EXCEPTIONAL CASE REPORT

blood advances

Phenotype description and response to thrombopoietin receptor agonist in *DIAPH1*-related disorder

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Key Points

- *DIAPH1*-related disorder has a bilineage hematological phenotype of macrothrombocytopenia and neutropenia associated with hearing loss.
- Eltrombopag increased proplatelet formation from cultured *DIAPH1*-related disorder megakaryocytes and improved platelet counts in vivo.

Introduction

The heritable thrombocytopenias (HTs) are genetically heterogeneous rare disorders in which reduced circulating platelet levels may be associated with nonhematological features.[1,2] Among recently discovered HTs, *DIAPH1*-related disorder (D-RD; OMIM #124900) was initially reported in 2 pedigrees with macrothrombocytopenia and hearing loss. This phenotype segregated with a heterozygous p.R1213* variant in *DIAPH1*, which encodes the cytoskeletal regulator diaphanous homolog 1 (DIAPH1).[3] This predicted truncation of the DIAPH1 C terminus diaphanous autoregulatory domain (DAD) and was proposed to confer gain-of-function, resulting in megakaryocyte (MK) cytoskeletal dysregulation and impaired proplatelet formation.[3] Macrothrombocytopenia and hearing loss have subsequently been reported in further isolated pedigrees with DAD *DIAPH1* variants,[4-6] suggesting that D-RD is a distinct syndromic HT. However, other descriptions of similar *DIAPH1* variants include hearing loss but not hematological findings.[7,8]

To provide a full phenotypic description of D-RD and the relationship with different *DIAPH1* variants, we report detailed hematological findings from 5 D-RD pedigrees, including the in vitro response and clinical outcome of treatment with the thrombopoietin (TPO) receptor agonist eltrombopag.

Case description

The 5 pedigrees consist of 16 available cases with heterozygous *DIAPH1* variants within the DAD (10 males, current ages 2-78 years; Figure 1A). Cases A-2, A-3, A-5, D-7, and E-3 (all with p.R1213*) have already been partially reported by us.[3,6] Pedigrees B and C are unreported. Abnormal bleeding was reported in 6 D-RD cases and was predominantly mild and mucocutaneous (Figure 1B). Three D-RD cases had previously received prophylactic platelet transfusions to prevent surgical or obstetric bleeding. The 3 D-RD cases from pedigree E had multiple hospital visits with respiratory tract or cutaneous infections. Bilateral sensorineural hearing loss was detected at

