bjh review

Advances in understanding the pathogenesis of hereditary macrothrombocytopenia

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Summary

Low platelet count, or thrombocytopenia, is a common haematological abnormality, with a wide differential diagnosis, which may represent a clinically significant underlying pathology. Macrothrombocytopenia, the presence of large platelets in combination with thrombocytopenia, can be acquired or hereditary and indicative of a complex disorder. In this review, we discuss the interpretation of platelet count and volume measured by automated haematology analysers and highlight some important technical considerations relevant to the analysis of blood samples with macrothrombocytopenia. We review how large cohorts, such as the UK Biobank and INTERVAL studies, have enabled an accurate description of the distribution and co-variation of platelet parameters in adult populations. We discuss how genome-wide association studies have identified hundreds of genetic associations with platelet count and mean platelet volume, which in aggregate can explain large fractions of phenotypic variance, consistent with a complex genetic architecture and polygenic inheritance. Finally, we describe the large genetic diagnostic and discovery programmes, which, simultaneously to genome-wide association studies, have expanded the repertoire of genes and variants associated with extreme platelet phenotypes. These have advanced our understanding of the pathogenesis of hereditary macrothrombocytopenia and support a future clinical diagnostic strategy that utilises genotype alongside clinical and laboratory phenotype data.

Keywords: platelets, macrothrombocytopenia, megakaryopoiesis, genomics, polygenic.

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Healthy adults produce and clear approximately 1×10^{11} platelets daily to maintain a stable platelet mass under steady state conditions.[1] Tight autoregulation of platelet mass is important to prevent bleeding and thrombotic complications from platelet counts that are too low or too high respectively. Platelet production is primarily dependent on thrombopoietin (TPO), a glycoprotein produced by the liver, kidneys and bone marrow.[1] The uptake of TPO by high-affinity TPO receptors (encoded by the *MPL* gene), drives the proliferation and differentiation of the haematopoietic stem cell (HSC) towards megakaryocytes (MKs) and supports the survival, proliferation and differentiation of MKs.[1,2] Simultaneously, the TPO receptors of platelets act as a sink, creating a negative feedback loop regulating platelet production in response to the level of free TPO.[1] The expression of TPO mRNA is itself regulated by the removal of platelets, which become desialylated at the end of their circulatory lifespan.[2] These desialylated senile platelets are bound by the Ashwell-Morell (asialoglycoprotein) receptor on the hepatocyte surface, which mediates platelet clearance from the circulation. Binding to the Ashwell-Morell receptor also induces hepatocyte signalling through the JAK2-STAT3 pathway, leading to upregulated TPO gene expression and secretion, thereby stimulating new platelet production by MKs[2]

The variation in platelet count (PLT) and mean platelet volume (MPV) between individuals is partly explained by individual-specific environmental exposures, but also by DNA sequence variations in the genes that regulate the platelet life cycle, including the fate decisions of HSCs. Disruption to the tight regulation of platelet mass can result in thrombocytopenia (low platelet count) or thrombocytosis (high platelet count). Macrothrombocytopenia, the frequent combination of enlarged platelets with thrombocytopenia, has many acquired causes, for example sepsis[3] and immune thrombocytopenia (ITP)[4], however, in this review we will focus on the pathogenesis of hereditary macrothrombocytopenia. We will first look at the technologies used for the

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clinical measurement of PLT and MPV. We will then consider how large-scale population cohorts, such as UK Biobank (UKB) and INTERVAL studies, can help delineate PLT and MPV reference intervals. We will discuss the results of recent genome-wide association studies (GWAS), showing that the aggregate additive effect of genetic variation at hundreds of independent loci can explain 19% and 27% of the variation in normalised representations of PLT and MPV respectively. These percentages are greater than those of most other published genetic scores for quantitative traits, for example those for weight and low-density lipoprotein concentration (both 8%).[5,6] Height is a notable exception (40%).[7] We will illustrate how the hundreds of GWAS associated genetic variants, many with small effects on PLT and MPV, modify the risk of macrothrombocytopenia conferred by variants, implicated in hereditary macrothrombocytopenia disorders. Finally, we will focus on the rare hereditary macrothrombocytopenia disorders and address some of the clinical implications of an accurate molecular diagnosis.

The measurement of platelet count and volume

The standard clinical reference range for PLT in healthy adults is 150–400 $\times$ 10^9/l.[8] The historical reference method for measuring PLT required manual counting of platelets using phase contrast microscopy.[8] This was superseded by an immunological flow cytometry method that uses fluorescently tagged antibodies to the platelet-specific glycoproteins GPIIb (CD41) and GPIIIa (CD61).[9] This is recommended by the International Council for Standardization in Haematology (ICSH) and the International Society of Laboratory Haematology (ISLH) for validation and standardisation of new methods of cell counting.[10] For clinical purposes, automated haematology analysers measure PLT as part of a full blood count (FBC). At present, the models of haematology analyser most frequently registered in the UK are the Sysmex XN-series, the Sysmex PocH-100i (Sysmex UK Ltd, Milton Keynes, UK), and the Siemens Advia (Siemens, Erlangen, Germany) (collectively 62%; National External Quality Assessment Service (NEQAS) exercise December 2019, unpublished data). Different analysers use different technologies to detect blood cells and to distinguish platelets, which has important implications for the interpretation of PLT (and MPV) because the method used can influence the accuracy and precision of measurements, particularly in macrothrombocytopenic samples. The impedance method (or 'Coulter principle') relies on the reduction in electrical conductivity caused by the displacement of electrolytes as cells pass through a sensing aperture or channel.[8] The increase in impedance is proportional to the volume of the traversing cell. Consequently, platelets are distinguishable from larger blood cell types, but not from similarly sized cells such as microcytic red blood cells (RBCs) or fragments.[8] If such cells/fragments are numerous, PLT can be overestimated.[8,11] Conversely, if unusually large platelets are present, these cannot be reliably distinguished from RBCs, and PLT can be underestimated.[8] Modern analysers often employ optical detection methods, in which the intensities of diffracted light scattered by a cell at different angles from an afferent light source are measured, sometimes together with the intensity of light fluoresced by the cell. These have improved ability to discriminate platelets, particularly in thrombocytopenic samples.[8,12] Optical counters classify cells according to the intensity of forward-scattered (FSC), side-scattered (SSC) and side-fluorescence (SFL) light. FSC measures cell volume, SSC provides information about cell complexity, including granularity, and SFL quantifies the RNA/DNA content of the cell. A division of the space of possible measured intensities into regions ('gates') corresponding to cell types is used to identify particular cell groups, for example, platelets. More than one type of counting instrument can be incorporated in the same analyser, with an automated algorithm that detects interference, for example from fragments in the impedance channel, or detects an abnormal optical platelet distribution (e.g. in the presence of white cell fragments in acute leukaemia or following chemotherapy).[11–13] If one counting method is deemed unreliable, the count from the alternative is reported.[11,13] Improvements to some optical counters allow them to detect platelets using monoclonal CD61 antibodies (e.g. Abbott Cell-Dyn Sapphire; Abbott Diagnostics, Santa Clara, CA, USA)[14] or cell-specific fluorescent dyes. The current Sysmex XN-series analysers (Sysmex, Kobe, Japan) have a dedicated channel for platelet analysis, PLT-F, which uses oxazine, a platelet-specific fluorescent dye that binds nucleic acids present in ribosomes and mitochondria.[15–17] The PLT-F method also incorporates an extended counting volume and time, to reduce the variance in measurement of PLT.[15] PLT-F correlates well with the ICSH/ISLH immunological reference method, even in markedly thrombocytopenic samples, which is critical to guiding platelet transfusions.[15,18]

The volume of an individual platelet is measurable from the magnitude of the change in impedance it induces or from its optical scatter properties. The distribution of platelet volumes within a blood sample is usually summarised by two quantities: the mean average volume (i.e. MPV) and the platelet distribution width (PDW), a measure of the variability of platelet volume within the sample. MPV is derived from the platelet crit (PCT; the percentage of blood volume occupied by platelets, calculated from the sum of the measured volumes of platelets in a fixed volume of blood) and PLT according to the formula MPV = PCT/PLT/100%. Unlike for PLT, there is no agreed standard clinical reference range for MPV, and this parameter is not currently included in the UK NEQAS haematology exercises. A large epidemiological study reported a reference range of 7·2–11·7 fL in healthy European ancestry individuals.[19] FBC samples were analysed within 6 h of venepuncture on a Cell-Dyn 3700 SL analyser (Abbott Diagnostics), which used impedance to derive MPV.[19,20] The comparability of MPV measurements against a standard reference range is, however, affected by

pre-analytical variability and differences between analyser methodologies for platelet detection and for calculation of PCT (and subsequent derivation of MPV).[19,21,22] Sources of pre-analytical variability include the choice of anticoagulant and the extent to which platelets swell between venepuncture and analysis; in EDTA, the majority of the increase in MPV occurs in the first 6 h, with an overall increase in MPV of 13·4% at 24 h.[21] The aggregate technical variation in MPV is such that measurements may vary by up to 25% between parallel analyses of the same sample by different instruments.[23] In macrothrombocytopenic samples, MPV derived by impedance can be systematically underestimated because the threshold used to discriminate platelets from other blood cell types excludes large platelets. Most analysers will flag samples for which the platelet size distribution is not demarcated and overlaps with that of other cell types. In such cases, MPV may be omitted from the analyser report, or it may be reported with a downward bias. Some instruments adopt a liberal platelet detection window to avoid this problem. For example, the Siemens Advia 2120 analyser, which uses optical detection, can detect platelets up to a volume of 60 fL and report an MPV up to 28 fL.[23] However, a liberal window risks upward bias in estimates of PLT and MPV, in the presence of microcytic RBCs.[23] A further practical approach to detect large platelets from the output of automated haematology analysers is by manual inspection of the platelet size distribution histogram. Macrothrombocytopenia typically manifests as a right shift or skew in the distribution, which is a helpful adjunct to considering the MPV and PDW and interpretation of the blood film.

