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Original research

Copy number variations in adult patients with chronic immune thrombocytopenia

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Abstract

Objectives: Immune thrombocytopenia (ITP) is an autoimmune disease with a heterogeneous background. *FCGR2C* mutations were defined in one third of the patients but exact genetic players have not been fully elucidated yet. Although childhood ITP present as benign and self-limiting, ITP in adulthood is a chronic disease with treatment challenges. This study aimed to focus on adult ITP patients by using a whole genome genotyping that is a valuable approach to identify the responsible genomic regions for the disease phenotypes of complex disorders.

Methods: Herein 24 adult primary-refractory for ITP patients were evaluated using HumanCytoSNP-12BeadChip, Illumina. Forty-six age and sex matched healthy individuals, and patients with non-hematological conditions were also analyzed as controls. Identified CNV regions were verified by qRT-PCR. T-cell receptor beta and delta (TCRB/TCRD) clonality were also assessed by heteroduplex analysis in mosaic cases.

Results: Several CNV losses and gains were defined (losses:2q,7q,17q,19p, and gains: 1q,2p,3q,4q,7q,10q,12p,13q,14q,15q,17p,20q,21p,22q,Xp). Mosaic structural changes of different sizes (0.2-17.77Mb) were identified in five patients and three of them showed TCRG/TCRD clonality. CNV regions that were unique to ITP patients were also identified for the first time and among these genes, those related to immune regulation, cellular trafficking, and cytoskeleton assembly were noteworthy.

Conclusion: Identified CNV regions harbor several candidate genes, the functions of which might shed light on the pathogenesis of chronic ITP

Key words

Immune thrombocytopenia, copy number variation, single nucleotide polymorphism, whole genome genotyping, clonality

1. Introduction

Immune thrombocytopenia (ITP) is a complex autoimmune disease characterized by a low platelet count resulting from destruction of platelets by platelet reactive autoantibodies and suppression of megakaryopoiesis [1]. Adult-onset ITP usually exhibits a relapsing chronic course and is often associated with other disorders of autoimmune or infectious origins, including systemic lupus erythematosus (SLE), lymphoproliferative diseases, common variable immunodeficiency (CVID) and human immunodeficiency virus (HIV) infection. Chronic ITP affects approximately 4 in 100.000 adults [2].

ITP is mainly caused by autoantibodies against platelet membrane glycoprotein (GP) complexes, GPIIb/IIIa (CD41/CD61) and GPIbIX (CD42b and CD42a). In some patients medullary megakaryopoiesis and platelet production are suppressed, while in others this might be normal. Evidence also suggests that antiplatelet antibodies not only lead to the destruction of platelets, but they also cause impairment in platelet production and function [3]. Among the proposed mechanisms of the platelet destruction in the spleen, the prevailing one is the F_C-$F_C$gamma receptor (FcγR) mediated phagocytosis by macrophages. However, treatment strategies targeting FcγR generally lead to transient responses or even fail totally in patients with anti-GPIba mediated ITP [4,5]. FcγR independent platelet destruction might underlie some of the refractory cases, including those that do not respond to splenectomy.

Studies in neonates, monozygotic twins and families enlightened the genetic background of ITP [7-9]. Breunis *et. al.* identified copy number variations (CNVs) on chromosome 1q23-24 where the Fcγ genes are located by using *FcγR* -specific multiplex ligation-dependent probe amplification assay and reported FcγRIIc activation in approximately 30% of ITP patients [10]. Given the fact that the familial cases comprise of 2% of all ITP patients only, and sporadic cases are highly heterogeneous it is not an easy task to study the genetic background of the disease.

Next generation sequencing findings in children showed novel mechanism and candidate genes that take place in the etiology of this complex disorder. Chronic ITP is a complex disorder with several immune deregulations, but contribution of genetic factors and their effects on progression and treatment are still unknown. SNP/CNV array is a valuable approach to identify the responsible genomic regions for the complex phenotypes. There is no previous report on genome wide CNV in adult ITP. In this study we focused on chronic ITP in adults to better understand the possible

molecular mechanisms for the susceptibility and our genome wide study identified unique CNV regions that suggest novel candidate genes and mechanisms.

2. Materials and methods

2.1. Patients

Blood samples of 24 adult patients (seven males and 17 females) with chronic ITP aged 23-80 years were enrolled. All patients underwent an extensive evaluation for secondary causes including bone marrow biopsy. At study entry, patients showed no clinical or laboratory findings associated with autoimmune diseases, no malignancy or infections. Patient characteristics are shown in Table 1. Ten patients underwent splenectomy for thrombocytopenia not controlled by corticosteroid therapy. Four of these patients showed steroid dependence with complete response, two of them showed steroid dependence with partial response and four of them were steroid resistant.

