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Interstitial 11q Deletions and Terminal 11q Duplications Cause a Bleeding Tendency due to Platelet Dysfunction That Is Similar to 11q Deletions Causing Jacobsen Syndrome

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ABSTRACT

Introduction: Jacobsen syndrome, resulting from a terminal deletion of chromosome 11 (11q), may lead to an increased bleeding tendency due to low platelet counts or platelet dysfunction. Currently, information on bleeding tendency and platelet function in patients with nonterminal 11q-aberrations such as larger deletions, interstitial 11q-deletions, or 11q-duplications is lacking.

Methods: We investigated the bleeding symptoms in relation to platelet numbers and function in 14 patients: 10 patients with a terminal 11q-deletion, one terminal 11q-duplication, two interstitial 11q-deletions, and one with an interstitial 11q-duplication.

Results: Twelve patients reported bleeding complications (12/14, 86%), most frequently perioperative bleeding (21%), hematomas (17%), epistaxis (13%), prolonged bleeding, and severe bruising (11%) and heavy menstrual bleeding in 4/5 postmenarcheal women (80%). Seven (50%) had platelet counts below normal values (median 62×10^9/L). Strikingly, the seven patients with a normal platelet count reported more frequent perioperative bleeding complications, as well as atypical bleeding such as

Abbreviations: 11q, long arm of chromosome 11; A, alpha granule; ADP, adenosine diphosphate; ATE, adenotonsillectomy; ATP, adenosine triphosphate; C, celsius; CNS, central nervous system; Del, deletion; Dupl, duplication; EM, electron microscopy; F, female; FDR, false discovery rate; fL, femtoliter; FVIII:Act, factor VIII activity; GI, gastrointestinal (tract); h, hour(s); HC, healthy control; HCD, higher-energy collisional dissociation; HMB, heavy menstrual bleeding; Im, intramuscular; IPF, immature platelet fraction; IQR, interquartile range; ISTH BAT, Bleeding Assessment Tool of the International Society of Thrombosis and Hemostasis; L, liter; LFQ, label-free quantification; LTA, light transmission aggregometry; M, male; Mb, megabytes; Min, minute(s); mL, milliliter; MPV, mean platelet volume; N, number; NA, not applicable; P, patient (number); PFA, platelet function analyzer; PFA-EPI, platelet function analyzer collagen-epinephrine; PRP, platelet-rich plasma; SD, standard deviation; Sec, second(s); TEM, transmission electron microscopy; TPO, thrombopoietin; TRAP, thrombin receptor-activating peptide; U, unit; VWF:Act, Von Willebrand Factor Activity; VWF:Ag, Von Willebrand Factor Antigen; µg, microgram; µM, micrometer.

intramuscular hematoma after injection than the group with low platelet counts. Platelet dysfunction was detected in all (100%) patients by light transmission aggregometry (LTA). Mass-spectrometry (MS) analysis showed an overrepresentation of NUBP2 and ATP5S, both involved in mitochondrial ATP synthesis. Electron microscopy (EM) confirmed abnormal granules in some.
Conclusions: We demonstrated increased bleeding tendency due to platelet disorders in the majority of patients with terminal 11q-deletions but also in nonterminal 11q-aberrations. This is confirmed by abnormal platelet function tests and MS. We therefore recommend screening of patients with all forms of 11q-disorders and, when indicated, preventive preoperative measurements and counseling for heavy menstrual bleeding in females.

1 | Introduction

Partial deletions or duplications of the long arm of chromosome 11 (11q) are rare and may involve various chromosomal regions. These chromosomal abnormalities range in size from 1 to 20 Mb [1], and can occur with concomitant abnormalities in other chromosomes [2, 3]. Most frequently, a terminal 11q-deletion occurs, but interstitial deletions as well as interstitial and terminal duplications of 11q are reported as well [4]. Breakpoints arising within or distal to subband 11q23.3 with a deletion extending to the telomere lead to a clinical entity known as Jacobsen syndrome [5, 6]. The estimated incidence of Jacobsen syndrome is 1:100000 births with a female: male ratio of 2:1 [1]. Incidences of other 11q-abberrations are unknown but are considered to occur even more rarely. The clinical phenotype of terminal 11q-deletions depends on the location and extent of the chromosomal abnormality and includes cognitive impairment, cardiac malformation, increased susceptibility to infections, and an increased bleeding tendency [1]. This bleeding tendency is most probably due to low platelet counts and/or platelet dysfunction and generally leads to mucosal and perioperative bleedings, but cerebral bleeding has also been reported [7]. Typically, giant alpha granules may be observed by electron microscopy (EM), known as Paris–Trousseau syndrome [8–11]. Abnormal dense granules [12], as well as microkaryocytes, have also been described in bone marrow specimens in a few cases with Jacobsen syndrome [10, 13]. Detailed data on bleeding phenotype, platelet numbers, and platelet function in other 11q-disorders is lacking. We therefore investigated the clinical phenotype and laboratory results of patients with a variety of 11q-aberrations.

2 | Methods

2.1 | Patient Population and Study Design

Patients were invited for this study by their treating physician or by the Dutch Chromosome 11 Network. Medical Ethical Committee approval was obtained (MEC-2013-026, Erasmus MC, University Medical Center Rotterdam). The study was performed according to the Declaration of Helsinki. All patients were included after patient or parental written informed consent according to national law and regulations.

Clinical data included patient characteristics, including diagnosis based on historical molecular analysis and medical history, including comorbidities, bleeding symptoms, hemostatic treatment, and transfusions. Data were derived from electronic patient files. Bleeding symptoms were documented in detail but also scored using the validated bleeding assessment tool of the International Society of Thrombosis and Hemostasis (ISTH-BAT). The ISTH-BAT contains 13 items. Per item, a score is given. This score reflects the presence (none = score 0) and severity of bleeding (score 1–4). The cutoff values for an abnormal bleeding score per individual are validated as the sum of all scored items and are defined as ≥ 3 in children, ≥ 4 in adult males, and ≥ 6 in adult females [14]. We also determined the sum of all individual bleeding scores in a predefined subgroup, leading to a bleeding score for the subgroup as a whole, covering both frequency and severity of the bleeding item.

