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The Incidence of Thrombocytopenia in Children with Cornelia de Lange Syndrome

Michele P. Lambert^{1,2}, Laird G. Jackson³, Dinah Clark⁴, Mani Kaur⁴, Ian D. Krantz^{1,4}, and Matthew A. Deardorff^{1,4}

¹Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA

²Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, PA

³Drexel University School of Medicine, Department of Obstetrics and Gynecology, Philadelphia, PA

⁴Division of Genetics, Children's Hospital of Philadelphia, Philadelphia, PA

Abstract

Thrombocytopenia was first reported in Cornelia de Lange Syndrome (CdLS) by Froster in 1993. Despite early reports, thrombocytopenia has been rarely reported in this disorder. We performed a retrospective analysis of a large cohort of patients with CdLS. We calculated prevalence of thrombocytopenia in 3 subsets of this cohort: the entire cohort (n=1740), a subset of subjects with substantial clinical records (n=695) and a subset of subjects with clinical information regarding platelet counts (n=85). This analysis revealed that 15 have had thrombocytopenia (18% of those with available blood counts); seven had Immune ThrombocytoPenia (ITP). The reported prevalence of pediatric ITP is between 5-13 per 100,000 persons. The prevalence of ITP in this cohort is between 7/1740 and 7/85, giving a relative risk of ITP of between 30 (CI 12 to 77) and 633 (CI 259-1549). Contrary to the reported cases in the literature, none of our patients have had progression of the thrombocytopenia nor have they developed other cytopenias. All 15 patients with thrombocytopenia had CdLS based on clinical criteria. Of the 10 patients tested for mutations in *NIBPL*, eight had mutations identified. These data support an increased incidence of thrombocytopenia and ITP in CdLS. Subsequently, patients are at risk for spontaneous hemorrhage, and likely increased risk secondary to the high frequency of self-injurious behavior. Although further studies are needed to better define the scope of the problem and to define the mechanisms of thrombocytopenia in CdLS, we would recommend screening for thrombocytopenia upon diagnosis and at five-year intervals thereafter.

Keywords

cornelia de lange; thrombocytopenia; ITP; immune thrombocytopenia

Introduction

Cornelia de Lange Syndrome (CdLS, OMIM 122470, 300590 and 610759) or Brachmann-de Lange Syndrome (BdLS) is a rare multisystem genetic syndrome occurring in approximately 1:10,000 live births [Opitz, 1985; Kline et al., 2007]. It is inherited in a dominant manner and shows considerable phenotypic heterogeneity [Liu and Krantz 2008].

Thrombocytopenia was first reported in a case of CdLS by Froster in 1993 [Froster and Gortner, 1993] and subsequently only two other examples have been reported [Fryns and Vinken, 1994]. These reports describe three patients, including two severely affected individuals with limb anomalies, who were diagnosed at birth with thrombocytopenia. Two of the patients subsequently progressed to pancytopenia and death in the second and third decades of life [Fryns and Vinken 1994]. Despite these reports, thrombocytopenia continues to be an infrequently noted manifestation of this disorder. Even recent reviews of CdLS do not mention thrombocytopenia as a finding [Kline et al., 2007; Liu and Krantz 2008].

One current model for pathobiology of the CdLS phenotype is based on data demonstrating gene dysregulation as a result of dysfunction of the Cohesin complex [Dorsett, 2007; McNairn and Gerton, 2008; Wendt et al., 2008]. This gene dysregulation may play a central role in the development of thrombocytopenia. Furthermore, several genetic disorders with immune dysregulation including the 22q deletion [Lawrence et al., 2003] and primary immunodeficiencies [Notarangelo, 2009] have been associated with Immune Thrombocytopenia (ITP), a common cause of thrombocytopenia in childhood.

