Case Report

ETV6: A Candidate Gene for Predisposition to “Blend Pedigrees”? A Case Report from the NEXT-Famly Clinical Trial

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Background. The identification of germline mutations in familial leukemia predisposition genes by next generation sequencing is of pivotal importance. Lately, some “blend pedigrees” characterized by both solid and hematologic malignancies have been described. Some genes were recognized as related to this double predisposition, while the involvement of others is still a matter of debate. ETV6 was associated with hematologic malignancies, in particular myeloid malignancies, and recently described as mutated also in oncologic patients. No clear evidences in its involvement in blend pedigrees are known.

Case Presentation. We present our recent experience in the identification of an ETV6-mutated “blend pedigree,” suggesting the involvement of ETV6 in the predisposition to both solid and hematologic neoplasia. The pedigree recognition started with a MDS case enrolled in the NEXT-Famly protocol. The patient presented 9 relatives affected by solid tumors and hematological malignancies. Following the clinical trial protocol, the patient underwent NGS analysis, which confirmed the presence of a mutation in the noncoding region of ETV6 both on tumor and on germline DNA. The mutation resulted was shared by the still alive affected relatives.

Conclusion. These evidences support the involvement of ETV6 in the predisposition to both solid and hematologic neoplasia and the importance of the investigation of the noncoding regions of the genes as recently suggested by different expert groups.

1. Introduction

Acute myeloid leukemias (AML) and myelodysplastic syndromes (MDS) are clonal disorders of hematopoietic stem cells. With the advent of next-generation sequencing (NGS) technology, significant progress has been made in furthering our understanding of the molecular pathophysiology and complexity of these diseases [1]. NGS has also led to an increasing clinical awareness of inheritable leukemia syndromes and the genes involved. It is well established that several inheritable single-gene syndromes are associated with AML and MDS, including bone marrow failure syndromes (BMFS), Li-Fraumeni syndrome, and Down syndrome [2], which tend to affect individuals at a younger age. Widespread incorporation of a detailed medical and family history into the clinical care of every patient with unexplained cytopenias, aplastic anemia, or MDS/AML will continue to identify an increasing number of individuals with these disorders.

Family pedigrees of inheritable MDS/AML have been previously reported [3–5]. Detailed genetic investigations of these pedigrees have led to the identification of a number of individual genes that predispose to the development of AML and/or MDS [6]. Other data suggest these genes, when
comutated with acquired somatic alterations, ultimately lead
to the development of overt myeloid disease. The identifi-
cation of additional potential candidate genes have been
recently published in limited reports, including SRP72 [7].

The clinical relevance of these syndromes is highlighted
by the updated World Health Organization (WHO) clas-
sification of myeloid neoplasia, which now recognizes
“Myeloid Neoplasm with Germline Predisposition” as a
separate entity [8]. These syndromes are often associated
with inherited mutations often affecting genes critical to
hematopoiesis [9], including RUNX1, CEBPa, and ETV6.
The WHO (Table 1) classifies three general categories of
germline myeloid neoplasms: “pure” familial MDS and AML
with no significant nonhematopoietic pathology, MDS/
AML-susceptibility mutations with concomitant platelet
abnormalities, and MDS/AML occurring in the setting of an
underlying clinical genetic syndrome, including those
characterized by BMFS (Table 2). For all these disorders,
MDS and AML are often the predominant hematopoietic
malignancies; however, lymphoid neoplasia as well as solid
tumors can also occur [10–14].

NGS has been essential to the identification of novel
molecular alterations in familial MDS/AML, and it has
contributed to further understanding of inherited leukemia
syndromes, and improved identification of inherited AML
predisposition alleles. This has opened the opportunity to
carry out a personalized management approach to affected
patients [15–17]. For example, because long-term outcomes
of MDS secondary to BMFS and familial MDS/AML are
historically poor, hematopoietic stem cell transplantation
should be considered sooner in the disease course, with
exception for patients with germline mutations in CEBPa
[18]. Nevertheless, a diagnosis of familial MDS/AML adds
several considerations to pretransplant planning, namely, in
the choice of the conditioning regimen and the selection of
the hematopoietic stem cell donor [19–21].