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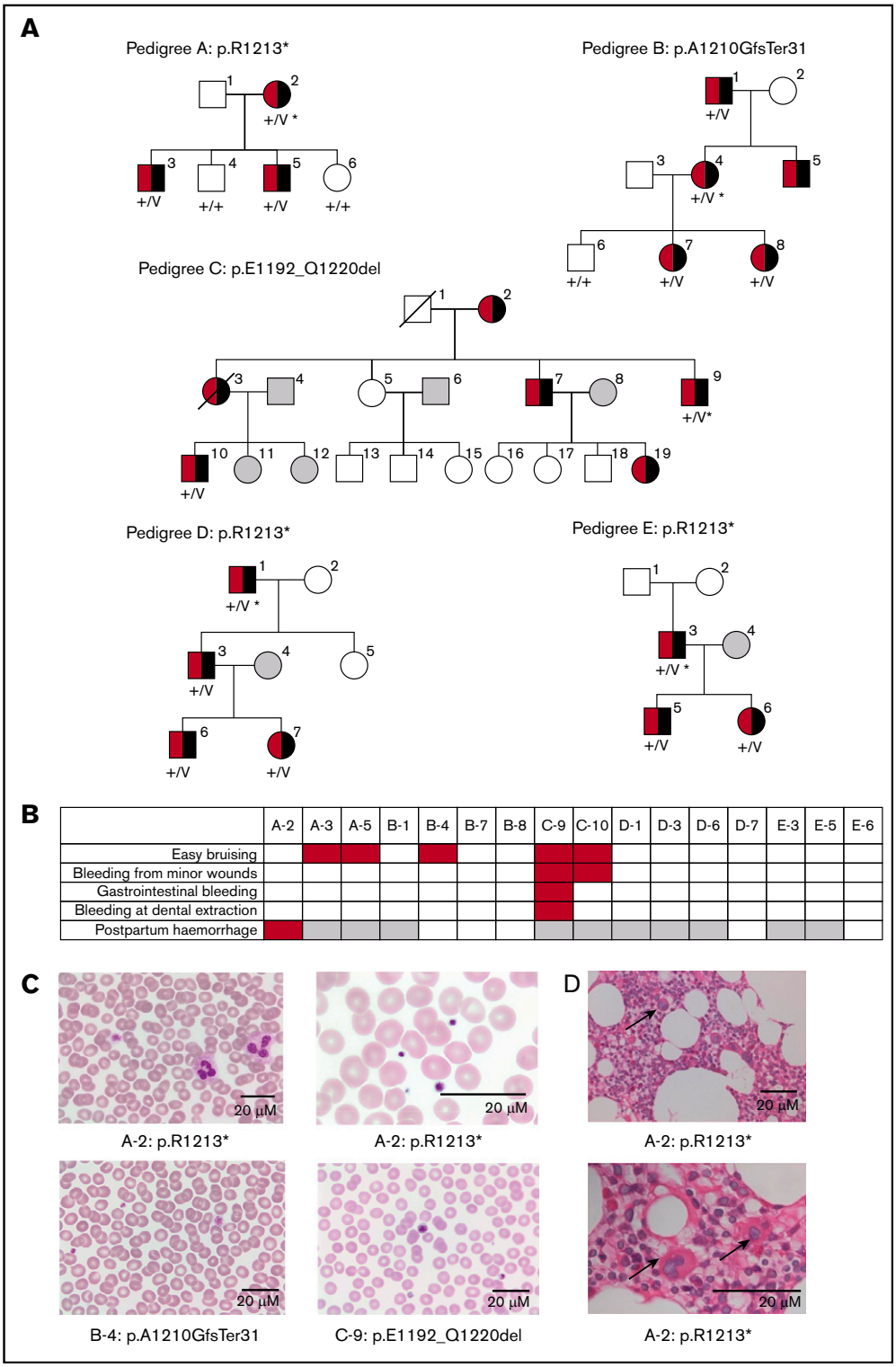

Figure 1. Variants in *DIAPH1* associated with D-RD. (A) Pedigree diagrams demonstrating cosegregation of the *DIAPH1* variants with sensorineural hearing impairment (black shading) and hematological abnormalities (red shading) in 5 pedigrees. The open symbols indicate unaffected pedigree members. The gray symbols indicate pedigree members with no data available. *Index cases. (B) Annotation of the 16 D-RD cases with Human Phenotype Ontology terms for bleeding symptoms. Red shading indicates the presence of the bleeding symptom. Gray shading indicates that a symptom was not applicable due to patient age or sex. (C) Representative May-Grünwald-Giemsa–stained peripheral blood smears from D-RD cases A-2, B-4, and C-9 representing each of the 3 observed *DIAPH1* variants. Original magnification ×40. (D) Hematoxylin and eosin–stained bone marrow biopsy from D-RD case A-2 (R1213*). Granulopoiesis was reduced, with few examples of mature neutrophils. MKs were normal in number but generally small, with hypolobated nuclei (arrows). Original magnification ×100. V, variant *DIAPH1* alleles; +, wild-type *DIAPH1* alleles.

