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Full title

Further delineation of the phenotype of severe congenital neutropenia type 4 due to mutations in *G6PC3*

Short title

Delineation of SCN4 phenotype

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Abstract

Severe congenital neutropenia type 4 (SCN4) is an autosomal recessive condition which was defined recently with identification of the causative mutations in *G6PC3*. To date there are only three reports in the literature describing patients with SCN4 with mutations in the *G6PC3* gene. We report four individuals with SCN4 who belong to a single large consanguineous kindred. We provide an overview of the non-haematological features of the condition with a focus on the adult phenotype which has not been previously described in detail. We show that the superficial venous changes seen in SCN4 patients can develop into varicose veins and venous ulcers in adulthood. We review the range of congenital anomalies associated with SCN4. We demonstrate that secundum atrial septal defect, patent ductus arteriosus and valvular defects are the most frequent cardiac anomalies in SCN4. Drawing parallels with type 1 glycogen storage disease, we propose that poor growth of prenatal onset, mild to moderate learning disability, primary pulmonary hypertension, delayed or incomplete puberty, hypothyroidism and dysmorphism likely represent features of this syndrome. We also suggest monitoring for lipid anomalies, and kidney and liver function in affected patients. Delineation of the SCN4 phenotype may help in appropriate treatment and management and provide further insights into the pathogenesis of this multisystem disease.

Keywords

Neutropenia

Severe congenital neutropenia

G6PC3

Glucose-6-phosphatase

Glycogen storage disease

Introduction

The congenital neutropenias are a group of heterogeneous disorders. To date, seventeen causative genes have been identified for syndromic and non-syndromic causes ¹. Severe congenital neutropenia type 4 (SCN4, OMIM 612541) was recently defined with identification of the causative mutations in *G6PC3* (Gene ID 92579) and to date there are only three reports in the literature describing fifteen patients in total ²⁻⁴. SCN4 has a distinct phenotype showing the presence of a prominent superficial venous pattern and an increased susceptibility to infection. Other described features include intermittent thrombocytopenia, hepatosplenomegaly, congenital heart defects, urogenital malformations, cleft palate, inguinal hernia, microcephaly, sensorineural hearing loss and myopathy ³.

We report four individuals from a single large consanguineous Israeli kindred of Arab-Muslim origin with severe congenital neutropenia, a prominent superficial venous pattern and additional clinical features. The pedigree is shown in Figure 1 and the clinical details are provided below.

Clinical description

Patient 1 (V-7) is a 29 year old female born to first cousin parents. She was small for gestation at birth. Her adult height is on the 2nd centile and her weight and occipito-frontal circumference (OFC) are on the 5th centile. She also has mild learning disability and

required special education. She has irregular menses. She has suffered from recurrent pneumonia, otitis media and perianal abscesses. Significant results of her haematological and biochemical investigations are summarized in table 1. On examination she has a prominent superficial venous pattern and varicose veins. She also shows mild kyphosis, tapering fingers and clinodactyly of the 4th and 5th fingers.

Patient 2 (V-13) is a 26 year old man. He is IV-7's second cousin on the maternal side and third cousin on the paternal side. At birth he was small for dates and failed to thrive in infancy. His current height is below the 0.4th centile and weight is on the 2nd centile. Agenesis of the left kidney and right hydronephrosis were noted in childhood. His puberty was delayed. He also has mild learning disability and went to a special school. He has experienced recurrent episodes of pneumonia and otitis media. His relevant haematological and biochemical investigation results are presented in table 1. On examination he has a prominent superficial venous pattern and varicose veins which have resulted in a venous stasis ulcer. He has poorly developed secondary sexual characteristics and gynaecomastia. He has full cheeks (Figure 2a) and fat deposition around his trunk. His anterior and posterior hairlines are low (Figure 2b) and he has broad thumbs.

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One of his younger brothers (V-10) died soon after birth due to a congenital heart defect, about which more details are not available. He has an older sister (V-14) who has learning difficulties but does not share any other clinical features of SCN4.

