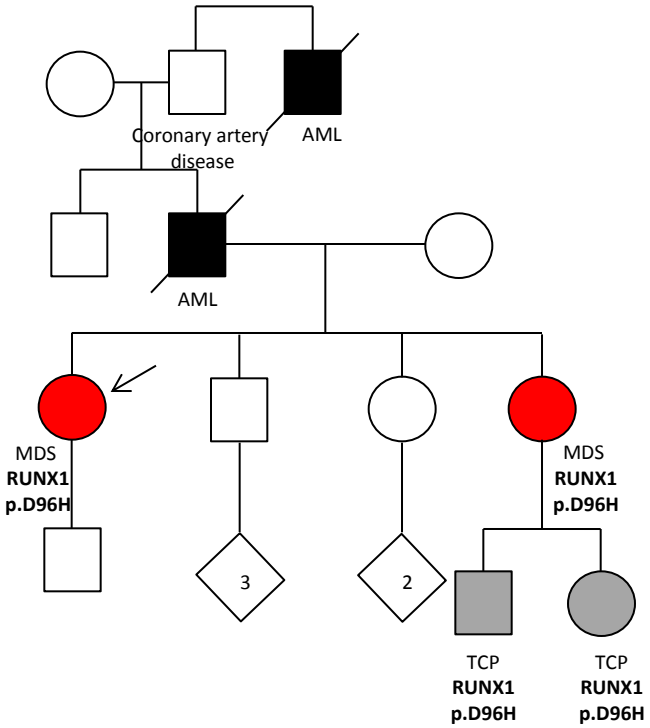
Our cohort of MDS/AML families – new Loci (Vulliamy, Dokal & Fitzgibbon)

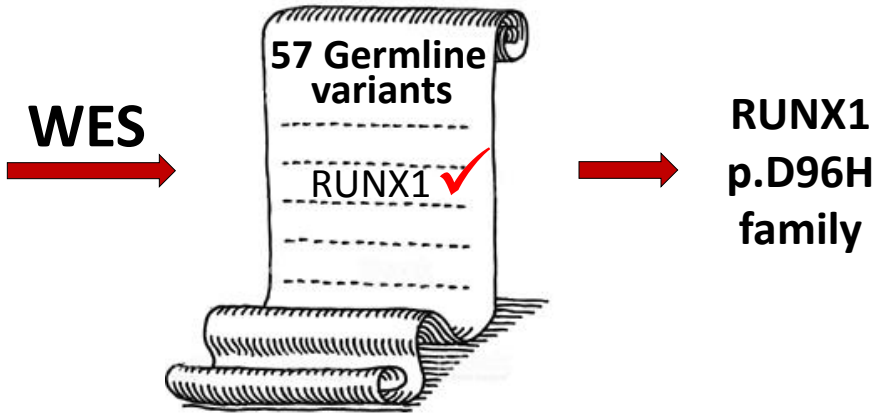


MUTATED GENE	NUMBER OF FAMILIES
CEBPA	7
DDX41	5
GATA2	7
RUNX1	12
SRP72	2
TERC	3
TERT	4
ETV6	1

Challenges in identifying a familial MDS/AML loci

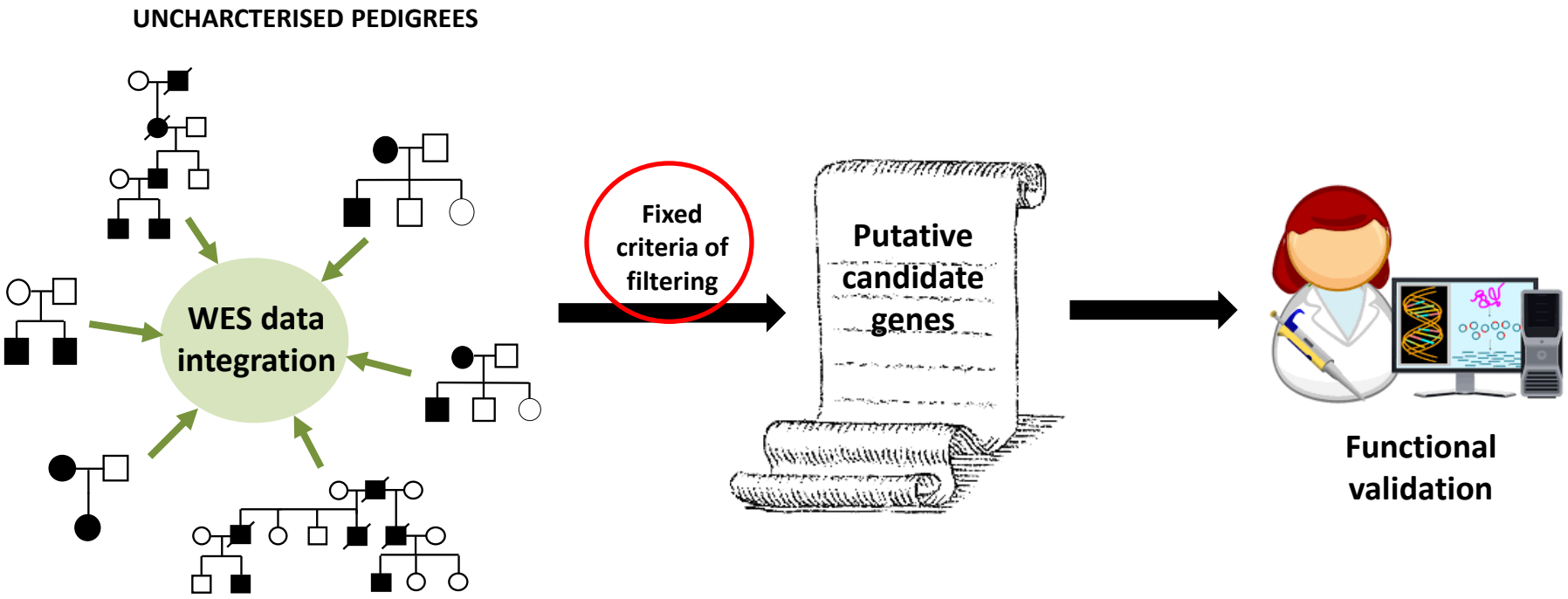
PEDIGREE 1





Challenges in identifying a familial MDS/AML loci

FAMILIES WITHOUT A CLEAR CANDIDATE



Challenges in identifying a familial MDS/AML loci

HOW TO FIX CRITERIA OF FILTERING?