2.2 | Laboratory Tests

Retrospectively, platelet counts and hemostatic test results were collected. Prospectively, the following laboratory results were measured at inclusion: blood count, platelet indices, thrombopoietin (TPO), hemostatic tests, platelet function, and platelet proteome. Blood cell count and platelet indices (Sysmex XN-9100, Netherlands) were measured in EDTA. Serum was used for measuring TPO analyzed with an in-house analysis (ELISA-assay, Sanquin). Citrate-blood was used for aPTT (Actin FS), PT (Thromborel S) and fibrinogen (Thrombin Reagent; all three Sysmex CS5100, Siemens Healthcare, Germany); von Willebrand factor antigen (VWF:Ag) levels were measured by an in-house ELISA assay using polyclonal rabbit antihuman VWF antibodies (DakoCytomation, Denmark) for capturing and detection; VWF activity (VWF:Act) on the Innovance VWF Act-assay (Sysmex CS-5100, Siemens); and VWF collagen binding (VWF:CB) activity by an in-house ELISA assay using bovine achilles tendon collagen type I for capturing (Sigma–Aldrich) and polyclonal rabbit antihuman VWF antibodies (DakoCytomation) for detecting. Factor VIII activity (FVIII:Act) was measured using one-stage clotting assays (APTT-based, Sysmex CS-5100, Siemens). Results were considered normal when test results were in the p5–p95 interval of the age references indicated.

Platelet function analyzer (PFA-200) was measured for collagen-epinephrine (PFA-EPI) and, following in-house protocol, when the closure time of the PFA-EPI was prolonged, collagen-ADP (PFA-ADP; INNOVANCE PFA-200 System, Siemens, Germany). Light transmission aggregometry (LTA) was performed on a Chrono-Log aggregometer 490 (Stago Benelux) using the following agonists: collagen (2 μg/mL), U46619 (4 μM), thrombin (40 U/mL) before July 2019 and TRAP (30 μM) from July 1st 2019, ADP (5 and 10 μM), epinephrin (5 μM), low and high concentrations of ristocetin (0.31 en 1.25 mg/mL), arachidonic acid (1 μM) [15]. Response was considered normal when the activation wave reached $\geq 50\%$ as final amplitude for weak agonists (ADP and epinephrin) and $\geq 70\%$ for strong agonists (Collagen, TRAP6, high

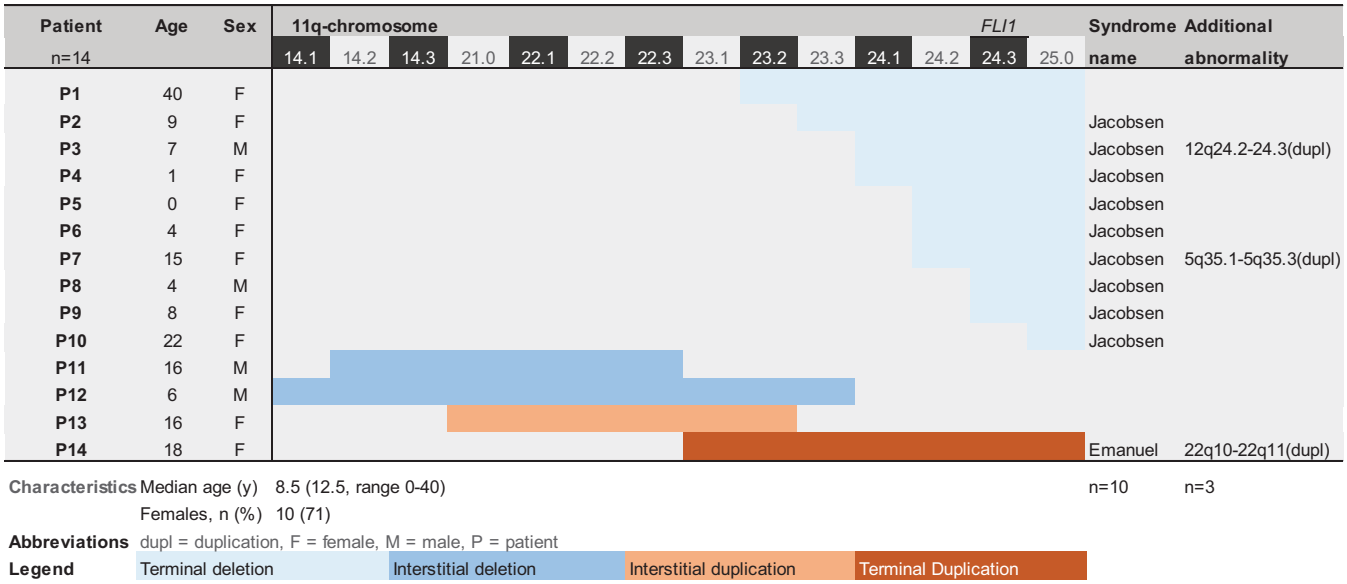

FIGURE 1 | Patient characteristics and genetic distribution of 14 patients with 11q disorders.

Ristocetin, Arachidonic acid, U46199) and the response was not reversible, no second wave was visible, and the response to ristocetin low was $<10\%$. A platelet-rich plasma (PRP) of $\geq 150\times10^9$/L was considered normal. In case of a PRP level of $<150\times10^9$/L, LTA test results were generated but interpreted with caution [15].

2.3 | Mass-Spectrometry (MS) and EM

Methods of MS and EM imaging are described in detail in Table S1.

2.4 | Statistical Analysis

Descriptive statistics were used to summarize baseline characteristics of the study population. In case of a skewed distribution, data are presented as median and interquartile range (IQR). In case of a normal distribution, data are presented as mean and standard deviation (SD) or range. Categorical data are presented as numbers with percentages and the range of minimum and maximum values. To identify the largest variation in the platelet proteome, a principal component analysis (PCA) is performed. Statistical data analyses were performed using SPSS version 21.0 (IBM, Armonk, NY, USA). For MS-data, files acquired from XCalibur software (Thermo Fisher Scientific) and processed with MaxQuant 1.6.2.10 software for MS-analysis were used.

3 | Results

3.1 | Patient Characteristics

Fourteen patients were included with a median age of 8.5 years (IQR 12.5; range 0–40). Ten were female (71%, Figure 1). Ten patients had a terminal 11q-deletion with a variable size of deletion (71%), and four had other 11q-aberrations: interstitial deletion, $n=2$ (14%); terminal duplication, $n=1$ (7%) and interstitial duplication, $n=1$. The localization and size of the chromosomal abnormalities varied, and in three patients additional chromosomal abnormalities were observed (See Figure 1, details in Table S2). Comorbidities were present in all patients; neurodevelopmental disabilities, cardiac abnormalities, and immunodeficiency were the most common (Table S3). During follow-up, one patient passed away unexpectedly.

