Supplemental methods and results

Title: Identification of novel *FLI1* and *RUNX1* mutations in six of thirteen families with excessive bleeding and platelet secretion defects

Running title: *FLI1* and *RUNX1* defects associated with bleeding

Authors: J Stockley,[1]* NV Morgan,[2]* D Bem,[2] GC Lowe,[2] M Lordkipanidzé,[2] B Dawood,[2] MA Simpson,[3] K Macfarlane,[2] K Horner,[4] VC Leo,[1] K Talks,[5] J Motwani,[6] JT Wilde,[7] PW Collins,[8] M Makris,[1] SP Watson[2] and ME Daly[1] on behalf of the UK GAPP Study Group

* Equal contribution

Supplemental Methods.

FLI1 constructs, cell culture and transfection

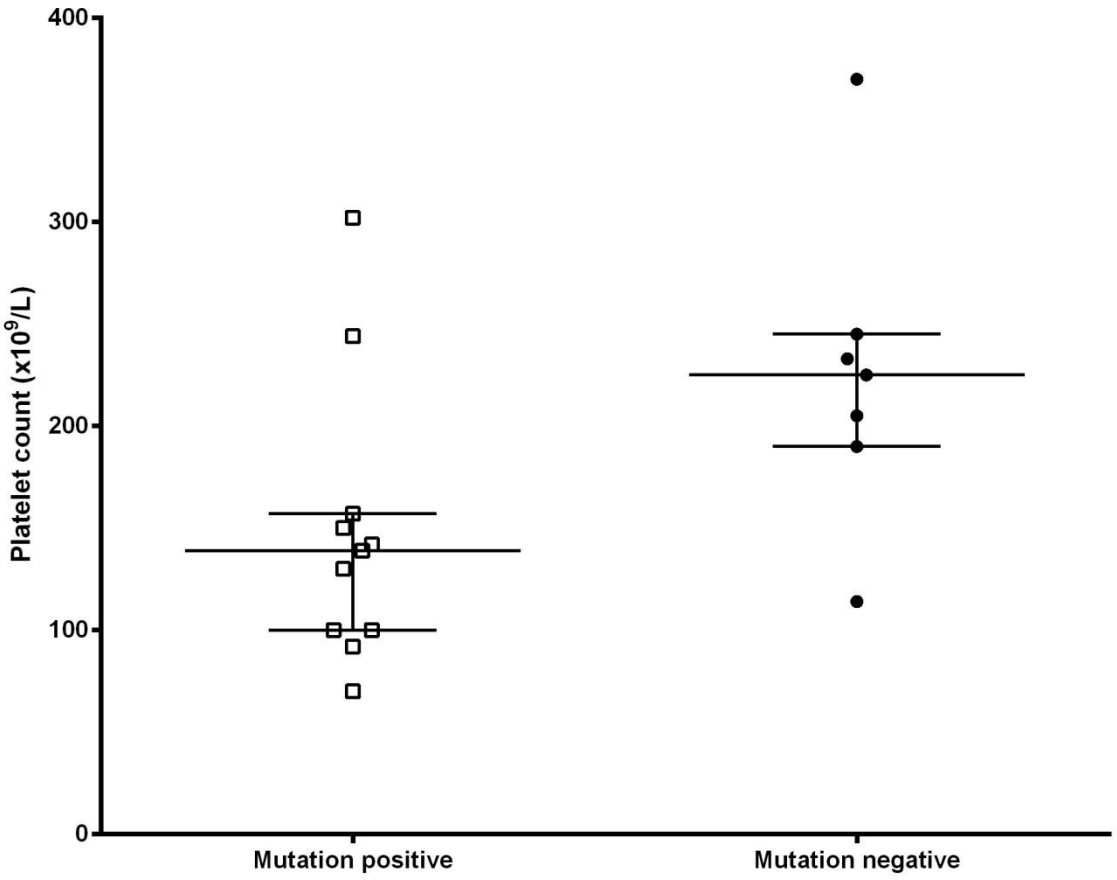
The QuikChange Lightening Site-Directed Mutagenesis Kit (Qiagen, UK) was used to introduce the p.R337W and p.Y343C substitutions into a wild-type (WT) FLI1 expression plasmid, pSG5-*FLI1*.[5] A DNA fragment corresponding to nucleotides -238 to -1 of the *GP6* promoter and including an ETS-1 binding site [nucleotides -73 to -69], was cloned into pGL3.10 upstream of the firefly luciferase gene to derive pGL3.10GP6[-238/-1].[6] HEK293T cells were co-transfected in 24-well plates with 100ng WT or mutated FLI-1 constructs, 200ng pGL3.10-GP6[-238/-1] and 200ng of Renilla luciferase reporter, pRLnull (Promega, UK) using Lipofectamine LTX (Life Technologies, UK) in a volume of 500μl according to the manufacturer's instructions. Forty-eight hours post-transfection, cells were lysed and *GP6* promoter activity assessed by luciferase assay (Promega, UK). Luciferase levels were normalised for transfection efficiency using Renilla activity and data analysed using a 1way ANOVA applying Tukey's post hoc test.

Legends to supplemental figures

Figure S1. Association of *FLI1* and *RUNX1* alterations with mild thrombocytopenia.
Platelet counts according to the presence or absence of a *FLI1* or *RUNX1* alteration in the 13 index cases, and affected family members studied. Bars represent median and interquartile range. A significant difference was found between the 2 groups ($p<0.05$) using the Mann-Whitney U test.

Figure S2. ATP secretion in patients studied.
Standardised ATP secretion in response to 100 μM PAR-1 specific peptide SFLLRN (or 1 U/ml thrombin, shown in red) in healthy volunteers, the 13 index cases and affected relatives with (mutation positive) or without (mutation negative) *FLI1* or *RUNX1* alterations, and other patients with platelet secretion defects enrolled in the UK GAPP cohort whose DNA was not sequenced in this study. Bars represent median and interquartile range. $p<0.001$ when comparing groups using Kruskal Wallis test with Dunn's adjustment for multiple comparisons. Significant differences ($p<0.05$) were found for healthy volunteers vs each of the other groups. For all other pairs no significant difference ($p\geq0.05$) was seen.

Supplemental Figure 1.



Supplemental Figure 2