Additionally, 46 age and sex matched healthy individuals (mean age 39.42 years (min:18-max:77) and patients with non-hematological conditions were also analyzed as controls. Seventeen of these individuals were diagnosed with intellectual disability with epilepsy, 11 of them were isolated epilepsy and five were diagnosed as cerebellar ataxia. The remainder of 13 individuals were healthy controls. The study was approved by the Clinical Research Local Ethics Committee of Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty (26 July 2016/ 270037).

2.2. Copy number variation analysis

Genomic DNA was extracted from the peripheral blood of the ITP subjects, and controls by using a Genomic DNA Purification Kit (Qiagen, Valencia, CA). Absorbance measurements were performed with NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) to verify DNA purity and adjust DNA concentration. SNP array was performed on 24 samples with primary ITP cases using Illumina HumanCytoSNP-12 BeadChip, with nearly 300,000 oligonucleotide probes and an average spacing of 9.7 kb across the whole array, and were processed according to the manufacturer's instructions. CNVs and LOH (loss of heterozygosity) were visualized in the Illumina GenomeStudio software (Illumina, San Diego, CA). The genotyping data was obtained by further analyzes in terms of CNV and CNV partition V1.3. Genes that overlapped with the CNV regions were reported according to UCSC genes (GRCh37/hg19). GeneMANIA (http://www.genemania.org) and STRING (https://string-db.org/) algorithms were used to produce functional predictions to prioritize the genes or pathways.

2.3. qRT-PCR validation of CNV regions

Increased and decreased CNVs were validated by gene dosage analysis. Quantitative real time PCR (qRT-PCR) was carried out on the Light Cycler 480 Instrument (Roche Applied Sciences, Mannheim, Germany). Gene dosage analysis was performed with LC480 SYBR Green I Master (Roche) as described in the protocol. For normalization *MRPL28* gene was used as a housekeeping gene. To validate the findings two representative regions, one copy and three copies of CNV regions (19q and 2p respectively) were selected. In 19q region *CYP2B6* locus, (forward 5'GCCATACACAGAGGCAGTCA and reverse 5'TCGGGGATTAAGAGAATCCA) and in 2p region *LCLAT1* locus (forward 5'TTCCTGTGGAATTGCCTGAT and reverse 5'AAATGGAATCGCTTTAATGCTT) was validated. A total of 50 ng of genomic DNA was used in each PCR reaction, which was conducted in triplicates. PCR conditions were as follows; 5 minutes at 95°C followed by 35 cycles of 30 seconds of denaturation at 95°C and 30 seconds of annealing 60°C and 30 seconds of extension at 72°C. Melting curve analysis was performed to check the product specificity. Delta Ct method is used to calculate the relative gene dosage.

2.4. Clonality Analysis for TCRG and TCRD

All the cases that were evaluated as mosaic were also evaluated for *TCRG* (T-cell receptor gamma) and *TCRD* (T-cell receptor delta) clonality. PCR amplifications were performed using BIOMED-2 protocol; for *TCRG* (*TCRGA:Vγl/γ10-Jγ* and *TCRGB:Vγ9/γ11-Jγ*) and for *TCRD (VD1-6/JD1-3)* regions [11]. For heteroduplex analysis, PCR products were denatured at 95^0C for 10 min. and renaturated at 4^0C for one hour. Samples were loaded on 8% non-denaturing polyacrylamide (acrylamide:bisacrylamide=29:1) gel and run at 100V for 50 minutes. Gel images were examined for clonality analysis. One or more discrete bands that are identified within the expected ranges (*TCRGA*:145bp.-255bp., *TCRGB*:80bp.-220bp. and TCRD:12bp-280bp) were interpreted as clonal.

3. Results

3.1. Common and unique CNV regions in adult ITP patients

In this study, using SNP/CNV analysis we detected several common regions as well as ITP related unique genomic regions. Regions that were previously described in the healthy population as polymorphic variants or identified in patients with autoimmune disorders are given in Supplemental data 1.

We identified 19 CNV regions that were solely present in 14 ITP patients (Table 2). None of these unique CNV regions were found in the two control groups consisting of patients with non-

hematological, non-autoimmune disorders, and healthy individuals, respectively. We used two-step exclusion pipeline to find out CNV regions that unique to ITP patients. Firstly in house data from healthy parents (mother and fathers) and patients with different neurological disorders including (mainly epilepsy) as mentioned above that was obtained from our previous studies was used to exclude the common CNV regions. These regions were further analyzed in detail for their probable role in ITP pathogenesis. The size of the rearrangements ranged from 0.06 Mb to 17.77 Mb. (Table 3). In six patients no CNV region was detected at standard resolution. Furthermore, three CNV regions contained no genes. Verification of the identified CNV regions (one, two and three copies) was done by gene dosage analysis for two genes (*CYP2B6* and *LCLAT1*), which were known to fall into these regions (Figure 1).

