bjh review

# Congenital neutropenia in the era of genomics: classification, diagnosis, and natural history

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## Summary

This review focuses on the classification, diagnosis and natural history of congenital neutropenia (CN). CN encompasses a number of genetic disorders with chronic neutropenia and, for some, affecting other organ systems, such as the pancreas, central nervous system, heart, bone and skin. To date, 24 distinct genes have been associated with CN. The number of genes involved makes gene screening difficult. This can be solved by next-generation sequencing (NGS) of targeted gene panels. One of the major complications of CN is spontaneous leukaemia, which is preceded by clonal somatic evolution, and can be screened by a targeted NGS panel focused on somatic events.

**Keywords:** G-CSF, severe congenital neutropenia, ELANE, Shwachman-Diamond syndrome, next-generation sequencing.

Congenital neutropenia (CN) is a family of genetic diseases associated with three main features: low neutrophil count and susceptibility to infection, various organ dysfunctions, and an extraordinarily high risk of leukaemic transformation. To date, 24 genes have been identified as being responsible for this syndrome (Table I). To add complexity, the same gene may be responsible for a 'constitutional defect' related to a germline mutation or, *via* a somatic genetic event, may be associated with leukaemogenesis. The phenotypic features and natural history of patients are important to consider, but they should be now interpreted in the context of molecular events. Genomics has aided in understanding CN, but involves costly techniques and cannot abolish the knowledge that comes from medical experience, such as the clinical and cytological evaluation that still should precede the molecular diagnosis. In addition to better classification, genomics offers a gateway to the pathophysiological mechanisms responsible for each disease and may offer the opportunity to develop adapted therapy. While genomics offers the hope of understanding the mechanisms of neutropenia, there is currently no clear or unifying genotype-phenotype correlation, disease by disease. In this review, we will not focus on any specific neutropenia but will attempt to unify this field of haematology.

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## Diagnosis and main features of congenital neutropenia

### *General definition of neutropenia*

Neutropenia is defined as a reduction in the absolute number of neutrophils circulating in the blood below $1{\cdot}5 \times 10^9$/l. The standard haematological examination is microscopic cell counting, which is necessary to confirm disorders identified by automated cell counters and to examine cell morphology. The neutropenia is said to be profound when $<0{\cdot}5 \times 10^9$/l and chronic if it lasts more than 3 months (intermittent or permanent).

The only exception is the first weeks of life, during which the number of neutrophils is physiologically elevated. Neutrophils are increased during the first 72 h, and then gradually decrease. Therefore, neutropenia in newborns is defined by a higher threshold than in adults, at least $2{\cdot}5 \times 10^9$/l (Schmutz *et al*, 2008). Physiological fluctuations of the neutrophil count have been known for more than 50 years (Maughan *et al*, 1973) and are chaotic in mathematic terms rather than random (Mackey, 2001).

Because nycthemeral and seasonal variations persist in pathological situations, neutropenia should be confirmed in several samples (minimum 3) over a 3-month period.

Neutropenia is said to be permanent when present in all samples and termed as intermittent when periods of normalization alternate with deep neutropenia. In the literature, cyclic neutropenia describes perfect sinusoidal variations every 21 days, which is almost never seen in practice (Donadieu *et al*, 2011). So far only a single study has thoroughly studied the real periodicity observed in patients, supposed to be cyclic, based on serial counts (Haurie *et al*, 1999). Among the


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**Table I.** List and main features of known genes in congenital neutropenia (as of May 2017).

| Subgroup of neutropenia | Gene/disease name (reference) | OMIM code | Main haematological features | Extra-haematopoietic features | Inheritance Gene localization | Normal function of the gene |
|---|---|---|---|---|---|---|
| CN usually without extra-haematopoietic manifestations | *ELANE* Severe congenital neutropenia/cyclic neutropenia (Horwitz *et al*, 1999; Dale *et al*, 2000) | 202700 162800 | Severe and permanent Maturation arrest Intermittent/cyclic with variable bone marrow features | No | Dominant 19q13.3 | Protease activity Antagonism with alpha 1 antitrypsin |
| | *CSF3R*/Germline mutation of CSF3R (Triot *et al*, 2014) | 202700 | Permanent Maturation arrest Unresponsive to G-CSF | No | Dominant 1p35-p34.3 | Transmembrane G-CSF receptor/intracellular signalling |
| | *WAS*/Severe congenital neutropenia (Devriendt *et al*, 2001) | 301000 | Severe and permanent Maturation arrest Monocytopenia | No | X Linked Xp11.4-p11.21 | Cytoskeleton homeostasis |
| | *CXCR2*/chronic neutropenia (Auer *et al*, 2014) | | Severe and permanent No maturation arrest Myelokathexis | No | Recessive 2q35 | Chemokine receptor (CXCL12) |
| CN with frequent extra-haematopoietic manifestations, including innate immunity deficiencies | *SBDS*/Shwachman-Bodian-Diamond disease (Boocock *et al*, 2003) | 260400 | Mild neutropenia Dysgranulopeosis, mild dysmegakaryopoiesis | Exocrine pancreas deficiency, bone: metaphyseal dysplasia, mental retardation, heart: cardiomyopathy | Recessive 7q11.22 | Ribosomal protein Regulation of RNA expression |
| | *EFL1* syndrome (Stepensky *et al*, 2017) | 260400 | Mild neutropenia dyserythropoeisis | Exocrine pancreas deficiency, bone: metaphyseal dysplasia, mental retardation, | Recessive 15q25.2 | Ribosomal protein Regulation of RNA expression |
| | *GATA2* syndrome (Collin *et al*, 2015) | 614038 614172 | Mild neutropenia, monocytopenia Macrocytosis | Lymphedema, deafness, mycobacteria HPV infections | Dominant 3q21.3 | Transcription factor |
| | *G6PC3*/Severe congenital neutropenia (Boztug *et al*, 2009) | 202700 | Maturation arrest | Skin-prominent superficial venous network, heart: atrial defect, uropathy | Recessive 17q21 | Glucose 6 – phosphatase complex catalytic unit |
| | *SLC37A4*/Glycogen storage type Ib (Veiga-da-Cunha *et al*, 1999) | 232220 | No maturation arrest | Hypoglycaemia, fasting hyperlactacidaemia and glycogen overload of the liver | Recessive 11q23.3 | Glucose 6 – phosphatase complex trans ER transporter |
| | *TAZ*/Barth disease (Barth *et al*, 1999) | 302060 | No maturation arrest | Hypertrophic cardiomyopathy, myopathic syndrome, 3 methyl glucaconic aciduria | X Linked Xq28 | Tafazzin, phospholipid membrane homeostasis |
| | *CXCR4*/WHIM syndrome (Gorlin *et al*, 2000) | 193670 | Severe and permanent No maturation arrest Myelokathexis | Lymphopenia, thrombocytopenia Cardiopathy type, Tetralogy of Fallot | Dominant 2q21 | Chemokine receptor (ligand CXCL12) |
| | *JAGN1*/Severe congenital neutropenia (Boztug *et al*, 2014) | 616022 | Variable | Bone abnormalities, exocrine pancreatic enzyme | Récessif 3p25.3 | ER protein |

 