1. Variant frequency (e.g. ExAC <0.0001)

2. Predicted to be damaging (*Polyphen, MutationTaster, SIFT, Provean* scores)

3. In at least 2 families (?)

4. Others: family phenotype, protein function, type of mutation, mutated in sporadic AML, other scores (PhyloP, GERP, and CADD)…

Some putative candidate genes

GENE	VARIANT FREQUENCY (ExAC)	PREDICTED TO BE DAMAGING	IN AT LEAST 2 FAMILIES	PROTEIN FUNCTION
ADA	0	YES	YES (2 families)	Adenosine deaminase (SCID related)
DHX34	< 0.0001	YES	YES (4 families)	DExH-box helicase (involved in NMD)
PDCD4	0	YES	YES (2 families)	Potential TSG (downregulated in AML)
PRR23B	0	Not all variants	YES (3 families)	Proline Rich 23B (regulated by *GATA2*)
VPS13C	< 0.0001	Not all variants	YES (5 families)	Vacuolar Protein (mitochondrial function)

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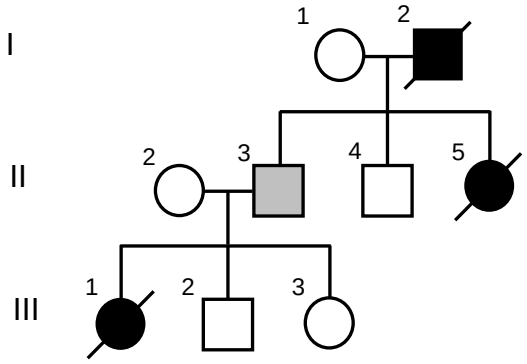
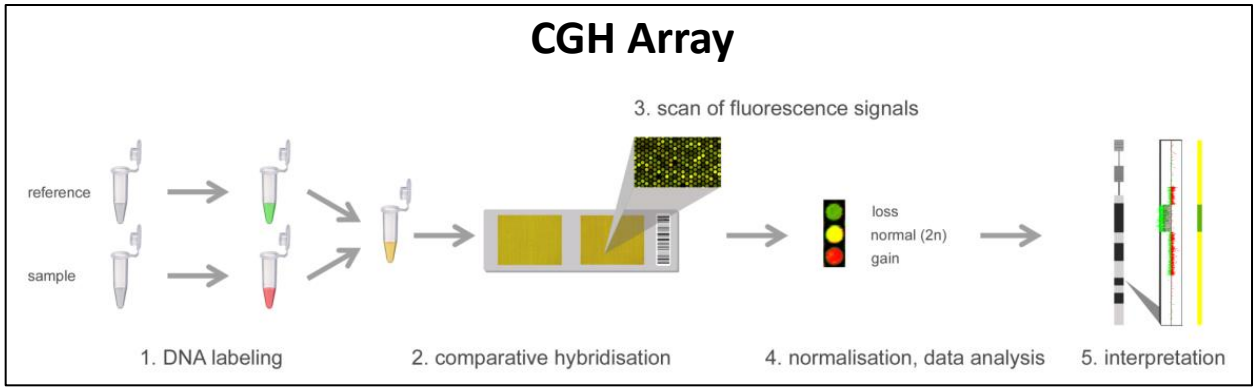
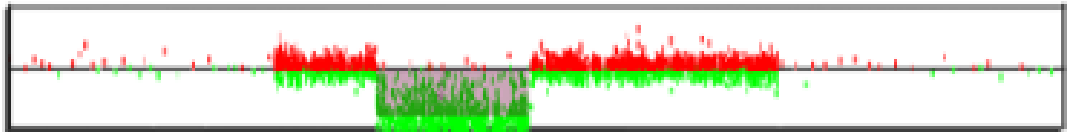
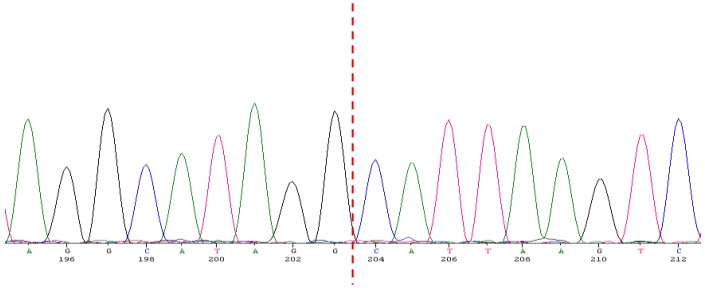
Atypical mutations: intragenic deletion in *RUNX1* – 20% of pedigrees







RUNX1 – Chr 21- 1.21Mb



Deletion breakpoints chr21:36389492-37056053 – 66,6561 bp deletion

Germ-line GATA2 – synonymous mutations

Major classes of germline mutations in cases ***GATA2* deficiency:**

1. Truncating (stop-gain, frameshift, splice-site);
2. Missense within zinc fingers;
3. Noncoding substitutions in the conserved EBOX-GATA-ETS regulatory region (intron 4);
4. In frame indels and whole gene deletions.
5. **Silent (synonymous) exonic substitutions – Splice defects**

2449 Systematic Assessment of GATA2 Genetic Variation Reveals the Presence of Novel Disease-Causing Synonymous Exonic Mutations

Bone Marrow Failure
Program: Oral and Poster Abstracts
Session: 508. Bone Marrow Failure: Poster II

Sunday, December 10, 2017, 6:00 PM-8:00 PM
Bldg A, Lvl 1, Hall A2 (Georgia World Congress Center)

Emilia J. Kozyra, MSc[1,2*], *Victor Pastor Loyola, MSc*[3,4*], *Claudia Wehr*[5,6*], *Sushree Sangita Sahoo, M.Sc.*[3,7,8*], *Rebecca Voss*[9*], *Enikoe Amina Szvetnik*[10*], *Shinsuke Hirabayashi, MD*[11*], *Albert Catala*[12*], *Henrik Hasle, MD, PhD*[13,14], *Marry M. Van den Heuvel-Eibrink, MD*[15,16], *Krisztián Kallay, MD*[17*], *Riccardo Masetti, MD, PhD*[18*], *Barbara De Moerloose, MD, PhD*[19*], *Markus Schmugge, MD*[20], *Owen Smith, MD*[21*], *Marek Ussowicz*[22*], *Jan Stary*[23*], *Ester Mejstrikova*[24], *Ramunė Pasaulienė*[25*], *Irith Baumann, Dr. med.*[26*], *Gudrun Göhring, MD*[27*], *Brigitte Schlegelberger, Prof. Dr.*[27], *Ulrich Salzer*[5,28*], *Michael Lübbert*[29], *Eirini Trompouki, PhD*[30*], *Charlotte M. Niemeyer, MD*[31] *and Marcin W. Wlodarski, MD, PhD*[32]