Patient 3 (V-17) was V-13's double first cousin. Patent ductus arteriosus (PDA) and secundum atrial septal defect (ASD) were noted at birth. Her adult height, weight and OFC were all on the 2nd centile. Menarche was not delayed but secondary sexual characteristics were slow to develop. She also had mild learning difficulties and went to a special-needs school. She suffered from recurrent urinary tract infections and herpes stomatitis. She died at the age of 25 years due to sepsis. Her peripheral blood counts and bone-marrow aspiration results are summarized in table 1. On examination she had prominent and widespread superficial venous pattern and varicose veins on the lower legs. She had acne on her face and trunk. Her breasts and pubic hair were between Tanner stages 3 and 4 in adulthood. She had bilateral keratoconus.

Patient 4 (V-21) is a two old year boy born to first cousin parents. His parents belong to the same kindred of the first three patients described above, although the exact relationship is not known. He was diagnosed prenatally to have pulmonary valve stenosis. At eight days of age he underwent pulmonic balloon valvuloplasty. PDA and secundum ASD were also noted. Soon after birth he developed pulmonary hypertension of unknown cause, which was treated with sildenafil until eight months of age. He had bilateral undescended testis and gastro-oesophageal reflux. He was also found to have raised gamma-glutamyl transferase and calcifications in the liver at seven days of age. At age three months he was diagnosed with choledocholithiasis and was treated with ursodeoxycholic acid. He is still failing to

thrive and has global mild to moderate developmental delay. His current length is on the 5th centile, weight below 0.4th centile and OFC on the 0.4th centile. He suffers with recurrent otitis media. His pre-treatment peripheral blood counts and bone-marrow aspiration results are provided in table 1. On examination he has plagiocephaly, sparse scalp hair, mid-face hypoplasia and a tented mouth. He also has a prominent superficial venous pattern on his head, a pectus carinatum and a small umbilical hernia. He has loose skin on the palms and soles and an uneven fat distribution.

Materials and Methods

Ethical approval for this study was obtained from the University of Manchester (06138) and NHS ethics committee (06/Q1406/52). DNA was extracted from lymphocytes from the four affected individuals using standard protocol. All six exons of *G6PC3* were amplified by polymerase chain reaction in the first three patients using the primers described by Boztug *et al.* In patient four, only exon six of *G6PC3* was amplified. V-14 has not been tested for the familial mutation. Genomic DNA sequence was analyzed using ABI 3130 and Staden software.

Results

All four patients were homozygous for the c.758G>A mutation in *G6PC3* (Figures 3a & b) which results in substitution of arginine to histidine at position 253 (p.R253H). This

mutation has been described previously ³. We were unable to establish if this mutation was on a common haplotype with previously reported cases or whether this residue represents a mutational hotspot. Previous cases with the same mutation have been of Turkish Aramean background, which would be consistent with a shared Mediterranean ancestry with the Israeli Arabs ⁵.

Discussion

Glucose-6-phosphatase catalyzes the final step in glycogenolysis, i.e. the conversion of glucose-6-phosphate to glucose. This step takes place in the endoplasmic reticulum (ER). Glucose-6-phosphatase is encoded by a gene-family consisting of *G6PC1*, *G6PC2* and, *G6PC3* ⁶. *G6PC1* is expressed in the liver, kidney and small intestine. Homozygous loss-of-function mutations in *G6PC1* cause type 1a glycogen storage disease (GSD1a) ⁷. GSD1a is characterized by fasting hypoglycemia, hepatomegaly, growth retardation, fasting lactic acidosis, hyperlipidemia and hyperuricemia. Long-term complications of GSD1a include hepatic adenomas, pancreatitis, gout, renal failure, pulmonary hypertension, polycystic ovaries, osteoporosis, platelet dysfunction, and mild to moderate learning difficulties ⁸.

The transport of glucose-6-phosphate from the cytosol to ER is facilitated by glucose-6-phosphate translocase, encoded by the ubiquitously expressed *SLC37A4* ⁹. Homozygous mutations in *SLC37A4* result in GSD1b, which has a more severe phenotype than GSD1a and, of note, also causes chronic neutropenia.

G6PC3 is expressed ubiquitously and is important in maintaining neutrophil viability³. Loss of *G6PC3* function results in increased susceptibility to apoptosis in skin fibroblasts, neutrophils and myeloid cells. Increased apoptosis is likely due to decreased intra-cellular glucose concentrations and mediated via an ER stress mechanism and may predispose to associated congenital malformations.

