**DNAJC21 Mutations Link a Cancer-Prone Bone Marrow Failure Syndrome to Corruption in 60S Ribosomal Subunit Maturation**

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A substantial number of individuals with bone marrow failure (BMF) present with one or more extra-hematopoietic abnormality. This suggests a constitutional or inherited basis, and yet many of them do not fit the diagnostic criteria of the known BMF syndromes. Through exome sequencing, we have now identified a subgroup of these individuals, defined by germline biallelic mutations in DNAJC21 (DNAJ homolog subfamily C member 21). They present with global BMF, and one individual developed a hematological cancer (acute myeloid leukemia) in childhood. We show that the encoded protein associates with rRNA and plays a highly conserved role in the maturation of the 60S ribosomal subunit. Lymphoblastoid cells obtained from an affected individual exhibit increased sensitivity to the transcriptional inhibitor actinomycin D and reduced amounts of rRNA. Characterization of mutations revealed impairment in interactions with cofactors (PA2G4, HSPA8, and ZNF622) involved in 60S maturation. DNAJC21 deficiency resulted in cytoplasmic accumulation of the 60S nuclear export factor PA2G4, aberrant ribosome profiles, and increased cell death. Collectively, these findings demonstrate that mutations in DNAJC21 cause a cancer-prone BMF syndrome due to corruption of early nuclear rRNA biogenesis and late cytoplasmic maturation of the 60S subunit.

**Introduction**

Inherited bone marrow failure (BMF) syndromes are a heterogeneous group of life-threatening disorders characterized by a hematopoietic defect in association with a range of variable extra hematopoietic features. Recognized syndromes include Fanconi anemia (MIM: 227650),1 dyskeratosis congenita (MIM: 305000),2 Shwachman Diamond syndrome (MIM: 260400),3 and Diamond Blackfan anemia (MIM: 105650).4 Cells from Fanconi anemia cases exhibit increased chromosomal breakage due to specific genetic and/or functional defects causing genome instability.1 Dyskeratosis congenita cases are characterized by diagnostic mucocutaneous features such as abnormal skin pigmentation, nail dystrophy, and leukoplakia together with the presence of very short telomeres.2 Shwachman Diamond syndrome is characterized by exocrine pancreatic insufficiency, and individuals with Diamond Blackfan anemia are typically reported to have craniofacial abnormalities, including clefting of the lip or palate, thumb abnormalities, cardiac malformations, and short stature.5 Importantly, these syndromes are also associated with an increased cancer risk.

In addition to the well-defined BMF syndromes, we have accrued a number of cases to our BMF registry that do not fit any definite diagnostic classification. These uncharacterized individuals have BMF together with one or more somatic abnormalities, suggesting a constitutional or inherited basis, but do not fulfill the clinical criteria of the known BMF syndromes. With the advent of whole-exome sequencing, it is now possible to unify subsets of these cases through the identification of their underlying genetic defect. This is the case here, as we identify a cohort of simplex BMF cases harboring biallelic mutations in DNAJC21. Through characterization of the encoded protein, we define a cancer-prone BMF syndrome caused by defective DNAJC21.

**Material and Methods**

**Study Approval**

All experiments were conducted with the approval of Barts and The London Hospital. Peripheral blood samples were obtained with written consent under the approval of our local research ethics committee (London – City and East).

**DNA Sequencing and Plasmids**

All samples were obtained with informed written consent and the approval of our local ethics committee. Genomic DNA was extracted from peripheral blood with Gentra reagents (QIAGEN). For exome sequencing, 50 ng of genomic DNA underwent library preparation and exome capture via the Illumina Nextera Rapid Preparation and exome capture.
Capture Exome kit. Sequencing was performed with the Illumina HiSeq 2000 system, and 100 bp paired-end reads were generated and the data processed through the Illumina pipeline. Variants were called as described previously. Resequencing of DNAJC21 was performed as a part of a 32 gene panel and amplified with a TruSeq Custom Amplicon reagent, which was then prepared for MiSeq sequencing according to the manufacturer’s instructions (Illumina). All relevant calls were confirmed by Sanger sequencing.