3.2 | Bleeding Symptoms

Bleeding symptoms are summarized in Figure 2, details in Table S2. Mean bleeding score was 6.7 (3.96 SD; range: 0–13). Only 2/14 (14%) patients had a bleeding score below the age-related cut-off: one patient with an interstitial 11q-duplication did not report any bleeding symptoms. She also had a normal platelet count. One child with a terminal 11q-deletion had a score of 2 and showed only a mildly lowered platelet count (range 82-105×10E9/L) as well as only a mild abnormal response in the LTA.

Twelve out of 14 (86%) patients with increased bleeding socre had a mean bleeding score of 7.1 (range: 4–13). Bleeding symptoms that were reported most frequently, were large hematomas (12/14), bleeding from small wounds (7/14), increased and prolonged bleeding during or after surgery (9/14), heavy menstrual bleeding (4/10 postmenarcheal women) and other bleedings (8/14). Ten patients (10/14) reported a total of 20 bleeding episodes that required consultation and even treatment (Figure 2A); reported bleeding for large hematomas, epistaxis, increased or prolonged bleeding during or after surgery as well as prolonged bleeding from small wounds, gastrointestinal bleeding, heavy menstrual bleeding and one eye-bleeding classified as “other.”

To illustrate these bleeding scores in more detail, the large hematoma (ISTH-BAT score 2) occurred in two patients after subcutaneous immunoglobulin replacement therapy. The perioperative bleeding complications were after adenotonsillectomy, circumcision, eyelid surgery, cardiac, and craniosynostosis repair

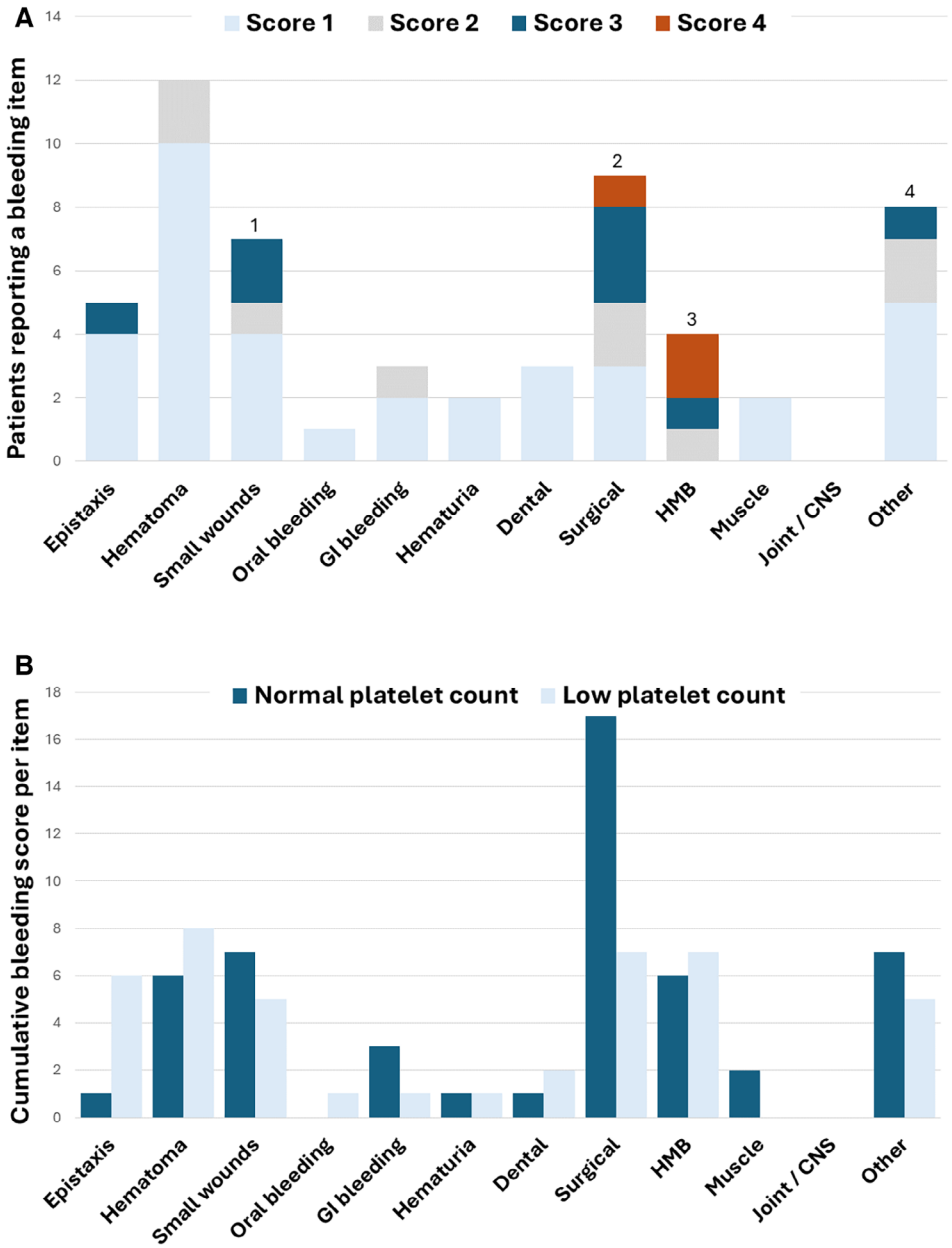

FIGURE 2 | Bleeding symptoms per bleeding item. [1]Treatment required for fibrinogen glue, stitching, local, and oral tranexamic acid; [2]consultation required in for postoperative bleeding after craniosynostosis repairment surgery or eye-lid correction; treatment required for postoperative bleeding after cardiac surgery and circumcision and blood transfusion needed after adenotonsillectomy; [3]consultation required in one of five postmenarcheal females with heavy menstrua bleeding and treatment required in three of five females with anticonceptive treatment, curettage (one of five, score 3), and hysterectomy for HMB (one of five, score 4); [4]consultation required for prolonged bleeding after venipuncture, treatment required for a spontaneous vitreous bleeding causing loss of sight despite timely ablation (1/14, score 3). CNS = central nervous system, GI = gastrointestinal, HMB = heavy menstrual bleeding.