Including the four patients presented here, a total of nineteen SCN4 cases have now been described in the literature²⁻⁴. Congenital neutropenia, frequent infections and prominent superficial veins are consistent features in these patients. We provide an overview of the non-haematological features of the condition with a focus on the adult phenotype which has not previously been described in detail. We draw parallels between the phenotype of SCN4 patients with GSD1a because both *G6PC3* and *G6PC1* code for the enzyme glucose-6-phosphatase and with GSD1b because of the known functional relationship of the enzyme with glucose-6-phosphate transporter. A summary of major clinical features is provided in table 2.

Venous anomalies: The prominent superficial venous pattern, which is the most distinctive non-haematological feature of SCN4, is less evident in infancy and gradually becomes more prominent with age. Considering the patients described here, it is clear that these

vascular changes can develop into varicose veins in adulthood resulting in venous ulcers.

Patients with SCN4 should be monitored for this complication.

Cardiac anomalies: Patient 3 and 4 were diagnosed with PDA and secundum ASD. Patient 4 also had PS. Combining our data with that presented by Boztug *et al* and Jun *et al*, 13/19 patients have at least one congenital cardiac malformation (see table 2 for details).

Secundum ASD and valvular defects are the most frequent cardiac anomalies in SCN4.

Pulmonary vasculature anomalies: One patient in the series presented by Boztug *et al* had pulmonary venous malformation. The congenital cardiac malformations (PS, ASD and PDA) observed in patient 4 do not normally result in pulmonary hypertension in early life. This leads us to conclude that the pulmonary disease in this patient was primary. Of note, Dursun *et al* recently reported a pair of Turkish siblings, one boy and one girl, with primary pulmonary hypertension, leucopenia and secundum ASD¹⁰. Both the siblings with Dursun syndrome (OMIM 613034) demonstrated a phenotype that is strikingly similar to that of the children described with SCN4. Primary pulmonary hypertension is also a well recognized feature of GSD1¹¹ and may be an additional feature of SCN4.

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Growth: Poor growth of prenatal onset may also be a core feature of SCN4. In four affected individuals presented here showed intrauterine growth retardation and

demonstrated a failure to thrive in infancy. Childhood stature and final adult height seem to be at the lower end of the normal range in all the four cases. The height and weight of the boy described by Arostegui *et al* were below the 3rd percentile ² and at least seven of the twelve patients reported by Boztug *et al* demonstrated growth retardation or height/weight at the lower end of the normal range ³. We note that female *g6pc3*^{-/-} mice exhibit growth retardation ¹², but in humans this features seems not to be sex-limited.

Development: All four patients described here have mild to moderate developmental delay and/or learning disability. All three adult patients required special education and none have ever been in paid employment. Patient 2 has a sibling with learning disability who does not share the rest of the SCN4 phenotype and therefore the possibility of a different cause for learning difficulties in this kindred cannot be ruled out. The male reported by Arostegui *et al* also had learning difficulties, while no specific comments were made about neurodevelopmental aspects in the series described by Boztug *et al* and Jun *et al*. We suggest that the neuro-developmental aspect of the SCN4 phenotype warrants further investigation.

Endocrine dysfunction: Of fifteen previously reported patients, only two were beyond pubertal age – a 22 year old male described by Arostegui *et al* and a 17 year old male described by Boztug *et al* ^{2,3}. The ages of the two patients described by Jun *et al* were not given ⁴. Three of the patients described here are over the age of 25 years, thus providing an

insight into the adult SCN4 phenotype. Raised TSH was found in patients 1 and 2, suggesting that hypothyroidism may represent a feature of SCN4. Poor secondary sexual development was seen in two of our patients (patient 2 and 3) and we note that *g6pc3^{-/-}* mice exhibit significant reduction in g6p hydrolytic activity in the testes, although without any known phenotypic consequence. We note also that delayed puberty is a known feature of GSD1. We suggest that evaluation of pubertal development in adolescents and thyroid function assessment should be considered in the follow-up of SCN4 affected patients.

Lipid abnormalities: Patients with GSD1 often have dyslipidemia, which is thought to be due to increased *de novo* lipogenesis and delayed conversion of Very low density lipoproteins to intermediate lipoprotein particles¹³. Patient 1, the oldest in our series, had low HDL levels. She also had persistently increased amylase activity. The risk of pancreatitis due to dyslipidemia is increased in GSD1¹⁴. Our findings suggest that lipid profile should be monitored in adults with SCN4.