The reference method for assessment of platelet size is morphological examination of platelets on a May-Grünwald-Giemsa stained blood film by a trained cytologist.[23] MPV cannot be measured directly from a blood smear image, but mean platelet diameter (MPD) and platelet diameter distribution width can be calculated by software assisted image analysis.[24] MPD correlates with MPV, and offers the advantage of standardisation between laboratories.[25] Blood microscopy is already part of the diagnostic pathway for thrombocytopenia in clinical diagnostic laboratories, to detect platelet clumping, indicating pseudothrombocytopenia, or to identify features such as leucocyte inclusions and agranular platelets respectively to diagnose MYH9-related disorders (MYH9-RD)[26] and gray platelet syndrome (GPS).[27,28] In principle, automated MPD measurement could be included in this evaluation.

Variation in PLT and MPV in representative populations

Our understanding of the distribution of platelet traits in the general population has improved because of data from large biomedical cohorts, specifically the measurement of participant FBCs at centralised laboratories using standardised protocols. These include the UKB, comprising 468 000 participants recruited through UK primary care registers, who were aged between 40 and 69 years at baseline (2006–2010),[29] and the INTERVAL study of 45 000 British blood donors aged 18 years or older at baseline (2012–2014).[30,31]. FBC data for the participants in UKB study were generated by Beckman Coulter LH 700 analysers (Beckman Coulter, CA, USA), which determined PLT and MPV by impedance.[32] FBC data for participants in the INTERVAL study were generated by Sysmex XN-1000 analysers, which determined PLT optically by flow cytometry and derived MPV through PCT by impedance.[32] Analysers are not calibrated as strictly or frequently for research as for clinical practice. Therefore, we present here technically adjusted data to remove variation explained by machine drift over time, the time of day of the measurement and the time between venepuncture and analysis.[32] The median PLT is similar across the two cohorts within both sex strata; however, PLT is approximately 30×10^9/l greater in females than males within both cohorts (Mann-Whitney test P-value $<10^{-15}$) (Table 1; Fig 1). The median MPV differs by less than 0·1 fL between sexes within each cohort, indicating that, on average, women have a greater circulating platelet mass than men. MPV is, in median, 2·1 fL higher in the INTERVAL cohort than the UKB cohort, which is not explained by the different age distributions of participants in these studies. It is possible that differences in the measuring technology used by the two analyser models are responsible, for example, the manufacturer-specific reagents used to prepare blood samples for analysis, which may cause differential swelling of platelets.[23,32] Irrespective of the differences between these studies, both illustrate the variation of PLT and MPV within large UK populations. The data highlight that PLT should perhaps be considered against sex-stratified reference ranges, and MPV needs to be considered against reference ranges specific to the model of analyser.

The large UKB collection illustrates the negative correlation between PLT and MPV (Fig 2) with similar correlation coefficients for males ($r = -0{\cdot}47$) and females ($r = -0{\cdot}48$).

Table 1. Statistical summaries of the distributions of platelet count (PLT) and mean platelet volume (MPV) after adjustment of the baseline measured values from the INTERVAL and UK Biobank (UKB) cohorts for technical noise. The corresponding densities are displayed in Fig 1.

	INTERVAL		UK Biobank	
	Sysmex XN-1000®		Beckman Coulter LH 700	
Platelet parameter	Median	Central 95% interval	Median	Central 95% interval
PLT ($\times 10^9$/l)				
M	232	155–348	234	145–356
F	263	172–398	261	164–393
MPV (fL)				
M	11·2	9·7–13·3	9·1	7·6–11·7
F	11·3	9·7–13·3	9·2	7·6–11·8

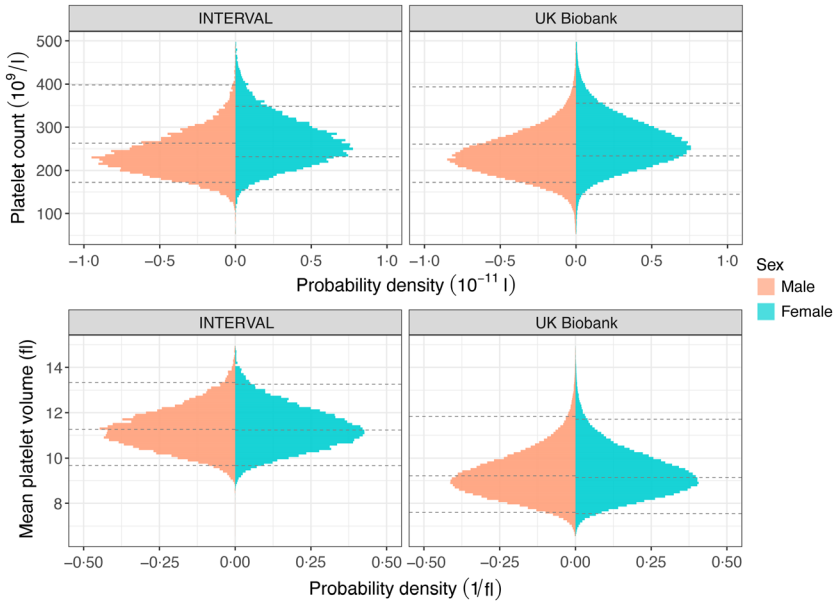

Fig 1. Sex-stratified distributions of platelet parameters in the INTERVAL and UK Biobank (UKB) cohorts. The histograms show the distributions of baseline measurements of platelet count (PLT) and mean platelet volume on participants in the INTERVAL (n = 45 000) and UKB (n = 468 000) cohorts, adjusted for technical artefacts. The horizontal dashed lines in each plot indicate the median, 2.5th and 97.5th percentiles of the distributions. More than 99·5% of participants had a PLT between 50 and 500 × 10^9/l in both studies; PLT values >500 × 10^9/l are not displayed.

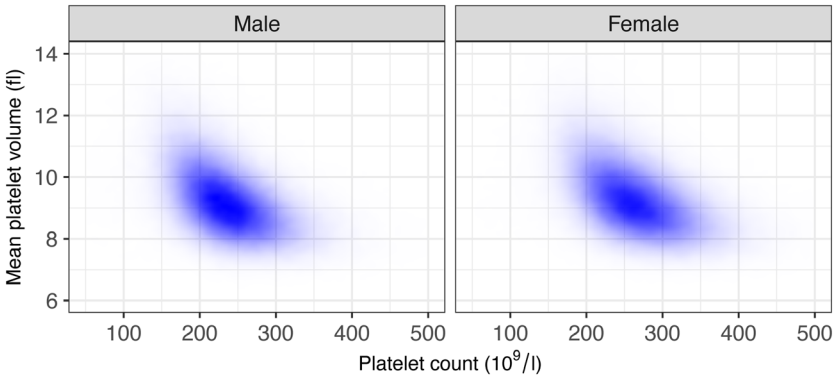

Fig 2. Relationship between platelet count and mean platelet volume in males and females. The plots show the bivariate distribution of baseline measurements of platelet count (PLT; X-axis) and mean platelet volume (MPV; Y-axis) in UKB, adjusted for technical artefact and stratified by sex. The depth of the blue colouring corresponds to a kernel density estimate. PLT and MPV are negatively correlated in both males (r = −0·47) and females (r = −0·48).

Interestingly, variance weighted log-linear regression of PLT on log(MPV), adjusting for sex and menopause and their interactions with age, indicates an exceptionally good fit to an inverse proportional dependence of mean PLT on MPV in a maximal unrelated subset of European ancestry UKB participants. The exponent of MPV is estimated as −0·998 (with standard error 0·003). This is consistent with biological control of the total percentage of blood volume occupied by platelets (i.e., PCT) around a common population average. When all UKB participants with at least one of PLT or MPV outside of their respective 95% reference intervals are considered, participants with both low PLT and high MPV are substantially more prevalent than expected, if PLT and MPV were distributed independently (0·524% males, 0·272% females; expected frequency 0·0625%; Fisher's exact test P-value <2·2 × 10^{-16}). This suggests that on a population scale, low PLT and high MPV together (macrothrombocytopenia) may be a composite trait representing one extreme of a range.