3.2. Mosaic patterns and TCRγδ clonality in ITP patients

In 20% of our patients (5/24) mosaic structural genomic rearrangements were detected. These mosaic duplications were found at 9q21.2-9q21.3(10.89Mb), 9q31.2-9q33.3(17.77Mb), 14q13.2-14q13.3(0.37Mb), 17p12(1.46Mb), 20q11.21-20q13.13(14.24Mb), and 22q11.21(0.16Mb) (Figure 2 and Table 2). Three of the mosaic cases showed complete/partial response to steroid treatment and no splenectomy was performed, whereas the two mosaic patients who were resistant to steroid therapy, were splenectomized and received additional IVIG and eltrombopag therapy. To understand if the mosaic patterns are associated with a clonal expansion of T- cells, we checked our patients with mosaic findings for TCRγδ clonality. Three out of five patients showed a clonal background; clonality for *TCRγ* was detected in patient #14 whereas *TCRδ* clonality was seen in patients #18 and #27 (Supplemental data 2). Two of our patients (patients #14 and #18) were resistant to steroid therapy and one (patient #27) showed a partial response.

3.3. Functional prediction of the identified de novo CNVs and ITP

We determined several protein coding and non-coding genes in the CNV regions that were solely found in ITP patients. Genes residing in these regions encode proteins, that are crucial components of different intracellular signaling pathways, which are involved in the immune response, cellular trafficking and endocytosis, cytoskeleton assembly, histone modification and chromatin remodeling, proteolysis and transport, metabolic processes and GTPase activity (Table 3).

4. Discussion

Immune thrombocytopenia is a heterogeneous disease, of which the genetic background is still unclear. Only 2% of the ITP cases were reported to be familial [9.12]. Here, we defined gains/losses, up to several mega bases in size, in different chromosomal regions of patients with chronic ITP. Although gains/losses involving large areas are mostly considered to be pathogenic, SNP/CNVs, recent studies using microarray analyses reported deletions (some 1.5Mb in size) in phenotypically normal individuals as well [13]. The rare and unique CNVs are usually disease or patient specific and hard to be interpreted as pathogenic [14]. Hence in this study, in addition to the public databases, e.g. DECIPHER (https://decipher.sanger.ac.uk/), we used independent in-house cohorts (consisting of individuals who have non-hematological and non-autoimmune diseases, as well as healthy individuals) to identify the ITP specific CNV regions. Most of the detected CNV regions were located in close proximity to or within the segmental duplication regions. These regions are containing the genes for immune system members and mediators, as previously shown [15].

Each ITP patient showed unique deviations from normal which was not shared by other ITP patients and was also not present in the control groups. First, we focused on the regions that have been shown to act in platelet destruction mechanisms in ITP. One of the most well known genes associated with the platelet destruction is *FCGR,* which is located on 1q23 [10]. However no CNVs could be identified in this region in any of our ITP patients and when the flanking regions were analyzed, CNVs were found in 1q21.3 (3 copies) and 1q25.3 (4 copies) regions but these were also detected in other control groups.

The FCGR mediated activation of *SRC* proto-oncogene (non-receptor tyrosine kinase) (20q11) has been reported to lead to platelet destruction by triggering phagocytic functions of macrophages [16,17]. In our study the 20q11 region was found in 3 copies in patient #18. The patient was also mosaic and showed *TCRD* clonality. He was resistant to steroid therapy, was given azathiopirin, splenectomy could not be performed and later he developed bladder and prostate cancer. Amplification of this region has been linked to malignant transformation in earlier reports [18]. This region harbors several genes that are mainly involved in cell trafficking, cytoskeleton constitution and immune response, i.e. important components of platelet function and ITP pathogenesis.

The ITP specific CNV regions consist of several candidate genes that might be related to platelet production, activation and immune system maintenance (Table 3). One of the most

interesting findings of this study was the frequent occurrence of genes responsible for primary immunodeficiency in the CNV regions. Recently a gain of function mutation in the *TNFRSF13B* (TNF Receptor Superfamily Member 13B) gene has been described in the members of an ITP family. This mutation was shown to lead to immune dysfunction and to induce megakaryocyte apoptosis [19]. *TNFRSF13B* encodes TACI protein, which is expressed on B-cells and is also mutated in patients with Common Variable Immune Deficiency (CVID) [20]. The TNF Receptor Superfamily Members; *TNFSF15 and TNFSF8*) that are involved in T-cell mediated immune response and IgG class switch reside in 9q21.2 region and were detected as 3 copies in our study. Another gene located on the same region was *AT-Hook Transcription Factor* (*AKNA)* which specifically activates the CD40 (TNFRSF5), is a known autoimmunity gene and is crucial for immune response modulation [21, 22] Cui et. al recently reported aberrant expression of CD40/40L in the peripheral blood samples of primary immune thrombocytopenia patients [23]. Other genes that were identified on that locus were *Ubiquitin Protein Ligase* (*ITCH)* and *SAM And HD Domain Containing Deoxynucleoside Triphosphate Triphosphohydrolase 1* (*SAMHD1)* were also previously associated with immune deficiency. They were both located on 19q13.13 and play a role in the regulation of innate immune response and there is previous evidence about *SAMHD1* involvement into the interferon-induced inflammation process in autoimmune diseases [24]. Another gene located on the same CNV region was *Adenosine Deaminase (ADA)* and heterozygous ADA mutations also reported in adult patients with immune deficiency or lymphopenia [25].