Lessons from familial leukaemia

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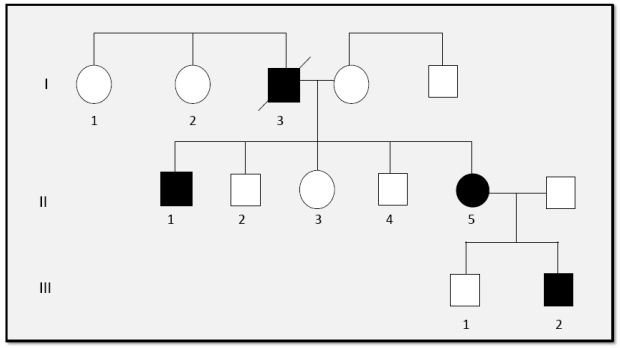
Clustering of same secondary mutations in pedigrees - *GATA2*

Acquired *GATA2* mutations in 3 relatives with germline *CEBPA* mutations



III.2

CEBPA III.2:
P23fs (germ-line)
K313dup (40%)
GATA2: **N317I (30%)**

Germline *CEBPA*

Acquired *CEBPA*

Acquired *GATA2*

II.1

II.1

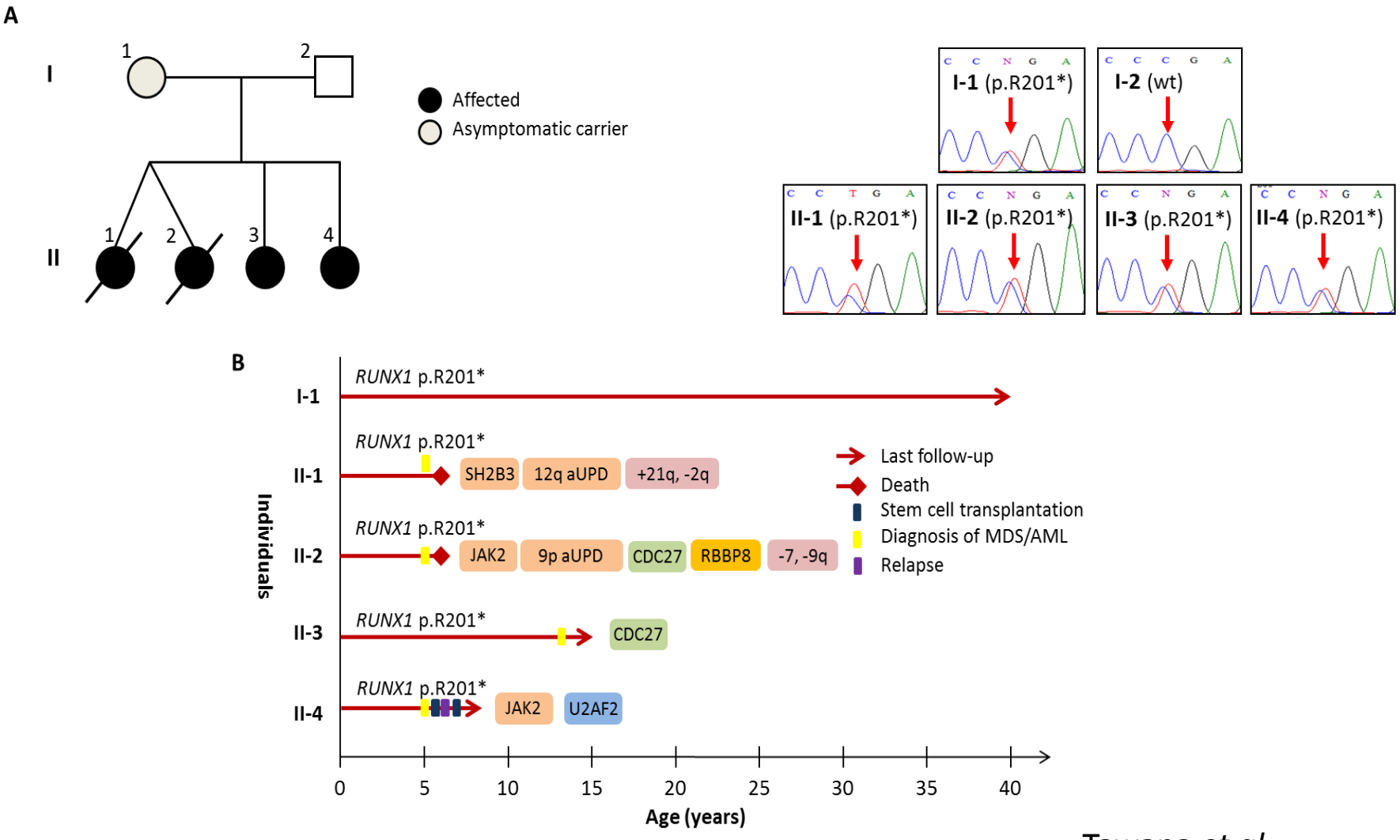
II.5

II.5

CEBPA II.1:
P23fs (germ-line)
K302_K313dup (38%)
GATA2: **L321F (31%)**

CEBPA II.5:
P23fs (germ-line)
Q305_K313dup (38%)
GATA2: **L321F (15%)**
R330Q (23%)

Disease Latency - *RUNX1*



Tawana *et al.*

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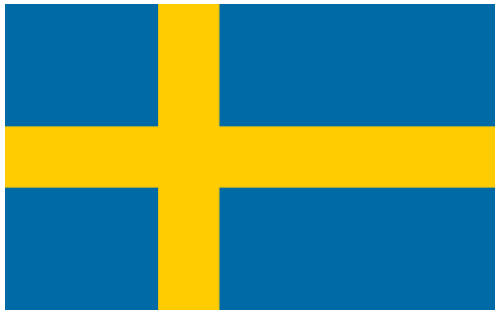
SAMD9L Mutations and Loss of Chromosome 7



blood®
2017 129: 2266-2279
doi:10.1182/blood-2016-10-743302 originally published online February 15, 2017

Gain-of-function *SAMD9L* mutations cause a syndrome of cytopenia, immunodeficiency, MDS, and neurological symptoms