The cases of MDS/AML with germline predisposition
also differ from typical sporadic MDS and AML in that they
are frequently associated with unique nonhematopoietic
manifestations, requiring more dedicated disease surveil-
ance [18].

In order to better recognize patients who may have an
inherited predisposition to leukemia and MDS, clinicians
must take a complete family history and be familiar with the
characteristics of familial MDS/AML syndromes. In this
scenario, evaluation of the family pedigree often reveals
multiple family members affected by both hematologic and
solid tumors. Moreover, other genes conventionally asso-
ciated with solid tumors inheritance, such as BRCA1 and
BRCA2, have also been more recently been implicated in
both pediatric and adult MDS/AML cases [22, 23].

In parallel with these well-established evidences, there is
a group of four genes still considered as associated with a
“possible tumor risk” that must be confirmed. PTPN11 is not
included in the ones recognized by the WHO classification,
while DDX41, GATA2, and ETV6 have been already linked
with defined familial MDS/AML [24].

ETV6 is located at chromosome 12p13 and encodes
a hematopoietic transcription factor involved in early
embryonic and adult hematopoiesis as well as in the reg-
ulation of differentiation and maturation of erythropoietic
and megakaryopoietic cell lineages [25, 26].

Alterations of ETV6 have been shown to be important
players in leukemogenesis since its recognition as a PDGFB
translocation partner in chronic myelomonocytic leukemia
[27]. ETV6 is also frequently found to be partnered in
numerous other variant translocations across hematologic
malignancies which affect ETV6 transcription and gene
regulation [28]. Moreover, deletions [29] and somatic
mutations in ETV6 have also been reported in myeloid
disease, including in MDS [30–32] and AML [33] as well as
in CML [34], and are generally associated with poor out-
comes [29].

More recently, germline mutations of ETV6 have been
identified in a small number of family pedigrees. Germline
mutations involving the ETV6 gene are often missense and
inherited in an autosomal dominant fashion (Table 3), even
if the penetrance of hematologic neoplasia depends both on
the different mutated locus and between families affected by
the same ETV6 variant [26]. Patients often have variable
thrombocytopenia with mild-to-moderate bleeding ten-
dency and are predisposed to develop both myeloid and
lymphoid malignancies. In particular, ETV6 is suggested to
be mostly predisposing to acute lymphoblastic leukemia
rather than to myeloid malignancies [35], even if it was not
reported in the conventional WHO classification.

Notably, additional nonhematological malignancies in
ETV6-mutant carriers have also been documented [36–41].
With the paucity of germline-mutated ETV6 cases pub-
lished, additional mutations are still being discovered. Given
this, we describe herein a novel mutation of ETV6 found in
an individual family pedigree linked to both hematologic
and solid tumor malignancy.

2. Case Presentation

We analyzed a 70-year-old woman who presented with
progressive thrombocytopenia that has been present for 8
years, and monocytopsis in June 2014. The bone marrow
biopsy performed at that time showed a MDS with mul-
tilineage dysplasia according to WHO classification [8],
with a bone marrow blast count percentage totaled <5%.
Cytogenetics revealed a translocation (3;21). Therefore the
patient falls within the intermediate 1 risk group, according to
IPSS. She was started on danazol 400 mg/day which was
increasingly increased to 600 mg/day, with a low
response in platelets count (30–40,000/mmc). The treat-
ment was well tolerated, and no major bleeding was ob-
served. After two years on therapy, she again developed
worsening thrombocytopenia (10,000/mmc). Repeat bone
marrow evaluation revealed MDS with excess blasts type 1
(MDS-EB 1). She was subsequently treated with 5-azaci-
tidine at a dose 75 mg (sqm)/day x 7 days monthly for 15
cycles, for which she achieved a complete hematological
response in platelets count (30–40,000/mmc). The treat-
mant was discontinued in November 2017 for hematological
toxicity and evolution of the disease to MDS-EB type 2 (IPSS intermediate 2). The
patient subsequently evolved to AML and died due to
disease progression. Her family history revealed several cases of hematological disease (2 cases of AML diagnosed at 62 and 48 years, 1 case of MDS diagnosed at 60 years associated with history of thrombocytopenia, and 2 cases of thrombocytopenia diagnosed at 49 and 57 years). In addition, several solid neoplasms distributed over 4 generations were also present (2 cases of gastric cancer diagnosed at 58 and 42 years, 1 pulmonary adenocarcinoma associated with history of thrombocytopenia diagnosed at 52 years, and 1 undetermined) (see Figure 1). Due to these