Platelets undergo dramatic shape changes during the platelet activation by the action of membrane skeleton, microtubule, and microfilament members. Changes in the platelet cytoskeleton play a crucial role in granule movement and exocytosis, which is not clearly understood. Platelet derived extracellular vesicles (EVs) have roles in mediating inflammatory response and have been attributed to cause autoimmune disease [26,27]. Furthermore, cytoskeletal proteins were reported to be essential for the proplatelet formation by megakaryocytes. Deficiencies of these proteins have been associated with morphological, qualitative and quantitative abnormalities of platelets [28]. Findings of this study also showed several genes coding cytoskeleton and migration (*DOCK4, CYLC2, EPB41L4B, PTPN3, FKBP15, KIF1, MDGA2, HS3ST3B1, DYNLRB1, PIGU*), calcium binding proteins (*TMEM38B, NECAB3*), intracellular proteases (*ABHD17B, PCSK5, EDEM2, PROCR*), and vesicular transport proteins (*LCLAT1, TRPM6, AMBP, TVP23C, CHMP4B, CPNE1, RPN2, RALGAPB, SLC32A1, FITM2, YWHAB*) that function in platelet granule secretion, were found to be very common in the CNV

regions that were detected only in ITP patients.

One of the most interesting findings of this study was the detection of mosaic patterns (20% of all the ITP cases), which are commonly found in clonal disorders. A large scale GWAS study showed that clonal mosaicism in the normal population was relatively low (<0.5%) between birth and 50 years of age. In older ages (>50yrs), peripheral blood mosaicism has been reported to occur at a rate of 2-3% and at regions that were previously linked to cancers including hematological malignancies [29]. Since patients with dysplasia and/or atypical cells in the bone marrow were excluded from this study, the detected clonality was assumed to be related to ITP. These patients will be monitored for long term malignancy development as well. There is evidence that T-cells have important roles in ITP pathogenesis [30]. Previous studies have shown an increased number of TCRγδ positive T-cells in children with acute and chronic ITP [31]. Also, it is reported that patients with clonality showed better therapeutic responses to immunosuppressive drugs or splenectomy than the ones without clonality. The presence of a clonal background might be associated with the therapeutic success rather than the severity of ITP [32]. Two of our ITP patients (#14 and #27) that were also found to be clonal were therapy resistant and unlike the previous findings, patients with clonality were complete/partial resistant to steroid therapy and needed additional treatments. To have a better understanding of the association between clonality and therapy response a larger cohort of refractory patients should be evaluated both for B-cell and T-cell clonality.

The heterogeneity of the clinical phenotype and the variable course of the disease are indirect signs for the multifactorial etiology of ITP. However, genetic predisposition is a prerequisite for ITP to develop, given the fact, that environmental factors help trigger the disease in genetically susceptible individuals only, i.e., not everyone facing with the environmental trigger acquires the disease. Furthermore, there is a wide range of inter-individual variability of response to immunosuppression and/or splenectomy, which is additional indirect evidence for the genetic heterogeneity that underlies the pathogenesis involved in deregulated immune function. Recently, genome wide studies done in familial or sporadic cases identified novel ITP related genes or mechanisms. Encouraged by this, we performed a genome wide approach for evaluating the genetic background of chronic primary ITP to identify unique chromosomal changes which would help explain the increased genetic susceptibility to autoimmunity, the mechanism of disease and the probable actors taking part in the immune dysfunction. This is the first genome wide SNP/CNV array study in adults that identified some rare unique CNV regions which seem to encode major actors in the pathogenesis of ITP. These regions

might have a modest impact on the disease development via minor genes and some rare variants unique for chronic ITP, which seem to encode major actors in the pathogenesis of ITP. One of the strengths of the SNP array was the detection of mosaic regions in this study; we detected mosaic patterns in the adult ITP patients more than the expected ratio, compared to the healthy population in a similar age group. Long term follow up of these patients would explain if this pattern will be associated with a malign transformation. Only half of the mosaic cases showed T-cell clonality and these also seemed to be correlated to steroid resistance (2 in 3) in our cohort, but this study should be extended with a larger ITP cohort with more refractory cases.