**Table I.** (*Continued*)

| Subgroup of neutropenia | Gene/disease name (reference) | OMIM code | Main haematological features | Extra-haematopoietic features | Inheritance Gene localization | Normal function of the gene |
|---|---|---|---|---|---|---|
| | *VPS13B*/Cohen syndrome (Kolehmainen *et al*, 2003) | 216550 | No maturation arrest | Psychomotor retardation, clumsiness, microcephaly, characteristic facial features, hypotonia and joint laxity, progressive retinochoroidal dystrophy, myopia | Recessive 8q22-q23 | Sorting and transporting proteins in the ER |
| | *GFI1*/Severe congenital neutropenia (Person *et al*, 2003) | 202700 | Permanent/severe or mild Sometimes maturation arrest | Internal ear (in mouse model), lymphopenia | Dominant 1p22 | Transcription factor Regulation of oncoprotein |
| | *HAX1*/Kostmann disease (Kostmann, 1956; Klein *et al*, 2007) | 202700 | Maturation arrest | Central nervous system: mental retardation/seizures | Recessive 1q21.3 | Anti-apoptotic protein located in the mitochondria and cytosol |
| | *AP3B1*/Hermansky–Pudlak syndrome type 2 (Huizing *et al*, 2002) | 608233 | No maturation arrest | Albinism | Recessive 5q14.1 | Cargo protein/ER trafficking with *ELANE* interaction |
| | *LAMTOR2*/Chronic neutropenia (Bohn *et al*, 2007) | 610389 | No maturation arrest | Albinism | Recessive 1q21 | Lysosome packaging |
| | *USB1*/Poikiloderma type Clericuzio (Volpi *et al*, 2010) | 604173 | No maturation arrest Minor dysgranulopoietic features | Skin: poikiloderma | Recessive 16q21 | Not known |
| | *VPS45*/Severe congenital neutropenia (Vilboux *et al*, 2013) | 615285 | Maturation arrest/myelofibrosis | Nephromegalia Hepato splenomegaly, mental retardation | Recessive 1q21.2 | Role in segregation of intracellular molecules into distinct organelles |
| | *TCIRG1*/Severe congenital neutropenia (Makaryan *et al*, 2014) | 202700 | Variable | Skin angiomatosis | Dominant 11q13.2 | |
| | *EIF2AK3*/Wolcott-Rallison syndrome (Delepine *et al*, 2000) | 604032 | Maturation arrest | Insulin-dependant neonatal diabetes | Recessive 2p11.2 | ER stress |
| | *CLPB* syndrome (Saunders *et al*, 2015; Wortmann *et al*, 2015) | 616254 | Maturation arrest | Mental retardation, congenital cataract 3 methyl glucaconic aciduria | Recessive 11q13.4 | |
| | *STK4* (*MST1*) syndrome (Abdollahpour *et al*, 2012) | 614868 | Intermittent neutropenia | Atrial defect | Recessive 20q13 | Serine/threonine protein kinase |
| | *SMARCD2* (Witzel *et al*, 2017) | | Dysplastic syndrome No granules in neutrophil | Chronc diarhea Bone abnomrlalites Low set ears | Recessive 17q23 | |

CN, congenital neutropenia; ER, endoplasmic reticulum; G-CSF, granulocyte colony-stimulating factor; HPV, human papilloma virus; OMIM, Online Mendelian Inheritance in Man; WHIM, warts, hypogammaglobulinemia, infections and myelokathexis.

10 patients supposed to have a cyclic neutropenia, periods of 18, 20 and 30 days were found in only three cases. And among the 28 patients supposed to have a severe and permanent CN or an idiopathic neutropenia, 6 showed regular variations. It suggests that there is a continuum between the two extremes – permanent neutropenia *versus* intermittent neutropenia. Indeed, the pathological processes that lead to neutropenia affect both the periodicity and amplitude of the neutrophil count. The terms 'permanent neutropenia' and 'intermittent neutropenia', which are more descriptive, should be used instead of cyclic neutropenia, which represents a kind of perfect biological clock and is almost never seen.

Monocytosis, hypereosinophilia and polyclonal hypergammaglobulinaemia appeared to be frequently associated with neutropenia and inversely proportional to its severity. A compensatory role of monocytes has been proposed to explain the good clinical tolerance of some profound CN.

### *Congenital neutropenia: an evolving definition*

The term 'congenital neutropenia' is not homogeneously used in the literature (Dale *et al*, 2003; Boxer & Newburger, 2007; Notarangelo *et al*, 2009; Donadieu *et al*, 2011). Some keep this term for chronic profound neutropenia without any immunological or extra-haematopoietic abnormalities, while others consider that genetic disease associating immunological or extra-haematopoietic abnormalities with neutropenia should belong to this family of disorders. Indeed, glycogen storage disease Ib, Shwachman-Diamond syndrome, warts, hypogammaglobulinemia, infections and myelokathexis (WHIM) syndrome, GATA2 syndrome and Barth disease have been included in the definition of CN. In this review, we have chosen not to restrict the definition of CN to disorders in which neutropenia is the only phenotypic manifestation, but have considered all genetic disorders comprising neutropenia as a chronic or recurrent manifestation. Despite such a broad definition, it seems reasonable to exclude lymphoid defects or other forms of primary immunodeficiency from this family of disorders (Notarangelo *et al*, 2009), which deserves specific therapy, as well as other bone marrow failure syndromes, limiting the definition to diseases with a predominant neutrophil count deficit.

## Classification of congenital neutropenia

The discovery of the genes responsible for different subtypes of CN induced a change in the disease classification. The term 'Kostmann syndrome' is commonly considered as a synonym of CN. This disease was first described in a Swedish publication in 1950, and was subsequently published in English (Kostmann, 1956). In this publication, we may find the key characteristics of CNs: profound neutropenia ($<0{\cdot}2 \times 10^9$/l) since birth, maturation arrest of granulopoiesis at promyelocyte stage, and death due to bacterial infections. Sixty years after the initial description, these patients' life expectancy routinely exceeds 30 years, and the molecular basis of this entity, *HAX1*, has been identified (Klein *et al*, 2007). In addition to the haematological consequences, Kostmann syndrome is frequently accompanied by neurological involvement (mental retardation and epilepsy), with usually late onset (Germeshausen *et al*, 2008). In the end, the morbid association of neutropenia and neurological disorder is a little far from the classical description of an isolated severe neutropenia, supposed to be the example of CN. Lastly, except in two geographic areas, Sweden and Asia Minor, *HAX1* mutations are extremely rare (<1%) among CN cases.

Another example of changes in disease classification induced by genetics comes from ELANE neutropenia. Cyclic neutropenia, a term coined in the 1940s (Reimann & DeBerardinis, 1949) was characterized by a biological clock responsible for a sinusoidal variation of the neutrophils every 21 days with autosomal dominant inheritance (Palmer *et al*, 1996). This pattern was supposed to be completely distinct from permanent neutropenia, the typical entity of which is described as Kostmann syndrome. Based on the segregation analysis of 13 pedigrees of patients with typical cyclic neutropenia, Horwitz *et al* (1999) identified mutations in the neutrophil elastase gene (*ELANE*). Shortly afterwards, the same team found that *ELANE* mutations were also identified in many patients with severe permanent CN (Dale *et al*, 2000). This suggested that the 2 entities were closer than initially expected and implied a continuum between severe CN and cyclic neutropenia. Reviews now associate the 2 phenotypes under the ELANE neutropenia entity, regardless of the pattern of variation in neutrophils (Germeshausen *et al*, 2013).

Lastly, GATA2 syndrome offers a challenge in any classification system. Clinical presentation varies from typical Emberger syndrome (deafness and lymphoedema), to families with both susceptibility to mycobacteria (MonoMac syndrome) (Hsu *et al*, 2011) and familial leukaemia. And in GATA2 syndrome a significant proportion of patients who later develop major complications present in youth with isolated chronic neutropenia (Pasquet *et al*, 2013). GATA2 syndrome belongs to several families of disorders, including the CN family. The biological characteristics are, in addition to a mild neutropenia, a monocytopenia ($<0{\cdot}2 \times 10^9$/l) and macrocytosis. Such examples can be given for all genes, as phenotypes do not perfectly match genotype. As the latter is much more reliable, any classification should take into consideration the implicated gene, as proposed in Table I.