Liver and kidney function: Findings of raised gamma-glutamyl transferase, liver calcifications and choledocholithiasis in patient 4, and elevated uric acid level in patient 1, indicates that liver function, kidney function and uric acid levels should be monitored in SCN4 similar to patients with GSD1.

Other congenital anomalies and minor dysmorphism: Inguinal hernia, cryptorchidism, genital dysplasia cleft palate and urachal fistula have already been described in patients with SCN4³. To this list we can now add renal agenesis, hydronephrosis (patient 2) and umbilical hernia (patient 4). Skeletal anomalies such as pectus carinatum, clinodactyly, broad thumbs and kyphosis could also be a part of this syndrome.

Additional dysmorphic features can provide useful clues towards a specific diagnosis. Proper delineation of this aspect of the SCN4 phenotype will require a larger series of patients from different ethnic backgrounds. Considering the features observed in patient 4, it may be that mid-face hypoplasia, sparse scalp hair and loose skin represent features of SCN4. It is not clear if loose skin is an independent feature or secondary to failure to thrive. In adolescents and adults, the fullness of cheeks is reminiscent of GSD1, and a truncal fat distribution might provide added clues to the diagnosis.

Conclusion

SCN4 is a recently described autosomal recessive condition whose phenotypic extent and natural history is still incompletely defined. It is important to recognize this syndrome so that appropriate treatment and monitoring can be instigated. Further studies into larger cohorts of patients are needed to determine the extent of possible complications, clarify associated dysmorphic features and determine if developmental delay and learning difficulties are consistent features of the syndrome. It is clear that significant inter- and

intra-familial variability can occur. Delineation of the phenotype will also provide further insights into the mechanisms of this disease.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Supplementary information is available at The European Journal of Human Genetics website.

References

1. Klein C: Molecular basis of congenital neutropenia. *Haematologica* 2009; **94**: 1333-1336.
2. Arostegui JI, de Toledo JS, Pascal M, Garcia C, Yague J, Diaz de Heredia C: A novel G6PC3 homozygous 1-bp deletion as a cause of severe congenital neutropenia. *Blood* 2009; **114**: 1718-1719.
3. Boztug K, Appaswamy G, Ashikov A *et al*: A Syndrome with Congenital Neutropenia and Mutations in G6PC3. *New England Journal of Medicine* 2009; **360**: 32-43.
4. Xia J, Bolyard AA, Rodger E *et al*: Prevalence of mutations in *ELANE*, *GFII*, *HAX1*, *SBDS*, *WAS* and *G6PC3* in patients with severe congenital neutropenia. *British Journal of Haematology* 2009; **147**: 535-542.
5. Arnaiz-Villena A, Karin M, Bendikuzze N *et al*: HLA alleles and haplotypes in the Turkish population: relatedness to Kurds, Armenians and other Mediterraneans. *Tissue Antigens* 2001; **57**: 308-317.
6. Hutton JC, O'Brien RM: Glucose-6-phosphatase Catalytic Subunit Gene Family. *Journal of Biological Chemistry* 2009; **284**: 29241-29245.

7. Lei K-J, Shelly LL, Pan C-J, Sidbury JB, Chou JY: Mutations in the Glucose-6-Phosphatase Gene That Cause Glycogen Storage Disease Type 1a. *Science* 1993; **262**: 580-583.
8. Janecke AR, Mayatepek E, Utermann G: Molecular Genetics of Type 1 Glycogen Storage Disease. *Molecular Genetics and Metabolism* 2001; **73**: 117-125.
9. Veiga-da-Cunha M, Gerin I, Chen Y-T *et al*: The putative glucose 6-phosphate translocase gene is mutated in essentially all cases of glycogen storage disease type I non-a. *European Journal of Human Genetics* 1999; **7**: 717-723.
10. Dursun A, Ozgul RK, Soydas A *et al*: Familial pulmonary arterial hypertension, leucopenia, and atrial septal defect: a probable new familial syndrome with multisystem involvement. *Clinical Dysmorphology* 2009; **18**: 19-23.
11. Humbert M, Labrune P, Simonneau G: Severe pulmonary arterial hypertension in type 1 glycogen storage disease. *European Journal of Pediatrics* 2002; **161**: S93-S96.
12. Wang Y, Oeser JK, Yang C *et al*: Deletion of the Gene Encoding the Ubiquitously Expressed Glucose-6-phosphatase Catalytic Subunit-related Protein (UGRP)/Glucose-6-phosphatase Catalytic Subunit-beta Results in Lowered Plasma

Cholesterol and Elevated Glucagon. *Journal of Biological Chemistry* 2006; **281**: 39982-39989.