Hereditary macrothrombocytopenia

The hereditary thrombocytopenia (HT) disorders are a heterogeneous group of rare diseases with an estimated

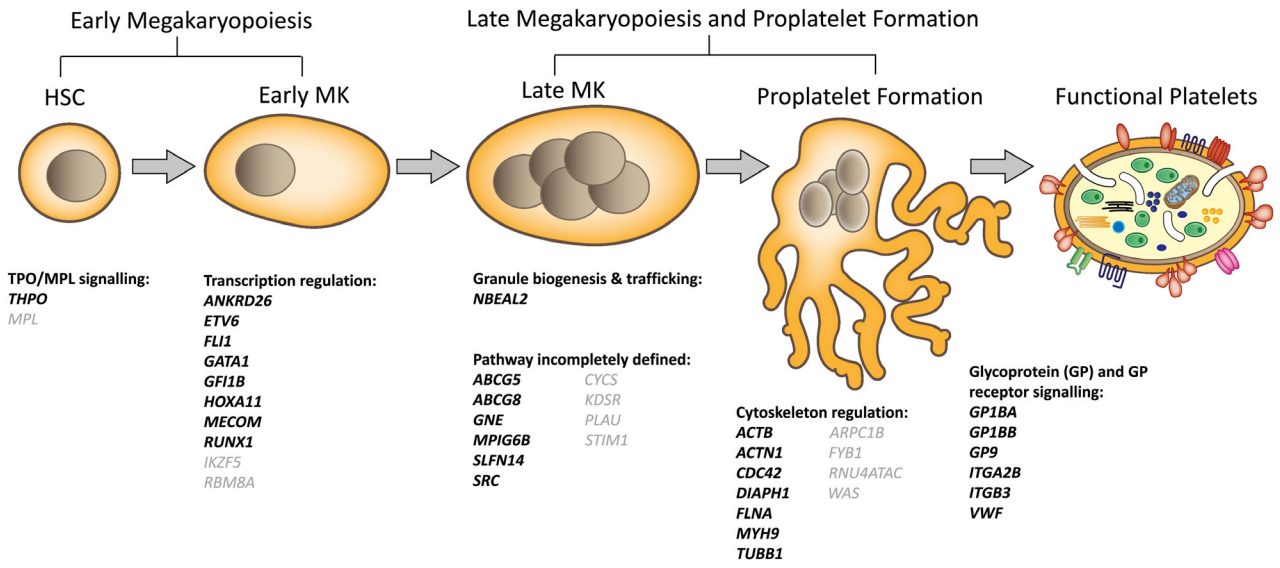

Fig 3. Genes associated with hereditary thrombocytopenia disorders. The genes names are shown beneath a schematic representation of the stages of differentiation from haematopoietic stem cells (HSC) to proplatelet forming megakaryocytes (MK). The genes in which rare pathogenic variants cause hereditary thrombocytopenia (HT; grey) and hereditary macrothrombocytopenia (HMT; bold) disorders are indicated, grouped into categories by pathogenesis. Adapted with permission from Lentaigne *et al.*[78]

combined incidence of 270 cases per million live births.[33] Initial genetic sub-classification of HT was achieved by candidate gene studies of groups of patients with distinctive common clinical or laboratory phenotypes, using Sanger sequencing to resolve their genotype. In 2004, only nine genes were known to carry aetiological mutations for HT disorders (*MYH9, GP1BA, GP1BB, FLI1, GATA1, RUNX1, HOXA11, MPL* and *WAS*).[34] The development of high-throughput sequencing (HTS) technology has enabled large-scale testing using diagnostic gene panels for HT[33,35–37] and parallel research studies using whole exome sequencing (WES)[38] and whole genome sequencing (WGS)[39] have substantially increased the repertoire of HT genes and the characterisation of pathogenic variants within these genes. The Scientific and Standardisation Committee on Genomics in Thrombosis and Haemostasis of the International Society on Thrombosis and Haemostasis (ISTH) have curated an evidence-based catalogue of diagnostic-grade (TIER1) genes that are causally implicated in bleeding, thrombotic and platelet disorders (BPD),[40] hereafter referred to as BPD genes. This assignment is based on specific criteria, including the number of reported independent pedigrees, the availability of functional experimental data and the existence of animal models. Presently, there are 40 TIER1 genes, in which mutations cause HT disorders (Fig 3).[40] In this review, we classify 29 of these (73%) as hereditary macrothrombocytopenia (HMT) genes (Fig 3; Table 2). Mutations in these HMT genes result in platelets across a size spectrum from 'normal/slightly increased' to 'giant', based on work by Noris *et al.*,[24] which we will discuss in more detail later. Rare variants in three additional genes, *PRKACG, TPM4* and *TRPM7,* co-segregate with macrothrombocytopenia in small numbers of pedigrees.[41–43] Further evidence from genetically unrelated patients is required before these can be re-classified as diagnostic-grade HMT genes.[40] It is significant that macrothrombocytopenia is observed in a large proportion of HT disorders, consistent with an extreme manifestation of the inverse population correlation between PLT and MPV (Fig 2). However, since platelets are not enlarged in all HT disorders, it is likely that some pathogenic mechanisms cause subversion of the normal PLT-MPV relationship.

The discovery of many new HMT genes has, in part, followed the inclusion of patients with BPDs in the thousands of people with rare diseases whose DNA has been sequenced as part of translational research programmes coordinated by the National Institute for Health Research (NIHR) BioResource. By July 2020, 13 037 patients with rare diseases and their close relatives had undergone DNA analysis by WGS as part of NIHR programmes; 1169 of these patients suffered from an unexplained BPD,[39] adding to an earlier cohort of patients with BPD who underwent WES.[38] Additionally, the ThromboGenomics (TG) HTS diagnostic panel test, developed to screen bleeding, platelet and thrombotic genes known to carry aetiological mutations, has been applied to more than 2,500 patients with a BPD.[37] Collectively, these BPD cases represent the largest cohort with detailed phenotyping and genotype sequencing data in the world. A multidisciplinary team (MDT) identified a causal variant in a HMT gene in 480 cases (Fig 4, Table 2). The four most frequently implicated genes were *MYH9*, *ACTN1*, *ANKRD26* and *TUBB1,* which together accounted for 48% of the genetically explained cases of HMT. Many HMT genes, including genes that represent aetiological discoveries from the past 6 years (*ACTB*,[44] *CDC42*,[45] *GNE*,[46,47] *MECOM*,[48,49] and

Table 2. The hereditary macrothrombocytopenia (HMT) disorders, organised by mechanism of pathogenesis.

Disorder name (OMIM ID)*	Gene symbol (HGNC)	Location	Platelet size classification	Inheritance	Case frequency		Bleeding phenotype	Additional features	Reference(s)
					TG	WGS/WES			
Early megakaryopoiesis: THPO/MPL signalling									
Thrombocytopenia progressing to trilineage bone marrow failure	*THPO*	3q27·1	Normal/slightly increased‡	AR	0	0	Mild	Possible progression to bone marrow aplasia	[73]
THPO–related thrombocytopenia	*THPO*	3q27·1	Normal/slightly increased‡	AD	2	0	Absent	None	[74,75]
Early megakaryopoiesis: transcription regulation									
Autosomal dominant thrombocytopenia 2 (188000)	*ANKRD26*	10p12·1	Normal/slightly increased†	AD	22	19	Absent – mild	Development myeloid malignancy (8%). Dysmegakaryopoiesis. Some patients have raised haemoglobin/white cell count.	[121]
Thrombocytopenia and susceptibility to cancer (616216)	*ETV6*	12p13·2	Normal/slightly increased‡	AD	8	6	Absent – moderate	Development Acute lymphoblastic leukaemia and other haematological malignancies (25%)	[122]
Platelet–type bleeding disorder 21 (617443)	*FLI1*	11q24·3	Large‡	AD; AR	3	1	Mild – moderate	None	[129,130]
Paris–Trousseau thrombocytopenia (188025); Jacobsen syndrome (147791)	11q23 deletion (including *FLI1*)	-	Normal/slightly increased†	AD	4	1	Moderate – severe	Developmental delay, skeletal abnormalities, congenital cardiac defects and CNS, gastrointestinal and renal malformations	[131,132,133]
X–linked thrombocytopenia with dyserythropoiesis (314050; 300367)	*GATA1*	Xp11·23	Normal/slightly increased†	XLR	5	2	Moderate - severe	Splenomegaly, haemolytic anaemia resembling beta-thalassemia, dyserythropoietic anaemia	[134]
Platelet-type bleeding disorder 17 (187900)	*GFI1B*	9q34·13	Large†	AD; AR	3	2	Moderate - severe	Red cell anisopoikilocytosis	[81,96,97]
Amegakaryocytic thrombocytopenia with radioulnar synostosis (605432)	*HOXA11*	7p15·2	Normal/slightly increased†	AD	0	0	Moderate - severe	Bilateral radio-ulnar synostosis, other skeletal abnormalities. Possible progression to bone marrow aplasia	[135]
Amegakaryocytic thrombocytopenia with radioulnar synostosis 2 (616738)	*MECOM*	3q26·2	Normal/slightly increased‡	AD	1	0	Moderate - severe	Bilateral radio-ulnar synostosis, other skeletal abnormalities. Possible progression to bone marrow aplasia	[48,49]

Table 2. (*Continued*)

Disorder name (OMIM ID)*	Gene symbol (HGNC)	Location	Platelet size classification	Inheritance	Case frequency		Bleeding phenotype	Additional features	Reference(s)
					TG	WGS/WES			
Familial platelet disorder with predisposition to AML (601399)	*RUNX1*	21q22·12	Normal/slightly increased†	AD	20	18	Absent - moderate	Acute myeloid leukaemia or myelodysplastic syndromes (>40%). Increased risk of T acute lymphoblastic leukaemia.	[123]
Late megakaryopoiesis: granule biogenesis and trafficking									
Gray Platelet Syndrome (139090)	*NBEAL2*	3p21·31	Large†	AR	33	3	Mild - severe	Myelofibrosis, splenomegaly, raised B12	[27]
Late megakaryopoiesis: pathway incompletely defined									
Sitosterolemia with macrothrombocytopenia (210250)	*ABCG5*	2p21	Large	AR	4	1	Mild	Xanthomas, accelerated atherosclerosis, premature coronary artery disease, arthritis	[136,137]
Sitosterolemia with macrothrombocytopenia (210250)	*ABCG8*	2p21	Large	AR	2	0	Mild	Xanthomas, accelerated atherosclerosis, premature coronary artery disease, arthritis	[136,137]
Myopathy associated with thrombocytopenia	*GNE*	9p13·3	Large	AR	2	1	Mild - severe	Myopathy (some cases)	[46,47]
Thrombocytopenia, anaemia and myelofibrosis (617441)	*MPIG6B*	6p21·33	Large	AR	0	1	Mild - moderate	Anaemia; myelofibrosis in childhood	[50,51]
Platelet-type bleeding disorder 20 (616913)	*SLFN14*	17q12	Large‡	AD	1	4	Mild - severe	None	[119]
Thrombocytopenia 6 (616937)	*SRC*	20q11·23	Large‡	AD	1	2	Moderate - severe	Congenital facial dysmorphism, juvenile myelofibrosis and splenomegaly, severe osteoporosis, premature loss of teeth	[118]
Late megakaryopoiesis and proplatelet formation: cytoskeleton regulation									
Baraitser-Winter syndrome 1 with macrothrombocytopenia	*ACTB*	7p22·1	Large	AD	0	4	Mild	Mild microcephaly, developmental delay and intellectual disability	[44]
Macrothrombocytopenia (615193)	*ACTN1*	14q24·1	Large†	AD	33	39	Absent - mild	None	[93]