surgery (scores 2–4). Concerning only 5 of 10 female patients who were postmenarcheal and could score on this item, it is remarkable that four out of five (80%) scored 2–4 and reported the need for medical consultation, tranexamic acid, contraceptives, and twice a gynecological intervention (curettage for heavy menstrual bleeding and hysterectomy after failure of anticonceptives). These four patients had terminal 11q-deletions (3/4, 75%) or a terminal 11q-duplication (1/4). Concerning the 'other' bleedings, one patient with interstitial 11q-deletion had a serious spontaneous bleeding in the vitreous gel of one eye leading to loss of vision despite timely laser photocoagulation. In our cohort, 2/14 reported an intramuscular bleeding, both after intramuscular vaccination was given at a young age, which is very atypical in patients with platelet disorders. After this bleeding complication, further vaccinations were given subcutaneously. In our cohort, joint or cerebral bleedings did not occur.

When comparing the bleeding scores of the seven patients (50%) with normal platelet counts with the scores of the seven patients with low platelet counts, there is a small difference in the mean bleeding score: 6.1 in the low platelet counts group versus 7.3 in the group with normal platelet counts. Remarkably, this is

A

Platelet counts	<150x10E9/L	≥150x10E9/L
11q-aberration	Terminal 11q-deletion, n=7	Terminal 11q-deletion, n=3 Non 11q-deletion, n=4
Laboratory tests, mean (range)		
Platelet count x10E9/L	67.0 (20-136)	230.9 (180-340)
IPF normal 1-5%	15.3 (11-29)	5.6 (3-8)
MPV normal 8-11fL	12.2 (11.8-12.5)	10.6 (9.4-12.2)
PFA EPI normal <150 sec	175.2 (91 - >300)	147.6 (79 - >292)
TPO normal 4-33 U/ mL	32 (18-59)	11.1 (7-13)
ATP/ADP-ratio normal 0-1.99	7.8 (6.8-8.8)	-
Abnormal LTA response	7	7

B

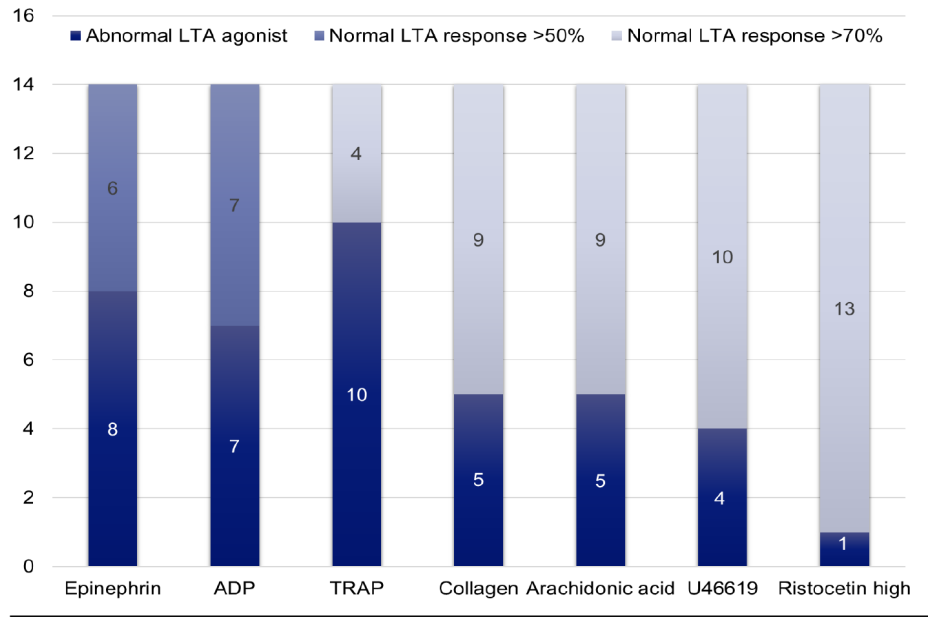

FIGURE 3 | Laboratory results of platelet count, indices, and function test. Blue lines in the box-plots visualize the 50% or 70% response lines, which are defined as the minimal adequate response. ADP = adenosine diphosphate; ATP = adenosine triphosphate; fL = femtoliter; IPF = immature platelet fraction; L = liter; LTA = light transmission agonist; mL = milliliter; MPV = mean platelet volume; *n* = number; PFA-EPI = platelet function analyzer-epinephrin cartridge; sec = seconds; TPO = thrombopoietin; TRAP = Thrombin receptor-activating peptide; U = unit.

mainly due to an increased frequency and/or severity of perioperative bleeding. In the seven patients with normal platelet counts, postsurgical bleeding occurred more frequently and/or more severely than in the group with low platelet counts (sum of all bleeding scores is 17 versus 7). Also, the intramuscular bleed occurred in two of seven patients with normal platelet counts. Vice versa, there is a higher frequency and/or severity of epistaxis in the group with the low platelet counts). We report the presence of heavy menstrual bleeding in four of five of the postmenarcheal female patients: two of five in the group with low platelet counts and two of five in the group with normal platelet counts (scores of 2 and 4, Figure 2B). Only the patient with the interstitial 11q-duplication reported no heavy menstrual bleeding.

3.3 | Platelet Count and Indices and TPO Levels

Results of most relevant laboratory findings are summarized in Figure 3. Median lowest platelet count is 149×10^9/L (range: 20–340), with seven patients (50%) having a congenital thrombocytopenia, ranging between 20 and 50×10^9/L in the neonatal period and increasing during childhood toward a range of $80–140 \times 10^9$/L at the time of inclusion. All seven patients with low platelet counts had a terminal 11q-deletion. All thrombocytopenic patients had an increased immature platelet count (IPF) with a mean of 15.3% (range: 11%–29%, normal value < 5%), compared to a mean IPF of 5.6% for the 7/14 with normal platelet counts (range: 3–8). This suggests an increased production of young platelets.

