Searching for genetic basis of congenital neutropenia

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FixNet
Fix Neutropenia

1 Introduction

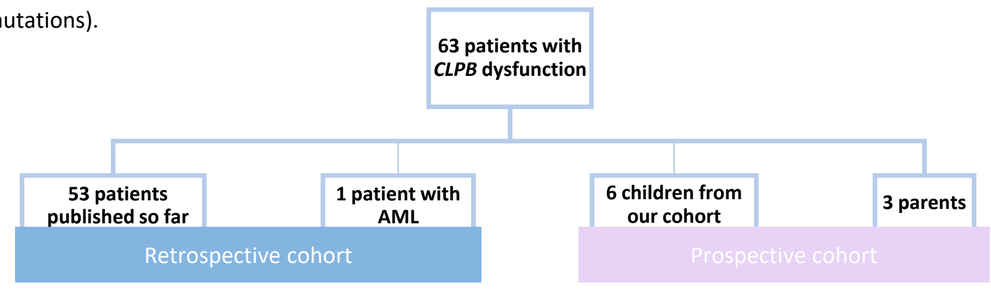
Neutrophils, which are part of the innate immune system, constitute the first line of defense against infection. They are produced in the bone marrow and differentiate from the multipotential progenitor cells. The term neutropenia is defined as an absolute neutrophil count (ANC) of less than 1500 per microliter (1500/µL). The low neutrophil count is associated with a high risk of developing serious infections, also life-threatening. Neutropenia can be divided into acquired (caused by i.a. chemotherapeutic agents) and congenital (genetic factors). The disease can develop as a result of mutation of one of many genes that play a key role in the maturation and proper functioning of neutrophils, thereby leading to disturbances in their activity or to apoptosis.

Recently, the *CLPB* gene has been identified as a novel cause of severe congenital neutropenia (SCN). While biallelic mutations in *CLPB* are associated with the 3-methylglutaconic aciduria, (MIM number: 616271 3), the heterozygous variants seem to induce neutropenia exclusively. The approach summarizes both unpublished data from our patients as well as all patients with *CLPB*- dysfunction published so far.

2 Materials and methods

The study group included 270 patients diagnosed under the FixNet project. Patients were analyzed using Next Generation Sequencing (Illumina NextSeq 550 System) and verificated by Sanger sequencing (Applied Biosystem 3100 Genetic Analyzer).

To assess the severity of clinical manifestation the scoring system was applied. We took into account the severity of the neurological symptoms, neuromuscular dysfunction, metabolic disorders, the onset of neutropenia, MDS/AML or death. We focused on the genotype-phenotype association as well as CLPB mutation position that disrupts different domains' function. The study included 65 patients who present clinical features of CLPB dysfunction (30 with monoallelic and 35 with biallelic mutations).



3 Results

Among analyzed patients were identified 49 pathogenic variants in congenital neutropenia genes and 46 pathogenic changes in other genes related with immune system which can be responsible for occurrence of neutropenia (Figure 1). Preliminary analysis of patients clinical data showed that the assessment of anti-granulocyte antibodies, whose presence has so far information about immune neutropenia, deserves special attention. It turns out that the presence of the above-mentioned antibodies may occur in patients with *ELANE* mutations (n=5) and should not stop the diagnostic process at this stage, especially due to the possible coexistence of genetic changes predisposing to the development of leukaemia (Table 1).

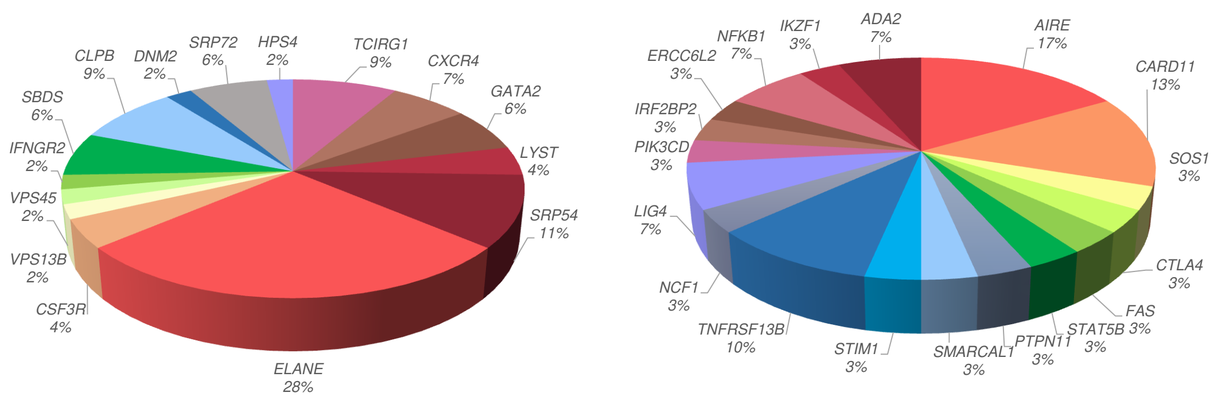


Figure 1. *Mutated genes found in analyzed patients.*

Table 1. *ELANE –SCN/CyN patients with neutrophil-specific antibodies,.*

Sex	Diagnosis (SCN/CyN)	*ELANE* variant (DNA) NM_001972.2	*ELANE* variant (protein)	Age at antibody testing (months)	Detected antibodies against neutrophil antigens
M	SCN	c.371A>C	p.Asn124Thr	84	Unspecific
M	SCN	c.163_165delTGC	p.Cys55del	17	HNA-1a
M	SCN	c.163T>C	p.Cys55Arg	12	HNA-1a
F	SCN	c.538delC	p.Leu180fs	6	HNA-1
F	CyN	c.575_589delGCCGGCAGGCCG	p.Gly192_Gly196	86	HNA-1a

We showed that the highest score, reflecting the most severe clinical state, was noted in patients with mutations in the ankyrin domain (p=0.00389). The median equals 60 points for ANK domaind compared to 41 points for AAA domains.