Table 2. (*Continued*)

Disorder name (OMIM ID)*	Gene symbol (HGNC)	Location	Platelet size classification	Inheritance	Case frequency		Bleeding phenotype	Additional features	Reference(s)
					TG	WGS/WES			
Takenouchi-Kosaki syndrome with thrombocytopenia (616737)	*CDC42*	1p36·12	Large	AD	0	0	None	Group I variants (based on residue position in protein) associated with macrothrombocytopenia and additional features of variable growth dysregulation, facial dysmorphism, cardiac malformations, neurodevelopmental and immunological abnormalities	[45]
Macrothrombocytopenia and sensorineural hearing loss (124900)	*DIAPH1*	5q31·3	Large‡	AD	1	3	Absent	Sensorineural hearing impairment, neutropenia	[116,117]
Syndrome with macrothrombocytopenia	*FLNA*	Xq28	Large†	XLD; XLR	3	0	Mild - moderate	Periventricular nodular heterotopia (OMIM 300049), otopalatodigital syndrome; nonsyndromic macrothrombocytopenia also described	[94,95,138]
MYH9-related disorders (155100)	*MYH9*	22q12·3	Giant†	AD	57	26	Absent - mild	Nephropathy, sensorineural hearing impairment, cataract, elevated liver enzymes (without liver dysfunction)	[26,87,88,89,90]
Macrothrombocytopenia (613112)	*TUBB1*	20q13·32	Large†	AD	29	11	Absent - moderate	None	[91,92]
Late megakaryopoiesis and proplatelet formation: glycoprotein signalling									
Bernard-Soulier syndrome (231200)	*GP1BA*	17p13·2	Giant†	AR	3	0	Severe	None	[99]
Mild macrothrombocytopenia (153670)	*GP1BA*	17p13·2	Large†	AD	14	0	Absent - mild	None	[100,101]
Platelet-type von Willebrand disease (177820)	*GP1BA*	17p13·2	Normal/slightly increased†	AD	5	0	Absent - mild	None	[104,105,106,107]
Bernard-Soulier syndrome (231200)	*GP1BB*	22q11·21	Giant†	AR	3	0	Severe	None	[99]
Mild macrothrombocytopenia	*GP1BB*	22q11·21	Large	AD	23	12	Absent - mild	None	[102]

Table 2. (*Continued*)

Disorder name (OMIM ID)*	Gene symbol (HGNC)	Location	Platelet size classification	Inheritance	Case frequency		Bleeding phenotype	Additional features	Reference(s)
					TG	WGS/WES			
DiGeorge syndrome (188400); Velocardiofacial Syndrome (192430)	22q11·2 deletion (including *GP1BB*)	-	Large	AD	1	1	Moderate - severe	Velopharyngeal dysfunction, craniofacial defects, congenital cardiac defects, hypotonia, immune deficiency	[103]
Bernard-Soulier syndrome (231200)	*GP9*	3q21·3	Giant†	AR	11	2	Severe	None	[99]
Platelet-type bleeding disorder 16 (187800)	*ITGA2B*	17q21·31	Large†	AD	11	1	Absent - mild	None	[111]
Platelet-type bleeding disorder 16 (187800)	*ITGB3*	17q21·32	Large†	AD	6	1	Absent - mild	None	[112,113]
Von Willebrand disease, type 2B (613554)	*VWF*	12p13·31	Normal/ slightly increased†	AD	17	0	Moderate	None	[108,109]
Molecular basis unknown									
Medich giant platelet syndrome (Orphanet ID 370127)	Unknown	–	Giant	AR	NA	NA	Severe	None	[55,56]
White platelet syndrome (Orphanet ID 370131)	Unknown	–	Slightly increased	AD	NA	NA	Mild - moderate	None	[53,54]

AML, acute myeloid leukaemia; AD, autosomal dominant; AR, autosomal recessive; NA, not applicable; XLD, X-linked dominant; XLR, X-linked recessive.
*Disorder name as per ISTH (Megy *et al.*[40]), OMIM number in brackets where available.
†size classification as per Noris *et al.*[24]
‡size classification as per Noris and Pecci;[80]if no label, classified as per information in individual reference. Case frequency shown in ThromboGenomics high-throughput sequencing gene panel test (TG) and by whole genome (WGS) or whole exome (WES) sequence analysis of 480 cases enrolled into the bleeding, thrombotic and platelet disorders (BPD) sub-study of the NIHR-BioResource rare diseases. In nine cases, variants were reported in two hereditary macrothrombocytopenia genes.

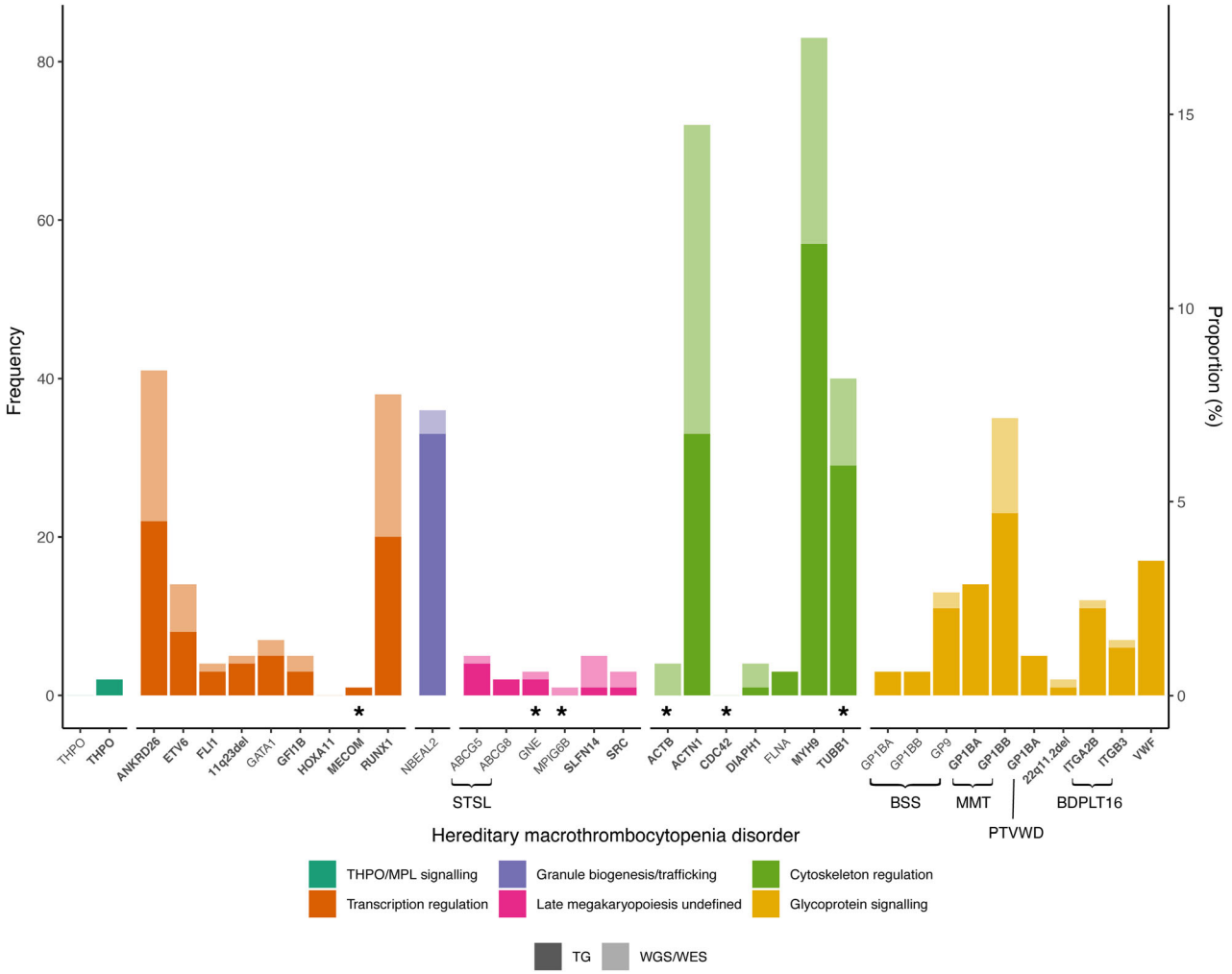

Fig 4. Genes containing rare causative variants in 480 cases undergoing next generation sequencing for diagnosis of hereditary macrothrombocytopenia (HMT). Genotype data for cases with unexplained HMT were measured using the ThromboGenomics high-throughput sequencing gene panel test (TG) or by whole genome (WGS) or exome (WES) sequence analysis of patients enrolled into the bleeding, thrombotic and platelet disorders (BPD) sub-study of the NIHR-BioResource rare diseases. Each bar represents an HMT disorder grouped by HMT gene, ordered and coloured by category of pathogenesis. In nine cases, variants were reported in two HMT genes. Gene names in bold are linked to disorders with an autosomal dominant mode of inheritance (MoI), and the remainder either autosomal recessive or X-linked MoI. An asterisk above the disorder/gene name depicts HMT genes discovered after 2015. *Y*-axes on the left and right give the absolute number and proportion of patients respectively. BDPLT16, platelet-type bleeding disorder 16; BSS, Bernard-Soulier syndrome; MMT, mild macrothrombocytopenia; PTVWD, platelet type von Willebrand disease; STSL, sitosterolemia with macrothrombocytopenia.