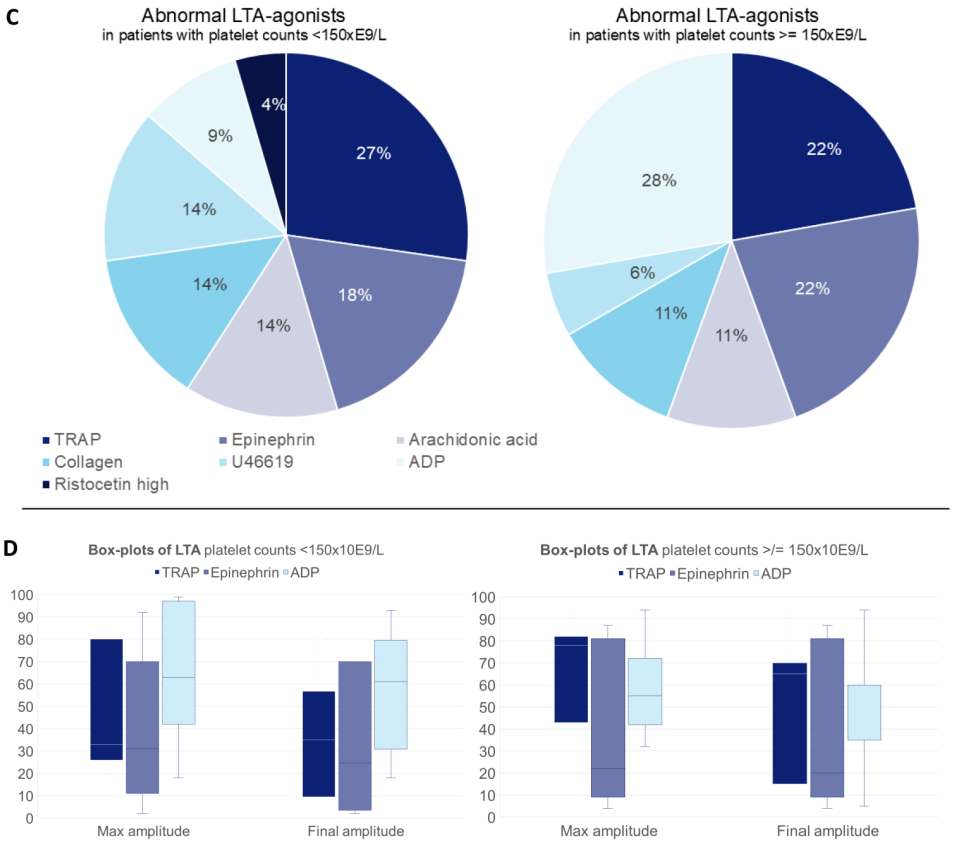

FIGURE 3 | (Continued)

Mean platelet volume (MPV) was normal in 6/7 patients with normal platelet counts (mean 10.6, only in one patient with interstitial 11q-duplication MPV > 12 fL was seen). In only three patients with low platelet counts MPV was measured due to measurement restrictions in severe thrombocytopenia, and in two, MPV was > 12 (mean 12.2 fL). The three patients with elevated MPVs ≥ 12 fL had no macroplatelets in the blood smear (3/10). Macroplatelets were reported in 3/14 (21.4%) on blood smear, but this did not lead to an elevated MPV. TPO was measured in 12 patients and elevated in two patients (16.7%) with congenital thrombocytopenia (TPO 46 and 59, normal range < 34 U/mL, Figure 3A). One patient underwent bone marrow aspiration to rule out (pre)malignant causes of neonatal thrombocytopenia before the diagnosis of terminal 11q-deletion was known (Figure 4). These images showed dysplasia in all megakaryocytes (> 10%), ranging from micromegakaryocytes to abnormal nuclei. Platelet ATP/ADP ratio was not routinely performed in this study, but was historically performed in three patients. The ratio was abnormal in 2/3 (66.7%), but results cannot be extrapolated to all patients due to possible selection bias.

3.4 | PFA, LTA, and Additional Coagulation Factor Deficiencies as Disease Modifier

Results are visualized in Figure 3. A PFA-EPI and ADP was performed in 11/14. It was prolonged ≥ 150 s in 3/11 (27.3%), without a clear correlation to platelet counts ≤ 80×10^9/L as this may bias the closure time. In all patients an LTA was performed for study purposes regardless of platelet count at inclusion. This resulted in a PRP that did not meet the standard of > 150×10E9/L in patients P6, P7, and P8 (PRP of respectively, 115, 51, and 98, with a platelet count of 80, 51, and 133×10^9/L). LTA was abnormal for all 14 patients (100%; Figure 3A). In 13/14, LTA test results were available for analysis of amplitudes (Figure 3B–D). On average, a combination of three abnormal responses on LTA agonists was seen (range: 1–7). The LTA was most often abnormal for TRAP in 10/14 (71%), for epinephrine in eight (57%), ADP in seven (50%), for collagen and arachidonic acid in five (36%), U46619 in four (29%), and ristocetin high in one patient (7%), but this patient had a PRP with only 51×10^9 platelets/L. In the seven patients with low platelet counts, the response on TRAP was more frequently abnormal than in the group with normal platelet counts, while abnormal ADP release was most often abnormal in the group with normal platelet counts. Details are depicted in Figure 3C. Additional factor deficiencies were also seen in 2/14 (14%): one patient with a low von Willebrand factor deficiency (mean VWF:Act activity of 0.50 U/L with normal FVIII activity) and one patient with FIX activity of 0.41 U/mL, which may be still physiological, as she was < 1 year at the time of inclusion.

3.5 | MS Results

We performed MS in the platelets of 10/14 patients (P1–3, 6–7, 9–13) (Figure 5). Platelet proteomics showed that in patients with 11q-disorders, the total amount of platelet proteins is significantly more abundant than in healthy controls. This did not correlate with clinical bleeding tendency, as patient 12 had the highest ISTH-BAT score and patient 13 the lowest; both were