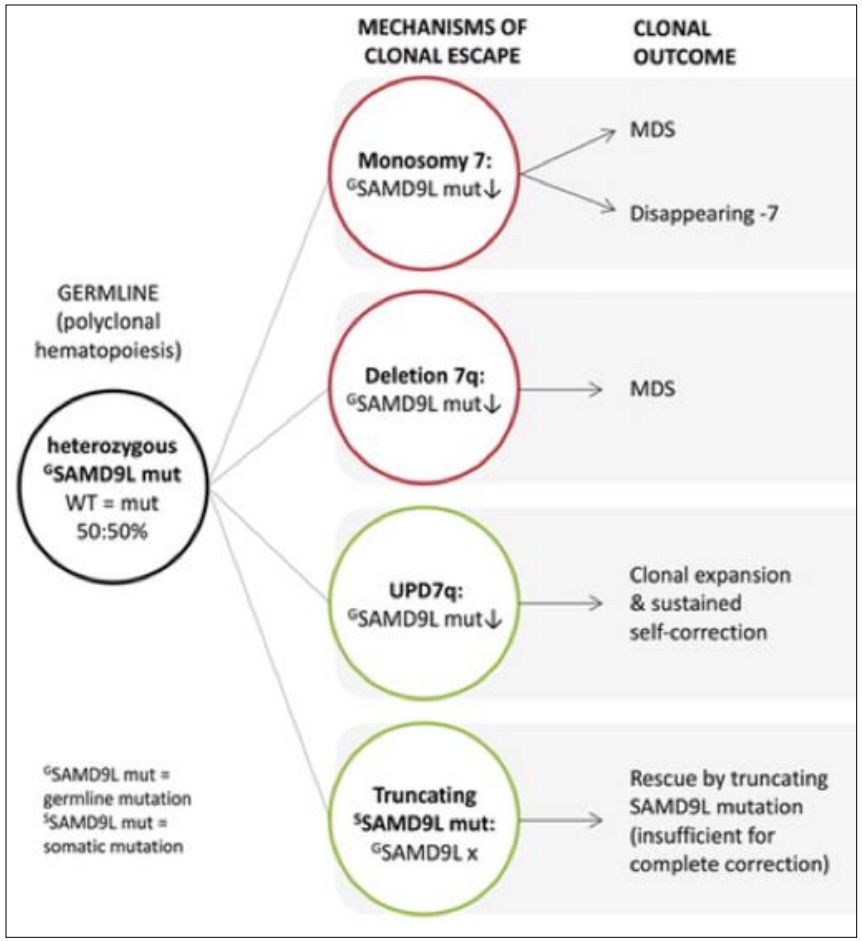
Bianca Tesi, Josef Davidsson, Matthias Voss, Elisa Rahikkala, Tim D. Holmes, Samuel C. C. Chiang, Jonna Komulainen-Ebrahim, Sorina Gorcenco, Alexandra Rundberg Nilsson, Tim Ripperger, Hannaleena Kokkonen, David Bryder, Thoas Fioretos, Jan-Inge Henter, Merja Möttönen, Riitta Niinimäki, Lars Nilsson, Cornelis Jan Pronk, Andreas Puschmann, Hong Qian, Johanna Uusimaa, Jukka Moilanen, Ulf Tedgård, Jörg Cammenga and Yenan T. Bryceson

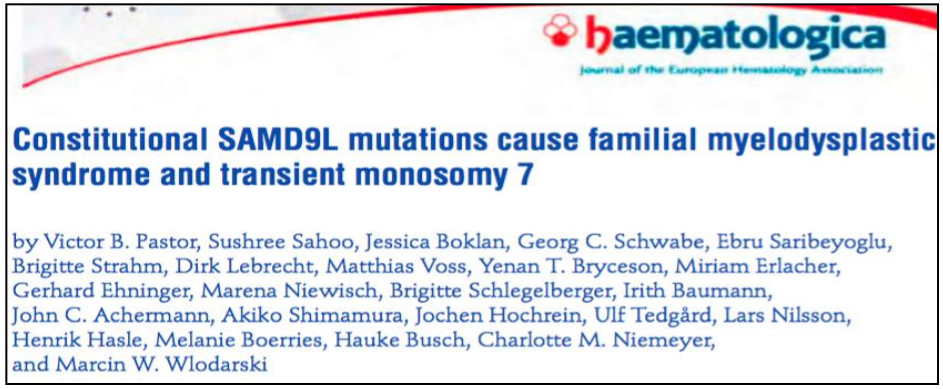
1. Heterozygous germline **gain-of-function (GoF)** mutations in *SAMD9L* are associated with cytopenia, immunodeficiency, MDS and neurological disease (e.g. ataxia-pancytopenia).

2. These **GoF** mutations cause **decreased cell proliferation** compared to WT protein and in turn, lead to **loss** of ***SAMD9L*-mutant allele** and **monosomy 7.**

So there is a section to lose the mutation (loss of Chromosome 7) to increase proliferation.

Familial MDS and Transient Monosomy 7 as a Sole Clinical Manifestation of *SAMD9L*-related Disease