MPIG6B[50,51]), explained only a handful of cases (Fig 4). Of the 480 cases, 95% of the reported variants were single nucleotide variants or small insertion/deletions in HMT genes. In 23 cases (5%), a copy number variant (CNV) was identified. Deletions were reported in *ABCG5* (1), *DIAPH1* (1), *ETV6* (1), *FLI1* (5), *GP1BB* (3), *GP9* (1), *ITGB3* (1) and *RUNX1* (10). In one case, an inversion in *RUNX1*, explained the patient's macrothrombocytopenia. CNV calling by HTS has improved significantly, specifically for deletions; however, identification of duplications and more complex structural rearrangements remains challenging (e.g. the *F8* inversion is not detectable by HTS). Nonetheless, analysis of structural variants is essential to perform an exhaustive molecular diagnostic, especially where a clear phenotype is present and no causative variant has been identified [or only one variant has been identified in a suspected autosomal recessive (AR) disorder].[37,39,52] The relative frequencies of HMT disorders in the case collection are representative of those expected amongst presentations at a tertiary referral haemostasis clinic, with one qualification. Patients carrying variants in *NBEAL2*, the aetiological gene for GPS, are over-represented in this study because of the proactive efforts by NIHR researchers to collate an international cohort of patients to investigate the specific pathology of this disease.[28] No causative variants were identified in 41% of patients with macrothrombocytopenia referred to TG,[37] a proportion similar to those of

other research programmes using HTS.[33,36] This illustrates the extent to which the genetic architecture of HMT remains incompletely understood. Indeed, there are two phenotypically distinct HMT disorders, White Platelet Syndrome[53,54] and Medich Platelet Syndrome,[55,56] for which no causal genes have yet been identified. New genes causing Mendelian forms of HMT are likely to be identified by ongoing research of large, rare disease cohorts. It is also possible that some HMT cases can be explained completely or partially by the non-Mendelian inheritance of an extreme polygenic load of low PLT predisposing alleles. Because of the potential diagnostic impact, in the following sections we will first address the recent advances in detecting common genetic variants regulating PLT and MPV, and then focus on the pathogenesis of the HMT disorders. Finally, we will cover the clinical implications of correctly identifying the cause of macrothrombocytopenia.

The genetic architecture of PLT and MPV in healthy populations

Quantitative blood cell traits have heritable components of variation.[57,58] Common genetic variation is estimated to generate 5–30% of the total variability in FBC traits measured in the European ancestry population.[32] Over the past decade, increasingly large GWAS analyses of FBC phenotypes have been performed to identify genetic variants perturbing haematopoiesis or blood cell clearance mechanisms.[32,59–62] The largest and most recent studies are by the Blood Cell Consortium (BCX), synthesising evidence from the UKB, INTERVAL and several other cohorts, in European ancestry and trans-ethnic meta-analyses.[63,64] Typically, each participant contributing to these studies had genotypes measured at a few hundred thousand variants, most of which were common, spread more or less uniformly across the genome. Subsequently, the genotypes for millions of variants not measured in the study participants were imputed computationally. Unmeasured genotypes can be imputed by matching measured genotypes to the haplotype structure observed in the panels of thousands of independent individuals with publicly available genotypes measured by WGS.[65–67] The BCX European ancestry GWAS had a sample size (n = 563 085) quadruple that of the largest preceding study and tested more than 41 million single nucleotide polymorphisms for association with each of 29 FBC phenotypes, including PLT and MPV.[32,63] The analysis identified 16 900 variant-trait association signals across the 29 FBC phenotypes.[63] These were assigned to at least 7,122 genomic tags, each either a distinct causal variant itself or strongly correlated (i.e., in strong linkage disequilibrium) to a distinct causal variant. Of these tags, 1,227 were associated with PLT and/or MPV. Of these, 117 (9·6%) were low frequency variants (minor allele frequency, (MAF) 1–5%) and 77 (6·3%) were rare variants[63] (MAF < 1%) (Fig 5A). In keeping with evolutionary theory, there is an inverse relationship between MAF and the maximum absolute effect size of a variant tolerated by natural selection (Fig 5A). Only variants with small effect sizes (<0·4 standard deviations (SD) per allele) have been able to attain a MAF of > 1%.[63] Conversely, rare variants, which on average have arisen more recently in evolution, exhibited absolute effect sizes as large as 0·86 SD per allele.[63] Interestingly, amongst the variants with genome-wide significant associations with one of PLT or MPV, there is a tendency for alleles associated with greater MPV to be associated with lower PLT and vice-versa (Fig 5B).[63] The annotation of variants by their computationally predicted transcriptional consequences, recapitulated observations from earlier GWAS that approximately 90% of variants associated with FBC phenotypes are located outside exons, in the non-coding regions of the genome and are, therefore, likely to exert a phenotypic effect by altering gene regulation.[32,63,68–71] Indeed, FBC associated variants were significantly enriched in open chromatin regions [(measured by assay for transposase-accessible chromatin using sequencing (ATAC-seq)] of 18 human haematopoietic progenitor cell types.[63,72] Variants associated with platelet phenotypes specifically were enriched in the open chromatin regions of MKs and in certain other myeloid precursor cell types.[63] However, platelet associations with coding and non-coding variants in, or physically adjacent to, BPD genes were enriched only in MKs. This suggests that the regulators of genes implicated in BPD act more specifically in MKs.[63] Common and low frequency variants associated with PLT or MPV are overwhelmingly (85%) intergenic or intronic.[63] By comparison, 60% of rare variants associated with PLT or MPV fall into these categories[63] and rare variants are much more likely than common variants to alter the amino acid sequence of a protein. There is a statistically significant overlap of 16 genes between the set of 29 HMT genes, and the set of 1185 genes in loci containing genetic associations with PLT or MPV (Fig 6). This illustrates that the same biological mechanism can be disturbed by multiple variants with effect sizes on a continuum. Variants with large effects on MPV or PLT are almost always rare and are more likely than variants with small effects to alter the structure of a protein rather than just disrupt the level of gene expression; variants with extreme effect sizes can disrupt biological function to such a degree that they cause disease. Consequently, genes with no known functions in MK or platelet biology, but in loci containing variants with large effects on PLT or MPV (e.g. *CKAP2L*, *PLEK* and *TNFRSF13B*),[63] are credible candidates to explain molecularly undiagnosed cases of HMT.

The polygenic risk of macrothrombocytopenia

Genome-wide association studies, such as the BCX studies, are now so large they can provide precise estimates of the effects on quantitative traits of single allele copies of rare variants known to cause AR disorders in homozygosity. Such studies have shown, in particular, that the population distributions of PLT and MPV in heterozygous carriers of alleles

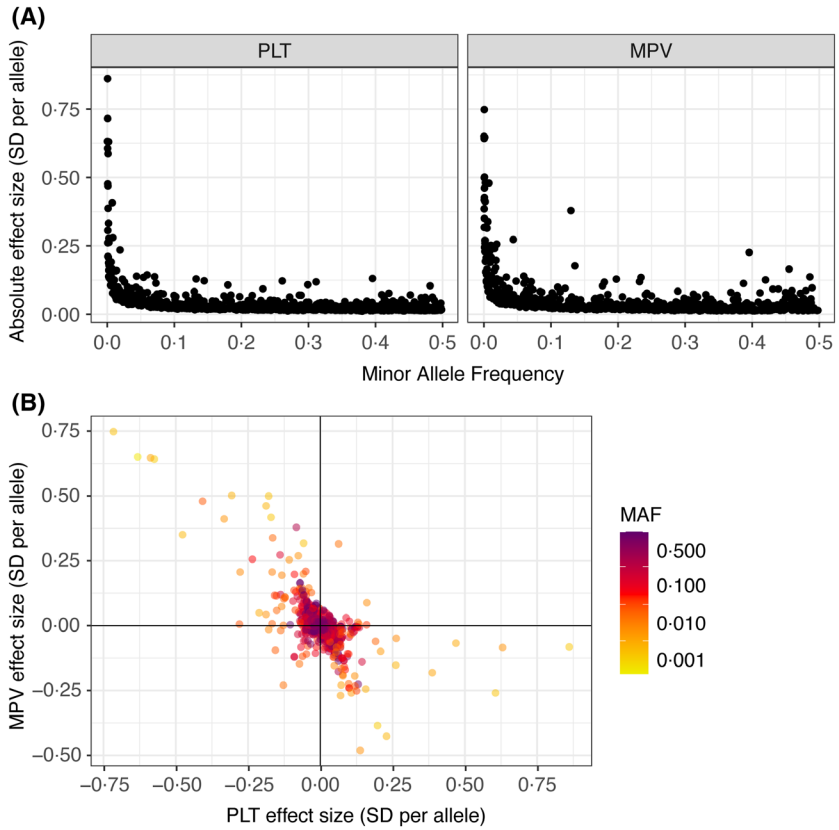

Fig 5. Phenotypic effect size of variants associated with platelet traits in the UKB genome-wide association studies (GWAS). (A) The scatterplots show the minor allele frequencies (MAF) of variants associated with platelet count (PLT) (left panel) and mean platelet volume (MPV) (right panel) in the UKB study on the *X*-axis. The effect size of each variant is shown on the Y-axis, expressed as the per allele effect of the variant on the mean of each parameter distribution expressed in standard deviations (SDs) of the respective distribution. (B) The inverse relationship between effect sizes for PLT and MPV. The colour gradient of the dots indicates the logit transformed MAF. Effect sizes are expressed as described above.