5. Conclusion

The findings of this study have to be evaluated in light of some limitations. Given the fact that ITP is an autoimmune disorder with considerable heterogeneity in terms of genetic susceptibility and environmental influence the non-homogenous composition and the relatively small size of the study group categorically precludes formation and statistical comparison of uniform clinical and/or genetic subgroups and hence make it difficult to draw solid conclusions about the findings. Consequently, results of this study should be regarded as the initial steps to define the research area in this field of medicine and therefore be cautiously evaluated and validated in homogenous patient populations with ITP. Lastly, findings in this study proposed novel candidate regions for the genetic susceptibility of ITP. The findings of this study can provide insights for the analysis of ITP patients by next generation sequencing which can detect specific variants in the genes that are residing in these regions. Future research with high-resolution approaches and a higher number of cases will shed light on revealing the possible roles of these genes and may serve as a potential target for diagnosis, treatment or prognosis.

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Author Contributions

Conception – M. Cem Ar, Muge Sayitoglu, Emrah Yucesan; Design – Emrah Yucesan, Ozden Hatirnaz Ng, Muge Sayitoglu; Supervision – Ugur Ozbek, Teoman Soysal, Zafer Baslar, Seniz Ongoren,; Fundings – M. Cem Ar, Muge Sayitoglu, Uguz Ozbek; Materials – Emrah Yucesan, Fevzi Firat Yalniz, Hulya Yilmaz, Ayse Salihoglu, Tugce Sudutan; Data Collection And/Or Processing – Emrah Yucesan, Ozden Hatirnaz Ng, Fevzi Firat Yalniz, Hulya Yilmaz, Ayse Salihoglu, Ahmet Emre Eskazan, Tugce Sudutan ; Analysis And/Or Interpretation – Emrah Yucesan, Ozden Hatinaz Ng, Muge Sayitoglu; Literature Review - Ugur Ozbek, Teoman Soysal, Zafer Baslar, Seniz Ongoren, Ahmet Emre Eskazan; Writer – Emrah Yucesan, Ozden Hatirnaz Ng, Muge Sayitoglu, M. Cem Ar.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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** of considerable interest

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Figure Captions

Figure 1: Gene dosage validation of two regions by qRT-PCR. The genes *CYP2B6* (A) and *LCLAT1* (B) were studied in triplicates, by qRT-PCR. The values were normalized to *MRPL28* housekeeping gene. Melting curve analysis was performed to check the product specificity. Delta Ct method is used to calculate the relative gene dosage.

Figure 2: Mosaic regions identified in only ITP patients. The black boxes are covering the regions that are found to be mosaic in ITP patients. A) 9q21.2-9q21.3(10.89Mb), B) 22q11.21(0.16Mb), C) 20q11.21-20q13.13(14.24Mb), D) 17p12(1.46Mb), E) 14q21.1 (0.32Mb)

Table 1: Characteristics of ITP patients. A total of 24 ITP patients with no autoimmune disease or infections were enrolled in the study. ***Complete response*:** platelet count >100x10^9/L, ***Partial response***: platelet count 30-100x10^9/L, ***Steroid dependent***: who need to use corticosteroids continuously or intermittently for at least 2 months to prevent bleeding or to increase the platelet count over 30X10^9. M: Male, F: Female, IVIG: Intravenous Immunoglobulin

Patient ID	Age at recruitment	Sex	Clinical presentation	Steroid response	Platelet count at recruitment (x10^9/L)	Splenectomy	Current medications	Prior treatments
ITP-1	47	F	Ecchymosis	Complete response	260	No	None	Steroid, IVIG
ITP-4	36	M	Ecchymosis	Complete response, steroid dependent	189	No	None	Steroid
ITP-5	23	F	Ecchymosis, hemorrhagic bulla	Complete response	10-20	No	Steroid	Steroid
ITP-6	26	F	Ecchymosis	Complete response	186	No	None	Steroid
ITP-9	41	F	Asymptomatic	Complete response, steroid dependent	40	Yes	Eltrombopag	Steroid, azathioprine
ITP-10	31	F	Vaginal bleeding	Partial response	115	No	None	Steroid, IVIG, azathioprine
ITP-11	35	F	Epistaxis	Partial response, steroid dependent	424	Yes	None	Steroid
ITP-13	32	F	Ecchymosis, mucosal bleeding	Steroid resistant	529	Yes	None	Steroid, IVIG
ITP-14	61	M	Ecchymosis, mucosal bleeding	Steroid resistant	205	Yes	Eltrombopag	Azathioprine, vincristine, cyclophosphamide, danazol
ITP-15	33	F	Menorrhagia	Complete response, steroid dependent	541	Yes	None	Steroid
ITP-18	80	M	Hematuria	Steroid resistant	27	No	Eltrombopag	Steroid, IVIG, azathioprine, eltrombopag
ITP-19	48	F	Ecchymosis	Steroid resistant	23	Yes	Steroid, mycophenolate, mofetil	Steroid, cyclophosphamide, androgens
ITP-23	37	M	Asymptomatic	Partial response, steroid dependent	90	Yes	None	Steroid
ITP-27	58	F	Ecchymosis, epistaxis	Partial response	87	No	None	Steroid
ITP-31	66	F	Menorrhagia, ecchymosis, epistaxis	Steroid resistant	50	Yes	Azathioprine	Steroid, cyclophosphamide
ITP-32	35	F	Mucosal bleeding	Complete response	186	No	None	Steroid
ITP-33	37	F	Asymptomatic	Complete response, steroid dependent	144	Yes	Azathioprine	Steroid, azathioprine
ITP-37	56	M	Petechiae, ecchymosis	Complete response	>100	No	None	Steroid
ITP-41	65	F	Ecchymosis	Complete response	144	No	None	Steroid