| Table 1: WHO-defined myeloid neoplasm with germline predisposition. |
|---------------------------------------------------------------|
| Myeloid neoplasm with germline predisposition without preexisting disorder or organ dysfunction |
| (i) CEBPA mutation |
| (ii) DDX41 mutation |
| Myeloid neoplasm with germline predisposition and preexisting platelet disorders |
| (i) RUNX1 mutation |
| (ii) ANKRD26 mutation |
| (iii) ETV6 mutation |
| Myeloid neoplasm with germline predisposition and other organ dysfunction |
| (i) GATA2 mutations |
| (ii) Telomere biology disorders |
| (iii) Bone marrow failure syndrome (Fanconi anemia, dyskeratosis congenita, severe congenital neutropenia, Swachman–Diamond syndrome, and Blackan-Diamond syndrome) |

| Table 2: Characteristics of AML/MDS predisposition syndromes. |
|---------------------------------------------------------------|
| Disease | Clinical characteristics | Mutated gene | Pattern of inheritance | Penetrance |
|---------------------------------------------------------------|
| Familial AML with mutated CEBPA | AML | CEBPA | AD | ≈100% |
| Myeloid neoplasm with germline DDX41 mutation | MDS/AML | DDX41 | AD | Unknown |
| Familial platelet disorder/AML | MDS/AML/T cell ALL, lifelong thrombocytopenia, bleeding propensity | RUNX1 | AD | 40% |
| Thrombocytopenia and predisposition to myeloid malignancies | Thrombocytopenia, platelet dysfunction, MDS/AML | ANKRD26 | AD | Unknown |
| Myeloid neoplasm with germline ETV6 mutation | Thrombocytopenia, platelet dysfunction, MDS/AML | ETV6 | AD | ≈100% |
| Familial MDS/AML with mutated GATA2 | MDS/AML, MonoMAC syndrome, Enderger syndrome | GATA2 | AD | 70% |
| Telomere biology disorders | MDS/AML, macrocytosis, mild to moderate single or multiple cytopenias, aplastic anemia | TERT, TERC | AD | Variable |
| Bone marrow failure associated with SRP72 mutations | Aplastic anemia, MDS | SRP72 | AD | Unknown |

AML = acute myeloid leukemia; MDS = myelodisplastic syndrome; AD = autosomic dominant.

| Table 3: Main hotspot mutations in the ETV6 gene and their impact on the protein. |
|---------------------------------------------------------------|
| Mutation | Domain | Effect |
|---------------------------------------------------------------|
| P214L | Central regulatory domain | (i) Repression of DNA binding by the ETS domain |
| | | (ii) Defective proplatelet formation and megakaryocyte maturation |
| | | (iii) Alteration of proplatelet spreading |
| | | (iv) Down regulation of several cytoskeletal proteins |
| | | (v) ETV6 delocalization |
| N385Vfs | ETS | (i) Reduction in repressive activity |
| | | (ii) Targeted proteins downregulation |
| Y401N | ETS | (i) Impaired interaction with corepressor |
| | | (ii) Defective proplatelet formation and megakaryocyte maturation |
| R369W/R369Q | ETS | (i) Reduction in repressive activity |
| | | (ii) Targeted protein downregulation |
| | | (iii) ETV6 delocalization |