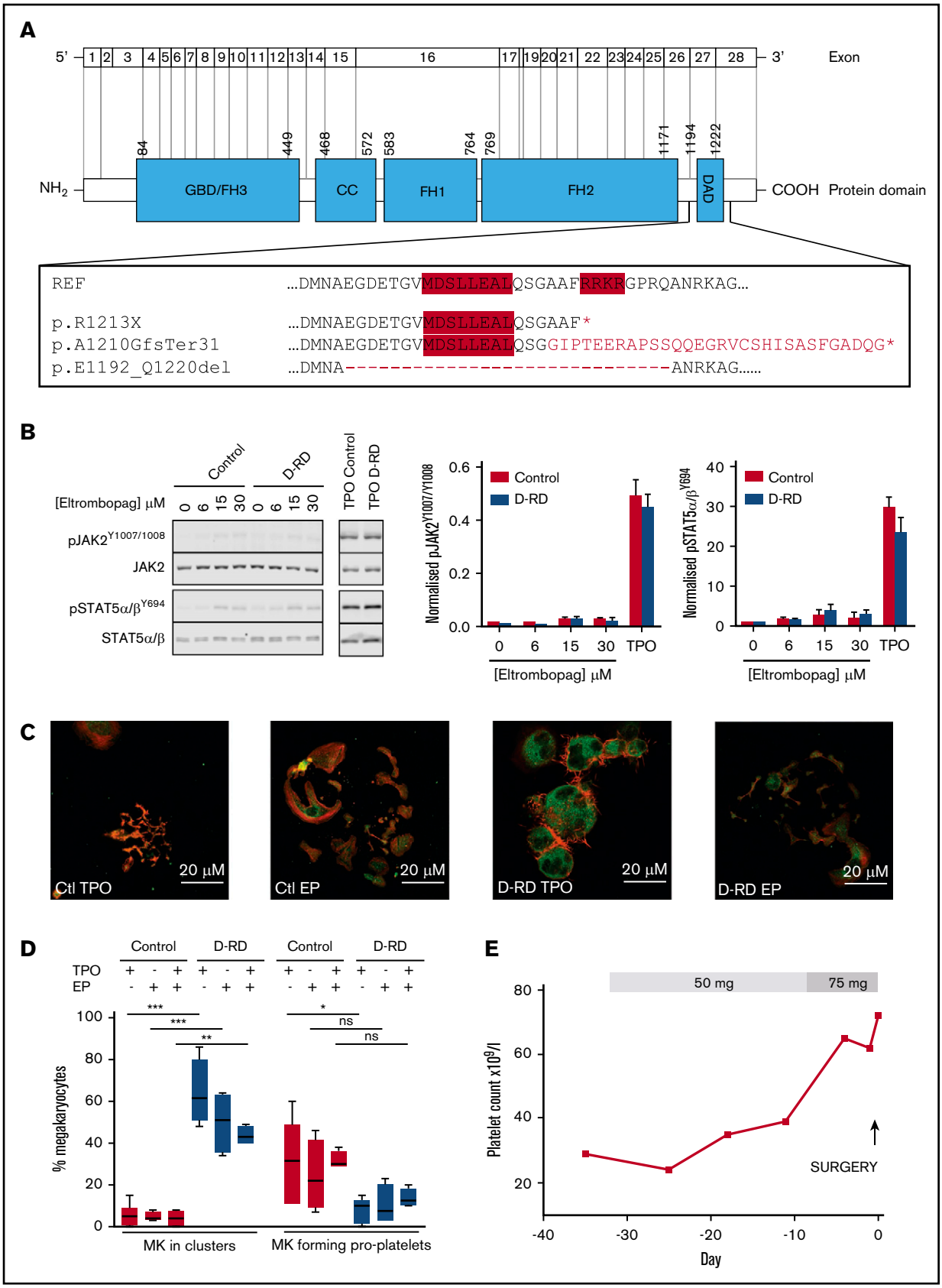

Figure 2. Detailed evaluation of D-RD cases. (A) Schematic representation of *DIAPH1* and DIAPH1 protein divided into functional domains, including the DAD near the C terminus. The expanded box shows the wild-type DAD amino acid sequence; the positions of the regulatory RRKR and MDxLLExL sequence motifs are

neonatal screening or in early childhood in all cases and progressed through childhood. Twelve cases required hearing aids, and 1 case underwent successful cochlear implantation. There were no other consistently reported clinical features.

Methods

Cases were identified through the National Institute for Health Research BioResource–Rare Diseases (pedigrees A-C; UK REC 13/EE/0325) and Functional and Molecular Characterization of Patients with Inherited Platelet Disorders (pedigrees D-E; Centro Regional de Hemodonación, Universidad de Murcia) programs. Phenotype collection and high-throughput sequencing were as reported previously.[6,9,10]