One alternative hypothesis is based on data that members of the Cohesin complex have been shown to play multiple roles in DNA damage repair [Kim et al., 2002; Yazdi et al., 2002; Watrin and Peters, 2009]. Several other disorders that have been linked to DNA damage repair can present with both skeletal limb anomalies and thrombocytopenia. Most notably, these include Fanconi anemia and some subtypes of VACTERL association [Shaw-Smith, 2006]. Interestingly, a number of additional diagnoses combine both of these findings including, thrombocytopenia with absent radii (TAR) [Hall et al., 1969], amegakaryocytic thrombocytopenia with radio-ulnar synostosis [Thompson et al., 2001] and several rarer reports combine limb anomalies and associated thrombocytopenias [von Bubnoff et al., 2004; Sahoo et al., 2005; Kranz et al., 2007]. Based on these observations, we also hypothesized that one of the common underlying mechanisms for these disorders of thrombocytopenia and limb anomalies, including CdLS, may also involve deficiencies in DNA damage repair. Although there is no published data demonstrating a direct role of sister chromatid cohesion in regulating immune function, there is an abundance of data both clinically and molecularly that connects immune dysfunction and defects in DNA damage repair (Figure 1).

Finally, the complex process that regulates thrombopoiesis is only beginning to be understood [Geddis and Kaushansky, 2004]. Megakaryocyte maturation requires the unusual process of endomitosis, also known as endoreduplication, which is the replication of sister chromatids without subsequent cell division, to become polyploid. Megakaryocytes that do not reach sufficient ploidy are markedly less efficient at platelet production [Odell et al., 1976; Mattia et al., 2002]. It is possible that this process may be controlled by allowing replication while inhibiting mechanisms involved in sister chromatid segregation and mitosis. Since mutations that cause CdLS have been identified in *NIPBL*, *SMC1A* and *SMC3*, genes that encode Cohesin and its regulatory proteins that regulate sister chromatid cohesion [Krantz et al., 2004; Musio et al., 2006; Deardorff et al., 2007], they may also be implicated in thrombopoiesis. Given the reports of thrombocytopenia in CdLS, the role of polyploidy-dependent maturation of megakaryocytes, the high proliferative rate of bone marrow and the disrupted activity of the Cohesin complex in CdLS, we hypothesized that patients with CdLS may be at risk for abnormal megakaryopoiesis (Fig 1).

The purpose of this study was to clarify the incidence of thrombocytopenia in a large, heterogeneous cohort of patients with CdLS, to clarify the clinical subtype of the thrombocytopenia and to begin to explore potential mechanisms for thrombocytopenia in CdLS.

Materials and Methods

The available records of subjects enrolled in an ongoing IRB-approved study of CdLS at the Children's Hospital of Philadelphia were retrospectively reviewed to further characterize the prevalence of thrombocytopenia.

Patients

There were 1740 patients in the registry. DNA samples were available for 500 probands and of these, 193 subjects had identified mutations: 177 in *NIBPL*, 15 in *SMC1A* and 1 in *SMC3*. Of the 1740 patients (Fig 2), significant medical records were independently available for 695 patients (data from interviews with families eliciting extensive comprehensive medical histories). Of these, 43 subjects had complete blood counts available for at least one visit. Forty-two other subjects had recommended blood counts without documentation of the results of these studies and were presumed to be normal.

Results

Of the 43 subjects with available blood counts, 15 (35%) had at least one CBC with platelet counts $< 100 \times 10^9/\text{mL}$ indicating thrombocytopenia. Four had transient thrombocytopenia noted during the neonatal period, which resolved prior to discharge from the neonatal intensive care unit. Four other subjects had transient thrombocytopenia later in life, with 1 subject having a platelet count $< 10 \times 10^9/\text{mL}$ in the setting of severe viral infection and then slowly improving platelet count without bleeding sequelae. Interestingly, the 7 remaining subjects had ITP.

ITP features

Six of the seven subjects with ITP had chronic ITP with thrombocytopenia of > 1 year duration. ITP was diagnosed in these subjects by severe thrombocytopenia with significant response to IVIgG and/or steroids or positive antiplatelet antibodies and bone marrow findings (if available) consistent with ITP. One subject had secondary ITP in the setting of systemic lupus erythematosus. One subject with mild CdLS features presented with severe thrombocytopenia and spontaneous intracranial hemorrhage. She had bone marrow findings consistent with ITP and had a complete response to IVIgG and steroids (with a good neurologic outcome), but has since required continued therapy for intermittent thrombocytopenia.

