The molecular basis of neutropenia in Israel – when to refer to NGS diagnosis

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מרכז שניידר לרפואת ילדים בישראל
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Introduction

- The molecular hematology laboratory in Schneider Children's Medical Center of Israel is the referral laboratory for molecular diagnosis of inherited hematological disorders

- We recently summarized our nation-wide experience studying 154 children presenting with persistent cytopenias from 12 pediatric hematology centers in Israel

The importance for achieving a genetic diagnosis

- Optimal treatment
- Monitoring for signs of clonal evolution –HSCT prior to leukemia development
- Identifying additional affected family members
- Genetic counseling
- Hematopoietic stem cell donor selection

Our aim

- To evaluate the genetic diagnosis in a cohort of pediatric patients with persistent cytopenia

Patients

- Children and adolescents (aged 0-20 years)
- Patients were evaluated between January 2016 and December 2019
- The patients were classified into 5 subgroups by their referring physicians:
  - Suspected IBMFS
  - MDS
  - Severe aplastic anemia (SAA)
  - Thrombocytopenia (platelet count <150X10^9/L)
  - Neutropenia (absolute neutrophil count <1/1.5X10^9/L)

Approach to molecular diagnosis

- Genes commonly mutated in suspected IBMFS were Sanger sequenced
  - *ELANE* and *G6PC3*, in patients with persistent neutropenia, *SBDS in* Schwachman-Diamond syndrome, *RPS19* in DBA

Approach to molecular diagnosis

- Our custom-made targeted NGS panel covers 226 genes known to be mutated in IBMFS, MDS predisposing syndromes, severe congenital neutropenia (SCN) and inherited thrombocytopenia

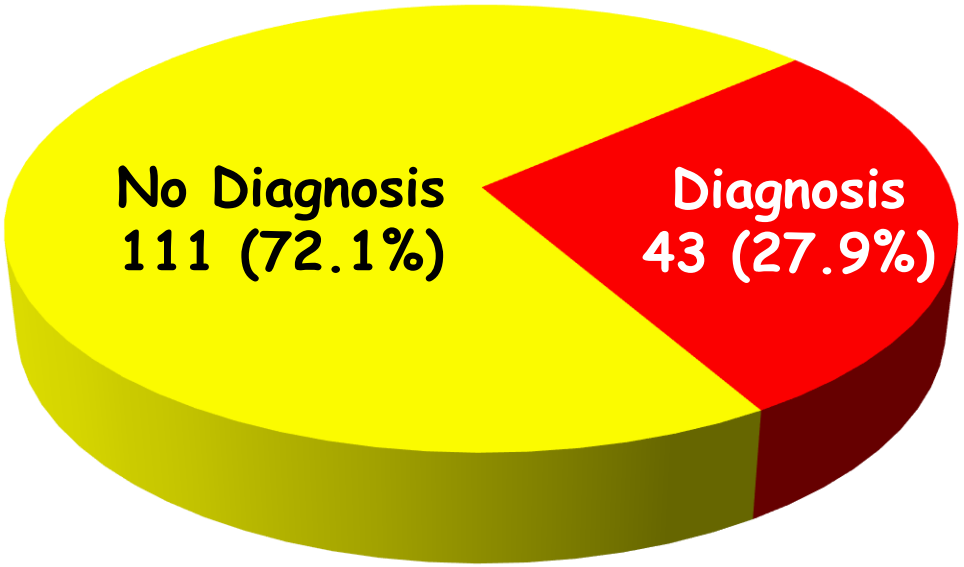
NGS targeted panel

- High depth of coverage across all genes of interest
- Interpretation is easier relative to WES
- Sequence cover of relevant non-coding regions
- Disadvantage - new genes of interest should be incorporated as they are reported

Gene variant analysis

- Variants were classified according to the American College of Medical Genetics (ACMG) guidelines
- Only pathogenic/likely pathogenic variants were considered as disease causing mutations
- Sanger sequence validation
- Family segregation
- Negative results using the NGS panel and prominent clinical presentation-further genomic evaluation