ETS = highly conserved C-terminal DNA-binding domain.
evidences, the patient provided written informed consent in accordance with the Declaration of Helsinki and she was enrolled in the Next-Family Italian Multicentric Study (NCT03058588). The written informed consent was attached to the patient’s medical record as recommended by the Good Clinical Practice (ICH) guidelines [42]. As part of the study, a gene panel deep sequencing (GPDS) was performed on isolated peripheral blood mononuclear cells (PBMCs) at time of diagnosis. Tumor DNA was first screened by using the MiSeq Illumina NGS platform for mutations in the following 25 genes: ASXL1, BCOR, NRAS, TP53, RUNX1, CEBPA, FLT3, EZH2, IDH1, IDH2, NPM1, DNMT3A, TET2, CBL, KRAS, ETV6, SF3B1, SRSF2, U2AF1, ZRSR2, GATA2, TERT, TERC, SRP72, and ANKRD26. The sequencing focused only on exons and/or regulatory domains of each gene. The library preparation was conducted following the manufacturer’s instructions, and DNAs were sequenced by MiSeq Illumina NGS platform using 2×150 sequencing (V2 kit, TruSeq). The coverage considered as acceptable for the consistence of the results was fixed at 1500x. The data analysis was performed using Web Annovar. During bioinformatics analysis, polymorphisms were discarded by comparison with NCBI, dbSNP, 1000 genomes, and EXAC, automatically investigated by using Web Annovar. However, since these databases contain known disease-associated mutations, all detected variants were compared with gene-specific mutation databases, ClinVar, and COSMIC. The ranking of unknown mutations was performed using Sift, Polyphen2, Mutation tester, FATHMM, ProVean, MetaSVM, M-CAP.

Review of NGS in this patient revealed a novel point mutation in ETV6 at c.514C>T in the 3′UTR locus not present in the interrogated database.

Since a suspected mutation was found, germline DNA from epithelial buccal cells was sequenced using traditional Sanger methods (SS). Germline DNA was extracted from buccal epithelial cells collected using the Isohelix SK-2 DNA Buccal Swab Collection Kit [43].

SS confirmed the presence of the variant c.514C>T of ETV6 on the germline DNA of the index case (Figure 2). Considering the presence of a germline mutation, affected relatives still living at time of analysis were enrolled in the study and tested for the presence of the identified mutation. The germline mutation was confirmed in two enrolled cousins. Unfortunately, DNA was not available for additional testing on family members affected by solid tumors as they had already deceased.

miRANDA (microRNA.org) and PolymiRST Database Version 3.0 analysis suggested that the affected ETV6 3′UTR sequence serves as an miRNA-binding site, specifically the regulatory microRNA hsa-miR5092. Notably, the mutated one was predicted to bind microRNAs, hsa-miR4717-3p and hsa-miR942-3p. microRNAs have a known role in gene regulation. In order to assess the potential effect of the mutation on ETV6 transcription in the index case, ETV6 mRNA was isolated from PBMCs and quantified on the QuantStudio 3D digital PCR system (ThermoFisher Scientific) using the Hs01045747_m1 Taqman Gene probe (ThermoFisher Scientific) with Hs039290997_g1 Taqman GAPDH probe (ThermoFisher Scientific) as a housekeeper gene. Ten AML cases presenting with wild-type ETV6 as well as 3 healthy individuals served as controls. The results of the quantification of ETV6, normalized for GAPDH transcript, are reported in Figure 3. Notably, affected relatives as well as the index case harboring the *514C>T mutation demonstrated a significant down regulation of ETV6 gene expression in
3. Discussion

The identification of some genes involved in inheritance of hematological malignancies allowed to recognition of an independent entity in last revision of WHO classification and also represents a further step toward the precision and personalized therapy [15, 24].