One subject has persistent ITP that had a transient, but short-lived response to IVIgG and did not respond to steroids. She had bone marrow findings consistent with ITP with increased megakaryocytes but also had subtle, mild myelodysplastic changes without any cytogenetic abnormalities. Her platelet count has remained $< 20 \times 10^9/\text{mL}$ and the family has opted for conservative observational management.

CdLS features

Of the fifteen patients with thrombocytopenia, 10 were tested for mutations and 8 were found to have *NIPBL* mutations. Of the seven subjects with ITP, all have typical CdLS facial features, although 3 have mild cognitive and structural anomalies, while 4 have more severe phenotypes.

Comparison of ITP in CdLS with General Pediatric Population Rates

The incidence of ITP in the general pediatric population is between 5 and 10 per 100,000 children/year [Fogarty and Segal 2007]. Because most (60-80%) children present with acute ITP that resolves in less than one year, the prevalence and incidence of childhood ITP are

considered to be essentially the same as was shown in a Norwegian study demonstrating a prevalence of ITP of 4.6 cases per 100,000 [Hedman et al., 1997]. Despite our sizeable cohort of patients with CdLS, we have few documented platelet counts and no mention of thrombocytopenia in most records. Given that most of these children have undergone some type of surgical intervention, it is reasonable to assume that if significant thrombocytopenia were present, it would have been noted. Therefore, we have conservatively assumed in our calculations that subjects without a documented abnormal platelet count had normal platelet counts. This assumption will likely underestimate the true prevalence of thrombocytopenia and ITP in this population. Nonetheless, the prevalence of ITP in this cohort of CdLS subjects is 7 in 1740 subjects. Therefore, the relative risk of ITP in subjects with CdLS is 30.95 (CI 12.36 to 77.49) even with a conservative estimate of the prevalence of ITP in the general pediatric population of 13 per 100,000 children (Figure 3). Using a denominator of 695 subjects with medical records available for review (to evaluate the highest possible risk), the relative risk of ITP increases to 77.48 (CI 31 to 193.6). Thus, the true risk is likely to be in between these two estimates. Using only those patients with mention of blood counts (85) results in a relative risk of 633.5 (259.1-1549) but it likely an overestimate of risk due to selection bias.

Discussion

Cornelia de Lange syndrome is a complex congenital disorder for which the incidence is estimated at 1 in 10,000 to 30,000. Many of the features in CdLS including synophrys, long eyelashes, small hands and feet, hearing impairments, vision abnormalities, gastroesophageal reflux, seizures, cleft palate, feeding issues, heart defects, limb abnormalities and developmental delays have been well studied and presented in a number of reports. However, the hematologic involvement in CdLS has been poorly ascertained. Since the reports of three subjects in the early 1990s [Froster and Gortner 1993; Fryns and Vinken 1994] there have been no further reports of thrombocytopenia in CdLS. Furthermore, there have been no systematic studies to examine the incidence/prevalence of thrombocytopenia in this population.

This is the first study to examine the prevalence of thrombocytopenia in children with CdLS. Given that self-injurious behavior is common in CdLS, thrombocytopenia compounds the life-threatening risk of intracranial hemorrhage (ICH).

The incidence of transient thrombocytopenia is poorly known in the general pediatric population and the lack of general knowledge regarding the prevalence limits studies like these. Specifically, thrombocytopenia is common in the neonatal intensive care nursery (estimated at up to 35% of patients in neonatal intensive care units) [Sola-Visner et al., 2009]. Thus, the observation of four in 1740 CdLS cases with neonatal thrombocytopenia is unclear as to its significance. Additionally, there were four subjects with transient thrombocytopenia outside of the neonatal period. Again, due to lack of data on the prevalence or incidence of this finding in the general pediatric population, it is difficult to ascertain whether this is different than the expected rate. Although our experience (M.L.) and published pediatric normal platelet values suggests that this may be increased.