Patients with and without genetic diagnosis

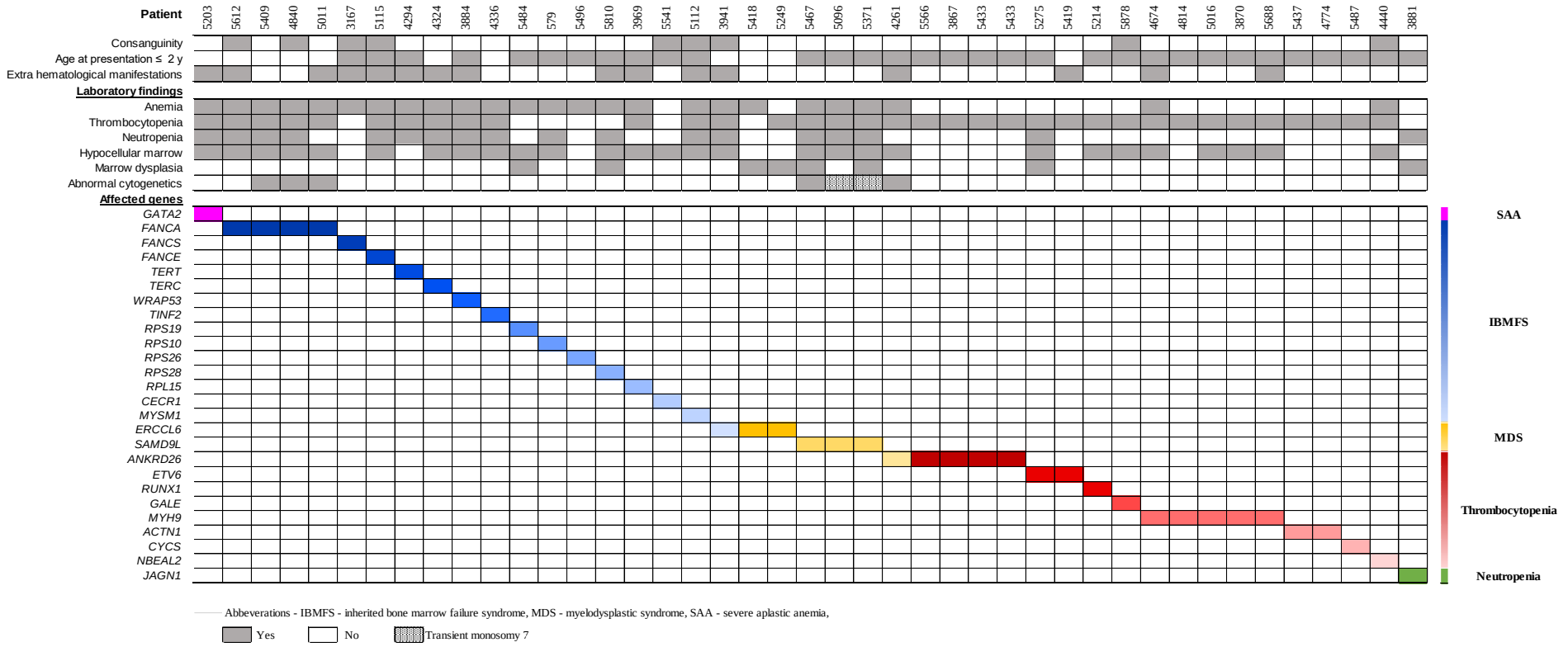


Additional 13 patients were genetically diagnosed through sanger sequencing

Referrals and genetic diagnoses in our cohort

Referral diagnosis	No of pts	No of genetically diagnosed pts (%)	Diagnosis according to molecular findings (No of pts)
IBMFS	36	18 (50.0)	IBMFS (18)
MDS	26	6 (23.1)	MDS predisposition (4)
			IBMFS (2)
SAA	28	1 (3.6)	IBMFS (1)
Isolated thrombocytopenia	33	17 (51.5)	Classical IT (9)
			IT with MDS predisposition (8)
Isolated neutropenia	31	1 (3.2)	SCN (1)
Total	**154**	**43 (27.9)**	