known to cause recessive BPDs can differ dramatically from the corresponding distributions in non-carriers.[32,63] Variation in PLT and MPV between individuals, due to background measurement and environmental and genetic factors, may explain why it has not been possible previously to demonstrate co-segregation of heterozygote effects within individual pedigrees, despite their relatively large population effect sizes. One example of this phenomenon is the *THPO* gene, which encodes TPO. AR inheritance of two loss-of-function (LoF) variants in *THPO* causes severe thrombocytopenia and, sometimes, tri-lineage bone marrow failure,[73] whereas monoallelic LoF variants in *THPO* cause a mild macrothrombocytopenia only.[74,75] Another example from the BCX GWAS, relates to the *GP9* variant rs5030764, which changes asparagine to serine at residue 61 of glycoprotein IX (GPIX), one of the proteins of the GPIb/IX/V platelet receptor for von Willebrand factor (VWF). In homozygosity or in compound heterozygosity with another pathogenic allele, the minor allele of rs5030764 causes Bernard-Soulier syndrome (BSS).[37] However, the allele also affects heterozygote carriers, for whom thrombocytopenia is three times more prevalent than usual in the UKB baseline FBC data.[63] The observation that heterozygote effects can be strong motivates the question, what is the consequence of inheriting AR alleles in heterozygosity in two different genes, and could this lead to a BPD phenotype, such as HMT? More generally, it can be hypothesised that some molecularly unexplained cases of HMT are due to the aggregate genome-wide polygenic effect of multiple alleles, each of which simultaneously predisposes the carrier to a lower PLT and greater MPV, perhaps compounded by pathogenic, or likely pathogenic, AR variants or by environmental exposures. This hypothesis is supported by recent studies of polygenic scores (PGS) for FBC traits.[63] A PGS is a numerical predictor of a quantitative trait or of a disease liability, constructed as a weighted sum of allele counts from numerous variants associated with the outcome of interest. The weights are usually chosen to be proportional to the marginal or joint effect of the variant on the trait of interest, estimated by regression. The effect of a one SD decrease in the PGS for PLT, developed by Vuckovic *et al.*, was comparable to the effect of a pathogenic rare variant in heterozygosity.[63] One way of presenting differences in

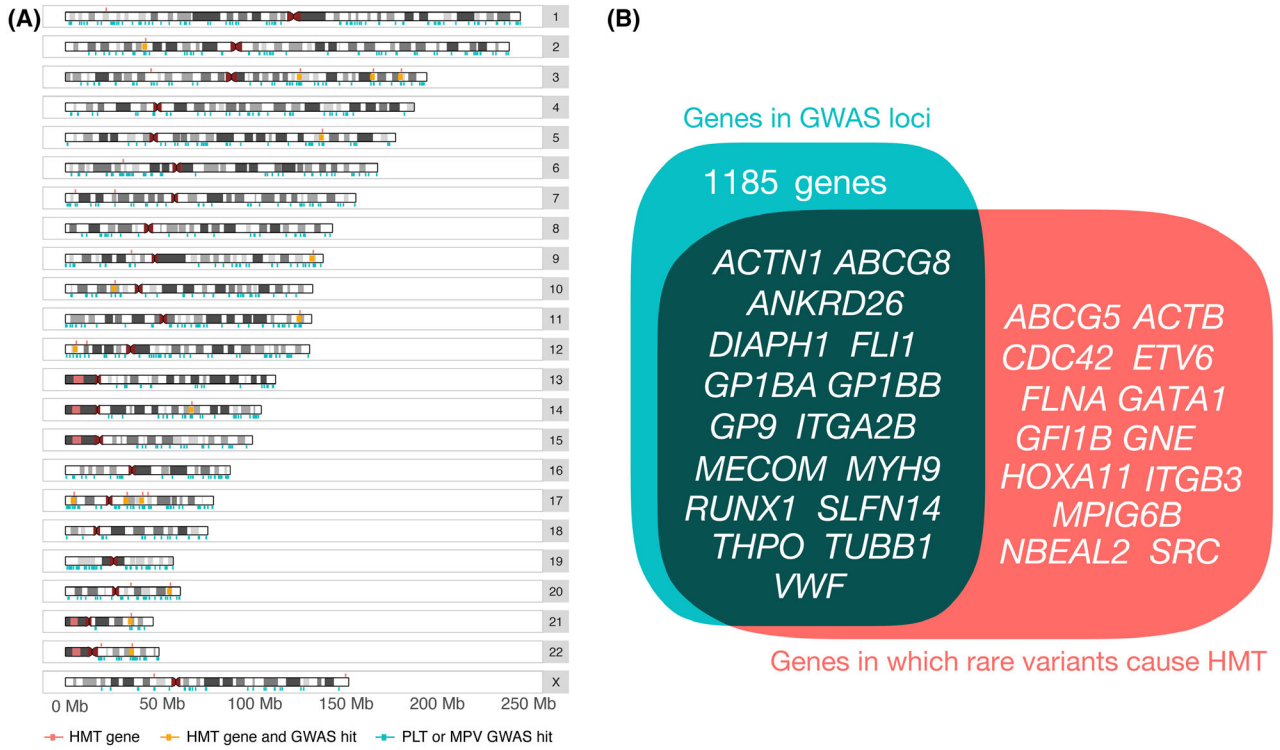

Fig 6. Overlap between genes identified by genome-wide association studies (GWAS) and genes implicated in hereditary macrothrombocytopenia (HMT) disorders. (A) Chromosome ideogram showing the positions of the 1185 gene loci with variants identified by GWAS as being associated with platelet count or mean platelet volume (blue bars) and the 29 genes implicated in HMT disorders (red bars). The 16 HMT genes falling within 5 kb of a GWAS locus are marked with an orange dot. The Y chromosome is not presented, as this was not included in the GWAS analysis. *ABCG5* and *ABCG8* lie in close proximity at 2p21 and are represented by only one red bar. (B) Diagram displaying the overlap between 16 genes in GWAS loci, in which rare variants cause HMT.

polygenic risk is to make comparisons between groups with a high or low PGS, defined by quantiles of the population distribution. The distributions of PLT in the subsets of UKB participants forming the lowest and greatest deciles of the PGS for PLT constructed by Vuckovic *et al.* are shifted left and right respectively, relative to the distribution of PLT in all participants (Fig 7A). Combined inheritance of a heterozygous pathogenic variant in *GP9* and a low PGS may, by an additive effect on PLT, manifest as macrothrombocytopenia. Conversely, inheritance of a high PGS may mitigate the increase in disease risk due to the pathogenic allele, resulting in a PLT within the normal range (Fig 7B). Risk modifying environmental and genetic variation, including that captured by PGS, can make pathogenicity assignment challenging. This is illustrated by the variant rs41303899 in *TUBB1* (Fig 7C), the minor allele of which is strongly associated with lower PLT (on average -33×10^9/l), and which when inherited in addition to a low PGS can have a joint effect on PLT sufficient to result in macrothrombocytopenia.[63] However, many carriers, in particular those with a high PGS, have a PLT within the normal range,[63] suggesting that this variant would be better classified as risk-associated rather than pathogenic. These examples illustrate how polygenic variation can modify the penetrance of rare variants with large effect sizes in the pathogenesis of HMT. In the future, it may be possible to diagnose a proportion of molecularly uncharacterised HMT cases, as caused by a very extreme PGS, perhaps in combination with a heterozygous pathogenic rare variant, although it is likely these patients will represent a small fraction of all cases.

The hereditary macrothrombocytopenia disorders

The characterisation of causal genetic variants in HMT has improved our understanding of the normal regulation of platelet production. Considering all HMT disorders, there are examples that result in dysfunction of several major steps in megakaryopoiesis and platelet formation, including MK differentiation, MK maturation and proplatelet formation (Fig 3). The clinical and genetic characterisations of HT and HMT disorders have recently been reviewed.[76–80] We will primarily focus on the pathogenesis of a subgroup of HMT disorders distinguishable by the presence of 'very large' platelets, in which there is preservation and exaggeration of the inverse relationship between PLT and MPV that is observed in healthy populations. In a diagnostic classification proposed by Noris *et al.*, based on a large study of 376 patients with 19 different

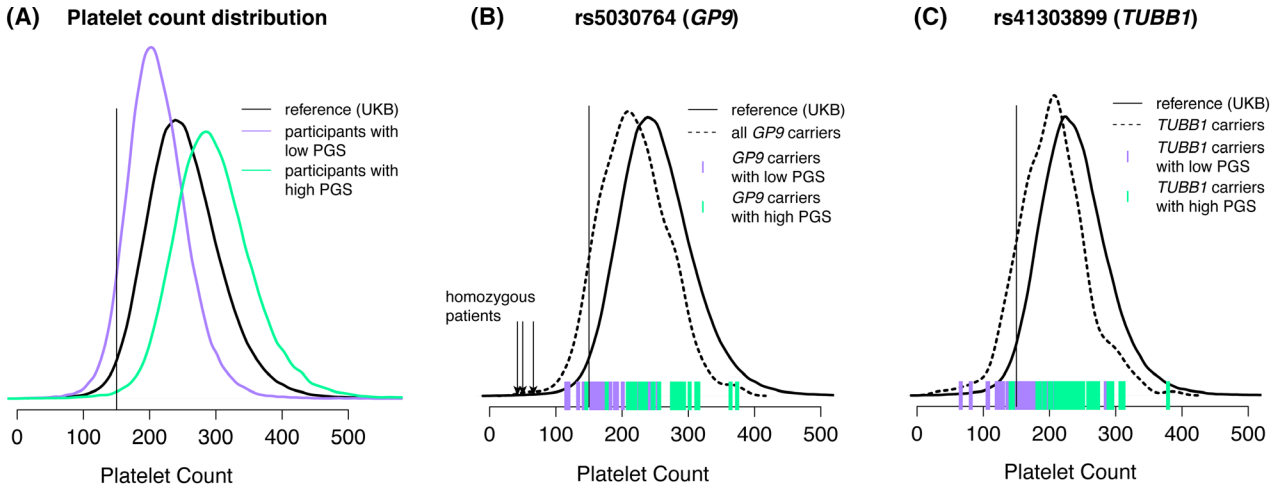

Fig 7. Representation of the interplay between polygenic trait scores (PGS) and the effect of monogenic variants casual of hereditary macrothrombocytopenia (HMT) disorders. (A) Platelet count (PLT) distribution for all participants in the UKB cohort (black line) and for those participants in the top 10% (green line) and bottom 10% (purple line) of the PGS for PLT respectively. (B) PLT distribution (black-line) for all UKB participants and UKB participants who are carriers of the *GP9* variant rs5030764 (dotted black line). Vertical lines in green and purple respectively represent the rs5030764 carriers with top 10% and bottom 10% PGS values. The black arrows indicate the PLT for three patients with Bernard-Soulier syndrome caused by rs5030764 in homozygosity. (C) Similar plots showing UKB participants who are carriers for the *TUBB1* variant rs41303899. The black vertical line in the three panels marks a PLT of 150 × 10^9/l, the lower limit of the normal range.