However, the incidence of ITP in the pediatric population has been well studied with an incidence of 5 per 100,000 children to as high as 13 per 100,000 [Fogarty and Segal, 2007]. Even with these estimates of incidence, the occurrence of ITP in 7 of 1740 subjects with CdLS is striking. Additionally, ITP in pediatrics is usually a benign, self-limited disease that resolves in 60-80% of patients by one year from diagnosis [Kuhne et al., 2001]. Not only is the incidence of ITP significantly higher than expected in this population ($p < 0.0005$), six of the seven subjects have chronic ITP lasting more than a year. Only one subject had ITP of

less than 1 year at the time of record review and this patient has been diagnosed for less than 1 year and has developed “persistent” ITP with poor response to therapy.

Although the mechanisms by which thrombocytopenia may be associated with CdLS are not clear at this time, we would suggest that of the three possible mechanisms envisioned at the outset of this study, gene expression dysregulation, failure of platelet production due to DNA damage repair or defects in megakaryocyte maturation, the former seems most likely.

Although this work does not directly assess the presence, gene dysregulation may result from dysfunction of the Cohesin complex [Dorsett 2007; McNairn and Gerton 2008; Wendt et al., 2008] and may alter gene expression in both B cell [Liu et al., 2009] and T-cell lineages [Hadjur et al., 2009]. Several genetic disorders with immune dysregulation including the 22q deletion [Lawrence et al., 2003] and primary immunodeficiencies [Notarangelo, 2009] have been associated with Immune Thrombocytopenia (ITP).

Alternatively, regulation of thrombopoiesis is only beginning to be understood [Geddis and Kaushansky, 2004]. Megakaryocyte maturation requires endomitosis to become polyploid. Megakaryocytes that do not reach sufficient ploidy are markedly less efficient at platelet production [Odell et al., 1976; Mattia et al., 2002]. Endomitosis may be controlled by allowing replication, while inhibiting mechanisms involved in sister chromatid segregation and mitosis. While less likely, it still may be possible that decreased rates of polyploidy-dependent maturation of megakaryocytes, the high proliferative rate of bone marrow and the disrupted activity of the Cohesin complex in CdLS, may contribute to abnormal megakaryopoiesis (Fig 1) to exacerbate suspected immune-mediated destruction of platelets or predispose to thrombocytopenia in the setting of acute illness.

This study is notably limited by the reporting of platelet counts in the collected records of our study population. Of the 1740 probands in our registry, only 43 patients had platelet counts documented. However, given that we have assumed that the remainder of subjects had normal platelet counts, this study likely underestimates the prevalence of ITP in CdLS. To characterize this further, larger, prospective studies are needed to document the both the incidence of thrombocytopenia as well as the prevalence of ITP. Through anecdotal experience, we would suggest that this decrease in platelets may have adverse consequences. We have cared for several children with severe intracranial hemorrhages, one in this study who developed it spontaneously and recovered and two who developed as a result of self-injurious behavior or falling and died. The latter of these had low-normal platelet counts.

Based on this data, we would recommend platelet counts at the time of diagnosis, with any unusual bleeding or bruising and, if asymptomatic, at five year intervals. If thrombocytopenia is noted, referral should be made to a hematologist for further characterization of the etiology and management of the low platelet count. Finally, further studies to examine the immune regulation in these patients will be required to lend insights into both the increased incidence of ITP in CdLS as well as to extend our understanding of ITP in the greater pediatric population.