Integrated matrix of the 43 patients diagnosed with inherited cytopenias



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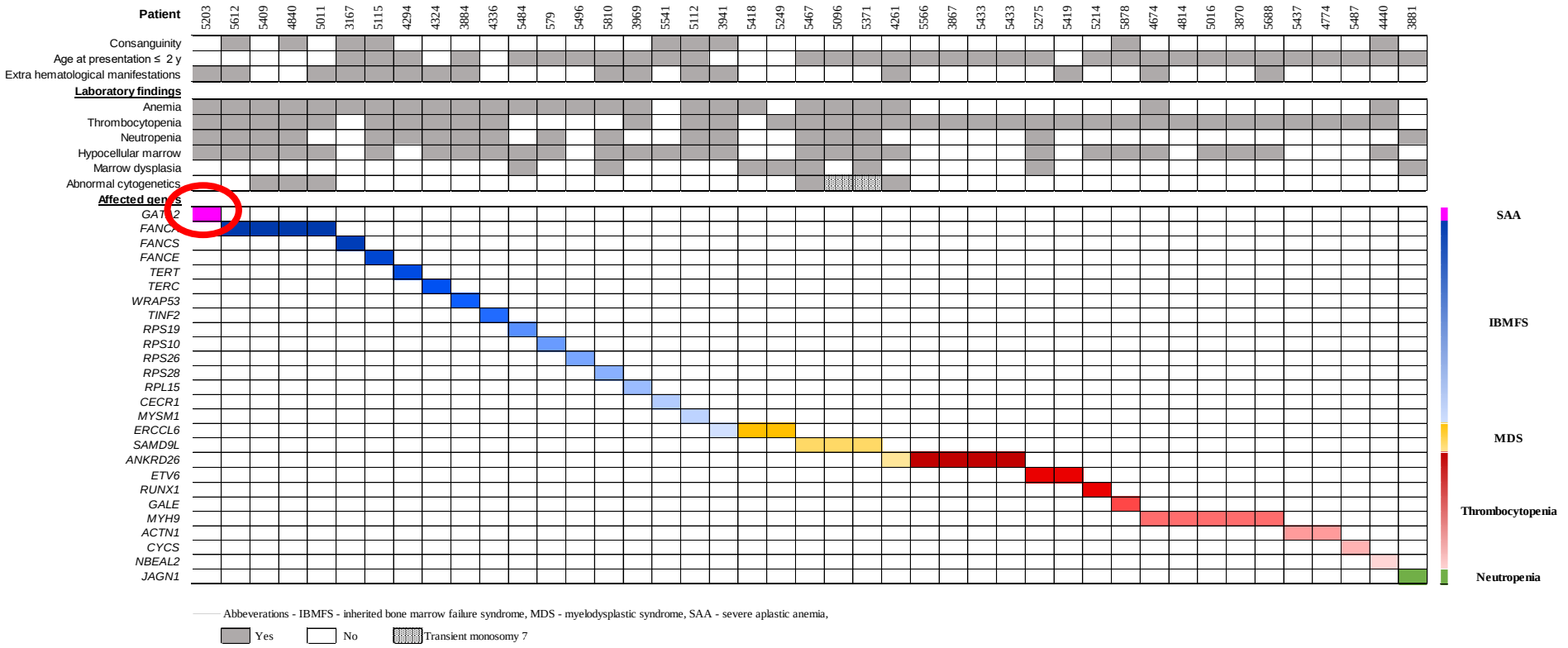

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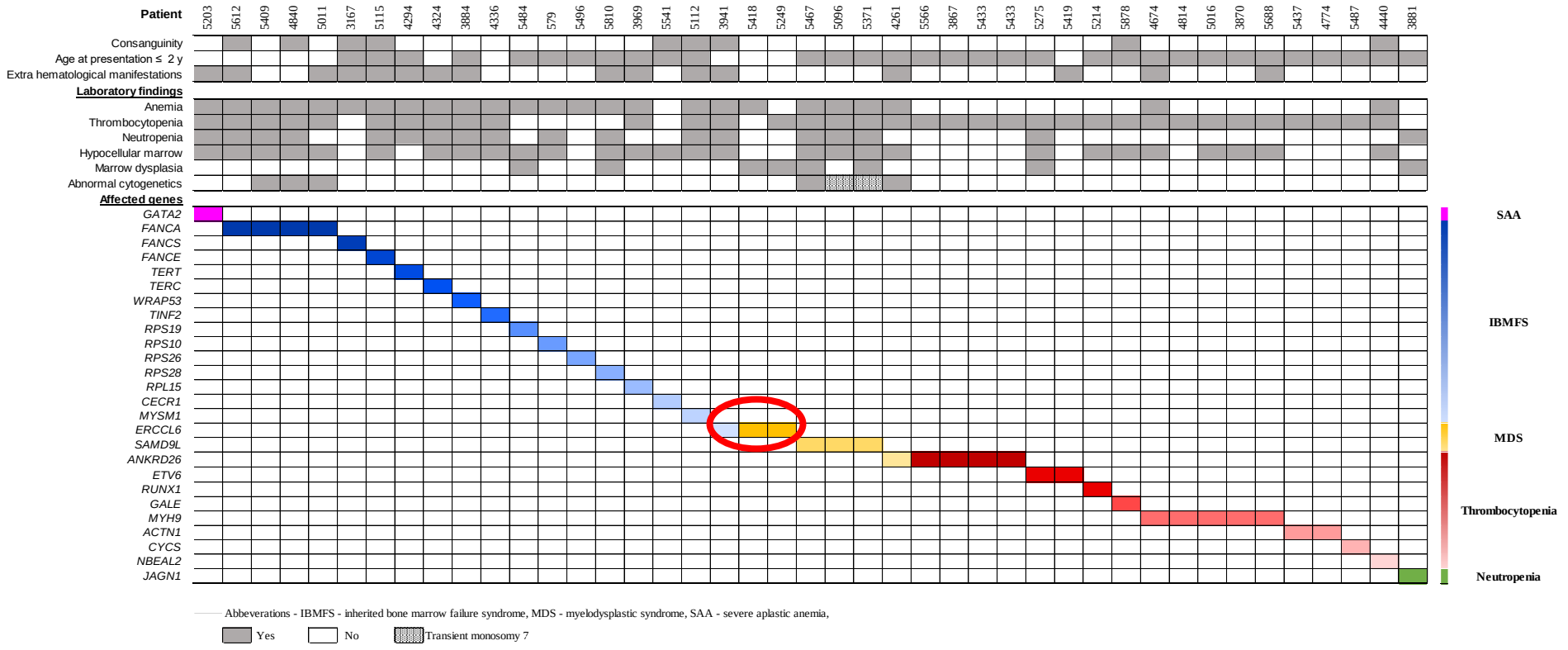