13. Bandsma RHJ, Prinsen BH, Van Der Velden MDS *et al*: Increased de novo Lipogenesis and Delayed Conversion of Large VLDL into Intermediate Density Lipoprotein Particles Contribute to Hyperlipidemia in Glycogen Storage Disease Type 1a. *Pediatric Research* 2008; **63**: 702-707.
14. Rake J, Visser G, Labrune P, Leonard J, Ullrich K, Smit G: Glycogen storage disease type I: diagnosis, management, clinical course and outcome. Results of the European study on glycogen storage disease type I (ESGSD I). *European Journal of Pediatrics* 2002; **161**: S20-S34.

Figure Legends

Figure 1: Pedigrees of one large and one small nuclear family are represented. Both the families belong to a single kindred although the exact relationship between them is not certain.

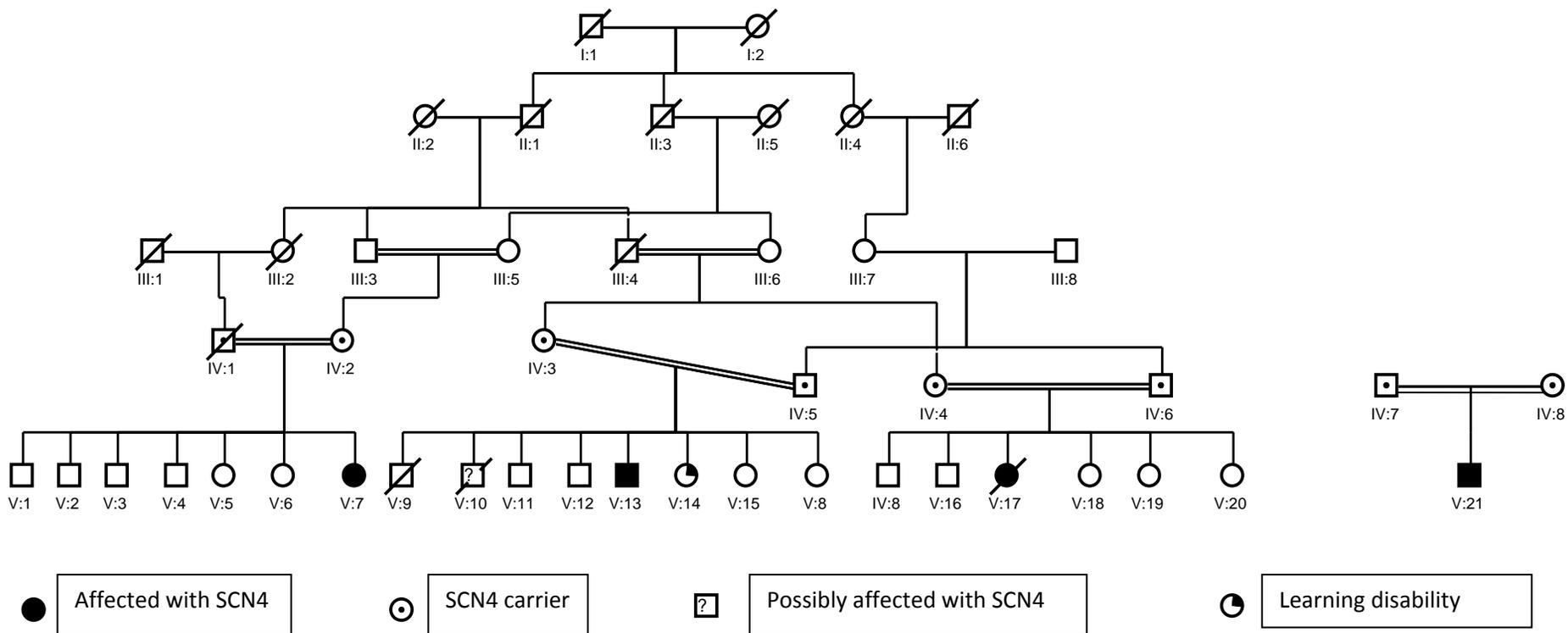
Figure 2a and 2b: Front and profile of patient 2 showing full cheeks (similar to what is seen in GSD1), prominent lips and low hair-lines.