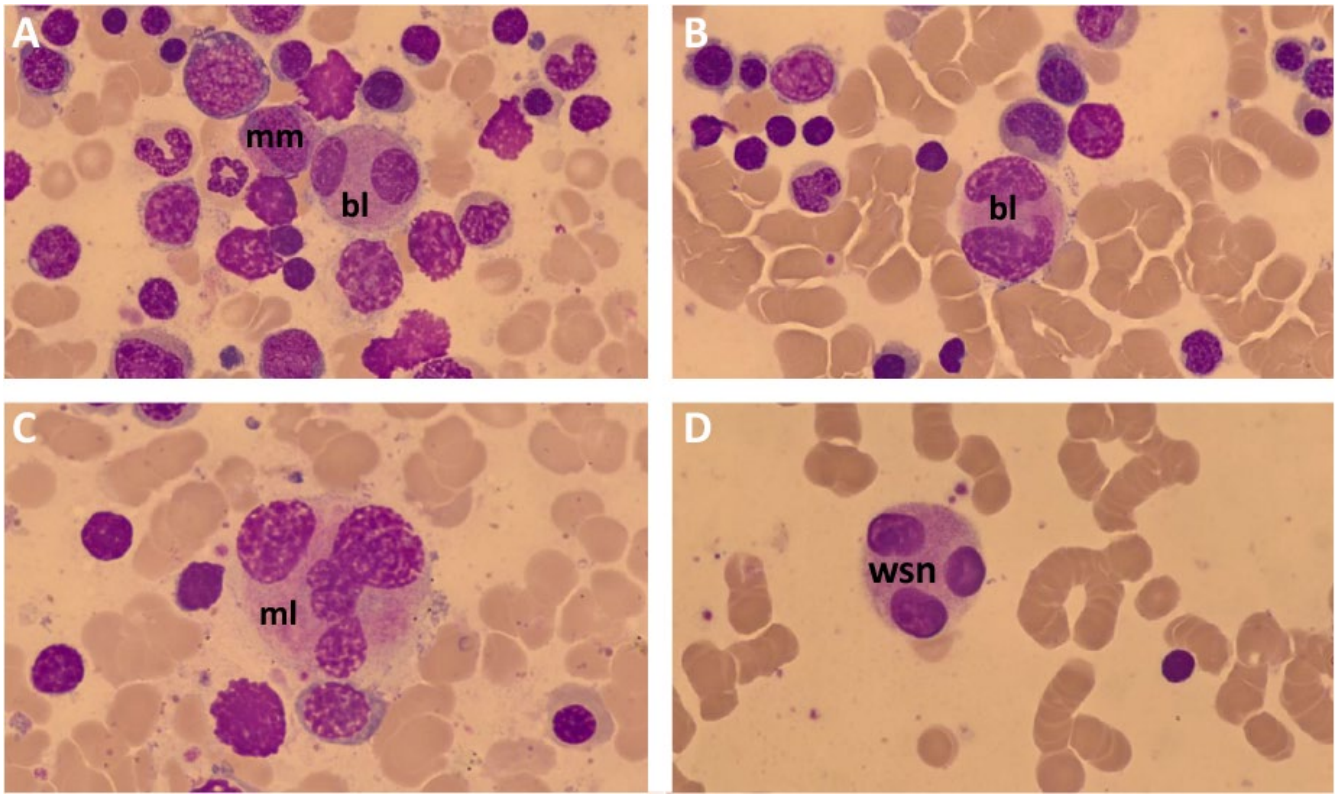

FIGURE 4 | Bone marrow morphology of one patient with a terminal 11q deletion. Bone marrow aspiration cytology (May–Grünwald Giemsa staining, magnification 1000×) shows > 10% of dysplastic megakaryocytes: Micromegakaryocytes < 12 μm, mono, bi-lobed, or multilobed megakaryocytes, as well as nonlobed nuclei megakaryocytes (not on image), and megakaryocytes with widely separated nuclei. bl = bilobed megakaryocytes; ml = multilobed megakaryocytes; mm = micromegakaryocytes; wsn = widely separated nuclei.

plotted near the healthy controls (Figure 5A). A volcano plot of identified proteins showed that multiple proteins were measured in lower or higher abundancy than in healthy controls. Only two proteins stood out: NUBP2 and ATP5S, which were significantly more present in platelets of patients with 11q-disorders than in controls (Figure 5B). Both are not located on 11q. NUBP2 is a member of the NUBP/MRP gene subfamily of ATP-binding proteins and is involved in mitochondrial function [16] ATP5S correlates with H(+)-ATP synthase, a multisubunit membrane-bound enzyme complex consisting of segments F0 and F1. F1 provides the catalytic activity for the interconversion of ADP to ATP and the H+-conduction of ATP-synthase, a very relevant step in platelet function [17] Although dysfunction of this process can be measured in abnormal ATP/ADP ratios, as is seen in two of three patients in which this ratio is tested, the exact role of NUBP2 and ATP5S in 11q-disorders needs more research.

3.6 | EM Results

We performed EM-images of platelets in 2/14 patients, one with a terminal 11q-deletion (P2) and one with an interstitial deletion (P11), Figure 6. In comparison with healthy controls, platelets of both patients showed multiple giant alpha-granules (a) as is observed in Paris–Trousseau thrombocytopathy, as well as enlarged compartments containing crystal-like structures of unknown origin (Figure 6C). These compartments are membrane enclosed, have an irregular shape, and content. Because the role of *FLI1* has been mentioned in the literature as the cause of the platelet dysfunction, we compared the EM-images of a patient with a pathogenic *FLI1* variant (c.279del, p.(Met100*)) with a low platelet count as well as platelet dysfunction to patients in our cohort. Transmission electron microscopy (TEM) analysis demonstrated the same unidentified structure and, in addition, increased amounts of open canalicular system with many fusion profiles. To our knowledge, the origin of the crystal-filled compartment is unknown, but it has been observed before by Saultier et al. [13]. Based on the description by Neumüller et al. [18] we hypothesize this is an abnormally shaped (alpha) granule, but more research is needed to confirm this hypothesis.

4 | Discussion

We present a case-series with results on bleeding symptoms in patients with a variety of 11q-disorders occurring in both female and male patients, with a 2:1 female: male ratio that is described by other authors as well [19, 20]. Our results confirm that there is an increased bleeding tendency which can already become apparent directly after birth as a result of platelet dysfunction and low platelet count in patients with terminal 11q-deletions [1, 10, 21–23]. We also demonstrate that these bleeding complications occur in nonterminal 11q-aberrations as well, by showing a platelet dysfunction in the patients with interstitial 11q-deletion and terminal 11q-duplication. So far, only one case report reported low platelet counts in a neonate with interstitial