## When to consider congenital neutropenia as a possible diagnosis and how to confirm it?

Neutropenia is a relatively frequent finding (Hsieh *et al*, 2007); approximately 1% of the population is living with a

mild to severe neutropenia and this rises up to 8% for some geographic origins, such as Africa. In contrast, CN is an extraordinarily rare condition, observed in less than 1/100 000 people (Donadieu *et al*, 2013). In other words, less than 1/1000 cases of observed neutropenia is congenital, i.e., genetic in origin. Such an enormous gap indicates that it is critical to consider the diagnosis of CN as a two-step process. The first step would consider some robust criteria in order to suspect or to exclude the CN diagnosis. This step should be performed by a physician and requires a minimal work-up (Bejjani *et al*, 2017). The second step is a very specialized approach and aims to provide a more accurate molecular diagnosis for a given patient.

For the first step, the initial interview and physical examination and the careful reading of the complete blood count are sufficient. The collection of information, such as consanguinity, geographic origin and familial history of neutropenia, is important. Patients with severe bacterial infections, any buccal recurrent disorders (ulceration, gingivitis) or obvious associated morbidities (Fig 1) should be considered to have a high risk of CN (Bejjani *et al*, 2017). Before embarking on an extensive and costly workup it is important to recall that neutropenia is often well tolerated and normalizes rapidly, in which case specialized investigations are not necessary. The initial work-up may also reveal a particular aetiology, such as a viral infection or malignant haemopathy, an iatrogenic cause, or an immune deficiency, warranting further specific investigations. Figure 1 presents a schematic evaluation tree.

Bone marrow examination is not mandatory for the diagnosis of all neutropenia, but should be performed to rule out malignant haemopathies in the case of additional

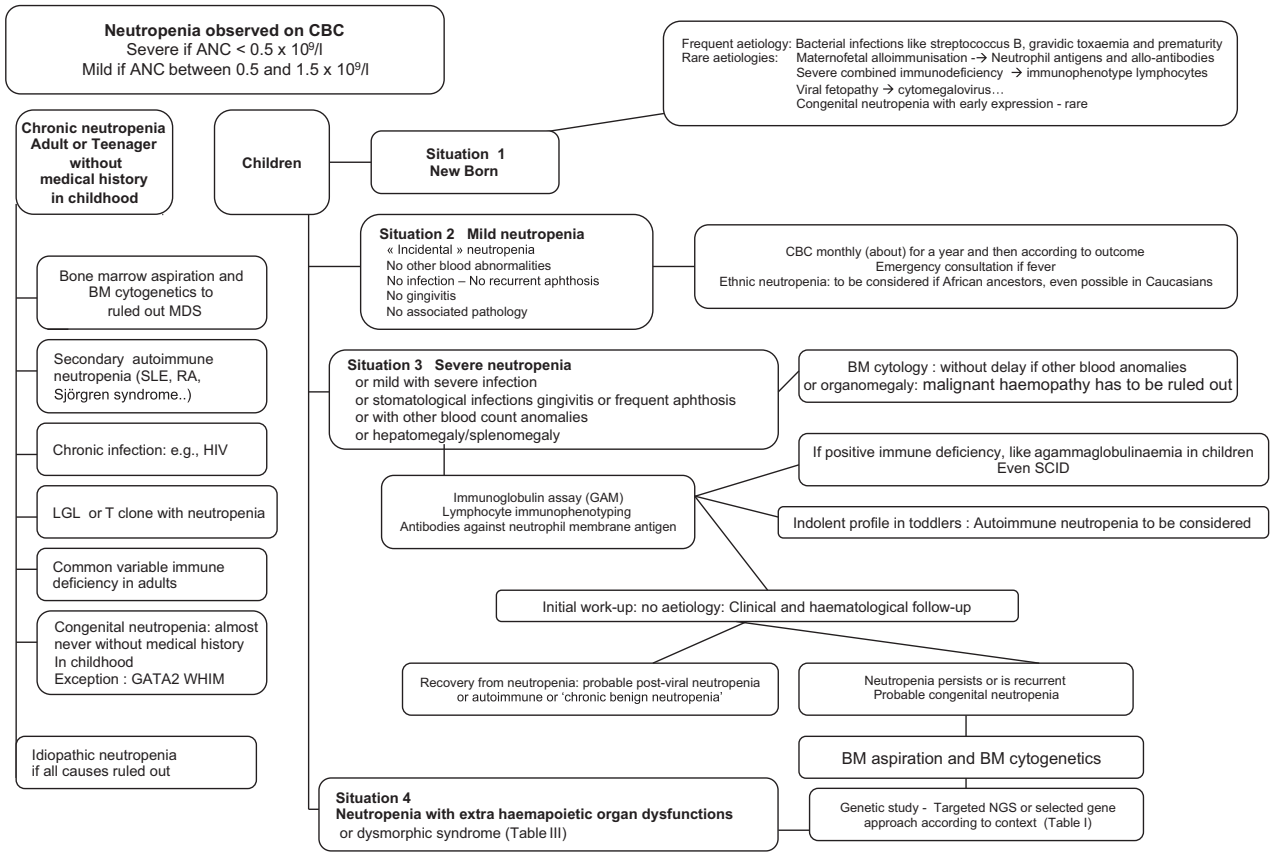


**Fig 1.** Chronic Neutropenia: diagnosis work-up in children and in adults. Several points should be taken into consideration (Bejjani *et al*, 2017): (A) Age at diagnosis (= age at first detection of neutropenia); (B) The indication that required performing a CBC(e.g., fortuitous; mild infection/fever; severe infection; fungal infection; aphthous, gingivitis stomatitis, or other symptoms, including developmental delay); (C) A family history of neutropenia and consanguinity; (D) The presence of any severe infections, bacterial or fungal, detectable at the time of diagnosis; (E) The presence of any stomatitis or gingivitis prior to the diagnosis; (F) The presence of any congenital malformation and/or any organ dysfunction; (G) The CBC with differential, performed at the time of diagnosis (including the ANC, absolute eosinophil count, absolute monocyte count, absolute lymphocyte count, haemoglobin levels and platelet levels). (H) Some specific cytological abnormalities observed on the blood, such as large granular lymphocytes. After this screening evaluation, bone marrow aspiration, immunological tests and auto-antibodies against neutrophils may help to determine the diagnosis. ANC, absolute neutrophil count; BM, bone marrow; CBC, complete blood count; GAM, IgG, IgA, IgM; HIV, human immunodeficiency virus; LGL, large granular lymphocyte; MDS, myelodysplastic syndrome; NGS, next generation sequencing; RA, rheumatoid arthritis; SCID, severe combined immunodeficiency; SLE, systemic lupus erythematosus; WHIM, warts, hypogammaglobulinemia, infections and myelokathexis.

