Article

PATIENTS WITH NOONAN SYNDROME PHENOTYPE: SPECTRUM OF CLINICAL FEATURES AND CONGENITAL HEART DEFECT

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ABSTRACT

Mutations in components of the RAS-MAPK signaling pathway have been reported to result in an expression of Noonan phenotype. This is actually a wide-spectrum-phenotype shared by Noonan syndrome and its clinically related disorders namely, the Cranio-facio-cutaneous (CFC) syndrome, Costillo syndrome as well as LEOPARD syndrome. Patients with Noonan Syndrome (NS) have mutations in PTPN11 gene in majority of cases. Recently, mutations in SOS1, RAF1, MEK1 and KRAS genes have been reported to cause NS as well.
Objective: To report patients with a Noonan phenotype followed in Rwandan University Teaching Hospitals, and to show the importance of the clinical diagnosis and challenges of making the diagnosis in resource limited settings where karyotype is almost the only genetic investigation accessible.
Patients and Methods: Here we are reporting 5 patients, all with relevant NS symptoms, whose morbidity is directly related to the severity of their congenital heart disease. Van der burgt et al diagnostic criteria have been used for the clinical diagnosis, karyotype studies have been performed to exclude chromosomal aberration disorders and patients DNA extraction for mutation studies have been obtained in some cases.
Results and Conclusion: we identified 5 patients with clinical features highly suggestive of NS and all of them had a normal karyotype, this excluding Turner syndrome, a clinically similar syndrome. As there are many as yet discovered mutations causing NS and the famous PTPN11 mutation being present only in 50% of cases, we maintain here that NS diagnosis should be a clinical diagnosis. The morbidity and mortality of our patients were directly correlated to the severity of their congenital cardiac defect. In conclusion, early management of such patients is highly recommended.

Key-words:Noonan syndrome - Rwandan patients - RAS-MAPK signaling pathway - genetic disease – karyotype - clinical features

RESUME

Les mutations impliquant la voie de transduction RAS-MAPK ont étaient reconnues identifiées comme causant un « phénotype du syndrome Noonan ». Ceci est en effet un phénotype de spectre très large, partagé entre le syndrome de Noonan et d'autres conditions cliniquement semblable notamment le syndrome Crânio-Facio-Cutané (CFC), le syndrome de Costillo ainsi que le syndrome de LEOPARD. Les patients atteints du syndrome de Noonan ont des mutations dans le gène PTPN11 dans la majorité des cas. Récemment, les mutations impliquant les gènes SOS1, RAF1, MEK1 et KRAS ont étaient caractérisées comme étant aussi impliquées dans le développement du syndrome de Noonan.
Objectifs : rapporter des patients atteints du syndrome de Noonan suivis dans nos hôpitaux universitaires et montrer l'importance du diagnostic clinique ainsi que le défi à faire le diagnostique dans un contexte où les ressources sont limitées, seul le karyotype étant presque le test génétique accessible.
Résultats et Conclusion : nous avons identifié 5 patients avec un tableau clinique suggestif du syndrome de Noonan et chez qui le karyotype a été normal, ceci excluant le syndrome de Turner qui mime la clinique du syndrome de Noonan. Compte tenu du fait qu'il y a beaucoup de mutations causant le syndrome de Noonan et les mutation du gène PTPN11 n'étant présentes que dans 50% des cas, nous plaidons pour l'importance du diagnostique clinique. La morbidité et mortalité des patients signalés dans cette revue étaient directement liées à la sévérité de leurs cardiopathies congénitales. En conclusion, une prise en charge précoce de tels patients devrait être recommandée.

Mots Clés: syndrome de Noonan - patients Rwandais - voie de signalisation RAS-MAPK - maladie génétique – caryotype - signes cliniques