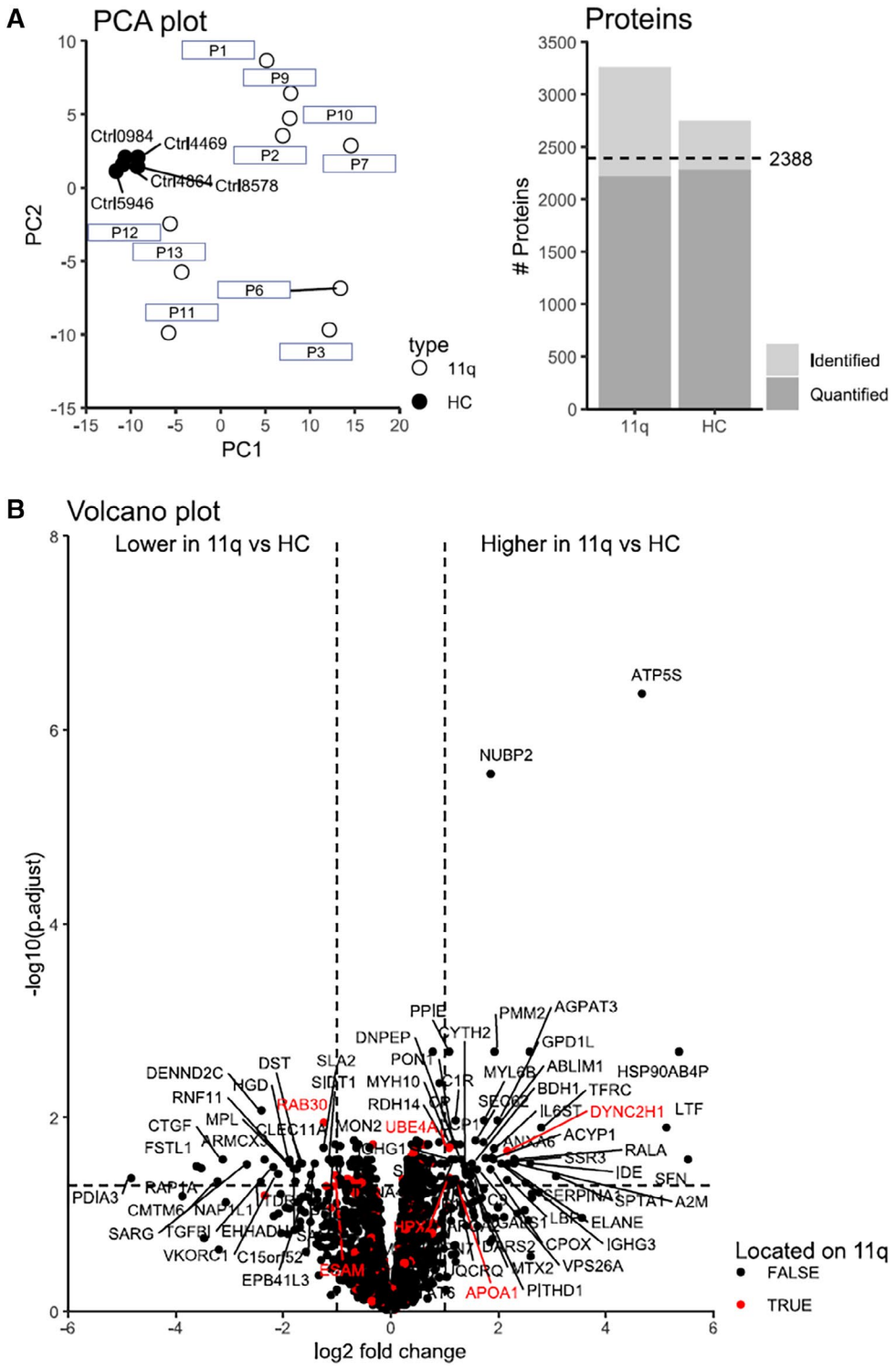

FIGURE 5 | Mass spectrometry of platelets from patients with 11q aberrations. HC = healthy control; *P* = patient; PC = principal component; PCA = principal component analysis.

11q-deletion ($30–50 \times 10^9$/L), but platelet function was not analyzed [24].

The bleeding complications for this cohort range between mild to severe. A special focus should be on the possibility of heavy menstrual bleeding in girls and on the risk of perioperative bleeding. This risk seems even higher in children with normal platelet counts. Rare but severe complications were a spontaneous vitreous bleeding and intramuscular hematoma after intramuscular vaccinations. No intracranial bleedings were reported in our cohort, but this is reported as a risk [7].

In our cohort it is remarkable that TRAP response was abnormal in 8 out of 12 abnormal LTA tests. TRAP is considered a strong agonist reflecting the cleavage of thrombin of platelet membrane proteases PAR1 and 2 and glycoprotein 1bα and V