DNAJC21 wild-type and mutant expression constructs were obtained by standard cDNA cloning and mutagenesis procedures into pcGFP-C1 (Clontech) plasmid.

Nuclear RNA Co-immunoprecipitation Assay
Dialyzed nuclear extracts from ~10^6 cells expressing eGFP alone or eGFP-tagged wild-type and mutant forms of DNAJC21 were subjected to immunoprecipitation with GFP-TRAP agarose beads (ChromoTek). After incubation and stringent washes, immune complexes were treated with DNase I (RNase-free) (Fermentas) (ChromoTek). After incubation and stringent washes, immune complexes were washed three times with IP buffer containing 150 mM KCl and two times with IP buffer containing 100 mM KCl, for each 5 min at 4°C. Immunoprecipitates were boiled in SDS-sample buffer (25 mM Tris-HCl [pH 6.8], 2% SDS, 10% glycerol, 0.05% bo- mophenol blue, 5% 2-mercaptoethanol) for 5 min, separated by SDS- PAGE, transferred to polyvinylidene membranes, and subjected to western blot analysis with a WesternBreeze Chromogenic kit (Thermo Fisher, cat. no. WB7106 and WB7104). Antibodies against DNAJC21 (Abcam, cat. no. ab86434, C terminal epitope corresponding to amino acids 400–450; Proteintech, cat. no. 23411-1-AP, N terminal epitope corresponding to amino acids 43–288), PA2G4 (Abcam, cat. no. ab119037), ZNF622 (Abcam, cat. no. Ab57859), and HSPA8 (Abcam, cat. no. EP1513Y) were used in the study. For immunocytochemical staining, HeLa cells and IMR-90 cells (primary lung fibroblasts obtained from ATCC CCL-186) were grown on coverslips, fixed in 4% paraformalde- hyde, permeabilized with 0.1% Triton X-100 (TX100) in PBS, quenched in 50 mM NH4Cl, and blocked in 10% goat serum and 1% BSA in PBS containing 0.05% TX100 for 1 hr. Cells were incubated with corresponding primary and secondary antibodies and mounted via vectashield containing DAPI (Vector Labs, cat. no. H-1200). Images were collected with an LSM710 laser scanning confocal microscope (Olympus) under relevant excitation, and the emitted signals were visualized with ZEN software (Zeiss). Nucle- olar co-staining was determined by mouse monoclonal antibody against nucleophosmin (Abcam, cat. no. ab40696) and DNAJC21.

Sub-cellular Fractionation
DNAJC21 knockdown and control cells were lysed in ice-cold HEPES containing 0.1% NP40 and centrifuged at 3,000 rpm, and the supernatant was collected for extraction of cytoplasmic protein. The pellet-containing nuclei were briefly washed three times in ice-cold HEPES containing 0.1% NP40, were added to the pellet ice-cold radioimmunoprecipitation assay (RIPA) buffer containing a cocktail of protease and phosphatase inhibitors (Roche, cat. no. 04693116001), and were sonicated twice for 10 s at 50% pulse to release nuclear proteins. The final mixture was shaken gently on ice for 15 min, and the nuclear fraction pro-tein supernatant was obtained by centrifugation at 14,000 g for 15 min. Fractionated lysates were verified with antibodies against cytoplasmic GAPDH (Abcam, cat. no. EPR16891) and nuclear TATA binding protein (Abcam, cat. no. EPR3826) by immunoblotting as described above.