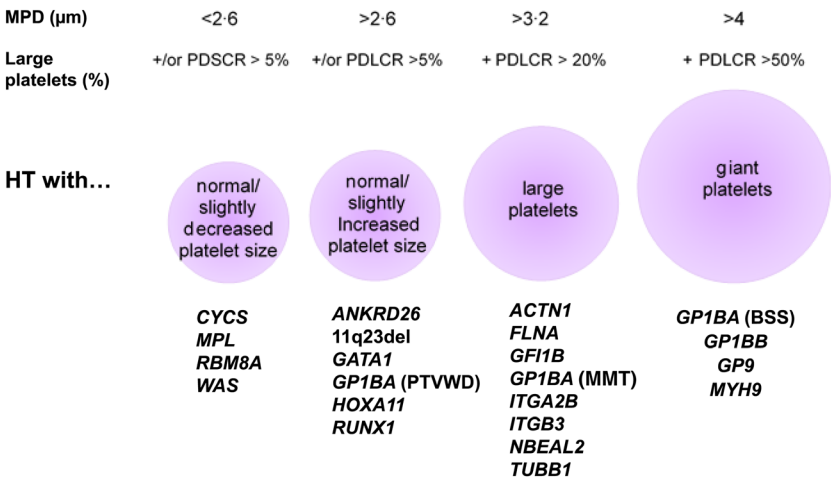

Fig 8. Proposed classification of hereditary thrombocytopenias (HTs) using platelet size parameters. In this classification proposed by Noris *et al.*,[24] 19 HTs associated with variants in 20 genes are sub-classified according to mean platelet diameter (MPD) and platelet diameter large and small cell ratio (PDLCR and PDSCR respectively; percentage of platelets above the 97.5th percentile and below the 2.5th percentile of the platelet diameter distribution in controls). HTs are listed by associated gene (the three HTs associated with *GP1BA* are also indicated in brackets). The platelet illustrations for each category are to a relative scale. BSS, Bernard-Soulier syndrome; MMT, mild macrothrombocytopenia; PTVWD, platelet type von Willebrand disease.

HT disorders, 'large' and 'giant' platelet disorders were those in which the MPD was greater than 3·2 µM [with a platelet diameter large cell ratio, (PDLCR) >20%] and 4·0 µM (with PDLCR >50%) respectively[24] (Fig 8; Table 2). Strikingly, the 11 genes, in which rare pathogenic variants lead to the HMT disorders in this subgroup[24] all have functional roles in pro-platelet formation. Nine genes have critical roles in cytoskeleton regulation (*ACTN1, FLNA, MYH9* and *TUBB1*) and expression of surface membrane glycoproteins at the later stages of MK differentiation (*GP1BA, GP1BB, GP9* encoding proteins GPIbα, GPIbβ, GPIX; *ITGA2B, ITGB3* encoding integrin αIIb and $\beta 3$)[79,80] (Figs 3 and 8; Table 2). The remaining two genes, *GFI1B* and *NBEAL2,* also have functional roles in cytoskeletal reorganisation.[81,82] In contrast, five genes associated with platelets that were 'normal/slightly increased' in size (*ANKRD26, FLI1, GATA1, HOXA11* and *RUNX1*)[24] have roles in transcription regulation in early megakaryopoiesis[79,80] (Figs 3 and 8).

The cytoskeleton is composed of tubulin microtubules, actin filaments and intermediate filaments constructed from different subunit proteins, which in combination provide shape and organisation to the MK and enable the cytoplasm to be packaged into multiple long protrusions called proplatelets.[83] Current models of platelet production suggest that platelets develop at the proplatelet tips and receive their granule contents through active cytoskeleton-mediated transport. Subsequent fission events enable the release of platelets into the circulation.[84] Rare variants in critical genes that disrupt cytoskeletal rearrangement and proplatelet formation appear to impair the release of mature platelets, and instead preferentially release immature, abnormally large platelets, resulting in macrothrombocytopenia.[85,86]

MYH9 is the most frequently implicated gene in patients with HMT, and underlies five historically recognised clinical disorders, May-Hegglin anomaly, Epstein syndrome, Fechtner syndrome, Sebastian syndrome and Alport syndrome with macrothrombocytopenia.[26,87,88] Patients with these autosomal dominant (AD) disorders had macrothrombocytopenia, giant platelets, leucocyte inclusions and a combination of additional clinical features (Table 2). Following the discovery of underlying pathogenic variants in *MYH9*, they became collectively termed the MYH9-related disorders (MYH9-RD) in which different clinical features are likely to represent variable expressivity.[89,90] *MYH9* encodes the heavy chain of non-muscle myosin IIA, which belongs to a family of cytoskeletal proteins that slide along actin filaments and generate mechanical forces to enable proplatelet formation, fragmentation and platelet release.[76] The association between cytoskeletal genes and HMT is further illustrated by *TUBB1*, *ACTN1* and *FLNA,* in which rare monoallelic variants lead to a mild-moderate reduction in PLT (typically 50–100 $\times$ 10^9/l in *FLNA*-related macrothrombocytopenia and >100 $\times$ 10^9/l in the others), large platelets and usually mild or absent bleeding symptoms.[80] We have already touched on *TUBB1* to illustrate the penetrance modifying effect of polygenic variation. *TUBB1* encodes β1-tubulin, the main tubulin isoform in MKs, critical to proplatelet extension, release of platelets and determination of platelet size.[91,92] *ACTN1* encodes α1-actinin, which acts to crosslink actin filaments[93] and *FLNA* encodes filamin A, which binds the intracytoplasmic domain of GPIbα to the actin filament network; these proteins act to stabilise the actin cytoskeleton.[80,94,95] *GFI1B* encodes a zinc finger protein, which is a transcriptional regulator necessary for development and differentiation of the MK and erythroid lineages.[81] Dominant negative mutations in *GFI1B* cause macrothrombocytopenia and a variable bleeding diathesis caused by a deficiency of platelet alpha (α)-granules and abnormal platelet activation responses including cytoskeletal dysregulation.[81,96,97] *NBEAL2* encodes a scaffolding protein, crucial to α-granule biogenesis; biallelic variants lead to GPS[27,28] and it is proposed that macrothrombocytopenia results from defects in the Rac1/Cdc42 pathway and subsequent dysfunctional cytoskeleton reorganisation.[82] Paradoxically, variants in other genes with critical roles in cytoskeletal regulation, such as *WAS* and *ARPC1B,* lead to microthrombocytopenia, possibly though an alternative effect on proplatelet formation or through increased rates of peripheral platelet clearance, resulting in small platelets.[98]

Genes involved in extracellular signalling to the cytoskeleton via membrane-bound receptors are also crucial to the regulation of platelet formation and release.[79] Biallelic LoF variants in *GP1BA, GP1BB* and *GP9* disrupt proplatelet formation by reducing or altering VWF binding to the GPIb/IX/V receptor and cause BSS, an AR disorder associated with severe mucocutaneous bleeding.[99] Platelets in BSS are historically described as 'giant', because, as in the MYH9-RDs, some platelets appear larger than RBCs on microscopy (Fig 8).[24] Monoallelic LoF variants in *GP1BA* or *GP1BB* result in a less severe phenotype, termed mild macrothrombocytopenia.[100–102] Deletion of a region of the long arm of chromosome 22 (22q11.2), which includes *GP1BB*, occurs in approximately 1:2000–6000 live births and leads to the disorders DiGeorge syndrome or Velocardiofacial syndrome.[103] Affected patients have macrothrombocytopenia, moderate-severe bleeding and a broad range of other clinical features[103] (Table 2). Gain-of-function (GoF) variants in *GP1BA* or *VWF,* which cause platelet-type von Willebrand disease[104–107] and Type 2B von Willebrand disease[108,109] respectively, result in enhanced receptor-ligand interaction and disrupted megakaryopoiesis. Platelets in these conditions are normal or only slightly increased in size[24] and likely to be related to increased platelet clearance; patients do not have any additional clinical features.