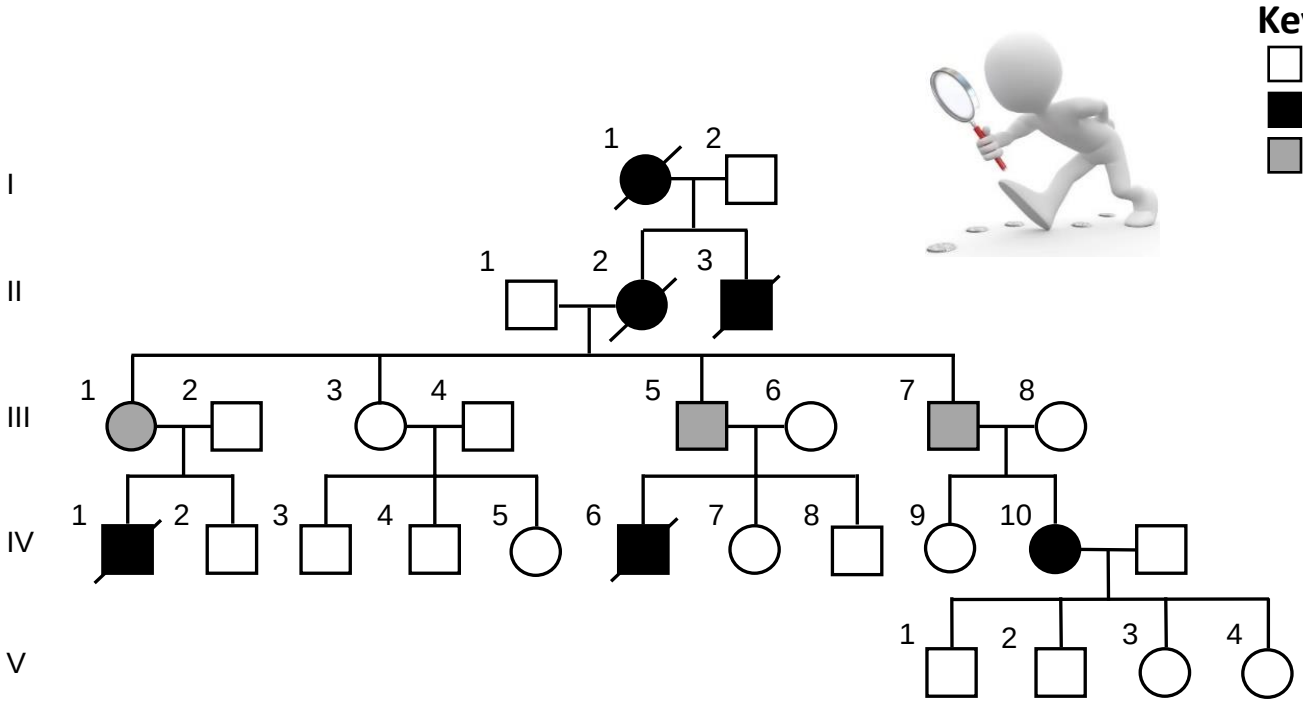

haematologica
Journal of the European Hematology Association

Constitutional SAMD9L mutations cause familial myelodysplastic syndrome and transient monosomy 7

by Victor B. Pastor, Sushree Sahoo, Jessica Boklan, Georg C. Schwabe, Ebru Saribeyoglu, Brigitte Strahm, Dirk Lebrecht, Matthias Voss, Yenan T. Bryceson, Miriam Erlacher, Gerhard Ehninger, Marena Niewisch, Brigitte Schlegelberger, Irith Baumann, John C. Achermann, Akiko Shimamura, Jochen Hochrein, Ulf Tedgård, Lars Nilsson, Henrik Hasle, Melanie Boerries, Hauke Busch, Charlotte M. Niemeyer, and Marcin W. Wlodarski

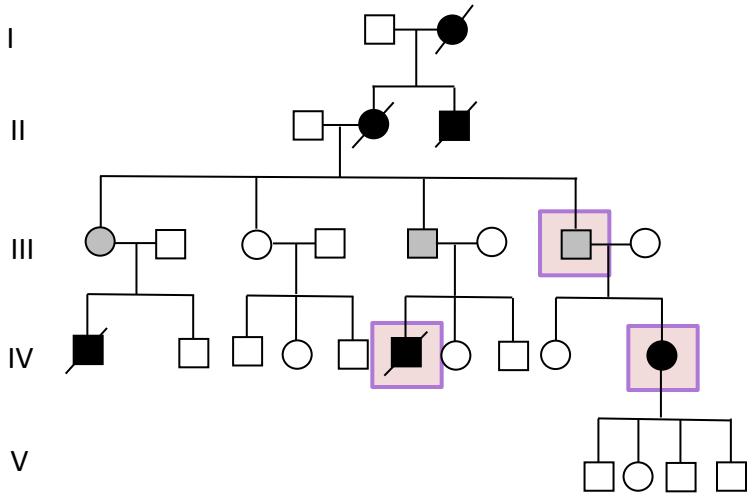
- Non-random loss of mutant *SAMD9L* allele can be achieved by:
  1. Complete (Monosomy 7)
  2. Partial (deletion 7q)
  3. UPD 7q
  4. Somatic truncating *SAMD9L* mutations

Reduced penetrance in *GATA2*-mutated MDS/AML - p.T354M mutations

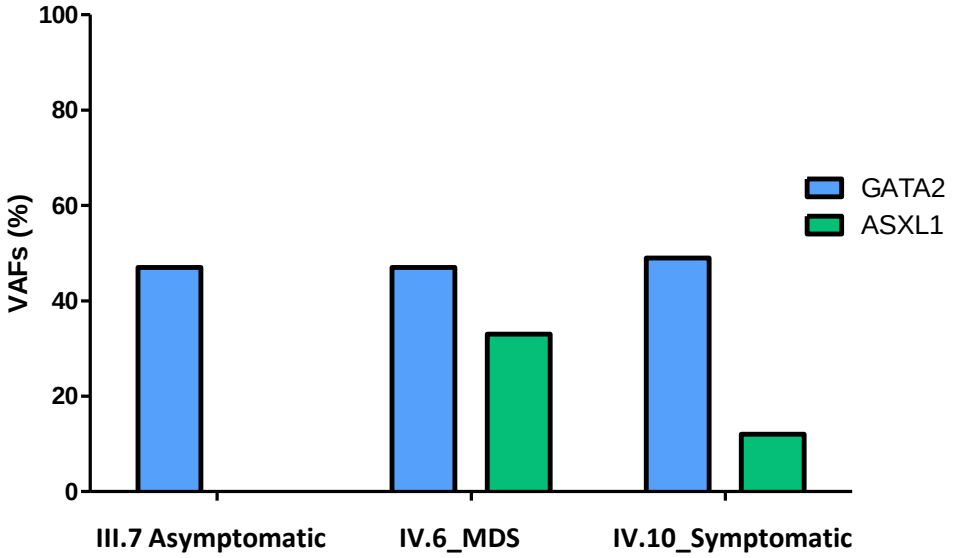


Bodor *et al*., *Haematologica* 2012

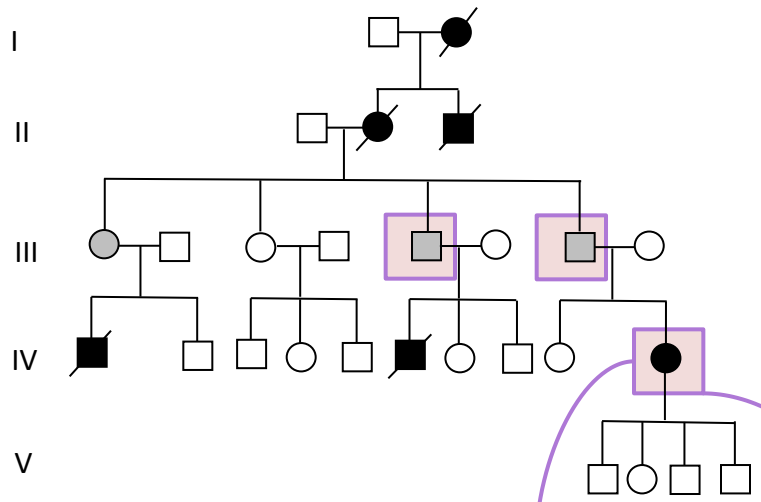
1. *ASXL1* mutation (p.Gly646TrpfsTer12) as a secondary genetic event in *GATA2* symptomatic carriers