ITP-44	42	M	Petechiae	Partial response	83	No	Steroid, azathioprine	Steroid
ITP-46	40	F	Mucosal bleeding	Complete response, steroid dependent	390	Yes	None	Steroid, IVIG
ITP-47	51	F	Asymptomatic	Partial response	>100	No	None	Steroid, IVIG
ITP-48	71	F	Asymptomatic	Complete response	165	No	None	Steroid
ITP-49	73	M	Ecchymosis	Complete response	247	No	None	Steroid

Table 2: **CNV Regions detected only in ITP patients.** CNV: copy number variation, Mb: megabase, ITP: immune thrombocytopenia, TCRG: T-cell antigen receptor gamma-chain, TCRD:T-cell antigen receptor delta-chain

Patient ID	Chr.	Gain/Loss (CNV Value)	Mosaic Pattern (Clonality)	Size (Mb)	Chromosomal Location	Chromosome Band
ITP06	2p	Gain (3)	No	0.34	2:30520464-30858736	2p23.2
ITP31	5q	Loss (1)	No	0.19	5:97333442-97522622	5q21.1
ITP01	7p	Loss (1)	No	0.09	7:17478404-17566650	7p21.1
ITP46	7q	Gain (4)	No	0.17	7:125671593-125841541	7q32.1
ITP48	7q	Loss (1)	No	0.13	7:111650835-111782891	7q31.1
ITP06	9q	Gain (3)	Yes	10.89	9:73281358-84167193	9q21.2-9q21.3
ITP06	9q	Gain (3)	Yes	17.87	9:104662585-122434053	9q31.2-9q33.3
ITP15	11q	Loss (1)	No	0.19	11:97757897-97945697	11q22.1
ITP19	13q	Gain (4)	No	0.15	13:105556018-105710754	13q33.1
ITP14	14q	Loss (1)	Yes (TCRG)	0.80	14:21888045-22690985	14q11.2
ITP27	14q	Gain (3)	Yes (TCRD)	0.37	14:43867179-44236516	14q13.2-14q13.3
ITP41	14q	Gain (4)	No	0.32	14:47200946-47516378	14q21.1
ITP13	17p	Gain (3)	Yes	1.46	17:14101029-15563393	17p12
ITP48	17q	Gain (3)	No	0.39	17:48578726-4898938	17q21.32
ITP31	19p	Loss (1)	No	0.17	19:21014999-21184533	19p12
ITP01	19q	Loss (1)	No	0.14	19:55035973-5517190	19q13.42
ITP33	19q	Loss (1)	No	0.06	19:41440014-41500213	19q13.13
ITP18	20q	Gain (3)	Yes (TCRD)	14.24	20:32007695-46252150	20q11.21-20q13.13
ITP49	22q	Gain (3)	Yes	0.16	22:18844632-19008108	22q11.21

Table 3: CNV regions and candidate genes in ITP patients. CNV: Copy Number Variation, Mb: Megabase