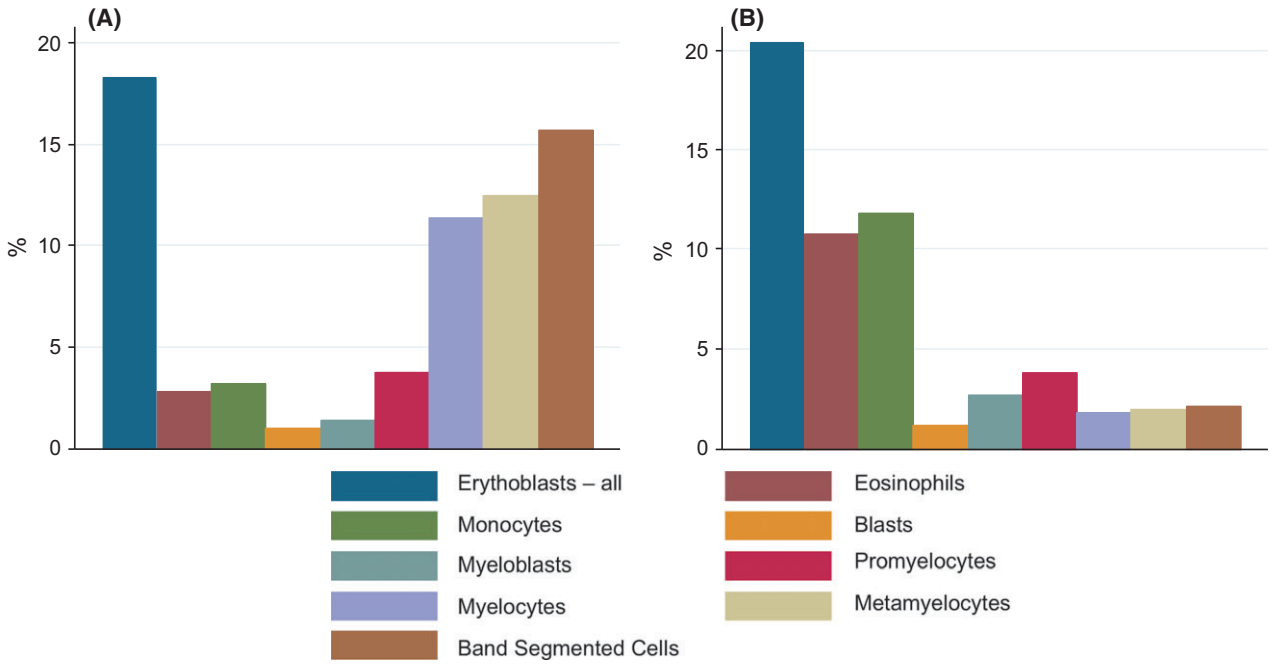


**Fig 2.** Schematic representation of a bone marrow smear differential count, showing the percentage of the different granulocyte precursors. (A) Normal bone marrow in a patient with a benign bone tumour without any blood abnormalities – shown as a control. (B) Bone marrow differential in a patient with severe congenital neutropenia and *ELANE* mutation: bone marrow myeloid arrest at promyelocyte stage with eosinophilia.

haematological abnormalities, and to determine cellularity, assess myeloid maturation and detect some features that are typical of a precise aetiology in the case of chronic neutropenia; this examination should performed before targeted next-generation sequencing (NGS).

Three pieces of information can be extracted from bone marrow cytology.

The first is the general overview of the bone marrow smear, which may give information on the overall cellularity of the sample (from poor to rich) and some obvious abnormalities, like infiltration by monomorphic cells, as observed in leukaemia.

The second investigation is the differential count of the myeloid lineage. Commonly, the myeloid cells show a progression from the most immature stage (haemoblast) to the mature neutrophils in the bone marrow. The normal percentage of myeloid cells from each sub category of myeloid cells are from less than 1% for haemoblasts, between 1% and 5% for promyelocytes and myelocytes, 10% for metamyelocytes and up to 15–20% for band cells and mature neutrophils (Rosse *et al*, 1977).

Figure 2 shows the normal pyramid of granulocyte precursor maturation in a control and early arrest at the promyelocytic stage in a patient with an *ELANE* mutation (Fig S1 plate 5). Maturation arrest at the promyelocyte stage is often associated with bone marrow hypereosinophilia and monocytosis.

Finally, bone marrow cytology can expose morphologically typical features that are strongly in favour of a particular aetiology:

1 Myelokathexis, defined by an increase in the granulocyte pool, with hypermature dystrophic neutrophils, indicating WHIM syndrome (Fig S1 plate 6) (Beaussant *et al*, 2012);
2 Condensed chromatin and hyposegmented neutrophils are in favour of Shwachman-Diamond syndrome whatever the nutritional status (Donadieu *et al*, 2012) (Fig S1 plate 7);
3 Haemophagocytosis of neutrophils is a sign of autoimmune neutropenia in young children (Dresch *et al*, 1980) usually associated with a normal myeloid maturation (Fig S1 plate 8);
4 Abnormal cytoplasmic granulations are suggestive of Chediak Higashi disease (Fig S1 plate 9);

But it is important to recall that, more frequently; the marrow smear may reveal non-specific dysgranulopoiesis or may be completely normal, even in some cases of CN.

Cytogenetic bone marrow studies are also crucial when investigating an isolated neutropenia that is suspected of being congenital. In contrast, a bone marrow trephine is usually not necessary, except in adults, when myelodysplasia is discussed.

Several other investigations can be of interest, especially antineutrophil antibody assay, immunoglobulin assay (for IgG, IgA and IgM; GAM), lymphocyte immunophenotyping, pancreatic markers (serum trypsinogen and faecal elastase) and liposoluble vitamin levels (vitamins A, E and D). In adults, Large granular lymphocyte-associated neutropenia should be ruled out (Lamy & Loughran, 2011) and a large panel of autoantibodies should be run to detect rheumatoid

disease, Sjögren syndrome, or systemic lupus erythematosus. The glucagon challenge test and studies of neutrophil demargination are rarely used, as they are not reliable and provide little information of practical use.

### *Differential diagnosis and some frequent causes of chronic neutropenia*

Post-viral, post-infectious and drug-induced causes are surely the most frequent aetiologies of neutropenia, but they usually have no specific features; moreover, such causes are transient. Table II summarizes the key information for six causes of chronic neutropenia that are important to consider this condition: alloimmune neutropenia in newborns, primitive autoimmunity in young children, secondary autoimmunity in adults, idiopathic neutropenia in adults and ethnic neutropenia.

### *Differential diagnosis: neutropenia in the course of lymphoid immune deficiency*

Because myeloid and immune cells interact both in their ontogeny and during their functional life and immune response, a great number of immune deficiencies may be associated with mild or severe neutropenia (Notarangelo *et al*, 2009; Parvaneh *et al*, 2013), classified in a distinct group of diseases from CN. Most of such cases of neutropenia are observed at diagnosis and recover once appropriate therapy is administered, such as parenteral immunoglobulin in B cell deficiency. In this subset, neutropenia can be very profound at onset, with maturation arrest at the promyelocyte stage. Therefore, immune evaluation is a logical part of the complete evaluation of chronic neutropenia, and such an evaluation ranges from routine tests (e.g., immunoglobulin GAM dosage, T and B immunophenotype) to more specialized tests, including gene screening, which is essentially more complex than for CN, as >300 genes associated with primary immunodeficiency have been identified. Humoral immunity should be thoroughly investigated first in patients with neutropenia. Bruton agammaglobulinaemia (*c.* 30% of cases), CD40 ligand deficiency (*c.* 50% of cases) and variable hypogammaglobulinaemia can be accompanied by neutropenia (Aghamohammadi *et al*, 2009). Severe combined immune deficiencies, like those associated with interleukin (IL) 2 receptor gamma mutation, can also include neutropenia. Lymphocyte defect, mainly affecting T cells, frequently includes neutropenia, which can be severe. In Wiskott-Aldrich disease, neutropenia usually accompanies the frequent autoimmune disorders (Dupuis-Girod *et al*, 2003) through a different mechanism than the underlying X-linked neutropenia and activating *WAS* mutations (Devriendt *et al*, 2001). Reticular dysgenesis, caused by a mutation in the adenylate kinase 2 (AK2) gene (Lagresle-Peyrou *et al*, 2009), is an autosomal recessive disease affecting haematopoietic lineages of both the innate and adaptive immune systems. Deficiencies in IL1 receptor-associated kinase 4 and myeloid differentiation factor (MYD88) are responsible for functional defects in innate immunity, which include marked susceptibility to pyogenic infections, but no other extra-haematological or infectious manifestations. These patients may have a moderate neutropenia, which tends to normalize during infections (Picard *et al*, 2010). The 22q11 syndrome is a complex malformative syndrome due to interstitial deletion of chromosome 22 at the q11 locus. Lastly, neutropenia is found in several disorders associated with haemophagocytic lymphohistiocytosis (HLH) and cellular exocytosis abnormalities (de Saint Basile & Fischer, 2003), sometimes with partial albinism, such as in Chediak–Higashi disease (Fig S1 plate 9) or in Griscelli disease.