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References

- Deardorff MA, Kaur M, Yaeger D, Rampuria A, Korolev S, Pie J, Gil-Rodriguez C, Arnedo M, Loey B, Kline AD, Wilson M, Lillquist K, Siu V, Ramos FJ, Musio A, Jackson LS, Dorsett D, Krantz ID. Mutations in cohesin complex members SMC3 and SMC1A cause a mild variant of cornelia de Lange syndrome with predominant mental retardation. *Am J Hum Genet.* 2007; 80:485–494. [PubMed: 17273969]
- Dorsett D. Roles of the sister chromatid cohesion apparatus in gene expression, development, and human syndromes. *Chromosoma.* 2007; 116:1–13. [PubMed: 16819604]
- Fogarty PF, Segal JB. The epidemiology of immune thrombocytopenic purpura. *Curr Opin Hematol.* 2007; 14:515–519. [PubMed: 17934361]
- Froster UG, Gortner L. Thrombocytopenia in the Brachmann-de Lange syndrome. *Am J Med Genet.* 1993; 46:730–1. [PubMed: 8362921]
- Fryns JP, Vinken L. Thrombocytopenia in the Brachmann-de Lange syndrome. *Am J Med Genet.* 1994; 49:360. [PubMed: 8209903]
- Geddis AE, Kaushansky K. Inherited thrombocytopenias: toward a molecular understanding of disorders of platelet production. *Curr Opin Pediatr.* 2004; 16:15–22. [PubMed: 14758109]
- Hadjur S, Williams LM, Ryan NK, Cobb BS, Sexton T, Fraser P, Fisher AG, Merkenschlager M. Cohesins form chromosomal cis-interactions at the developmentally regulated IFNG locus. *Nature.* 2009; 460:410–413. [PubMed: 19458616]
- Hall JG, Levin J, Kuhn JP, Ottenheimer EJ, van Berkum KA, McKusick VA. Thrombocytopenia with absent radius (TAR). *Medicine (Baltimore).* 1969; 48:411–39. [PubMed: 4951233]
- Hedman A, Henter JI, Hedlund I, Elinder G. Prevalence and treatment of chronic idiopathic thrombocytopenic purpura of childhood in Sweden. *Acta Paediatr.* 1997; 86:226–227. [PubMed: 9055900]
- Kim ST, Xu B, Kastan MB. Involvement of the cohesin protein, SMC1, in Atm-dependent and independent responses to DNA damage. *Genes Dev.* 2002; 16:560–70. [PubMed: 11877376]
- Kline AD, Krantz ID, Sommer A, Kliewer M, Jackson LG, FitzPatrick DR, Levin AV, Selicorni A. Cornelia de Lange syndrome: clinical review, diagnostic and scoring systems, and anticipatory guidance. *Am J Med Genet A.* 2007; 143A:1287–1296. [PubMed: 17508425]
- Krantz ID, McCallum J, DeScipio C, Kaur M, Gillis LA, Yaeger D, Jukofsky L, Wasserman N, Bottani A, Morris CA, Nowaczyk MJ, Toriello H, Bamshad MJ, Carey JC, Rappaport E, Kawauchi S, Lander AD, Calof AL, Li HH, Devoto M, Jackson LG. Cornelia de Lange syndrome is caused by mutations in NIPBL, the human homolog of *Drosophila melanogaster* Nipped-B. *Nat Genet.* 2004; 36:631–635. [PubMed: 15146186]
- Kranz C, Basinger AA, Gucsavas-Calikoglu M, Sun L, Powell CM, Henderson FW, Aylsworth AS, Freeze HH. Expanding spectrum of congenital disorder of glycosylation Ig (CDG-Ig): sibs with a unique skeletal dysplasia, hypogammaglobulinemia, cardiomyopathy, genital malformations, and early lethality. *Am J Med Genet A.* 2007; 143A:1371–1378. [PubMed: 17506107]
- Kuhne T, Imbach P, Bolton-Maggs PH, Berchtold W, Blanchette V, Buchanan GR. Newly diagnosed idiopathic thrombocytopenic purpura in childhood: an observational study. *Lancet.* 2001; 358:2122–2125. [PubMed: 11784627]
- Lawrence S, McDonald-McGinn DM, Zackai E, Sullivan KE. Thrombocytopenia in patients with chromosome 22q11.2 deletion syndrome. *J Pediatr.* 2003; 143:277–8. [PubMed: 12970648]
- Liu J, Krantz ID. Cohesin and human disease. *Annu Rev Genomics Hum Genet.* 2008; 9:303–320. [PubMed: 18767966]
- Liu J, Zhang Z, Bando M, Itoh T, Deardorff MA, Clark D, Kaur M, Tandy S, Kondoh T, Rappaport E, Spinner NB, Vega H, Jackson LG, Shirahige K, Krantz ID. Transcriptional dysregulation in NIPBL and cohesin mutant human cells. *PLoS Biol.* 2009; 7:e1000119. [PubMed: 19468298]
- Mattia G, Vulcano F, Milazzo L, Barca A, Macioce G, Giampaolo A, Hassan HJ. Different ploidy levels of megakaryocytes generated from peripheral or cord blood CD34+ cells are correlated with different levels of platelet release. *Blood.* 2002; 99:888–97. [PubMed: 11806991]
- McNairn AJ, Gerton JL. Cohesinopathies: One ring, many obligations. *Mutat Res.* 2008; 647:103–111. [PubMed: 18786550]