Patient ID	Chromosome	CNV Copy	Mosaic pattern / Clonality	Size (Mb)	Genomic Location	Chromosome Band	Candidate Gene-s/Functio
ITP06	2p	(Gain) 3	No	0.34	2:30520464-30858736	2p23.2	***LCLAT1;*** functions as an acyltransferas reticulum and required for establishmen and endothelial lineages
ITP31	5q	(Loss) 1	No	0.19	5:97333442-97522622	5q21.1	No gene
ITP01	7q	(Loss) 1	No	0.09	7:17478404-17566650	7q31	No gene
ITP46	7q	(Gain) 4	No	0.17	7:125671593-125841541	7q32.1	***GRP8;*** G-protein coupled receptor activ
ITP48	7q	(Loss) 1	No	0.13	7:111650835-111782891	7q31.1	***DOCK4;*** Plays a role in cell migration. zinc finger transcription factor, involve chromatin remodeling and associated wi ***IFRD1***; encodes a protein related to inte function as a transcriptional co-activator the growth and differentiation of specifi embryonic development and tissue reger leucine rich single pass membrane prote
ITP06	9q	(Gain) 3	Yes	10.89	9:73281358-84167193	9q21.2-9q21.3	***ABHD17B***; hydrolase activity and serin activity, ***ZFAND5;*** inhibits tumor necro and TLR4-induced NF-kappa-B activati manner. Overexpression sensitizes cells apoptosis. ***ANXA1***; AnxA1 is able to re and thrombosis via the suppression of in binding transcription factor activity. ***TR*** epithelial magnesium transport**,** ***PCSK5***, endopeptidase activity and functions in t regulated secretory pathway.
ITP06	9q	(Gain) 3	Yes	17,77	9:104662585-122434053	9q31.2-9q33.3	***CYLC2***; structural constituent of cytosk transmembrane protein that is localized compartment. ***TMEM38B***; intracellular TLR4; pathogen recognition and activat channel that functions in maintenance o release. ***RAD23B;*** nucleotide excision r ubiquitin mediated proteolytic pathway scaffold protein and a regulator for three involved in proinflammatory signaling. constituent of cytoskeleton and cytoskel promotes cellular adhesion, migration a may play a role in wound healing and m mediating cytoskeletal changes associat cell differentiation. ***PTPN3;*** phosphatas cytoskeletal protein binding. ***LPAR1;*** G receptor activity, phospholipid binding a biologic functions, including proliferati cell differentiation, chemotaxis. ***UCGC***; glucosyltransferase activity.***FKBP15***; m microfilament dynamics and has a role i early endosomes. ***PRPF4;*** mRNA splici apoptosis in response to prolonged ER s polyubiquitination.***POLE3;*** is a histone- combine within larger enzymatic compl

							transcription, replication, and packaging proteins that may play a role in the regu processes and vesicle-mediated transpor the kinesin superfamily of microtubule-a motors that play important roles in intrac cell division. ***AKNA;*** Centrosomal prote transcription factor that specifically acti the CD40 receptor and its ligand CD40L surface molecules on lymphocytes that a dependent-B-cell development. ***TNFSF*** biologic functions, including cell prolife regulation, and inflammation. ***TNFSF8*** that expressed on B cell surface plays an modulating Ig class switch. ***TLR4;***patho activation of innate immunity.
ITP15	11q	(Loss) 1	No	0.19	11:97757897-97945697	11q22.1	No candidate gene
ITP19	13q	(Gain) 4	No	0.15	13:105556018-105710754	13q33.1	No gene
ITP14	14q	(Loss) 1	TCRG	0.80	14:21888045-22690985	14q11.2	***SUPT16H;*** transcription of protein-codi reconstituted on naked DNA with only t factors and RNA polymerase II. ***CHD8;*** encodes a member of the chrom binding protein family, which is charact domain and two chromatin organization encoded protein also contains brahma an which are common to the subfamily of c DNA binding proteins to which this prot
ITP27	14q	(Gain) 3	Yes/TCRD	0.37	14:43867179-44236516	14q13.2-14q13.3	No gene
ITP41	14q	(Gain) 4	No	0.32	14:47200946-47516378	14q21.1	***MDGA2;*** may be involved in cell-cell in
ITP13	17p	(Gain) 3	Yes	1.46	17:14101029-15563393	17p12	***COX10;*** the terminal component of the r respiratory chain, catalyzes the electron cytochrome c to oxygen. ***HS3ST3B1;*** G metabolism, sulfotransferase activity and glucosamine 3-sulfotransferase 3 activity component of ciliary and flagellar micro directed 5'-3' RNA polymerase activity. network vesicle protein, ***CDRT1;*** protei ***TRIM16;*** nucleic acid binding
ITP48	17q	(Gain) 3	No	0.39	17:48578726-48968938	17q21.32	***ABCC1;*** transporter activity and ATPas transmembrane, movement of substance binding and associated to cisplatin resist contains an immunoglobulin domain, pr ***TOB1;*** members of this family are anti-p have the potential to regulate cell growth
ITP31	19p	(Loss) 1	No	0.17	19:21014999-21184533	19p12	***ZNF66;*** nucleic acid binding. ***ZNF85;*** r transcription corepressor activity and as thrombocytopenia
ITP01	19q	(Loss) 1	No	0.14	19:55035973-55171920	19q13.42	***LILRA1-LILRA2;*** a family of immunor expressed predominantly on monocytes is thought to control inflammatory respo help focus the immune response and lim