## Congenital neutropenia: the best strategy for finding germline mutations

More than 24 genes have been identified in CN in the last ten years (Table I) including 5 in the last 3 years. Until recently, genes were sequentially analysed by Sanger sequencing according to the clinical history. This approach was time-consuming and often restricted to the analysis of a small number of genes according to clinical history. However, the clinical and genetic heterogeneity of CN makes it difficult to select the genes for analysis.

The integration of NGS technologies into diagnostic practice allows the simultaneous analysis of multiple genes in a single assay at a similar cost to testing a few genes by Sanger sequencing. Targeted NGS panels, including the protein coding regions and conserved splice sites of the known genes involved in isolated or syndromic CN (e.g., the 18-gene diagnosis panel used in the Department of Genetics, Pitié-Salpétrière Hospital, Table SI) have been developed for the diagnosis of CN. The design of these panels can easily and regularly be updated by integrating the genes most recently reported in literature.

The challenge of the NGS approach is the interpretation of identified variants and their classification following the guidelines of the American College of Medicals Genetics and Genomics (Richards *et al*, 2015). Variants are classified based on a five-class terminology score as pathogenic (class 5), probably pathogenic (class 4), uncertain significance (class 3), probably benign (class 2) or benign (class 1). Only pathogenic variants (classes 4 and 5) can be used in genetic counselling. Nonsense, frameshift, canonical $\pm 1$ or $\pm 2$ splice sites, single or multi-exon deletions are considered as pathogenic variants, together with reported mutations of any type for which analyses indicate an alteration of the function (class 5). All other sequence variants (missense, in-frame deletions/insertions, intronic variants not affecting the canonical splice sites and promoter variants) are classified taking into account (i) variant allele frequency in geographically-matched population databases (ExAC [http://exac.broadinstitute.org/]; dbSNP [www.ncbi.nlm.

**Table II.** Non-monogenic chronic neutropenia: differential diagnosis and main features.

| Type of neutropenia (reference) | Age | Context | Diagnosis method | Outcome |
|---|---|---|---|---|
| Alloimmune (Curtis *et al*, 2005). | Neonate | Typically very profound neutropenia at birth but no severe infection | Identification of the neutrophil goup of the father and mother and alloantibodies in the mother | Recovery between 3 and 9 months of age<br>No or few infections related to the neutropenia |
| Post prematurity neutropenia | Prematurity <36 weeks; Maternal gravidic hypertension | Mild neutropenia<br>No infection | Exclusion of alloimmune neutropenia and context | Recovery by end of the first year of life<br>No or few infections related to the neutropenia |
| Autoimmune neutropenia – primitive<br>Chronic benign neutropenia (Bux *et al*, 1998) | Betweeen 6 months and 2 years | Very frequent<br>Profound neutropenia<br>No associated pathologies<br>Can occur after community viral infections | Autoantibodies<br>If bone marrow smear, no maturation arrest and sometimes neutrophagocytis (Dresch *et al*, 1980) | Very limited severe infections<br>Common viral infections are difficult to manage, and usually no deleterious consequences<br>Spontaneous recovery after 1–6 years |
| Autoimmune neutropenia – secondary (Palmblad & dem Borne, 2002) | Young adult | Systemic Lupus erythromatosis<br>Rhumatoid disease<br>Sjorgen | Specific autoantibodies | Depending on the underlying disease |
| Idiopathic neutropenia (Kyle & Linman, 1968; Sicre De Fontbrune *et al*, 2015) | Typically young women | Profound neutropenia<br>Low infection risk | All work up is negative. Sometimes minor sign of auto-immunity, such as thyroiditis or vitiligo | No infection, no malignancy. Usually chronic |
| Ethnic neutropenia (Forbes & Johnson, 1941) | Typically in African people and those of African descent, but can be observed in other populations (Denic *et al*, 2009) | No infection<br>Chronic<br>Mild between 0·5 and 1·5 × $10^9$/l | African origin, frequent Duffy antigen receptor for cytokines null (Grann *et al*, 2008) | Chronicity but no infection |

 

nih.gov/snp]) (ii) data from literature (iii) segregation and family history, and finally (iv) *in silico* analysis based on predictive algorithms of pathogenicity for missense mutations (SIFT, PolyPhen-2, Align-GVGD, CADD) and for splice site defects (MaxEntScan and Splice site Finder). The majority of identified sequence variants are novel and in the absence of functional tests, most of them are classified as variants of uncertain significance (class 3).

After analysis based on targeted NGS, about 40% of CN remain without molecular aetiology (unpublished observations). Whole exome sequencing (WES) is a powerful strategy for the identification of novel genetic aetiologies, as shown by several groups that discovered novel causative genes in rare forms of syndromic CN such as *VPS45*, *CLPB*, *EFL1*, *SMARCD2* (Table I). Until now, WES is performed through research projects but, due to the reduced costs associated with NGS technologies and the ever-increasing number of genes, we could expect WES will be rapidly incorporated into diagnostic practice as the first-intention diagnostic strategy.

In addition, the search for pathogenic copy-number variants or for regions of homozygosity in case of consanguineous individuals should be considered.

Finally, mutations in some genes such as C*SF3R* and *GATA2* can be either germline or somatic. The germline status of a mutation should therefore be confirmed by analysing DNA extracted from non-haematopoietic tissue, such as nails, hair follicles or fibroblasts.

## The phenotypes of congenital neutropenia and natural history

### *A common consequence of neutropenia: infections*

Whatever the underlying causes of neutropenia, a central consequence of the phenotype of CN is severe infections. *In vitro*, there exists a strong correlation between antibacterial activity and the concentration of neutrophils, which can be depicted by a simple dilution curve (Li *et al*, 2002). *In vivo*, the correlation between chronic neutropenia and infections cannot be considered as linear. Because CN is rare, physicians usually consider the risk of infections in this subset of patients as analogous to drug-induced neutropenia, where the most typical context for deep and prolonged neutropenia is after induction therapy for acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) (Castagnola *et al*, 2010). In this subset the risk is low at neutrophil counts $>1 \times 10^9$/l, increases moderately between 1 and $0{\cdot}2 \times 10^9$/l, and is very high at $<0{\cdot}2 \times 10^9$/l. The risk of infection also depends on the duration of neutropenia, with the risk of fungal infections increasing after several weeks. In CN, this risk cannot be extrapolated simply from drug-induced neutropenia. The most determinant factor linked with infection risk is the residual capacity to mobilize neutrophils from the site of production to the site of infection and this parameter is both variable with regards to the exact causes of neutropenia, and poorly known. For example, auto immune neutropenia or idiopathic neutropenia present a limited risk of infection, compared to ELANE neutropenia, at the same level of neutrophils (Fioredda *et al*, 2013). In typical CN with profound neutropenia, the risk is not as severe as in drug-induced neutropenia and the risk of fungal infections is very low, far lower than in other phagocyte disorders, such as chronic granulomatosis disease.