T Cell Isolation and Culture
Blood samples were obtained from individual 4 and her unaffected heterozygous parents. Peripheral blood mononuclear cells were separated with lymphocyte separation medium (Lonza) according to the manufacturer’s instructions, washed twice with complete
Table 1. Features of Subjects with Biallelic Mutations in DNAJC21

<table>
<thead>
<tr>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype (c.)</td>
<td>517C&gt;T</td>
<td>983+1G&gt;T</td>
<td>94C&gt;G</td>
</tr>
<tr>
<td>Protein (p.)</td>
<td>Arg173*</td>
<td>Gly299Alafs*24</td>
<td>Pro32Ala</td>
</tr>
<tr>
<td>Gender</td>
<td>F</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Age when sample received (years)</td>
<td>3</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Country of origin</td>
<td>France</td>
<td>Algeria</td>
<td>Pakistan</td>
</tr>
</tbody>
</table>

F, female; M, male; Y, yes; N, no; IUGR, intrauterine growth restriction.
*Inferred from splice-site variant.
*The bone marrow failure was global and was associated with a reduction in all cell lineages in the peripheral blood (pancytopenia). Subject 3 developed acute myeloid leukemia, sub-type megakaryocytic (AML-M7).
Growth hormone defect.
Dental abnormalities (microdontia), hyperkeratosis, and retinal dystrophy with poor vision.
Skin pigmentation abnormalities on feet and dental abnormalities.
Dysphagia and/or oral ulceration.
After treatment with diepoxybutane or mitomycin C.
*Measured by monochrome multiplex qPCR.

RPMM medium containing 15% FCS, and resuspended at 1·0 χ 10⁶ cells/mL in RPMI-1640 medium and 20% FCS (Invitrogen). T cell mitosis was stimulated by the addition of 10 μg/mL of phytohemagglutinin (PHA; Roche Applied Science). After 48 hr, the medium was replaced with fresh RPMI medium containing 10 units/mL of recombinant human interleukin-2 (Invitrogen) and harvested after 14 days. Cells were counted on a NucleoCounter YC-100 automated cell counter (ChemoMetec). Epstein-Barr virus (EBV)-infected lymphoblastoid cells (LCLs) were established and grown in RPMI-1640 medium supplemented with penicillin and streptomycin, 2 mM L-glutamine, and 20% (vol/vol) FBS (Invitrogen).

Polysome Analysis
For analysis of monosomes and polysomes, HeLa cells and LCLs were grown to confluence, harvested 10 min after treatment with 100 mg/mL cycloheximide (CHX; Sigma), and resuspended in PBS containing 100 μg/mL CHX. Equal numbers of cells were lysed in 425 μL of hypotonic buffer (5 mM Tris-HCl [pH 7.5], 2.5 mM MgCl₂, 1.5 mM KCl, and 1× protease inhibitor cocktail [EDTA-free]), with 5 μL of 100 μg/mL CHX, 1 μL of 1 M DTT, and 100 U of RNase inhibitor. Lysates were vortexed for 5 s, followed by addition of 25 μL of 10% Triton X-100 (final concentration 0.5%) and 25 μL of 10% sodium deoxycholate (final concentration 0.5%), and vortexed again for 5 s. Lysates were then centrifuged at 16,000 g for 2 min, and supernatants were layered on 15% to 45% (wt/vol) sucrose gradients. The gradients were centrifuged at 36,000 rpm for 4 hr at 4°C in a Beckman SW41Ti rotor. The fractionated lysates were serially collected, and polysome profile peaks were obtained by measuring absorbance at 254 nm. Proteins from these fractions were precipitated with 20% (vol/vol) trichloroacetic acid, separated on SDS-PAGE gels, and transferred to polyvinylidene membranes for immunoblotting.

Cell Death and Rescue by Wild-Type DNAJC21
After 72 hr of RNAi treatment, cells were washed in PBS; propidium iodide (PI) was added to the cell suspension to a final concentration of 5 μg/mL and analyzed on an LSRII Flow Cytometer (BD Biosciences). Plasmids encoding WT-eGFPDNAJC21 and peGFP-C1 alone were transfected into DNAJC21 3’ UTR shRNA expressing stable cell lines. 48 hr after transfection, cells were fluorescein-activated cell (FAC) sorted by gating for eGFP, collected, and re-plated in 96 wells in equal number. It is important to note that the shRNA system targets the 3’ UTR of the endogenous DNAJC21 mRNA. This UTR is absent from the plasmid-derived transcript, resulting in shRNA depletion of the endogenous DNAJC21 only upon dox induction. DNAJC21 3’ UTR shRNA 1 (Figure S3) was preferred because it showed DNAJC21 knockdown upon dox induction at a lower concentration, 100 ng/mL.