- Musio A, Selicorni A, Focarelli ML, Gervasini C, Milani D, Russo S, Vezzoni P, Larizza L. X-linked Cornelia de Lange syndrome owing to SMC1L1 mutations. *Nat Genet.* 2006; 38:528–530. [PubMed: 16604071]
- Notarangelo LD. Primary immunodeficiencies (PIDs) presenting with cytopenias. *Hematology Am Soc Hematol Educ Program.* 2009:139–143. [PubMed: 20008192]
- Odell TT, Murphy JR, Jackson CW. Stimulation of megakaryocytopoiesis by acute thrombocytopenia in rats. *Blood.* 1976; 48:765–775. [PubMed: 974268]
- Opitz JM. The Brachmann-de Lange syndrome. *Am J Med Genet.* 1985; 22:89–102. [PubMed: 3901753]
- Sahoo T, Naeem R, Pham K, Chheng S, Noblin ST, Bacino CA, Gambello MJ. A patient with isochromosome 18q, radial-thumb aplasia, thrombocytopenia, and an unbalanced 10;18 chromosome translocation. *Am J Med Genet A.* 2005; 133A:93–98. [PubMed: 15637724]
- Shaw-Smith C. Oesophageal atresia, tracheo-oesophageal fistula, and the VACTERL association: review of genetics and epidemiology. *J Med Genet.* 2006; 43:545–554. [PubMed: 16299066]
- Sola-Visner M, Sallmon H, Brown R. New insights into the mechanisms of nonimmune thrombocytopenia in neonates. *Semin Perinatol.* 2009; 33:43–51. [PubMed: 19167581]
- Thompson AA, Woodruff K, Feig SA, Nguyen LT, Schanen NC. Congenital thrombocytopenia and radio-ulnar synostosis: a new familial syndrome. *Br J Haematol.* 2001; 113:866–870. [PubMed: 11442476]
- von Bubnoff D, Kreiss-Nachtsheim M, Novak N, Engels E, Engels H, Behrend C, Propping P, de la Salle H, Bieber T. Primary immunodeficiency in combination with transverse upper limb defect and anal atresia in a 34-year-old patient with Jacobsen syndrome. *Am J Med Genet A.* 2004; 126A:293–298. [PubMed: 15054845]
- Watrin E, Peters JM. The cohesin complex is required for the DNA damage-induced G2/M checkpoint in mammalian cells. *EMBO J.* 2009; 28:2625–35. [PubMed: 19629043]
- Wendt KS, Yoshida K, Itoh T, Bando M, Koch B, Schirghuber E, Tsutsumi S, Nagae G, Ishihara K, Mishiro T, Yahata K, Imamoto F, Aburatani H, Nakao M, Imamoto N, Maeshima K, Shirahige K, Peters JM. Cohesin mediates transcriptional insulation by CCCTC-binding factor. *Nature.* 2008; 451:796–801. [PubMed: 18235444]
- Yazdi PT, Wang Y, Zhao S, Patel N, Lee EY, Qin J. SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint. *Genes Dev.* 2002; 16:571–582. [PubMed: 11877377]

Figure 1. Schematic representation of potential mechanisms for thrombocytopenia

(A) Cohesin mediated direct effects on sister-chromatid adhesion result in dysregulation of endomitosis and dysfunctional megakaryopoiesis. This results in not only decreased platelet production but also inability to respond appropriately to transient insults to platelet count resulting in an increased incidence of thrombocytopenia. (B) Cohesin effects on gene expression result in dysregulated B-cell function resulting in autoantibody production and ITP.

Figure 2. Schematic representation of chart review

Schematic representation of fate of 1740 charts identified for review. Gray boxes with heavy borders are emphasized in this report.

Figure 3. Incidence of ITP in subjects with CdLS versus healthy control
Relative risk of ITP in subjects with CdLS was 30.95 (CI 12.36 to 77.49).