INTRODUCTION

Noonan syndrome is a heterogeneous autosomal dominant relatively common genetic disorder (1:1000 to 1:2500 lives births worldwide), formerly called the "Male Turner's syndrome" though equal prevalence in males and females [1, 2, 3].
Noonan syndrome is a well known syndrome of a large spectrum of clinically related syndromes recently put under the umbrella of the Neuro Cardio Facial Cutaneous syndromes [4]. These include Costillo syndrome, LEOPARD syndrome, Neurofibromatosis 1 syndrome as well as the Cranio Facial Cutanous (CFC) syndrome [4]. All of them are autosomal dominant congenital disorders, with a penetrance of almost a 100% [4]. They all share the same spectrum of cranio-facial abnormalities (a broad forehead, hypertelorism, down-slanting palpebral fissures, a high arched palate, low-set posteriorly rotated ears, epicanthic folds and webbed neck), chest deformity (either pectus excavatum or pectus carinatum), wide spaced nipples, scoliosis, bleeding diathesis and short stature latter in life [1, 2, 3], and most of these syndromes present with congenital heart defects. In NS, the most common is pulmonary valvar stenosis [2, 3, 5, 6], but in some few cases NS patients are reported to have hypertrophic cardiomyopathy [7].
Lymphatic anomalies may present prenatally as nuchal edema, pleural effusions or hydrops fetalis, and postnatally with hand or foot edema. More than half of the individuals affected by NS show some delay in developmental milestones (motor and/or speech delay), and roughly one third exhibit learning difficulties and require special educational support.

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However, mental retardation (IQ<70) is uncommon in patients with NS [4]. Clinical signs associated with NS, as described in clinical synopsis available at the Online Mendelian Inheritance in Man website (OMIM 163950) are reported in Table 1.

In about 50% of cases, NS is caused by missense mutations in the PTPN11 gene (protein-tyrosine phosphatase, nonreceptortype 11) on chromosome 12q24.1, coding for SHP-2. The SHP-2 gene product is a nonreceptor protein tyrosine phosphatase containing two SH2 domains (N-SH2, C-SH2), a PTP domain, and a C-tail with tyrosine phosphorylation sites and a proline-rich motif [5]. The SHP2 protein is a component of several intracellular signal transduction pathways involved in embryonic development that modulate cell division, differentiation, and migration, including that mediated by the epidermal growth factor receptor [6]. The latter pathway is important in the formation of the cardiac semi lunar valves [5]. Other genes responsible for NS have been already reported. These include mutations in KRAS [8], mutations in RAF1 [9], and recently mutations in SOS1 and MEK1 have also been reported to cause the syndrome [10, 11, 12].

However, in about 30% of patients with Noonan Syndrome signs who fill the Van der Burgt et al. criteria (Table 2), no mutation is seen [11]. This is why some resources characterize NS as a clinical diagnosis [11].

In this review, we report on 5 patients followed in our Teaching Hospitals for NS and related pathologies and/or complications, 2009 through 2011 and support the practice of retaining the diagnosis of NS based on clinical criteria.

CLINICAL CASES

Patient 1, was a 7 month-old male when clinically diagnosed with NS. He was born by normal delivery to normal and non consanguineous Rwandan parents. At birth, he was a preterm baby, weighed 1.250 kg, and had a weak cry. Right in the neonatal period, pediatricians at Kigali University Teaching Hospital [KUTH] had noticed dysmorphic features of the face and a transient tachypnea of the newborn (TTN). At time of our assessment, he was 7 month-old, his weight was 3.99 kg, his height 56 cm (less than –3 Z-score). On physical examination, the general condition was fair, we noted some dysmorphic features of the facies; hypertelorism, epicanthic folds and exophthalmia. He had a pectus excavatum and cardiopulmonary examination revealed a lower left sternal border (LLSB) pansystolic murmur and ronchi bilaterally. Chest radiography showed an enlarged cardiac silhouette. Chromosomal studies have been done on the patient's cells and showed a normal male karyotype (46, XY). Patient's genomic DNA was extracted in order to search for mutations in PTPN11, RAF, SOS1 genes involved in NS. We went ahead and confirmed the diagnosis of NS as it is mainly a clinical one. The patient met 3 major criteria in the Van der Burgt et al. criteria for NS diagnosis.

Patient 2, was a 2 year-old female toddler when clinically diagnosed with NS at KUTH. She was born by normal delivery to normal and non consanguineous Rwandan parents with birth weight of 3.8 kg. At birth she presented dysmorphic features and transient respiratory distress. Her development progress was slow; she couldn't walk nor speak (2 word – sentence) at 2 years. Her clinical features included hypertelorism, down slanting eyes, epicanthic folds, exophthalmia, anterior fontanel not yet closed, short webbed neck, short philtrum, pectus carinatum and scoliosis. On cardiopulmonary examination, she had a respiratory distress, an ejection systolic murmur at upper left sternal edge and bronchial congestion (Figure 1). Echocardiography showed atrial septal defect and pulmonary valvar stenosis. Cytogenetic tests showed a normal female karyotype (46, XX), and a genomic DNA extraction was performed to search for mutations in PTPN11, RAF and SOS1 genes involved in NS. Additionally, she met 3 major (including typical face) and 2 minor criteria of the Van der Burgt et al. criteria for NS diagnosis.