Results and discussion

All 16 D-RD cases displayed mild thrombocytopenia on ≥1 occasion (median platelet count, 111 × 10^9/L; range, 13-209 × 10^9/L) and enlarged platelets (median mean platelet volume, 12.7 fl; range, 9.3-19.8). Eleven cases also displayed neutropenia on ≥1 occasion (median neutrophil count, 1.33 × 10^9/L; range, 0.50-4.30) (supplemental Table 1). There were no other morphological abnormalities by light microscopy (Figure 1C). Bone marrow biopsies from cases A-2 and D-3 revealed a normal distribution of cells but reduced granulopoiesis in case A-2. MKs were present in normal numbers but were small with hypolobated nuclei (Figure 1D). Neutrophil adhesion, degranulation, reactive oxygen species generation, and extracellular trap formation were the same in D-RD cases with the p.R1213X and p.A1210GfsTer31 DIAPH1 variants as controls (supplemental Table 2). There were no consistent abnormalities in immunoglobulin concentrations, lymphocyte subset numbers, or lymphocyte proliferation responses (supplemental Table 2). There was no consistent red cell phenotype.

The DIAPH1 variants included the previously reported p.R1213*[3,6] and the unreported p.A1210GfsTer31 arising from the dinucleotide deletion *DIAPH1* c.3771_3772delAG (pedigree B). Cases from pedigree C harbored an unreported inversion with breakpoints in *DIAPH1* introns 26 and 27, predicting in-frame skipping of exon 27 (p.E1192_Q1220del). Consistent with this, a platelet complementary DNA amplicon corresponding to *DIAPH1* exons 26-28 was smaller in pedigree C compared with controls and did not contain exon 27 (supplemental Figure 1). All of the *DIAPH1* variants predict truncation within the DIAPH1 DAD (Figure 2A), resulting in loss of the RRKR motif and, for p.E1192_Q1220del (pedigree C), also the MDxLLExL motif. These conserved regulatory sequences within the DAD mediate DIAPH1 autoinhibition by competitive binding at the Rho GTPase activation site in the GBD/FH3 domain.[11] Cases from pedigree C had more bleeding symptoms and the lowest overall platelet counts in the cohort, suggesting a relationship between the extent of the DAD truncation and phenotype.

To evaluate the safety of the TPO receptor agonist eltrombopag (Novartis, Frimley, United Kingdom) as a potential therapy to increase platelet count in D-RD, we first evaluated its effect on TPO receptor signaling in D-RD platelets. In platelets from healthy controls, eltrombopag activates TPO receptor signaling pathways to a lesser extent than TPO and does not enhance agonist-mediated activation.[12] In keeping with this, immune thrombocytopenia patients receiving eltrombopag show no increase in platelet activation in vivo.[13] In platelets from D-RD cases with all 3 variants, clinically relevant eltrombopag concentrations stimulated only weak phosphorylation of $pJAK2^{Y1007/1008}$ and $pSTAT5\alpha/\beta^{Y694}$ compared with TPO (Figure 2B). This response was similar in healthy controls and indicated no effect of the *DIAPH1* variants on the TPO receptor pathway in platelets.

We reported previously that blood cell–derived $CD34^+$ MKs from case A-2 with the DIAPH1 p.R1213* variant displayed abnormal clustering and reduced proplatelet production, as well as abundant and disorganized actin, when cultured with TPO.[3] This finding was similar in MKs from cases B-4 (p.A1210GfsTer31) and C-9 (p.E1192_Q1220del) (supplemental Figure 2), supporting a common effect from the different DAD variants (Figure 2C-D). For all 3 cases, there was a trend toward reduced MK clustering and increased proplatelet production after culture of MKs with eltrombopag instead of TPO and further improvement after culture with TPO plus eltrombopag (Figure 2C-D).

We monitored the clinical effect of eltrombopag in case C-9 (DIAPH1 p.E1192_Q1220del) who, unrelated to D-RD, required right hip arthroplasty. However, because of previous platelet transfusions, case C-9 had multiple anti-HLA immunoglobulin G alloantibodies and platelet refractoriness. Eltrombopag (50 mg) was administered once daily from day −29 before surgery, which was increased to 75 mg once daily from day −9 until day −1. The platelet count, which was determined using the PLT-F detection