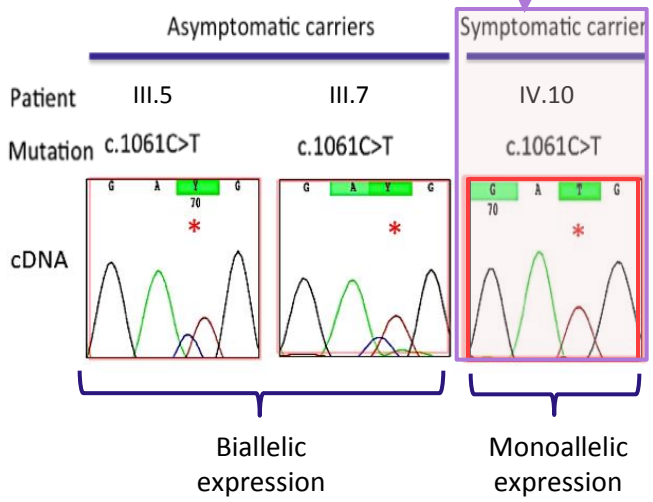


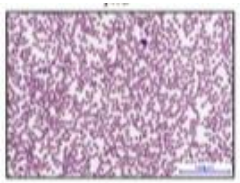
ASXL1 mutations are insufficient to promote onset of MDS.



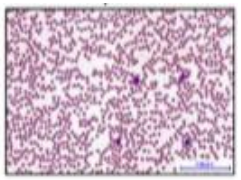
2. *GATA2* monoallelic expression discriminates between symptomatic and asymptomatic carriers and correlates with clinical parameters







Monocytopenia[0.1x10^9/L]
Neutropenia [0.8x10^9/L]



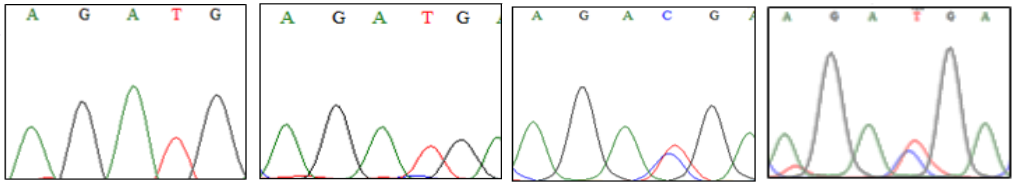
Normal monocyte count
Neutrophils[>1x10^9/L]

IV.10

Years since presentation

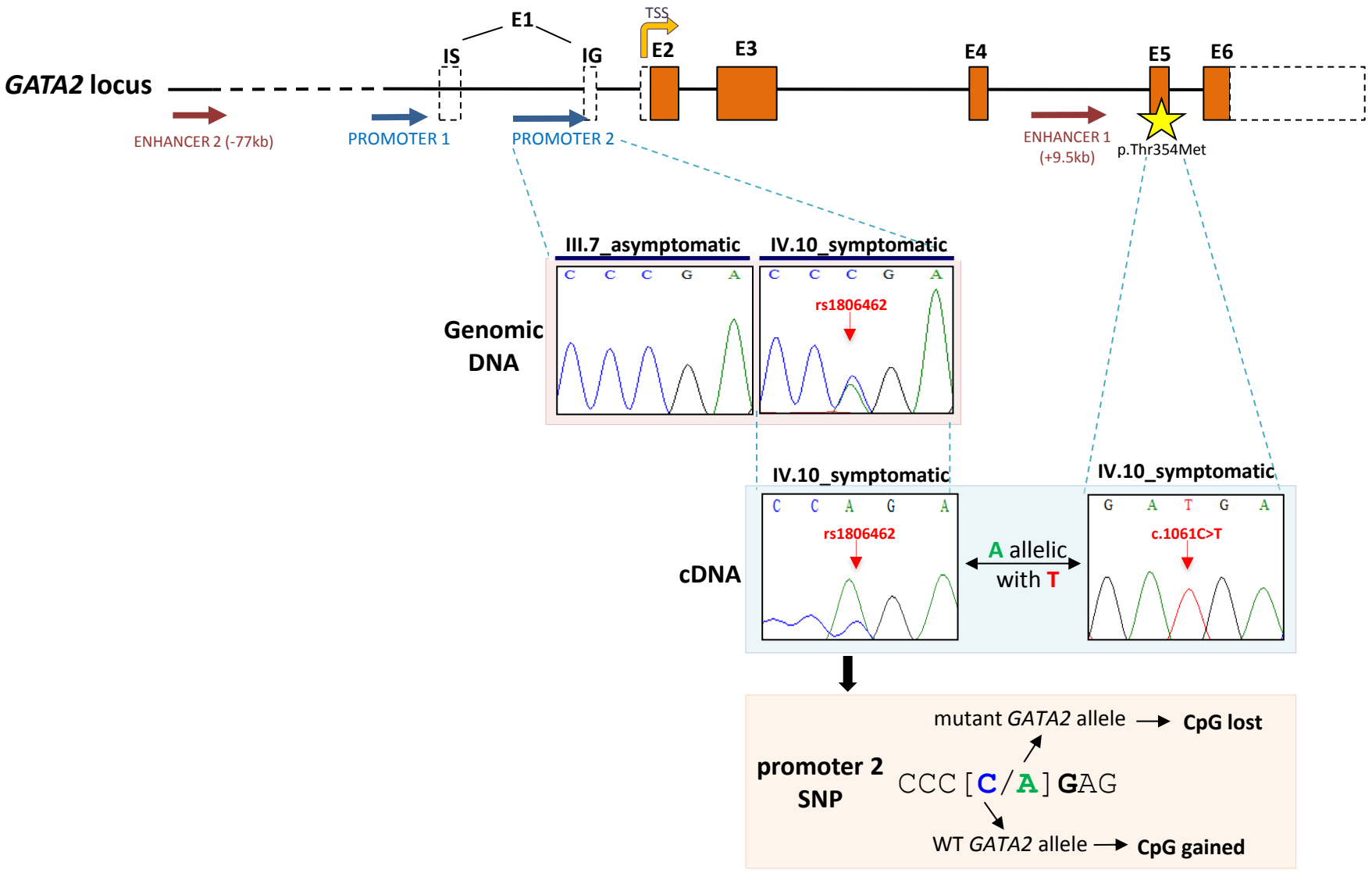
1	3	4	6
Monoallelic Expression	Monoallelic Expression	Biallelic Expression	Biallelic Expression