Summary of our findings in children with cytopenia (1)

- Molecular diagnosis was achieved in 43/154, (27.9%) of children with persistent cytopenias
- 79% of children with inherited cytopenias have predisposition to MDS/AML
- This may direct close monitoring and intervention prior to the development of overt leukemia

Summary of our findings in children with cytopenia (2)

- Compared to other similar studies
  - Only children
  - Report only pathogenic and likely pathogenic variants
- Low yield with genetic diagnosis of SCN

Pediatr Blood Cancer 2015;62:103–108

Genetic Analysis and Clinical Picture of Severe Congenital Neutropenia in Israel

Asaf Lebel, MD,[1] Joanne Yacobovich, MD,[2] Tanya Krasnov, MD,[3] Ariel Koren, MD,[4] Carina Levin, MD,[4] Chaim Kaplinsky, MD,[5] Shoshana Ravel-Vilk, MD,[6] Ruth Laor, MD,[7] Dina Attias, MD,[7] Ayelet Ben Barak, MD,[8] Dalia Shtager, MD,[9] Jerry Stein, MD,[10] Amir Kuperman, MD,[11] Hagit Miskin, MD,[12] Orly Dgany, MD,[3] Neelam Giri, MD,[13] Blanche P. Alter, MD,[13] and Hannah Tamary, MD[2,3]*

TABLE III. Prevalence of Mutated Genes in SCN Patients in Literature Reports

Country	No. of patients or kindreds	No. of patients with mutation (%)					No mutation	Reference, year
		ELANE	*HAX1*	*G6PC3*	*GFI1*	*WAS*		
North America	25	22 (88)	ND	ND	ND	ND	3 (12)	[23], 2000
French	54	19 (35)	ND	ND	ND	ND	35 (65)	[24], 2004
Great Britain*	109	33 (30)	4 (4)	4 (4)	ND	2 (2)	66 (60)	[13,25], 2008
North America	160	90 (56)	0 (0)	2 (1)	1 (1)	1 (1)	66 (41)	[26], 2009
Sweden	21	9 (43)	4 (19)	ND	ND	ND	8 (38)	[27], 2012
Iran	27	4 (15)	11 (41)	2 (7)	0 (0)	0 (0)	10 (37)	[29], 2013
Germany*	395	175 (41)	ND	ND	ND	ND	251 (59)	[28], 2013
Israel*	26	10 (38)	0 (0)	5 (19)	0 (0)	0 (0)	11 (42)	Current study

ND, not done. *Reported by kindred.