Structural Analysis of the DNAJC21 p.Pro32Ala Variant
The J domain crystal structure (PDB: 1FAF) was obtained from the Protein Data Bank and visualized with Swiss PDB Viewer. The predicted effects of the DNAJC21 p.Pro32Ala substitution were generated with this program.

Statistics
Analyses were performed with GraphPad Prism 5.0 software (GraphPad Software). Mann-Whitney U test and one-way ANOVA with Tukey’s post hoc tests were used when appropriate, as indicated within the text or figure legends. Data are presented as the means ± SEM unless otherwise indicated. A two-tailed p value of <0.05 was considered significant.

Results
Biallelic Mutations in DNAJC21 Cause a BMF Syndrome
In a cohort of 28 unrelated individuals with BMF and non-specific somatic features from our BMF registry (Table S1), we sought an underlying genetic basis through exome sequencing. Three of these individuals, none of whom had any family history of disease, were found to have homozygous likely pathogenic variants in DNAJC21 ([GenBank: NM_001012339.2] Table 1, Figures 1A and 1B). Specifically, these were a nonsense variant, c.517C>T, p.Arg173*, in individual 1, a splice variant, c.983+1G>T, in individual 2, and a missense variant, c.94C>G, p.Pro32Ala, in individual 3. Targeted resequencing of DNAJC21 in a second cohort of 23 similar individuals (Table S2) identified one further individual (individual 4) with a homozygous nonsense variant, c.793G>T, p.Glu265* (Table 1). Sanger sequencing of parental DNA revealed
that this variant segregated as an autosomal-recessive trait. The four individuals with biallelic DNAJC21 mutations are similar in clinical presentation (Table 1): they have global BMF associated with peripheral pancytopenia and are characterized by intrauterine growth restriction and/or short stature. One of them (individual 3) developed acute myeloid leukemia, sub-type megakaryocytic (AML-M7), at the age of 12 years (Table 1).

Three of the variants are predicted to cause loss of function (LOF, defined here as nonsense or splice variants), and the transcripts containing these nonsense and predicted frameshift mutations are likely to undergo nonsense-mediated mRNA decay. In the fourth individual, a missense variant (p.Pro32Ala) disrupts the highly conserved histidine-proline-aspartic acid (HPD) motif that lies at the heart of the J domain, which defines a family of proteins (Figures 1B and 1C). In silico analysis of the p.Pro32Ala substitution on the tertiary structure of the E. coli J domain (PDB: 1FAF) reveals the loss of the distinctive cyclic structure of proline in a loop between two α helices (Figure 1D). This structural alteration to the HPD motif is likely to disrupt the interaction of the J domain with its cognate heat shock protein 70 and the subsequent stimulation of ATPase. To our knowledge, none of the variants have been reported previously apart from the splice variant (c.983+1G>T), which is present only in the heterozygous state in the Exome Aggregation Consortium (ExAC) database at an allele frequency of 5/120,170.

The ExAC database estimates the frequency of LOF alleles in DNAJC21 (combining frameshift, nonsense, and splice donor- and acceptor-site variants) at about 0.1% (123 LOF alleles in ~120,000 chromosomes). Hence, the frequency of LOF homozygotes and compound heterozygotes is expected to be in the range of one per million individuals. Not including the likely damaging homozygous missense variant, the observed frequency of LOF homozygous cases in our combined cohort (3 out of 51 individuals) significantly exceeds this expectation (binomial test p < 10⁻¹⁰). We conclude that the allelic series of mutations that we have identified here defines a subgroup of
individuals with constitutional BMF caused by defective DNAJC21.