cDNA



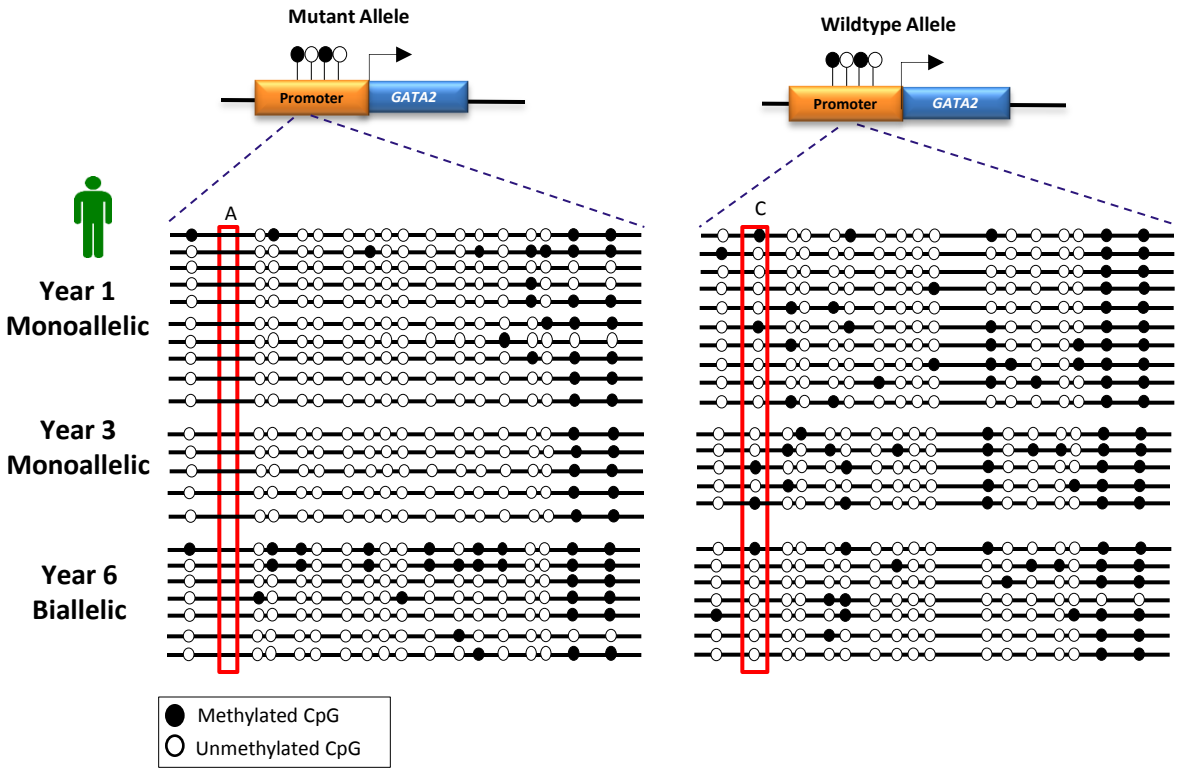
Reactivation of the wildtype allele expression

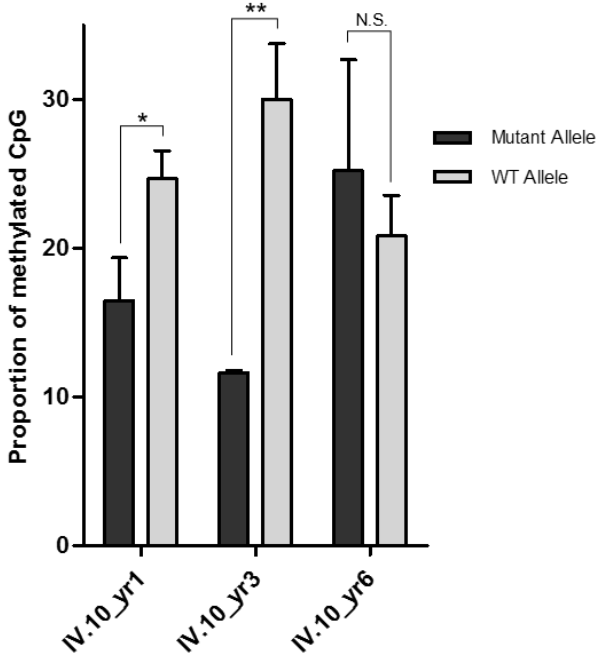
3. A CpG-SNP provided a means of distinguishing between alleles