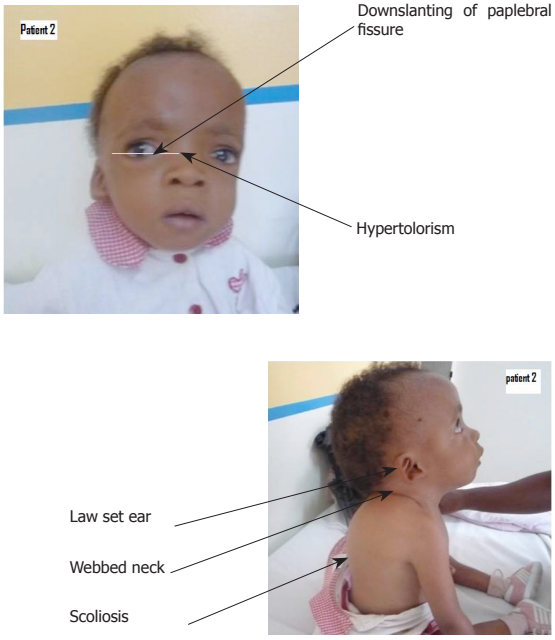
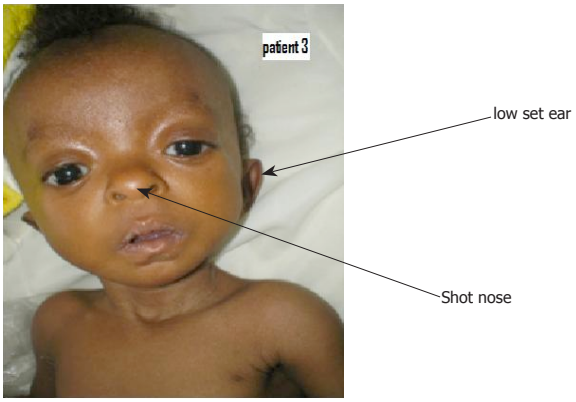


Figure 1. A, B : Photograph of patient 2 with definite Noonan Syndrome, 2 year-old

Patient 3, was a 10 month-old female infant when we clinically confirmed the previously suspected diagnosis of NS. Born by normal delivery with a birth weight of 2.580 kg. She did not cry immediately. At birth she had feeding problems and was hypotonic. At one month of age, she presented with severe respiratory distress, failure to thrive and physical examination found a baby with good color though in respiratory distress with a 4/6 ejection systolic murmur at left upper sternal edge (Figure 2). At four months of age, she had an echocardiography and this was in favor of a severe pulmonary valvar stenosis and large peri membranous ventricular septal defect (PMVSD) with an unrestrictive left to right shunt. At seven months, she underwent an open heart surgery at King Faysal Hospital



(KFH) for VSD repair and pulmonary valvar stenosis

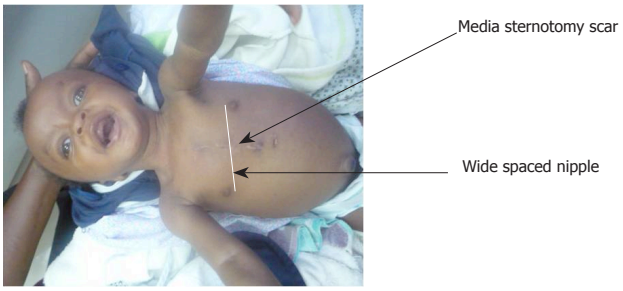
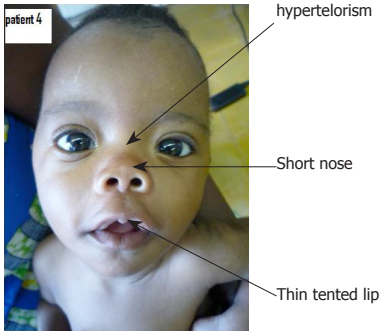