The preferential sites of infections are highly variable, but the most frequent are the skin and mucosa; the ear, nose, and throat; and the lungs. Stomatological infections are very frequent after 2 years of age in patients with profound central neutropenia, and are characterized by erosive, haemorrhagic, and painful gingivitis associated with papules (aphthae-like oral furuncles) of the tongue and cheek mucosa (Fig S1 plates 1, 2 and 3). The ultimate consequence of such recurrent stomatitis and parodontal infections is tooth loss early in life.

Digestive tract could be the site of diffuse mucosal lesions, leading to abdominal pain and diarrhoea, and sometimes mimicking Crohn disease in radiological studies (Roe *et al*, 1992). Such Crohn-like disease is very frequent with the two glucose-6-phosphatase defects, like Glycogen storage type Ib disease and *G6PC3* neutropenia. These lesions may also be related to bacterial enteritis and inflammation.

Infections in neutropenic patients may be atypical with an absence of pus and a local inflammation, possibly complicated by a necrotic tendency (Fig S1 plate 4). One particular aspect is ecthyma gangrenosum (infectious perianal ulceration). Bacterial infections are most frequent and generally involve *Staphylococcus aureus* and epidermidis, streptococci, enterococci, pneumococci, *Pseudomonas aeruginosa* and Gram-negative bacilli.

In addition to the risk of bacterial infections, two very specific opportunistic infections may be found in a limited group of disorders belonging to this family; human papilloma virus (HPV) infections are very frequent in WHIM syndrome, GATA2 syndrome and STK4 deficiency, as well as in tuberculosis and atypical mycobacterial infections. In such conditions, the risk of HPV infection seems to be related to a defect in the CXCR4 – CXCL12 axis, whereas the mycobacterial infection appears to be related to a monocyte/macrophage defect.

### *Extra-haematopoietic involvement in congenital neutropenia*

Extra-haematopoietic organ dysfunction or morphological abnormalities (Table III) may be observed, contributing to the definition of several syndromes. Almost all organs can present a dysfunction. Extra-haematopoietic involvement may have a very strong impact on CN patients' lives, such as the neurodevelopmental disorders observed in Kostmann syndrome (Roques *et al*, 2014), Shwachman-Diamond syndrome (Kerr *et al*, 2010), and Cohen disease (Kivitie-Kallio

**Table III.** Main haematological and associated organ dysfunctions observed in congenital neutropenia with associated genetic subtypes.

| System | Haematological or extra-haematopoietic features | Disease | Gene |
|---|---|---|---|
| Blood/bone marrow maturation | Maturation arrest | ELANE neutropenia | *ELANE* |
| | | Kostman disease | *HAX1* |
| | | Wiskott-Aldrich | *WAS* |
| | | G-CSF receptor | *CSF3R* |
| | | CLPB syndrome | *CLPB* |
| | No maturation arrest | GSDIB | *SLC37A4* |
| | | WHIM syndrome | *CXCR4* |
| | | Shwachman-Diamond disease | *SBDS* |
| | | Cohen disease | *VPS13B* |
| | | Hermansky Pudlak type 2 | *AP3B1* |
| | | TCIRG1 | *TCIRG1* |
| | | Neutropenia G6PC3 | *G6PC3* |
| | | Jagunal 1 | *JAGN1* |
| | Myelokathexis | WHIM syndrome | *CXCR4* |
| | Myelofibrosis | VPS45 syndrome | *VPS45* |
| | Macrocytosis | GATA2 syndrome | *GATA2* |
| | Monocytopenia | WHIM syndrome | *CXCR4* |
| | | GATA2 syndrome | *GATA2* |
| | | STK4 (MTS1) | *STK4 (MST1)* |
| | | Wiskott-Aldrich syndrome | *WAS* |
| | Thrombocytopenia | Shwachman-Diamond disease | *SBDS* |
| | | GATA2 syndrome | *GATA2* |
| Pancreas | External pancreatic insufficiency | Shwachman-Diamond disease | *SBDS* |
| | | Wolcott-Rallison | *EIF2AK3* |
| | | Neutropenia G6PC3 | *G6PC3* |
| | | Jagunal 1 neutropenia | *JAGN1* |
| Digestive tract | Crohn disease chronic diarrhea | Glycogen storage type I | *SLC37A4* |
| | | G6PC3 neutropenia | *G6PC3* |
| | | SMARCD2 | *SMARCD2* |
| Eyes | Congenital cataract | CLPB syndrome | *CLPB* |
| | | Charcot-Marie-Tooth | *DNM2* |
| | Retinochoroidal dystrophy | Cohen disease | *VPS13B* |
| Heart | Heart: arrhythmias | G6PC3 Neutropenia | *G6PC3* |
| | Dilated cardiomyopathy | Barth' diseases | *TAZ* |
| | Cardiomyopathy | Shwachman-Diamond disease | *SBDS* |
| | Various cardiac abnormalities | Shwachman-Diamond disease | *SBDS* |
| | | WHIM syndrome (TetralogyFallot) | *CXCR4* |
| | | Neutropenia G6PC3 | *G6PC3* |
| | | STK4 (MST1) deficiency | *STK4* |
| Skin | Skin xerosis eczema | Shwachman-Diamond disease | *SBDS* |
| | Prominent superficial veins | G6PC3 Neutropenia | *G6PC3* |
| | Poikiloderma | SCN with poikiloderma type Clericuzio | *USB1* |
| | Partial or complete albinism | Hermansky Pudlak type 2 | *AP3B1* |
| | | AP14 defect | *LAMTOR* |
| | | Chediak Higashi disease | *LYST* |
| | | Griscelli disease | *RAB27A* |
| | Fine, sparse and light-coloured hair | Cartilage hair hypoplasia | RMRP |
| | Lymphoedema | GATA2 syndrome | GATA2 |
| | Skin angiomatosis | TCIRG1 | *TCIRG1* |
| Bone | Metaphyseal dysplasia | Shwachman-Diamond disease | *SBDS* |
| | | Cartilage-hair hypoplasia | RMRP |
| | Facial dysmorphia | Cohen disease | *VPS13B* |
| Central nervous system | Mental retardation | Kostmann disease | *HAX1* |
| | Epilepsy | Shwachman-Diamond disease | *SBDS* |

**Table III.** (*Continued*)

| System | Haematological or extra-haematopoietic features | Disease | Gene |
|---|---|---|---|
| | | Cohen disease | *VPS13B* |
| | | CLPB syndrome | *CLPB* |
| | | VPS45 syndrome | *VPS45* |
| Muscle | Weakness | G6PC3 Neutropenia | *G6PC3* |
| | | Axonal Charcot-Marie-Tooth disease | *DNM2* |
| | | Shwachman-Diamond disease | *SBDS* |
| Metabolic pathway | Type I diabetes | Wolcott-Rallison | *EIF2AK3* |
| | Fasting intolerance and glycogenesis | Glycogen storage disease type Ib | *SLC37A4* |
| | 3-methyl glucagonic acid | Barth syndrome | *TAZ* |
| | | CLPB syndrome | *CLPB* |
| Inner ear | Inner ear defect | GFI1/severe chronic neutropenia | *GFI1* |
| | | GATA2 syndrome | *GATA2* |
| Urogenital tract | Uropathy | G6PC3 Neutropenia | *G6PC3* |
| | | GATA2 syndrome | *GATA2* |
| | Cryptorchidism | Cohen disease | *VPS13B* |
| | | G6PC3 Neutropenia | *G6PC3* |
| | Nephromegalia | VPS45 syndrome | *VPS45* |
| Dysmorphy | Palatal cleft | Shwachman-Diamond disease | *SBDS* |
| | Hyperlaxity | Cohen disease | *VPS13B* |
| Non-bacterial infections | HPV | WHIM syndrome | *CXCR4* |
| | | GATA2 syndrome | *GATA2* |
| | | STK4 deficiency | *STK4 (MST1)* |
| | Mycobacterial | GATA2 syndrome | *GATA2* |
| | | WHIM syndrome | *CXCR4* |

HPV, human papilloma virus; SCN, severe congenital neutropenia; WHIM, warts, hypogammaglobulinemia, infections and myelokathexis.