ITP33	19q	(Loss) 1	No	0.06	19:41440014-41500213	19q13.13	***CYP2B6;*** a family of monooxygenases reactions involved in drug metabolism a cholesterol, steroids and other lipids.
ITP18	20q	(Gain) 3	Yes/TCRD	14.24	20:32007695-46252150	20q11.21-20q13.13	***CBFA2T2;*** DNA-binding transcription transcription corepressor activity**.** ***NEC*** binding and localizes to the golgi appar zinc finger transcription factor that regu STAT3, a central regulator of immune h associated with Hyper IgE Syndrome. ***C*** part of the endosomal sorting complex, sorting of endocytosed cell-surface recep endosomes. ***RALY;*** mRNA splicing ***AH*** in the regulation of biologic methylation protein plays a role in multiple cellular p erythroid and lymphoid cell differentiati immune responses. Mutations in this ge syndromic multisystem autoimmune dis microtubule motor activity. ***MAP1LC3A*** associated proteins which mediate the pl between microtubules and components ***PIGU;*** a predicted integral membrane p in cell division control. ***TP53INP2;*** enc protein that acts as a nuclear coactivator autophagy. ***GGT7;*** gamma-glutamyltran glutathione hydrolase activity. ***GSS***; pro oxidative damage by free radicals, detox and membrane transport. ***EDEM2***; misf retrotranslocated to the cytosol and degr ***PROCR;*** a receptor for activated protein activated by and involved in the blood c ***ERGIC3;*** plays roles in ER stress-induc growth ***CPNE1;*** membrane trafficking. role in regulation of the innate immune integral membrane protein found only in reticulum. ***BLCAP;*** a protein that reduc stimulating apoptosis. ***TGM2;*** GTP bind glutamine gamma-glutamyltransferase a protein heterodimerization activity and activity and vesicle mediated transport. membrane protein involved in gamma-a (GABA) and glycine uptake into synapt positive regulator of pulmonary endothe function and involved in the regulation signaling pathway, angiogenesis and en proliferation . ***FITM2***; Plays an importa accumulation. Plays a role in the regulat and cytoskeletal organization. ***ADA;*** an the hydrolysis of adenosine to inosine in pathway and involved in immundodefici group of highly conserved proteins that vital cellular processes such as metaboli signal transduction, apoptosis and cell c Heparan sulfate proteoglycans (HSPGs) numerous heparin-binding growth factor involved in cell signaling.

ITP49	22q	(Gain) 3	Yes	0.16	22:18844632-19008108	22q11.21	No candidate gene

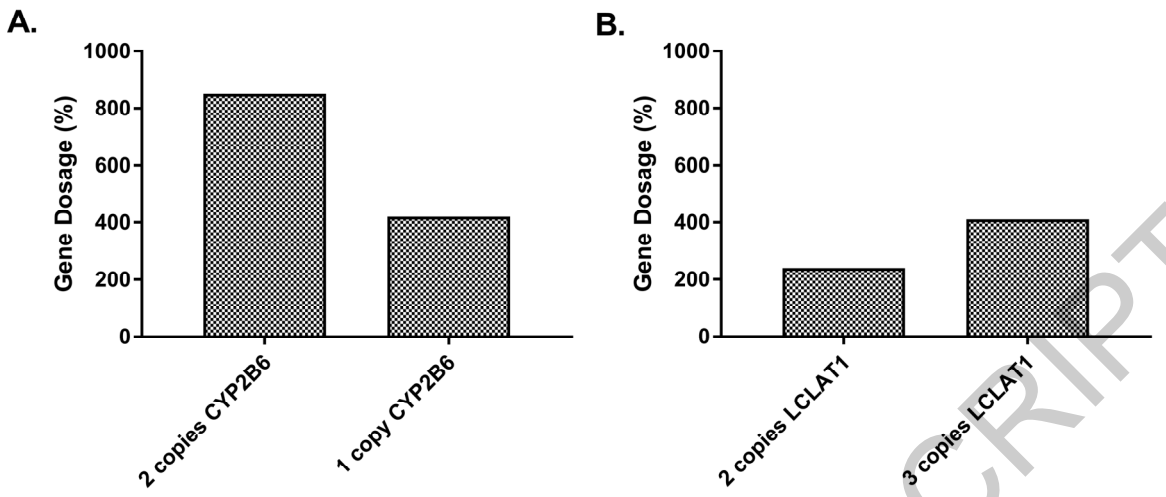

Figure 1

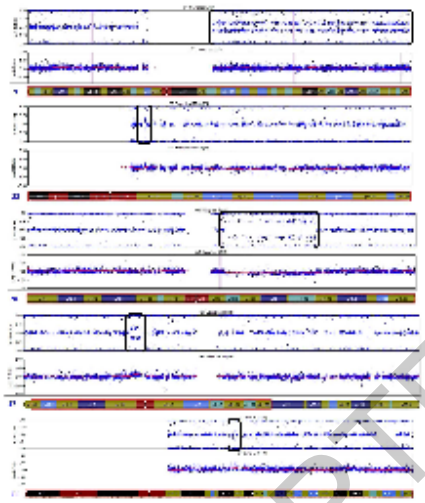

Figure 2