DNAJC21 Is Implicated in rRNA Biogenesis

DNAJC21 belongs to the family of DnaJ (heat shock protein 40) chaperone proteins and is ubiquitously expressed in human tissues (Figure S1). We found that DNAJC21 was present in both cytoplasm and the nucleus of HeLa and 293T cells. (Figure 2A) Within the nucleus, it localized primarily to the nucleolus in three different cell lines tested (Figure 2B). T lymphocytes obtained from one affected individual (individual 4) revealed a lack of DNAJC21 immunoreactivity to an antibody recognizing an N terminal epitope, whereas the parental T cells stain positive. Nucleophosmin (NPM1) is used as a control. Images display NPM1 (green), DNAJC21 (red), and DAPI (blue). Scale bar, 20 μm.

(C and D) DNAJC21 translocates to the nucleus after actinomycin D treatment. NPM1 is used as positive control.

(E) LCLs from individual 4 and asymptomatic heterozygous parent controls (1 and 2) were plated in the presence of increasing concentrations of actinomycin D for 48 hr and assayed for cell viability by staining with neutral red. Assays were performed in octuplets per experiment and repeated for a minimum of two independent experiments. Data points represent mean ± SEM.

(F) Individual 4 LCLs show reduced expression for rRNA in nuclear extracts analyzed when compared to both those of parents and three unrelated samples as controls. Expression of SNORA63 and SNORA68 was determined as internal controls. All genes are normalized relative to expression of GAPDH mRNA. Data represent mean ± SD, n = 2, performed in triplicates.

Given its nucleolar localization and response to ActD, we sought to investigate a role for DNAJC21 in 45S precursor rRNA binding. We therefore performed native RNA co-immunoprecipitation experiments on nuclear extracts from HeLa cells expressing wild-type and mutant forms of GFP-tagged DNAJC21. Amounts of ectopically expressed GFP-tagged proteins were established by immunoblotting with an anti-GFP antibody (Figure 3A). qRT-PCR analysis on GFP-TRAP immunoprecipitates showed that DNAJC21...
associates with the precursor 45S rRNA. The truncated DNAJC21 mutant (p.Arg173*) failed to bind to precursor 45S rRNA (Figure 3B). Taken together, these results support the notion that DNAJC21 is indeed a pre-rRNA processing factor, given that it associates with precursor 45S rRNA and is involved in rRNA biogenesis.

**DNAJC21 Interacts with 60S Ribosome Maturation Factors**

The yeast ortholog of DNAJC21, Jjj1 (Figure S2A), has been studied in some detail. In addition to its role in rRNA processing in the nucleus, Jjj1 has been shown to act in the final stages of 60S ribosome subunit maturation in the cytoplasm. Specifically, Jjj1 is responsible for the eviction of the 60S nuclear export receptor, Arx1 (human orthologs, proliferation-associated protein 2G4 [PA2G4], also known as Erbb3-binding protein 1 [EBP1], and IRES-specific cellular transacting factor 45 [ITAF45], Figure S2B). In order to execute this function, Jjj1 acts in concert with Rei1 (human orthologs, zinc finger protein 622 [ZNF622], Figure S2C), binding to the 60S subunit. Together, Jjj1 and Rei1 recruit Ssa1p (human ortholog, heat shock 70 kDa protein 8 [HSPA8], Figure S2D) to stimulate ATPase activity. This takes place in the cytoplasm, allowing Arx1 to recycle back into the nucleus to aid in further rounds of 60S nuclear export (Figure S3).