Clinical features of patients referred with isolated neutropenia (n=31)

- Patients were referred with isolated neutropenia
- 13 (42%) - consanguinity
- 9 (29%) - family history of neutropenia
- Clinical presentation:
  - Median age of presentation -3 months
  - Tendency of infections
    - 21 (67.7%) -recurrent infections
    - 3 (9.7%) –recurrent aphthous stomatitis
    - 6 (19.4%) – asymptomatic
  - 6 (19.4%) patients had congenital anomalies

Laboratory findings of patients referred with suspected congenital neutropenia

- Peripheral blood count
  - mean ANC $0.35 \pm 0.26 \times 10^9/L$
- BM studies (26 patients)
  - 7 (29.1%) - early myeloid maturation arrest
  - 9 (37.5%) - late myeloid maturation arrest
  - 8 (33.3%) – normal maturation
- Anti neutrophil antibodies
  - 7 patients of 20 (35%) tested positive

Genetic diagnosis of patients referred with neutropenia (n=31)

- Only one patient referred with neutropenia was found by NGS panel to have a mutation (*JAGN1*)
- 2 patients were subsequently diagnosed by WES

  SRP54 -a gene not known at the time of panel diagnosis

  MUNC13 rarely described as cause of neutropenia

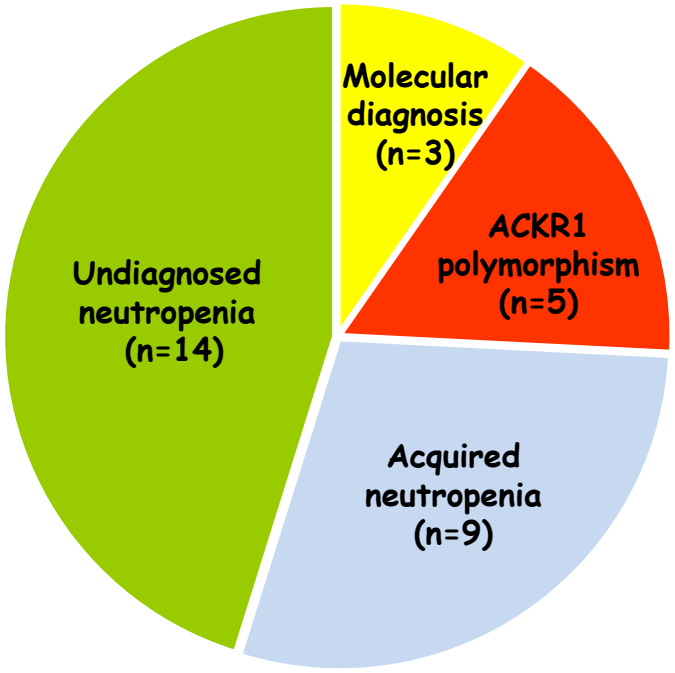
Patients with neutropenia and with no apparent genetic diagnosis (n=28)

- Most patients (28/31, 90.3%) referred with prolonged neutropenia had no genetic diagnosis

Possible reasons for low yield of diagnosis of inherited neutropenia

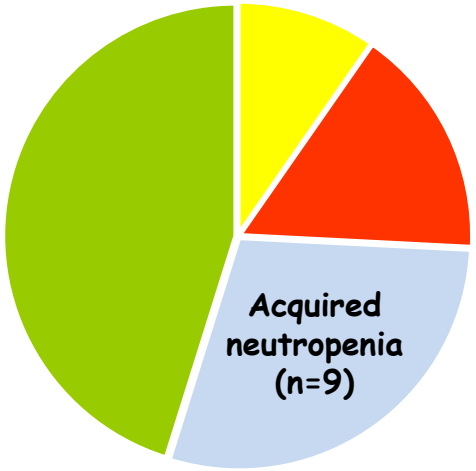
- Acquired disorder
- Genes not initially on NGS panels;
  - Ethnic neutropenia
  - Mutated genes primarily considered to be causing immune deficiency
- Still unknown genes

Patients referred for evaluation due to neutropenia (n=31)



Acquired neutropenia (n=9)

- 7/20 (35%) patients had anti neutrophil antibodies
- Clinical presentation was consistent with autoimmune neutropenia
- At present, 3/7- recovered



- Two patients suffered from profound neutropenia (ANC 0-200x10^9/L) but no infections
- Both went through WES – negative
- In both patients – neutropenia resolved

Mutated genes in supposedly autoimmune neutropenia

- **Andrea Beccaria (Early Career Investigator's Workshop, Genoa, Sep.2021)**
  - 46 pediatric patients
  - Median Age 11.2 years
  - 5 pathogenic variants; TACI, CARD11 mutations