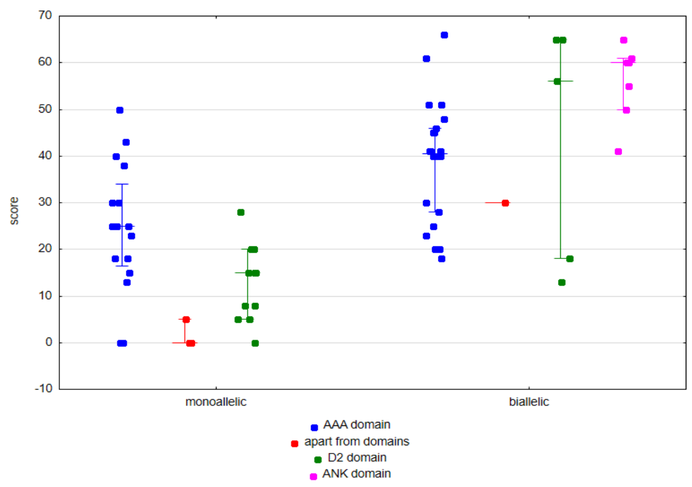


Figure 2. *The clinical severity manifestation score level in groups of patients with various location of CLPB mutation .*

Among this group (n=7) all had developmental/intellectual delay and 3-methylglutaconic aciduria, n=2 developed MDS/AML, n=6 died including n=4 during infancy. From all analyzed patients, 4 developed MDS/AML regardless of the localization of the mutation.

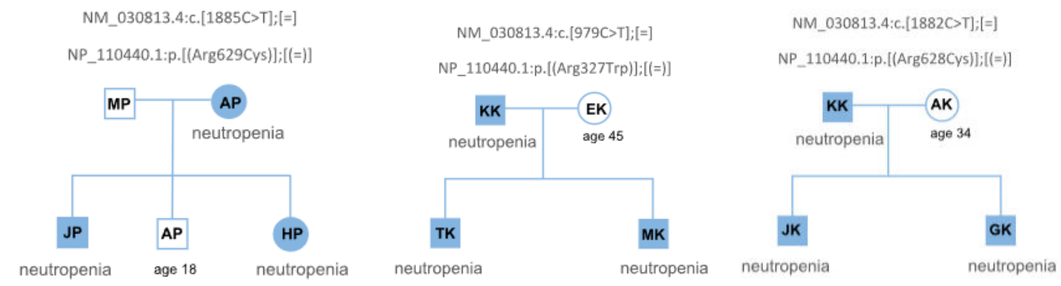


Figure 3. *Pedigree charts for 3 families with CLPB-deficiency (prospective cohort).*

Looking at hematological features suggesting BMF, in our cohort patients with SCN, we have identified 6 patients and their families (n=10), including one patient who developed MDS (with germline *CLPB* variant p.Arg629Cys, and somatic changes in *MPL* (p.Arg592*) and *RUNX1* (p.Ile337Val fs*23),) and one patient who developed AML (spliced site variant c.646+1G>A).

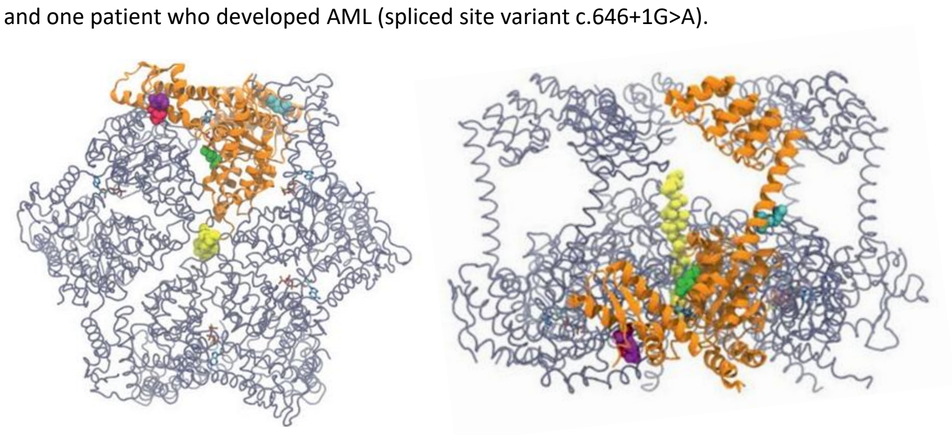


Figure 4. *The predicted 3D structure of CLPB protein with marked variants: Arg327Trp (blue), Arg628Cys (red), Arg629Cys (purple). The ATP molecule is marked with yellow.*

The localizations of all mutations identified in our cohort are visualised in the structure of CLPB protein (Cryo-EM structure, PDBid:7TTS (25)) is presented. The Arg628Cys (red) and Arg629Cys (purple) mutations are in D2 domain which is essential for oligomerization. In addition, the position of Arg327Trp (blue) mutation which is apart from the functional domains, allows us to assume that it may also disrupt forming of a hexameric ring-shaped structure.

4 Conclusions

The antigranulocytic antibodies may occur in patients with defined congenital neutropenia and should not stop the diagnostic process at this stage, especially due to the possible coexistence of genetic changes predisposing to the development of leukaemia.Our findings point out that changes in the CLPB ankyrin domain lead to the most severe phenotype. We revealed that mutations localized apart from the active domains are also responsible for the development of pure neutropenia. Moreover, we found that the risk of MDS/AML transformations persists regardless of the severity of BMF clinical presentation in patients from mild to severe phenotype.

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