To determine whether these interactions also occur in humans, we expressed eGFP-tagged wild-type and mutant forms of DNAJC21 in HeLa cells. Co-immunoprecipitation experiments revealed the predicted interactions of DNAJC21 with PA2G4, ZNF622, and HSPA8 (Figure 3C). These interactions were not mediated by nucleic acids given that they were detected in both DNase-I- and RNase-A-treated cell lysates (Figure 3C). In contrast, expression of the truncation mutant (p.Arg173*) revealed no detectable interaction with any of the aforementioned proteins (Figure 3D). We note that it is likely that in vivo, the transcript encoding p.Arg173* undergoes nonsense-mediated decay, and the variant is included in these studies as a negative control. The missense variant (p.Pro32Ala) did interact with PA2G4 and ZNF622, but failed to interact with HSPA8 (Figure 3D). This is consistent with studies in yeast, which have shown that mutation of the HPD motif in Jjj1 results in loss of interaction with its HSP70 binding partner, Ssa1p.

Because these mutants failed to interact with 60S maturation factors, we further investigated the role of DNAJC21 in ribosome biogenesis. Immunostudies revealed a dramatic accumulation of PA2G4 in the cytoplasm of DNAJC21-knockdown cells as well as T lymphocytes from individual 4, in comparison to the predominantly nuclear localization observed in the relevant controls (Figures 4A–4C). These data suggest that loss of DNAJC21 perturbs PA2G4 traffic and is consistent with previous studies in yeast, which showed that Arx1 fails to traffic back to the nucleus in strains that lack Jjj1. However, there was no change in the relative abundance of PA2G4, ZNF622, and HSPA8 in individual 4 LCLs compared to those of parent controls (Figure 4D). Sucrose density centrifugation analysis revealed abnormal polysome profiles in individual 4 LCLs, as well as in DNAJC21-knockdown cells, in comparison to controls (Figure 4E). Furthermore, a stark increase in the amount of PA2G4 bound to 60S and 80S peak fractions is observed in individual 4 LCLs (Figure 4E).
Collectively, these observations indicate that human DNAJC21 plays a prominent role in the maturation of the 60S ribosome subunit.

Loss of DNAJC21 Disrupts Cell Morphology and Inhibits Cell Growth

Defective ribosome biogenesis could disrupt protein translation, impairing cellular growth. In line with this, knockdown of DNAJC21 induced cell death in HeLa cells (Figures 5A and 5B). DNAJC21-knockdown cells also exhibited an unusual morphology with markedly elongated cellular morphology (Figure 5C). T cells obtained from individual 4 also showed a significant impairment in growth rate after mitogenic stimulation with phytohemagglutinin and interleukin-2, relative to parental controls (Figure 5D, p < 0.001, Student’s t test).

To confirm that the cell death, cytoplasmic accumulation of PA2G4, and altered morphology were a consequence of DNAJC21 deficit, we performed experiments using a dox-inducible shRNA system that targeted the 3’ UTR of DNAJC21. Cells expressing DNAJC21 3’ UTR shRNA were transfected with either eGFP-DNAJC21 or the eGFP vector alone. Knockdown of the endogenous protein and the ectopic expression of eGFP-tagged wild-type DNAJC21 (which escapes the effect of the shRNA) were verified by immunoblotting (Figure 5E). As expected, re-introduction of wild-type DNAJC21 rescued cell viability and restored normal PA2G4 traffic and morphology (Figures 5F and 5G), demonstrating that the observed effects were a specific consequence of DNAJC21 depletion.

Discussion

Although the inherited BMF syndromes have several distinguishing hallmarks, their heterogeneous presentation and overlapping features have often confounded their clinical diagnosis. However, as the underlying disease associated genes have been discovered, our understanding of BMF pathogenesis has improved considerably. Now, in the era of next-generation sequencing, it is possible to identify subgroups of cases, unified by their underlying genetic defects. In this study, we have used whole-exome sequencing to identify a distinct subgroup of individuals presenting with constitutional BMF characterized by defective DNAJC21.