Figure 3a: Homozygous mutation c.758G>A in exon 6 of *G6PC3* of Patient 1.

Figure 3b: Corresponding normal sequence from a control subject.

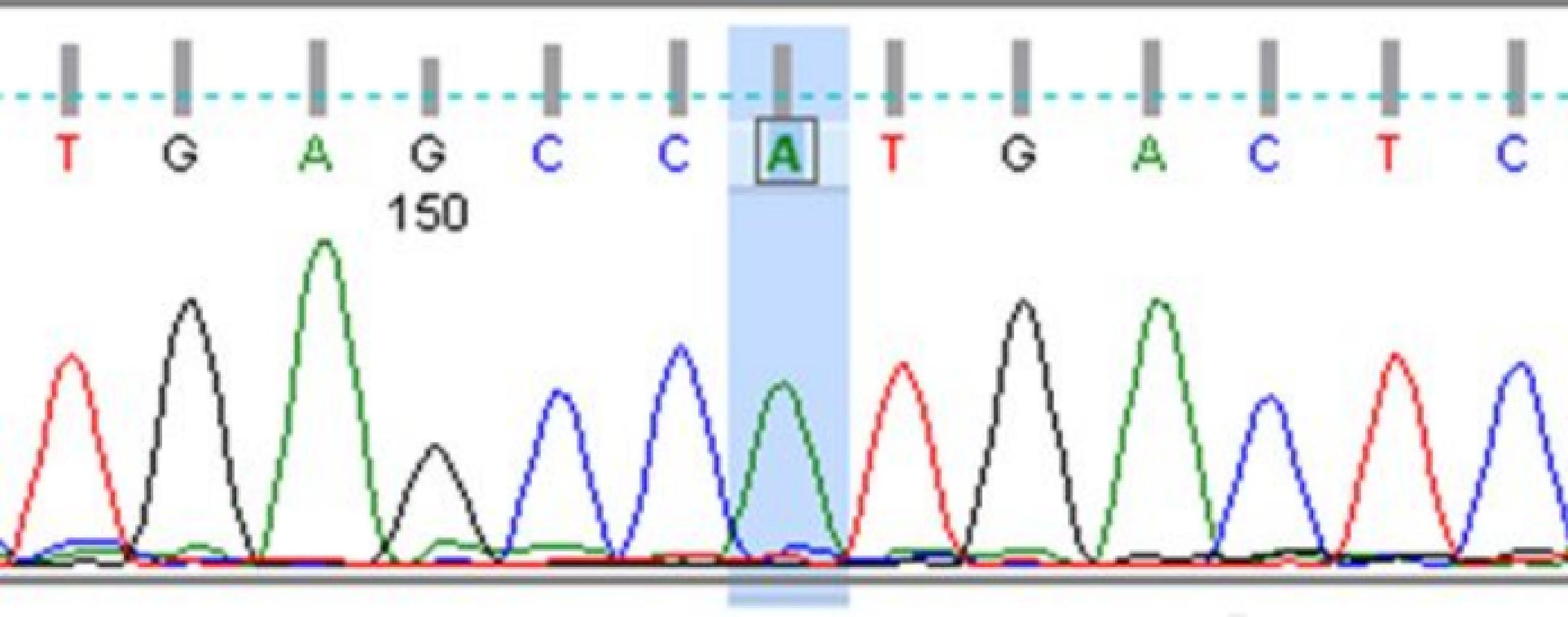
Supplemental Figure 1: Wright's stain bone marrow aspirate at 500X magnification of patient 4 showing presence of all the stages of myelopoiesis without any evidence of maturation arrest.