Biallelic LoF variants in either *ITGA2B* or *ITGB3* cause Glanzmann thrombasthenia, in which patients typically have severe mucocutaneous bleeding due to absent surface expression of the integrin αIIbβ3, resulting in defective aggregation and outside-in platelet signalling, but PLT is typically within the normal range.[110] *ITGA2B* and *ITGB3* may also harbour rare monoallelic GoF missense variants, in which the main manifestation is macrothrombocytopenia, sometimes causing bleeding.[110–113] These GoF variants are thought to lead to constitutive activation of αIIbβ3, which impacts actin cytoskeleton reorganisation and results in defective proplatelet and subsequent platelet formation.[114,115]

Three more recently discovered HMT genes further support the importance of cytoskeletal dysfunction in the HMT phenotype: *DIAPH1, ACTB* and *CDC42*. Patients with rare causative variants in *DIAPH1* have moderate macrothrombocytopenia without bleeding complications, but severe sensorineural deafness and often mild neutropenia.[116,117] *DIAPH1* encodes a member of the formin protein family, which controls GTPase dependent assembly of actin and microtubule regulation during cytoskeletal remodelling.[116] The variants underlying *DIAPH1*-associated macrothrombocytopenia lead to constitutive activation of the DIAPH1 protein, which disrupts cytoskeletal and microtubule function and impairs proplatelet formation.[116] Rare variants in *ACTB* were only very recently discovered as causative of HMT,

resulting in platelets with an MPD in the 'large platelet' disorder range.[24,44] *ACTB* encodes β-cytoplasmic actin and pathogenic variants in the 3' region disrupts microtubule organisation in MKs.[44] Finally, *CDC42,* which encodes a member of the Rho family of small GTPases, has a major role in cytoskeletal remodelling and intracellular signalling.[45] A subgroup of monoallelic missense variants in *CDC42* lead to macrothrombocytopenia and a variety of developmental and multisystem features, but no apparent haemostatic abnormality[45] (Table 2).

There are a number of additional HMT genes that are understood to play a role in late megakaryopoiesis but with incompletely defined pathways. For example, monoallelic variants in *SRC* and *SLFN14* disrupt MK maturation and result in reduced proplatelet formation, causing macrothrombocytopenia with variable bleeding phenotypes.[80,118,119] In the case of *SRC* variants, there are myriad other clinical features including bone marrow fibrosis[118] (Table 2).[112] The recent discoveries of variants in *MPIG6B* and *GNE,* as causes of AR HMT disorders, provide new insights into the pathogenesis of proplatelet formation. *MPIG6B* is located in the major histocompatibility complex locus and encodes a transmembrane receptor of the immunoglobulin superfamily.[50,51] *MPIG6B* is expressed on the surface of MKs and platelets and when non-functional, leads to impaired proplatelet formation.[51] *GNE* encodes an enzyme (glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase), involved in the sialic acid biosynthesis pathway that is expressed in all haematopoietic cells.[46,47] Biallelic LoF variants in *GNE* reduce sialic acid biosynthesis, impairing platelet formation and increasing platelet clearance.[46,47]

Clinical implications

It is critical to distinguish between acquired and hereditary macrothrombocytopenia, to enable appropriate management. This includes the avoidance of treatments that are not indicated and potentially harmful in patients with HMT disorders. The most common example is the use of immunosuppressants following a misdiagnosis of ITP.[34] Additionally, patients have been inappropriately treated with 5-azacytadine for myelodysplastic syndrome, following the observation of dysmegakaryopoiesis on bone marrow biopsy, and prior to receiving a genetic diagnosis of AD thrombocytopenia 2 due to *ANKRD26* mutations.[120]

Accurate molecular diagnosis in HMT is crucial to provide patients and clinicians with an accurate diagnosis and prognosis. There is an association between some HMT disorders and the development of malignancy, for example those caused by variants in *ANKRD26*,[121] *ETV6*,[122] and *RUNX1*.[123] Patients with these disorders can present with only mild macrothrombocytopenia, and absent or mild bleeding symptoms, therefore, a high index of clinical suspicion is required to resolve the diagnosis. Of 29 patients with a working diagnosis of 'ITP refractory to treatment', referred to TG, seven were diagnosed with HMT disorders, including *ANKRD26* and *ETV6* mutations.[37] Monitoring of patients with a 'pre-malignant' genetic profile for the emergence of detrimental somatic mutations also has potential to improve survival.[124]

Many HMT genes are expressed in other blood cells and tissues, explaining the additional clinical features displayed in a number of HMT disorders, which may have a greater impact on a patient's health than bleeding complications (Table 2). For example, approximately 30% of patients with MYH9-RDs are at risk of end stage renal failure (ESRF) secondary to glomerulonephritis, 60% acquire a sensorineural hearing defect and 16% develop pre-senile cataracts.[125] There is genotype–phenotype correlation between the molecular defect and these disease manifestations; variants modifying arginine at position 702 of the protein, located in the head of myosin IIA, lead to glomerulonephritis, whereas variants affecting the non-helical portion of the tail almost never associate with this phenotype.[125] Early pharmacological intervention with angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers can prevent deterioration to ESRF.[125] Therefore, a precise molecular diagnosis assists in best management of this condition.

Treatment of HMT disorders currently focuses on prophylaxis prior to high-risk procedures and treatment of acute bleeding. For example, for patients with BSS, the mainstay of prophylactic treatments are tranexamic acid and transfusions of HLA class I matched platelets.[126] There is little evidence for the effectiveness of desmopressin and recombinant human activated factor VII for bleeding, which is licensed for patients with Glanzmann thrombasthenia but not BSS.[126] Patients with both MYH9-RDs and macrothrombocytopenia with sensorineural hearing loss due to *DIAPH1* mutations have responded to treatment with the TPO receptor agonist (TPO-RA) eltrombopag.[117,127] In a recent prospective phase II clinical trial, 24 patients with five different HT disorders were treated with eltrombopag.[128] The average increase in PLT from baseline was $64{\cdot}5 \times 10^9$/l, with more than 90% of the patients responding to treatment, and patients with MYH9-RD and BSS achieving the greatest response. These results are encouraging for the use of TPO-RAs to prepare patients with HMT disorders for elective procedures, without the need for platelet transfusion, although further clinical data is needed.[128] Currently, HSC transplantation is the only curative treatment for severe HMT disorders, including BSS and amegakaryocytic thrombocytopenia with radioulnar synostosis.[125] As our knowledge of the molecular pathways regulating megakaryopoiesis and platelet formation advances, we pave the way for identification of new therapeutic targets. It is also conceivable that gene therapy may become an option in the future for HMT disorders associated with severe pathologies.

The effect of polygenic variation on PLT has aetiological relevance for HMT and might have diagnostic implications. In the previous *TUBB1* example, we demonstrated that a person who inherits a rare monogenic variant in an HMT gene may also, by chance, inherit a high PGS for PLT,

resulting in aggregate to a typical risk of macrothrombocytopenia, i.e., the polygenic variation masks or compensates for the rare monogenic variant. This phenomenon is unlikely to have harmful clinical consequences in the case of *TUBB1* because, other than mild macrothrombocytopenia, rare variants have no known phenotypic consequences. However, if we consider genes in which rare pathogenic variants cause thrombocytopenia and also confer a risk of malignancy, in theory, an extreme polygenic compensation that makes thrombocytopenia less likely to be detected and investigated could potentially have clinical implications. In addition, a low PGS for PLT combined with an environmental trigger, such as a viral infection or pregnancy, could have clinical consequences, for example, by increasing the likelihood of PLT dropping to below safe thresholds for invasive procedures. On the other hand, a high PGS for PLT may lead to an individual being extensively investigated for a reactive or genetic cause of thrombocytosis. Therefore, in the clinical setting, understanding an individual's PGS could potentially influence the management of patients presenting with both low and high PLT.

Conclusion

The majority of HT cases are accompanied by enlarged platelets, that is, macrothrombocytopenia. In some cases, platelets are only subtly increased in size, including in HMT disorders caused by variants in transcription factor genes (e.g. *ANKRD26*). More frequently, macrothrombocytopenia is pronounced and a common causative mechanism is dysregulation of proplatelet formation. These HMT disorders lie at one extreme of the PLT and MPV variation, which is negatively correlated at a population level. There are technical challenges in measuring PLT and MPV, particularly relevant in macrothrombocytopenic blood samples. However, accurate and precise measurements are fundamental to the work-up of thrombocytopenia, to identify the underlying pathology. This is to help to distinguish between acquired and hereditary causes and because platelet volume/size measurements, which provide a well-defined clinical phenotype alongside other haematological, morphological and organ system abnormalities, enable the MDT to assign pathogenicity to variants identified by HTS-based testing. On the TG platform, the diagnostic yield for patients with macrothrombocytopenia was 59%, compared to 40% for patients with thrombocytopenia (where either MPV was normal or a value not provided) and 37% across all patients with a BPD.[37]

Huge advances in the availability and diagnostic capacity of HTS platforms, such as TG,[35,37] the UK Genotyping and Phenotyping of Platelets study,[33] and the Spanish HTS platform for the diagnosis of hereditary platelet disorders,[36] make early incorporation of genetic testing appropriate and feasible. Where targeted HTS panels cannot identify a causative gene, enrolment into a WGS project, such as the follow up to the 100 000 Genomes Project, is important for identification of new causative genes to expand our knowledge of the pathogenesis of HMT disorders.[39] In England, these new NHS-based diagnostic services are being made available through a network of seven NHS Genomic Laboratory Hubs. The contribution of the polygenic predisposition to macrothrombocytopenia may explain a proportion of patients without a molecular diagnosis. However, further research is needed in patient cohorts in order to confirm the effects detected in healthy populations.

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Author contributions

Janine Collins performed the literature search and wrote the manuscript draft. Janine Collins, William Astle, Karyn Megy and Dragana Vuckovic performed data analysis. Janine Collins, William Astle and Dragana Vuckovic generated the figures. William Astle, Karyn Megy, Andrew Mumford and Dragana Vuckovic provided expert input to critically review and edit the manuscript. All authors read and approved the final manuscript.

Conflict of interest

AM has received speaker fees, research funding or consultancy fees from Shire, Sanofi-Genzyme, NovoNordisk and AstraZeneca. All other authors declare no conflicts of interest.

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