Moreover, the study of some pedigrees highlights the possible involvement of some genes in both tumorigenesis and leukemogenesis [44]. The definition of intermediate phenotype typically defined as “blend pedigree” leading to a high prevalence of both solid and hematological neoplasia is mandatory to clarify both the biological processes involved in the development of these different malignancies and for the establishment of an efficient treatment strategy and clinical survival.

Some “blend pedigrees” have already been reported in the literature and some genes have been linked to this condition. The implication of some of them in the pathogenesis of genetic predisposition to neoplasia is still a matter of debate. Recent evidences highlighted the importance of sequencing by NGS in these patients and also of investigating the regulatory and intragenic portions instead of the only coding regions which are conventionally queried [17, 24]. This approach is still mandatory for the study of ANKRD26 since the known mutations associated with MDS/AML pedigrees affect the 5′UTR of the gene [45].

The family presented in this paper has been recognized thanks to the investigation of the noncoding sequences of all the genes included in our panel. The mutation was detected in 3′UTR of ETV6 and no variants in this locus has been described before as causative of MDS/AML familiarity.

ETV6 is conventionally associated to hematological diseases both of lymphoid and myeloid lineages [35, 45], but more recently it has been implicated in both liquid and solid tumor malignancies (so-called “blend families”). The lack of acute lymphoblastic leukemia cases in the described family is uncommon and it might be due to the mutated locus, never described before. The mutation is associated with a downregulation of ETV6 transcript, while the previously reported mutations modify the ETV6 protein structure. This different pathogenetic mechanism may affect lesser the lymphoid lineage than the myeloid one. Moreover, ETV6 *514C>T seems to be associated with double predisposition to both hematologic and solid tumor malignancies and not to the conventional predisposition to hematologic disorder described in the literature [8, 37, 40, 41]. The pedigree we studied and reported was strongly suspected to be a “blend pedigree” in that the ETV6 mutation–identified mutation might have led to both hematological and solid tumor malignancies. Recent studies reinforce this hypothesis since ETV6 has been reported as a tumor suppressor also in primary cutaneous mammary analog secretory carcinoma [46], of pediatric papillary thyroid cancers [47], and in comparison to AML ETV6 wild-type (P = 0.0004) and healthy controls (P = 0.02).
gastric cancer [48]. In particular, this last paper is particularly interesting due to the presence of 2 cases of gastric cancers in the described family. Unfortunately, due to the lack of the deceased patients’ permission for further investigation, it was not possible to analyze the presence of the ETV6 mutation in relatives affected by solid tumors.

Another important perspective that could be investigated in the future is the relationship between the t(3; 21), detected in the reported case, and ETV6 3’ UTR mutations. A possible cooperation of ETV6 *514C>T with this or other lesions may be evaluated. In fact, in absence of WGS analysis, we cannot be sure that *514C>T in ETV6 is the only causative mutation of the high susceptibility of the described family’s members, but considering the evidences in the scientific literature, the reduction of the ETV6 expression and the presence of the mutation in all alive affected family members, we may assess that the described mutation is strongly suspected to predispose to hematologic and solid malignancies. Nevertheless, further investigation on cell and animal models will give us pivotal data among the leukemogenesis process, since no evidences concerning this aspect have been reported.

Since the role of this variant in leukemogenesis and tumorigenesis has to be confirmed, we suggest to always carry out a careful family history because many pedigrees, strongly suspected to be “blend pedigree,” could be misunderstood.

Our experience adds an indication of a possible involvement of ETV6 in the “blend pedigrees” field, but new studies have to be conducted to investigate familiar predisposition to malignancies with a special focus on these entities, which are still an unexplored scenario.

Abbreviations

AML: Acute myeloid leukemia
MDS: Myelodysplastic syndrome
NGS: Next-generation sequencing
BMFS: Bone marrow failure syndromes
WHO: World Health Organization
IPSS: International Prognostic Scoring System
MDS-EB: Myelodysplastic syndrome with excess of blasts
SS: Sanger sequencing
GPDS: Gene panel deep sequencing.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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