Figure 2. (continued) indicated by red shading. The predicted impact of the variants associated with D-RD is shown compared with the reference sequence. The abnormal C terminus amino acid sequence predicted from the p.A1210GfsTer31 variant is indicated in red type. The position of exon 27 residues that are absent with the p.E1192_Q1220del variant is indicated by the dashed line. *Premature stop codon. (B) Representative immunoblot using monoclonal antibodies recognizing $p\text{-}STAT5\alpha/\beta^{Y694}$, total STAT5α/β, $pJAK2^{1007/1008}$, and total JAK2 of lysates from case A-2 and control platelets stimulated with eltrombopag (0-30 μM) or TPO (100 ng/mL) (left panels). Bar graphs of the ratio of phosphorylated/total densitometry signal of 3 D-RD cases (A-2, B-4, and C-9) combined show that eltrombopag causes markedly reduced STAT5α/β and JAK2 phosphorylation compared with TPO and that the extent of phosphorylation in D-RD platelets is the same as controls (middle and right panels). The data are representative of 3 independent experiments expressed as mean ± standard error of the mean. (C) Representative immunofluorescence confocal microscopy images (case A-2 and control) of differentiated peripheral blood–derived $CD34^+$ MKs at day 12 of culture, visualized using anti-integrin β3 (green; CD61) and phalloidin (red; F-actin) staining. In the presence of TPO, D-RD MKs show abnormal clustering, reduced proplatelet formation, and abnormal distribution of F-actin when compared with controls. Reduced proplatelet formation and cluster formation are partially rescued in culture conditions containing eltrombopag (EP). (D) Corresponding bar graphs of aggregate data from duplicate MK-differentiation experiments from 3 unrelated healthy controls and cases A-2, B-4, and C-9, with each of the 3 D-RD variants cultured with TPO (5 μM), EP (3 μM), or TPO (2.5 μM) + EP (2 μM). Data are expressed as mean and standard error of the mean of the percentage of all cultured MKs that associate in clusters and the percentage that are forming proplatelet extensions, as specified in supplemental Methods. (E) Time course of the hematological response to oral eltrombopag administered to case C-9 before elective hip arthroplasty at day 0. Platelet counts were determined using a Sysmex XN analyzer using the fluorescence end point. ***$P < .0001$, **$P < .001$, *$P < .05$, 1-way analysis of variance. ns, not significant.

end point (XN-Series analyzer; Sysmex, Kobe, Japan), increased from a baseline of 29 $\times$ 10^9/L to 72 $\times$ 10^9/L on day -1 before surgery (Figure 2E). The patient also received tranexamic acid for 72 hours from the start of surgery but not platelet transfusion. Hemostasis was satisfactory (total estimated blood loss 500 mL vs 493 mL for controls undergoing similar surgery[14]), and there were no adverse events.

This largest reported series of 16 cases illustrates that D-RD is a dominant disorder characterized by macrothrombocytopenia, neutropenia, and hearing loss. This analysis revealed no defects in neutrophil function, and no lymphoid or red cell abnormalities. Although neutropenia potentially accounted for the recurrent infections observed in 1 D-RD pedigree (E), clinical immunodeficiency was absent in the other cases. No cases had renal disease, cataracts, or neutrophil inclusions, distinguishing D-RD from *MYH9*-related disorder in which there may also be macrothrombocytopenia and hearing loss.[15-17] The D-RD phenotype was associated with chain truncation variants close to the DIAPH1 C terminus, resulting in loss of conserved regions responsible for autoinhibitory interactions within the DIAPH1 protein.[11] This suggests a distinct molecular pathogenesis for D-RD, arising from a gain-of-function effect.

This report also illustrates that eltrombopag partly rescues defective proplatelet formation in D-RD MKs cultured in vitro but that TPO receptor signaling responses in D-RD platelets are unaltered. We provide proof of concept that, similar to in *MYH9*-related disorder,[18,19] short-term eltrombopag may allow temporary correction of platelet counts in D-RD cases before surgery, enabling avoidance of platelet transfusion.

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Authorship

Contribution: S.K.W. and A.D.M. wrote the manuscript with assistance from K.D., K.F., and J.R.; C.B., M.L.L., S.G.O., T.S., and C.H.T. provided samples and clinical data; W.N.E. provided bone marrow and blood smear analyses; N.V.M. provided DNA sequencing and analysis for pedigree E; C.K., S.F.M., C.T., and S.K.W. performed laboratory experiments and analyzed data; K.D. and K.G. managed and chaired the ThromboGenomics program, respectively; S.P. coordinated the NIHR BioResource–Rare Diseases Bleeding and Platelet Disorders project, including ethics and governance; and K.F., M.A.L., W.H.O., and A.D.M. contributed to the study design.

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