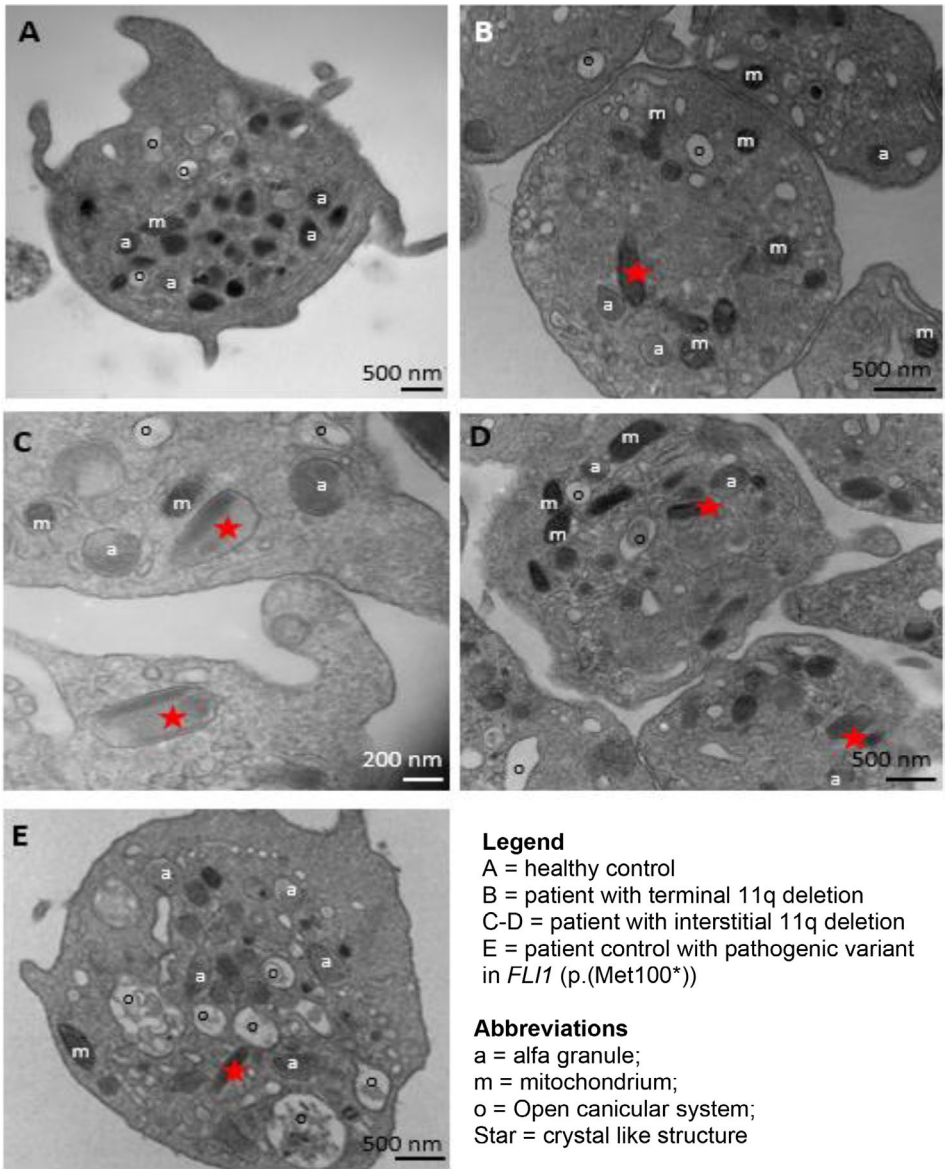

FIGURE 6 | Electron microscopic images of two patients with 11q aberrations. (A) healthy control; (B) patient with terminal 11q deletion. (C, D) patient with interstitial 11q deletion. (E) patient control with pathogenic variant in *FLI1* (p.(Met100*)). a = alpha granule; m = mitochondrion; o = open canicular system; star = crystalline structure.

[25]. Abnormal responses in TRAP have been correlated with abnormal alpha granule function [26, 27]. We also hypothesize that there is also an abnormal ATP-ADP formation in platelet mitochondria and delta granules, as we have found significantly increased NUBP2 and ATP5S in the platelet proteome, as well as the abnormal ATP/ADP ratio in three patients [9, 13]. More research to confirm this finding is needed.

The platelet dysfunction in patients with terminal 11q-deletion has been reported more often and is classified as Paris-Trousseau thrombocytopathy due to abnormal alpha-granules [1, 8, 10, 12, 28]. Our EM images confirm this relation in a patient with terminal 11q-deletion, but also demonstrate abnormal alpha granules in a patient with interstitial 11q-deletion. This has not yet been described and it confirms the clinical and hemostatic diagnosis of platelet dysfunction in the two patients in this cohort.

It has been suggested that the gene *FLI1*, located on 11q24.3, could be associated with platelet dysfunction as well as abnormal megakaryopoiesis leading to low platelet counts [8, 29, 30]. For the role of *FLI1* in the abnormal alpha granules formation, there is one case report that demonstrated abnormal alpha granules in a patient with a homozygous pathogenic single nucleotide variant in *FLI1* as well [30]. What remains unclear is why platelet dysfunction and abnormal alpha granules formation occurred in the patients with an interstitial 11q-deletion not involving the 11q23.3 region. For platelet numbers, monosomy of *FLI1* has been related to thrombocytopenia as well [29, 31, 32]. In our cohort, monosomy of *FLI1* correlated with thrombocytopenia in only 67% (six of nine) of patients with a terminal 11q-deletion involving 11q23.2. And one patient with a microdeletion after the locus of *FLI1* (11q25.0 deletion) also had low platelet counts. This discrepancy of *FLI1* and both platelet number and

function is reported by other authors as well [1, 5, 33]. Some authors therefore suggest that more than one critical region or gene is involved and that thrombocytopenia only occurs when at least three out of the following four genes are involved: *FLI1, ETS1, JAM3*, and *NFRKB* [6, 33]. But to date, it remains unclear and more research is needed.

Our study has several limitations. This study comprises a small cohort with a variety of chromosomal defects, as could be expected with a rare continuous gene syndrome. For the even more rare non-11q-deletion syndromes, more research is needed before definitive conclusions can be drawn. We can also only report on reported incidences. Due to the low numbers of patients, we cannot correct for confounders such as gender, age, or frequency of surgery performed. Lastly, although we followed a preset protocol, we have missing values. Especially when venipuncture was complicated, for example, due to young age, choices regarding further analyses were necessary. In three patients, LTA was performed when platelet counts of PRP were $<150\times10^9$/L. Although these results were interpreted with caution as guidelines underline, results may have influenced study results. Future developments regarding low-blood volume tests that are able to test platelet function in patients with very low platelet counts are warranted and may overcome these issues in the near future. However, for most patients, we obtained information on the clinical course of disease, and for the majority of patients, we could perform LTA and MS to further explain their disease trajectory.

To conclude, based on this case series, we recommend screening patients with all forms of chromosome 11q-disorders as soon as diagnosis is made and certainly before surgery is indicated. Screening should include platelet count and platelet function. When platelet function tests are difficult to obtain or results are still pending at a young age, we recommend subcutaneous vaccinations and avoidance of drugs that influence platelet function like nonsteroid anti-inflammatory drugs. Special attention should be paid to counseling female patients with 11q-disorders on potential heavy menstrual bleeding.

Author Contributions

All authors contributed to this article. E.J.H. and V.A.S.H.D. designed the study for 11q patients. E.J.H. included patients and collected data. I.C.L.K.H. and F.J.S. provided patient data from referred patients. M.J. provided and supervised the genetic data. L.P., H.J., and A.H. performed and analyzed laboratory tests. E.J.H., N.N.v.d.W., and A.H. performed analyses for figures and tables. E.J.H., V.A.S.H.D., M.d.H., and M.H.C. wrote the manuscript. All authors critically revised the manuscript, agreed with its content, and approved submission.

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Ethics Statement

The study protocol has been approved by the Medical Ethical Committee of the Erasmus MC before start of patient selection or inclusion (MEC-2013-026).

Consent

All patients or their legal care keepers have consented in participation in this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data remains available in the Erasmus MC for 15 years according to national law and regulations.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.