We have shown that DNAJC21 associates with precursor 45S rRNA. Cells from affected individuals harboring biallelic DNAJC21 mutations had reduced amounts of precursor 45S and mature 28S rRNA and exhibited increased sensitivity to ActD, implicating its role in rRNA biogenesis.
In addition, DNAJC21 participates in coordinating nucleocytoplasmic shuttling of PA2G4, to aid in nuclear export of the 60S subunit. Our findings from DNAJC21 RNAi studies in HeLa cells and cells from an affected individual provide compelling evidence in support of the hypothesis that loss of DNAJC21 perturbs an essential late cytoplasmic step of the 60S subunit maturation, thereby corrupting ribosome biogenesis. Previous studies reported that PA2G4 binds to FG-repeat nucleoporins through its methionine amino peptidase activity and aid in 60S nuclear export.23 In eukaryotic cells, PA2G4 is shown to regulate diverse functions such as cell growth and differentiation, interaction with transcription factors,22 ribosome biogenesis, and rRNA processing20,24 as well as IRES-mediated translation.21 Based on these reports, it is conceivable that cytoplasmic accumulation of PA2G4 in absence of DNAJC21 would affect this wide range of diverse functions in human cells. Together, our data clearly establish that the function of DNAJC21 in ribosome biogenesis is highly conserved from yeast to humans.14–19

Altered or reduced ribosome biogenesis has long been implicated in BMF syndromes.5 We now add DNAJC21 to the growing list of ribosomopathies, which are a class of disease caused by mutations that affect the biosynthesis and or function of the ribosome.25 Ribosomopathies are intriguingly enigmatic given that they initially present with pathognomonic features of too few cells in the marrow but can (in ~10%–20% of cases) later give rise to hyper-proliferative disorders such as acute myeloid leukemia.5,26 It is notable that this holds true for one of the four individuals presented here, although with such a small number of affected individuals, it is difficult to extrapolate this proportion to a larger cohort. Although it is likely that these cancers arise from a selection pressure...
resulting in the acquisition of mutations, we also believe that a dearth of the 60S ribosome subunit could exert pressure on cells to use defective ribosomes. Over time, these cells might become error prone, leading eventually to transition from a hypo- to a hyper-proliferative phenotype.\textsuperscript{26,27}

We have identified two distinct areas of operation of DNAJC21, one in nucleolar rRNA biogenesis and a second in cytoplasmic recycling of nuclear export factor PA2G4 for 60S subunit maturation. We note that the yeast ortholog of human DNAJC21 (Jjj1) acts just upstream of the release of Tif6 by Sdo1 in 60S maturation\textsuperscript{28} and that the human ortholog of Sdo1 is SBDS, the protein defective in Shwachman Diamond syndrome, the BMF is more variable and often only involves isolated neutropenia.\textsuperscript{34} This is in contrast to Shwachman Diamond syndrome cases, where no homoyzogotes for a recurrent nonsense variant have been reported, suggesting that null mutations in SBDS are embryonic lethal.\textsuperscript{33} We do note, however, that the small numbers of DNAJC21 cases presented here have global BMF by the age of 12 years, whereas in Shwachman Diamond syndrome caused by mutations in DNAJC21 the BMF is more variable and often only involves an isolated neutropenia.\textsuperscript{34}

In conclusion, our study defines a cancer-prone BMF syndrome caused by mutations in DNAJC21. Characterization of the encoded protein shows that it plays a highly conserved role in ribosome biogenesis. We have therefore identified a distinct ribosomopathy, highlighting the significance of corrupted ribosomes in the etiology of both hypo-proliferative and hyper-proliferative disorders.

\textbf{Accession Numbers}

Variants identified in DNAJC21 were deposited under accession numbers ClinVar: SCV000257530, SCV000257531, SCV000257532, and SCV000257533.

\textbf{Supplemental Data}

Supplemental Data include four figures and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.05.002.

\textbf{Acknowledgments}

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\textbf{Web Resources}

1000 Genomes, http://www.1000genomes.org

\textbf{References}

J. Biol. Chem. 271, 9347–9354.
6. Swanson, A.W. (2010). Defining the pathway of cytoplasmic matura-
2. Lo, K.Y., Li, Z., Bussiere, C., Bresson, S., Marcotte, E.M., and John-