*et al*, 1999). Cardiac dysfunction may be very severe in Shwachman-Diamond syndrome (Hauet *et al*, 2013) and WHIM syndrome (Badolato *et al*, 2012), and is almost always observed in Barth syndrome, leading to early death (Rigaud *et al*, 2013). Skin lesions are prominent in Clericuzio neutropenia (Piard *et al*, 2012), and pancreatic exocrine insufficiency is a major morbidity in Shwachman-Diamond syndrome that is sometimes responsible for severe nutritional perturbation (Donadieu *et al*, 2012). The medical management of such patients is always multidisciplinary.

### *Myelodysplasia and leukaemia*

The haematological malignancies presented by patients with CN are classified as myelodysplasia, but frank leukaemia may be observed, AML in the majority, but sometime ALL. The most common cytogenetic feature is monosomy 7, which is detectable in approximately two-thirds of malignancies, but other recurrent cytogenetic abnormalities are also observed, such as trisomy 21 or trisomy 18.

Such malignant complications are exceptionally discovered prior to the diagnosis of CN. The sole exception is the GATA2 syndrome, which is frequently discovered at the time of myelodysplasia or leukaemia, even if mild neutropenia has been diagnosed years before the outset of malignancy in some cases (Pasquet *et al*, 2013; Collin *et al*, 2015).

The rate of haematological malignancy in CN, regardless of genetic subtype, is far higher than that observed in the general population (10–60% in CN compared to 1/10 000 inhabitants in the general population). The leukaemic transformation rate varies considerably according to the genetic cause of the neutropenia and, by 30 years of age, this rate is estimated to be roughly 60% in patients with *GATA2* mutations (Pasquet *et al*, 2013; Spinner *et al*, 2014), 30% in patients with *SBDS* mutations (Donadieu *et al*, 2012), and 15% in patients with *ELANE* mutations (Bellanne-Chantelot *et al*, 2004; Germeshausen *et al*, 2013). In other genetic subtypes, the rate of transformation is not precisely documented, but leukaemic transformation has been reported in patients with *WAS*, *HAX1*, *G6PC3* or *SLC37A4* gene mutations, whereas no transformation has been observed in patients with *VPS13B* or *CXCR4* mutations so far.

In addition to leukaemia, solid tumours may develop early in life, such as kidney tumours (*SLC37A4*) (Donadieu *et al*, 2000) or HPV-induced carcinoma (*GATA2*, *CXCR4*) (Beaussant *et al*, 2012; Pasquet *et al*, 2013).

Leukaemogenesis in CN is a multi-step process. In addition to germline mutations, several genetic mutations occur in myeloid cells, resulting in the final transformation. Acquired *CSF3R* mutations have been described since 1995, but have been observed almost exclusively in patients receiving granulocyte colony-stimulating factor (G-CSF), mainly in

*ELANE* neutropenia patients. These mutations may be transient and have never been observed in patients with *SBDS* (usually not treated with G-CSF) or *GATA2* mutations. More recently, in one *ELANE* patient, several other acquired mutations were identified by NGS in the following genes: *ASXL1*, *CCDC155*, *EP300*, *FBXO18*, *LAMB1*, *LLGL2*, *MGA*, *RUNX1*, *SUZ12*, *ZC3H18* (Beekman *et al*, 2012). These mutations occurred sequentially during leukaemic transformation, with distinct genetic events arising early in the disease and others occurring at later stages. In a survey of 31 cases with germline *ELANE*, *HAX1* or *WAS* mutations, acquired *CSF3R* and *RUNX1* mutations were present in 70% of the cases at the time of transformation (Skokowa *et al*, 2014). In contrast, *ASLX1* mutations are reported in one-third of patients with GATA2 syndrome (West *et al*, 2014) while *TP53* appeared to be specific of Shwachman syndrome (Welch *et al*, 2016). In addition to the condition itself, G-CSF exposure may contribute to leukaemic progression. After myeloid stimulation, G-CSF therapy induces various transient cryptic mutations (mutagenic effect) (Nagler *et al*, 2004), and G-CSF specifically promotes a monosomy 7 clone in bone marrow cultures (Sloand *et al*, 2006). Because G-CSF may promote leukaemic transformation, patients requiring high doses of G-CSF to prevent infection are candidates for haematopoietic stem cell transplantation. However, G-CSF is not sufficient to explain the high risk of leukaemia observed in CN. Patients with *SBDS* or *GATA2* mutations are usually not treated with G-CSF, but present a very high incidence of leukaemia/myelodysplasia transformation.

As the genes involved in CN are not usually considered to be oncogenes, we speculate that the myeloid defect responsible for neutropenia is responsible for various haematopoiesis compensatory stimulation, and is the cause of acquired molecular events, some of which may result in leukaemic transformation. Several models of myelodysplasia and myeloid leukaemia suggest that a large panel of genes is required to induce clonal progression and to favour mutated clones implicated in the leukaemic transformation (Hirsch *et al*, 2016; Papaemmanuil *et al*, 2016). Targeted panels of genes involved in the development of leukaemia have been developed for the follow-up of myeloid malignancies and are currently tested through research project in CN (Hirsch *et al*, 2016; Papaemmanuil *et al*, 2016). In contrast to NGS, applied for the search of germline variants, a depth of reads of about 500–1000× is necessary to pick out variants with a variant allele frequency above 5–10%. These on-going studies will be of utmost importance to identify markers of prognosis among the analysed genes.

## Pathophysiology: genes open the door of understanding and personalized therapy

CN represents a model for studying granulopoiesis. In the past 10 years, 24 genes have been identified as being responsible for CN (Table I). Each mutation is responsible for a very peculiar molecular defect. Surprisingly, most known molecular abnormalities responsible for neutropenia do not involve genes with a transcriptional role in granulopoiesis, but rather translation or cell trafficking. Intracellular regulation of translation, via endoplasmic reticulum (ER) stress pathways, is one of the key functions altered in this process.

### *Translation and post-translation defects: inside the cellular machinery*

Protein translation is performed by ribosomes on the cytosolic surface of the ER, and the unfolded polypeptide chains are translocated into the ER lumen via the Sec61 complex. In the ER lumen, these chains are often N-glycosylated and folded into secondary and tertiary structures that are stabilized by disulfide bonds and prepared for release via granules to the cell surface. The ER produces transmembrane proteins and lipids for most cell organelles, and is involved in the synthesis of almost all secreted proteins. The transport system inside the ER is dependent on chaperone proteins, including cargo proteins and, mainly later in the Golgi apparatus, with the SNARE proteins.

All of these processes occur in the basic metabolism of all cells and are more or less active according to the functional needs of the cells.

Neutrophils are highly dependent on this process for two reasons:

1 Neutrophils have a higher rate of renewal in the body, as *c.* $5 \times 10^6$ neutrophils/kg are renewed every hour.
2 Neutrophils have several functions, including mobility, phagocytosis and extracellular traps, which are all strongly related to a large panel of (>100) proteins, including proteases (Borregaard *et al*, 2007; Korkmaz *et al*, 2010) such as elastase, proteinase 3, cathepsin G and LL37.