4. Epigenetic reprogramming regulates monoallelic *GATA2* expression

1. DNA methylation as a mechanism of silencing the WT allele.



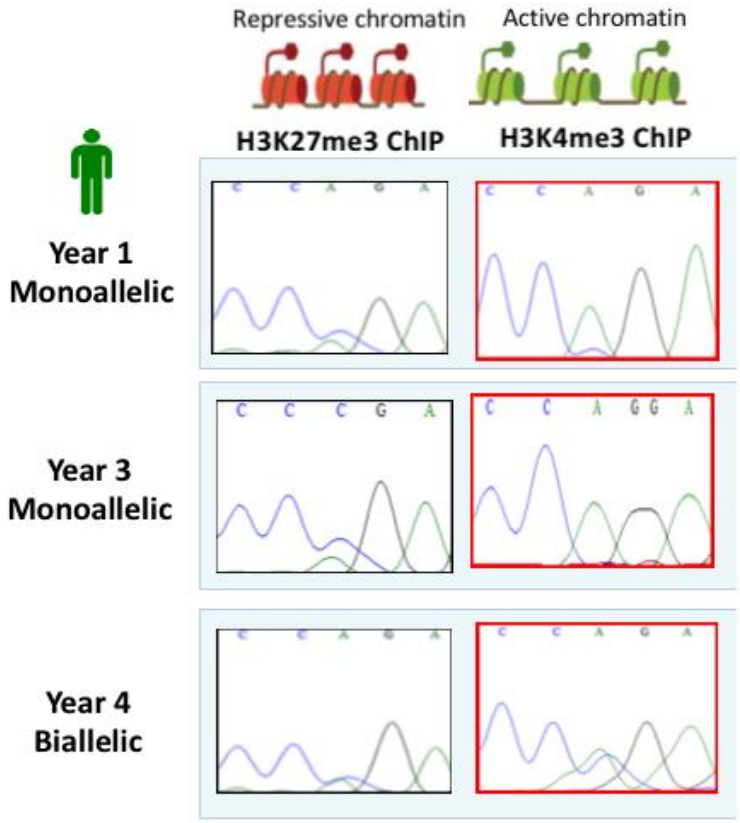


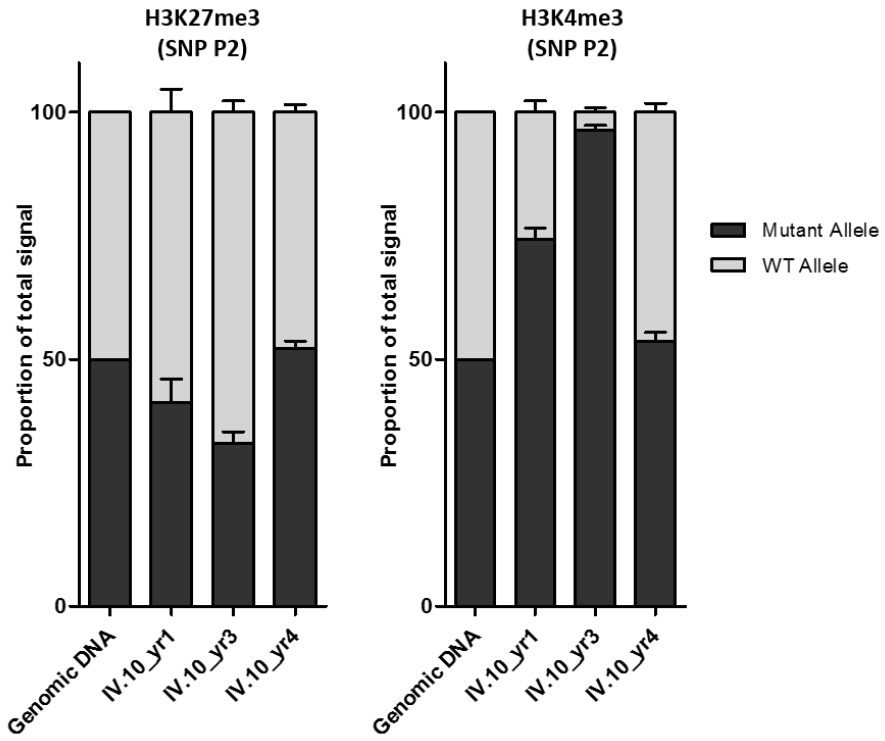
4. Epigenetic reprogramming regulates monoallelic *GATA2* expression

1. DNA methylation as a mechanism of silencing the WT allele.

2. Enhanced H3K4me3 promoter deposition on the mutant allele.

Mutually exclusive





Collaboration is Key

How to improve diagnosis, treatment and management?

Molecular diagnostics, 14 predisposition loci

COLLABORATIONS

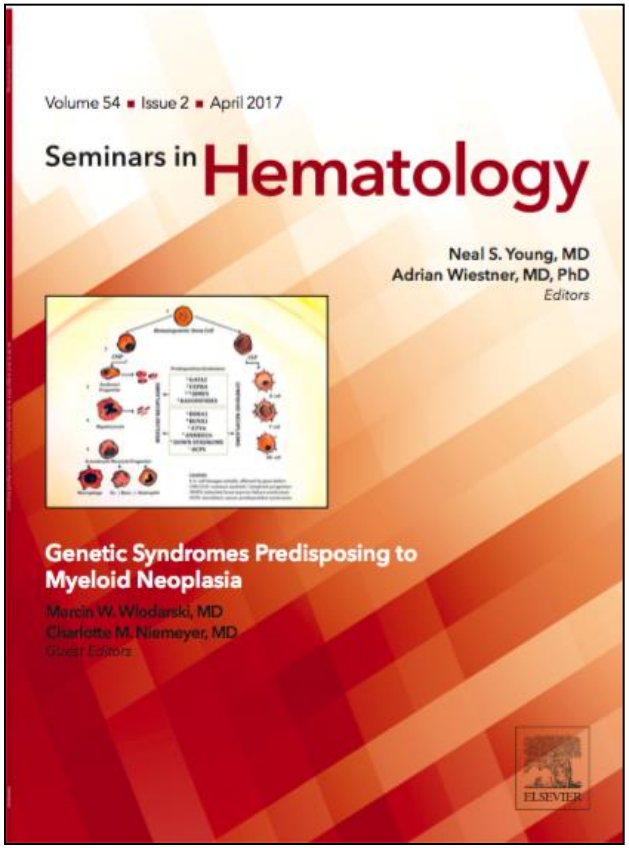
Tissue bank (Data collection)

New disease genes and functional validation

Identification of new familial cases, Individual at risk

NOVEL GUIDELINES AND IMPROVED MANAGEMENT

Seminars in Hematology – Special Issue 2017
Edited Marcin W. Wlodarski and Charlotte M. Niemeyer



- Recognition of familial myeloid neoplasia in adults
- Practical considerations for diagnosis and management of patients and carriers
- RUNX1 deficiency (familial platelet disorder with predisposition to myeloid leukemia, FPDMM)
- GATA2 deficiency and related myeloid neoplasms
- Familial CEBPA-mutated acute myeloid leukemia
- DDX41-related myeloid neoplasia
- ETV6 in hematopoiesis and leukemia predisposition
- Classical inherited bone marrow failure syndromes with high risk for myelodysplastic syndrome and acute myelogenous leukemia
- Cancer predisposition syndromes associated with myeloid malignancy

Acknowledgements

Barts Cancer Institute

Ahad Al Seraihi
Ana Rio-Machin
Kiran Tawana
Inderjeet Dokal
Tom Vulliamy

local care: global impact

Birmingham Women's NHS
NHS Foundation Trust

UNIVERSITY OF BIRMINGHAM

Gavin Ryan
Yvonne Wallis
Susanna Akiki
Joanne Mason

Csaba Bodor

Semmelweiss - Hungary

Our Global Collaborators

Some Key Groups Worldwide

Hamish Scott (Australia)
Charlotte Niemeyer (Germany)
Inderjeet Dokal/Tom Vulliamy (UK)
Lucy Godley (USA)

Familial Initiatives

Cristina Mecucci (Perugia)
Brigitte Schlegelberger (Hannover)

FUNDERS:

Bloodwise
Beating blood cancer since 1960

CHILDREN with CANCER

CANCER RESEARCH UK