Figure 1









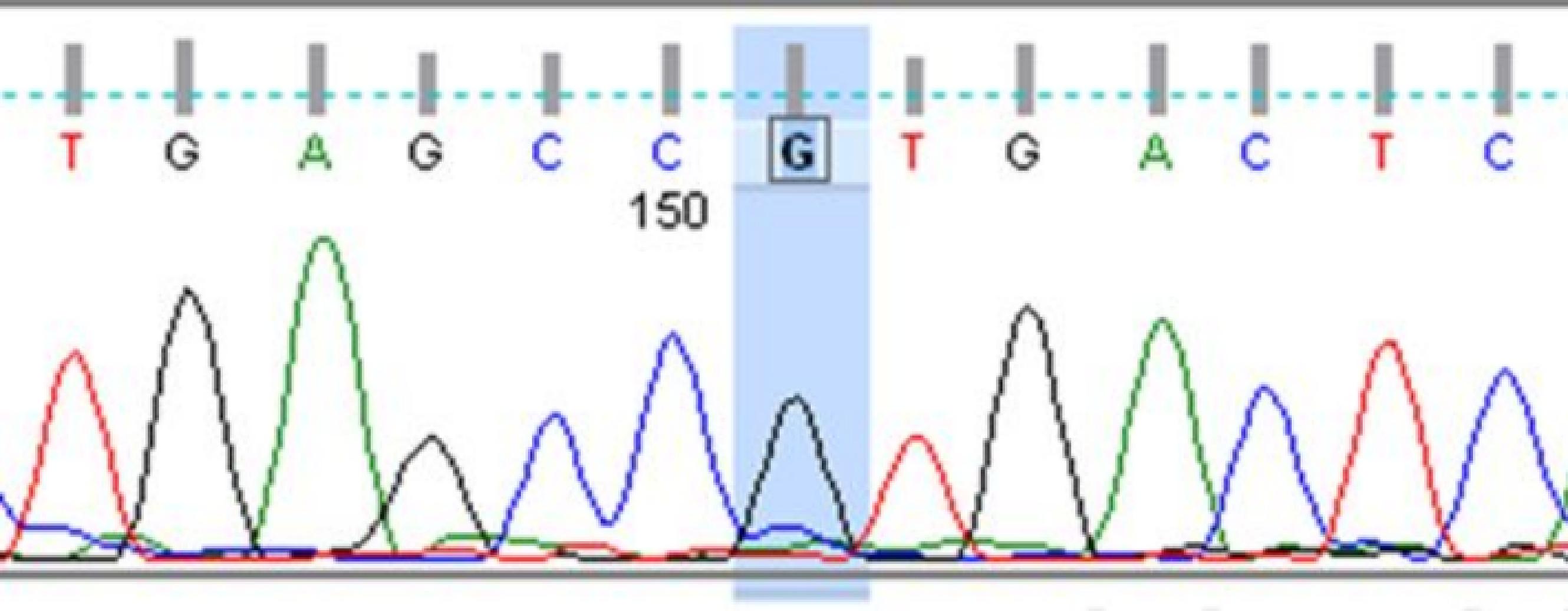


Table 1: Summary of haematological and biochemical investigations of the four patients.

	Complete peripheral blood count											Bone marrow aspiration results	Significant results of other biochemical investigations
	WC	N	L	M	E	B	Hb	RC	MCV	MCH	P		
RR	4.5 to 12.0	2.0 to 7.5	1.0 to 5.0	0.16 to 1.0	0 to 0.8	0 to 0.2	See foot- note	4.0 to 5.0	80 to 90 fl	27 to 31 pg	150 to 350		See foot-note
Pt 1	<u>2.0</u> to <u>4.0</u>	<u>0.7</u> to <u>1.3</u>	2.2 to 2.3	0.2 to 0.4	0.01	0.01 to 0.02	<u>11.4</u> to <u>13.0</u>	4.5 to 4.9	77.3 to 81.8	<u>25.1</u> to <u>26.5</u>	<u>38</u> to <u>140</u>	Hypercellular marrow with myeloid hyperplasia. Increased number of megakaryocytes and blast-like forms.	Reduced HDL - 22 mg/ml. Elevated uric acid - 7.2 mg/ml. Elevated pancreatic amylase - 124 to 148 U/L. Raised TSH - 4.73 IU/ml.
Pt 2	<u>1.7</u> to <u>4.0</u>	<u>0.2</u> to <u>0.6</u>	1.1 to 2.5	0.17 to 0.94	0 to 0.02	0 to 1.9	<u>9.9</u> to <u>11.6</u>	3.87 to 4.5	<u>77.5</u> to <u>80.5</u>	<u>24.0</u> to <u>26.3</u>	233 to 356	NA	Raised TSH - 4.99 IU/ml.
Pt 3	<u>2.1</u> to <u>3.2</u>	<u>0.2</u> to <u>1.5</u>	0.9 to 1.7	0.3 to 0.8	0.02 to 0.12	0 to 0.01	<u>10.8</u> to <u>12.5</u>	4.5 to 4.9	<u>75.0</u> to <u>81.9</u>	<u>23.2</u> to <u>25.2</u>	260 to 428	Decreased erythropoiesis, dysmyelopoietic changes with reduced granules in the cytoplasm and increased megakaryocytes.	None
Pt 4	<u>3.7</u> to <u>16.3</u>	<u>0.4</u> to <u>7.7</u>	<u>1.9</u> to <u>9.5</u>	<u>0.5</u> to <u>3.3</u>	0.01 to 0.3	0.01 to 0.59	<u>10.6</u> to <u>12.6</u>	<u>3.6</u> to <u>4.8</u>	<u>72.7</u> to <u>86.1</u>	<u>23.2</u> to <u>29.1</u>	<u>368</u> to <u>819</u>	All stages of myelopoiesis seen without any myeloid maturation arrest (See Supplemental Figure1)	None