The ER is extremely sensitive to alterations in homeostasis, and proteins formed in the ER may fail to acquire their correct conformation due to a lack of chaperones or cellular energy to promote chaperone-protein interactions, $Ca^2$ depletion, disrupted redox state, protein mutations that hamper adequate folding, or reduction of disulfide bonds. Misfolded proteins aggregate and accumulate in the ER lumen, causing ER stress and the activation of the unfolded protein response (UPR). The aims of the UPR are to reduce ER stress, restore ER homeostasis and prevent cell death.

The UPR restores normal cell function by halting protein translation and activates the signalling pathways that lead to increased production of molecular chaperones involved in protein folding. If these objectives are not achieved within a certain time period, or if the disruption is prolonged, the UPR initiates programmed cell death (apoptosis). Three ER-localized protein sensors are known: inositol-requiring (IRE) 1 alpha, protein kinase (PKR)-like ER kinase (PERK) and activating transcription factor 6 (ATF6).

In cases of ER stress, these sensors are activated and trigger a complex series of events designed to maintain ER homeostasis and to promote protein folding, maturation, secretion, and ER-associated protein degradation. BCL2 and CEBP alpha are involved in these processes.

Genetic changes in the shape of the protein, or a change in its function, such as a gain of function, may be one trigger of ER stress, as shown for *ELANE* (Kollner *et al*, 2006; Grenda *et al*, 2007), *JAGN1* (Boztug *et al*, 2014), *EIF2AK3* (Wolcott-Rallison syndrome) (Delepine *et al*, 2000), and *VPS13B* mutations (Cohen syndrome) (Duplomb *et al*, 2014).

Many other types of CN present with an abnormal translation process, such as SNARE protein defects (*VPS45*), change in membrane polarity (*HAX1*, *TAZ*), or change in the glycosylation pattern of proteins (*G6PC3*, *VPS13B*, *JAGN1*).

### *Deficit in cell migration: CXCR4 – CXCL12 axis*

An excess of cell death is not the only way to become neutropenic. WHIM syndrome indicates that the regulation of neutrophil traffic from the bone marrow to the blood (and back) is an important pathway and may be a cause of neutropenia. The circulating pool of neutrophils represents only 1–4% of the morphologically mature neutrophils and the bone marrow reserve is the larger pool of mature neutrophils. The release of the bone marrow pool is dependent on the receptor CXCR4 and its ligand CXCL12. Convincing data on the role of this pathway come from the therapeutic use of CXCR4 inhibitor in normal subjects and animal models (Balabanian *et al*, 2012). CXCL12, which is produced by osteoblasts, is able to bind CXCR4 to the neutrophil membrane. In this way, CXCL12 exerts a negative signal on neutrophils, actively retaining these cells in the bone marrow. In mice, when CXCL12 levels are reduced by the administration of G-CSF over 3–5 days, the rate of neutrophil release from the bone marrow increases. In WHIM syndrome, increased Gαi protein-dependent signalling (e.g. CXCL12-induced chemotaxis) was associated with the inability of CXCR4 to be uncoupled from G proteins (i.e. desensitized) and internalized in response to CXCL12 (Balabanian *et al*, 2005). Furthermore, administration of the CXCR4 antagonist, plerixafor, stimulates a rapid mobilization of neutrophils from the bone marrow within 1 h (McDermott *et al*, 2011).

### *Miscellaneous mechanisms*

Lymphoid enhancer factor 1 (LEF1) is a 48-kD nuclear protein expressed in pre-B and T cells and myeloid cells. A low level of this factor has been observed in the myeloid cells of patients with CN with maturation arrest at the promyelocyte level (Skokowa *et al*, 2006). The decrease in transcription factor expression is difficult to interpret because *LEF1* is normal and LEF1 expression is dependent on the type of CN. This suggests that the decreased expression of LEF1 is a protein consequence of mutations causing CN. Horwicz's team, who initially showed the involvement of LEF1 in this pathway, has shown that LEF1 cooperates with core-binding factor-α to activate *ELANE in vivo* (Li *et al*, 2004). They also raised the possibility that up-regulating promoter mutations may contribute to CN.

Pro-LL37 is an antibacterial peptide packaged in neutrophil granules. Its level is usually low, regardless of the genetic background of the CN (Karlsson *et al*, 2007). Low pro-LL37 levels may be responsible for the persistence of parodontopathy in patients with CN treated with G-CSF. Lastly, vitamin B3 (nicotinamide) participates in a regulatory loop controlling the transcriptional expression of G-CSF and induces a peripheral increase in neutrophils (Skokowa *et al*, 2009).

## Conclusion

Congenital neutropenia encompasses a family of neutropenic disorders, both permanent and intermittent, severe (neutrophil count $<0{\cdot}5 \times 10^9$/l) or mild (neutrophil count between 0·5 and $1{\cdot}5 \times 10^9$/l), which may also affect other organ systems, such as the pancreas, central nervous system, heart, bone, muscle and skin. As of May 2017, 24 genes have been identified as a cause of such diseases. The general understanding of these diseases has benefited enormously from the gene discovery. Because of the clinical and genetic heterogeneity of CN, identifying a genetic diagnosis is becoming more complex, and an NGS approach based on the initial analysis of targeted panels of genes and secondarily on whole-exome sequencing appears to be the most efficient strategy to identify the molecular aetiology of a CN. Treatment of severe chronic neutropenia should focus on prevention of infections, the management of associated organ dysfunction and the prevention of leukaemic transformation. Such risks may be monitored by targeted NGS, which should detect somatic mutations involved in myeloid leukaemia or myelodysplasia.

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## Author contributions

JD conceived the study design and the first draft of the manuscript. OF collected and presented the cytological iconography. All authors critically reviewed the initial draft and participated in the final version of the manuscript.

## Conflict of interest

The authors declare that they have no competing interests.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig S1.** Supplementary figure 1 including:

**Plate 1.** Large aphthae on inner lip of a patient with severe CN.

**Plate 2.** Inflammatory gum lesion in a 12-year-old body with severe CN. Note the enamel damage and loss of parodontal tissue.

**Plate 3.** Gingival hypertrophy, with limited inflammation and no pain, in teenager with ELANE mutation.

**Plate 4.** Cellulitis of the leg at different day of outcome. At D1 (not shown) the patient, who is followed for a severe permanent neutropenia with ELANE mutation, had presented a simple insect bite. At D2, the lesion turns to a cellulitis with a central necrosis and no pus. The patient was treated by Intra venous Antibiotics, high dose of GCSF – she was on a preventive mild dose before – and surgery.

**Plate 5.** Bone marrow smear (May-Grunwald-Giemsa stain; original magnification ×100) Aspects of maturation arrest at the promyelocytic stage associated with hypereosinophilia and monocytosis in a patient with ELANE severe CN.

**Plate 6.** Bone marrow smear (May-Grunwald-Giemsa stain; original magnification ×100) from a patient with WHIM syndrome. Myelokathexis is observed over 50% of neutrophils with abnormal nuclei encompassing 3 to 5 lobes connected by long thin chromatin filaments, and less than 10% vacuolated mature neutrophils.

**Plate 7.** Bone Marrow smear in a patient with Shwachman-Diamond syndrome (May-Grunwald-Giemsa stain; original magnification ×100). Neutrophils frequently (>20%) present a condensed chromatin and nuclear hyposegmentation whatever their nutritional status.

**Plate 8.** Bone Marrow smear (May-Grunwald-Giemsa stain; original magnification ×100) in a young patient with autoimmune neutropenia showing phagocytosis of a neutrophil by a histiocyte.

**Plate 9.** Bone Marrow smear (May-Grunwald-Giemsa stain; original magnification ×100) in a patient with Chediak–Higashi Syndrome: Voluminous inclusions in the cytoplasm of myeloid precursors are observed.

**Table SI.** List of genes studied in the genetic laboratory, Department of Genetics, Pitié Salpétriére hospital.

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