Mutated genes primarily considered to be causing immune deficiency

- **Miano et al, Am J Hematol; 2021**
  - 97 patients, median age 5 years (0-32)
  - 17% were found to have an underlying primary immunodeficiency
  - *CD40l mutation, TNFRSF13B mutation*

Ethnic neutropenia – ACKR1/DARC null polymorphism

- Shoenfeld et al, described (Eur. J. Haematology, 1995) Benign ethnic neutropenia in several ethnic groups including; Yemenite Jews, Africans in South Africa, West Indians and Arab Jordanians

- The linkage between a homozygous polymorphism in DARK (or ACKR1) promoter and benign neutropenia in African Americans was described by Tournamille et al (Nat Genet. 1995)

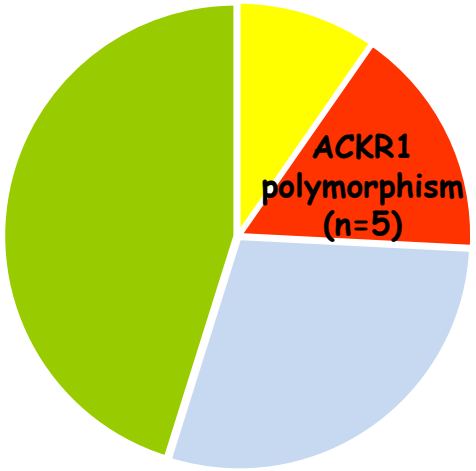
Ethnic neutropenia – ACKR1/DARC null polymorphism

- Nalls et al (2008), and Reich et al (2009), later described the presence of the polymorphism in people of Yemenite Jewish and Arab ancestries

- The phenomena is described as 'Benign neutropenia' because the neutropenia is mild and does not predispose patients to infections

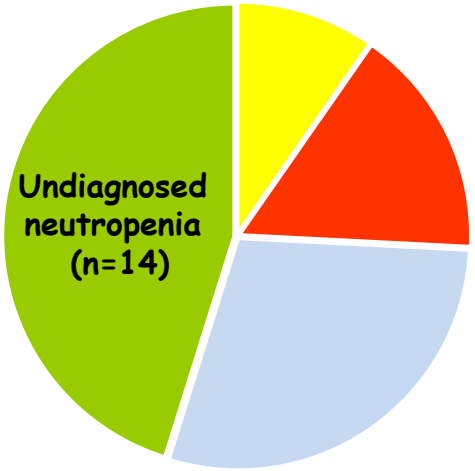
ACKR1/DARC null polymorphism (5 patients)

- 6 patients were homozygous
- 5 had (ANC) >0.5 x10^9/L and no recurrent infections
- One patient suffered from profound neutropenia and recurrent infections



Undiagnosed neutropenia (n=14)

- 9 had a family history of neutropenia
- 6 patients had congenital anomalies
- 10 suffered from recurrent infections, 2- recurrent aphthous stomatitis, 2– asymptomatic



- 6 underwent successful HSCT, additional 5 patients are treated with GCSF
- WES was recommended for all undiagnosed patients; 4 already underwent WES – negative results

Recent studies using NGS/WES for evaluating persistent neutropenia

Blombery et al, Haematologica 2021

- Methods – NGS and than WES
- Out of 11 children (median age 10) with isolated neutropenia – only one was genetically diagnosed

Galvez et al, Hemasphere 2021

- Methods - NGS (sporadic use of WES)
- Out of 25 patients with isolated neutropenia – only 3 was genetically diagnosed

Summary

- Selection of candidates for genetic work up of neutropenia
  - No/mild infections & positive for presence of antineutrophil antibodies –no need for further evaluation
  - Specific ethnic background with mild/moderate neutropenia and no/mild infections –*ACKR1/DARC* null polymorphism
- Molecular diagnosis in terms of NGS/WES is indicated in all children with neutropenia and high clinical suspicion of SCN

Our proposed workflow of patients with neutropenia

Sanger sequencing for *ELANE, G6PC3 or SBDS*

(In areas where ethnic neutropenia is common, check for polymorphism in the *ACKR1/DARC* gene)

↓

Targeted next-generation-sequencing

The panel includes all genes known to cause inherited neutropenia and is continuously updated

↓

Whole exome sequencing, WGS, RNAseq

Acknowledgments

- Prof. Hannah Tamary
- Dr. Orna Steinberg Shemer
- Dr. Joanne Yacobovich
- Dr. Orly Dgany
- Dr. Sharon Noy-Lotan
- Tanya Krasnov

- Referring physicians
- Patients and families