The highest and lowest available values of cell counts (prior to granulocyte colony stimulating factor administration, if treated) are given for each patient in multiples of 1000 cells per mm³. Counts which were detected to be outside the reference range on a majority of occasions have been underlined.

Key : B – Basophils, E - Eosinophils, Hb – Haemoglobin, HDL – High density lipoprotein cholesterol, L - Lymphocytes, M - Monocytes, MCH – mean corpuscular haemoglobin, MCV – Mean corpuscular volume, N – Neutrophils, P – Platelets, Pt – Patient, RC – Red cells, RR – Reference range, NA – Not available, TSH - Thyroid stimulating hormone and WC - White cells.

Hb is given in g/dl with a reference range of 13.5-18.0 for adult males and for 11.5-16.0 for adult females. Reference range for HDL is 40 to 120 mg/ml; for uric acid is 2.6 to 6.0 mg/ml, for pancreatic amylase is 28 to 100 U/L and for TSH is 0.4 to 4.0 IU/ml.

Table 2: Summary of major non-haematological features of all nineteen patients described in the literature with SCN4 and G6PC3 mutations.

Features		This paper n ₁ = 4	Boztug <i>et al</i> n ₂ = 12	Arostegui <i>et al</i> n ₃ = 1	Xia <i>et al</i> n ₄ = 2	Total n = 19	
Congenital anomalies	Cardiac	Secundum ASD	2	7	1	2	12
		PDA	2	1	0	0	3
		Valvular defects	1 (pulmonary stenosis)	2 (1 pulmonary stenosis & 1 mitral insufficiency)	0	0	3
		Others	0	1 (cor triatriatum)	0	1 (coronary aneurysm)	2
	Pulmonary anomaly		1 (pulmonary hypertension)	1 (pulmonary venous malformation)	0	0	2
	Urogenital anomalies	Bilateral Cryptorchidism	1	4	1	0	6
		Others	1 (unilateral renal agenesis & hydronephrosis)	2 (urachal fistula & genital dysplasia)	0	0	3
	Inguinal hernia		1	1	0	0	2
	Cleft palate		0	1	0	0	1
Features of infancy and childhood	Prominent venous pattern		4	10	1	NA	15
	Poor growth		4	7	1	NA	12
	Developmental delay/learning difficulties		4	NA	1	NA	5
	Sensorineural hearing loss		0	2	0	NA	2
	Relative microcephaly		0	2	0	NA	2
	liver calcifications and/or choledocholithiasis		1	0	0	NA	1
Features of adolescence	Varicose veins		3/3	NA	NA	NA	3/3
	Delayed/incomplete puberty		3/3	NA	NA	NA	3/3

and adulthood	Sub-clinical hypothyroidism	2/3	NA	NA	NA	2/3
	Lipid abnormalities	1/3	NA	NA	NA	1/3
	Elevated uric acid	1/3	NA	NA	NA	1/3

Key: NA - denotes that the feature was either not applicable due to age or not given or could not be inferred from the given data;

ASD - atrial septal defect; PDA - patent ductus arteriosus, n - total number of cases in the series.