Figure 2. A, B: Photograph of patient 3 with definite Noonan Syndrome, 10 month-old

balloon dilatation by catheterization. The operation was successful with satisfactory control echocardiography. The patient was discharged stable on furosemide. Genetic consultation was done at ten months of age, and she weighed 3.10 kg, height of 54 cm and a head circumference was 37cm (all less than – 3 Z score). Her both parents and her 2 brothers were healthy. Clinical features included hypertelorism, large philtrum, delayed closure of the anterior suture, high peaks of upper lip vermillion border, low set ears and wide spaced nipple. As we saw the patient after the open heart surgery, the cardiac and pulmonary examination were normal and she was hemodynamically stable. Workup included karyotype study which was normal (46,XX) and DNA extraction for mutation studies have been performed. The patient met 3 major and 1 minor van der burgt et al. criteria for NS diagnosis. We went ahead and made a definite diagnosis of NS.

Patient 4, was a 5 month-old female when clinically diagnosed with NS at Butare University Teaching Hospital (BUTH). She was born by normal delivery to normal and non consanguineous Rwandan parents with a birth weight of 2.6 kg (-2 Z score), height of 51 cm (0 Z score) and head circumference 35 cm (0 Z score). At time of our assessment, her development milestones at 5 month of age was poor, she weighed 4kg (less than -3 Z score) and height of 56 cm (less than -3 Z score). Her clinical features included hypertelorism, depressed nasal bridge, large philtrum, webbed neck, pectus excavatum (Figure 3). Blood sample was taken for karyotype studies. Her karyotype formula was 46, XX, a normal female karyotype



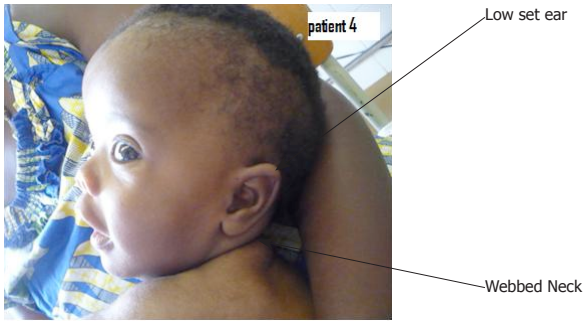


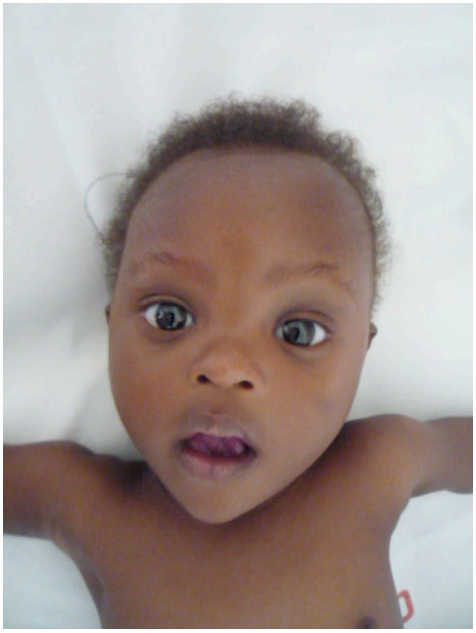
Figure 3. A, B: Photograph of patient 4 with definite Noonan Syndrome, 5 month-old

and a genomic DNA extraction was done to search for mutations in PTPN11, RAF and SOS1 genes involved in NS. Additionally, she met 2 major (including typical face) and 2 minor criteria of the van der burgt et al. criteria for NS diagnosis.

Patient 5, was a 8 month-old male when clinically diagnosed with NS at KUTH. Born by normal delivery to normal and non consanguineous Rwandan parents with normal birth weight of 3.5 kg. At time of our assessment, his development milestones at 8 months were poor. He had a poor weight gain, weighed 5.570 kg (-3 Z score), height 69cm (-1 and 1 Z score) and head circumference of 41 cm. His clinical features included hypertelorism, discrete strabismus, large philtrum, wide spaced nipples, and small umbilical hernia (Figure 4). Pan systolic murmur at lower left sternal edge was noted on cardiac examination. Workup included chest radiography that showed an enlarged cardiac silhouette; Echocardiography was in favor of complete atrioventricular septal defect (AVSD) and pulmonary hypertension. The karyotype was normal (46, XY) and his genomic DNA extraction was performed to search for mutations in PTPN11, RAF and SOS1 genes involved in NS. He met 1 major and 1 minor criteria of the Van der Burgt et al. criteria for NS diagnosis.

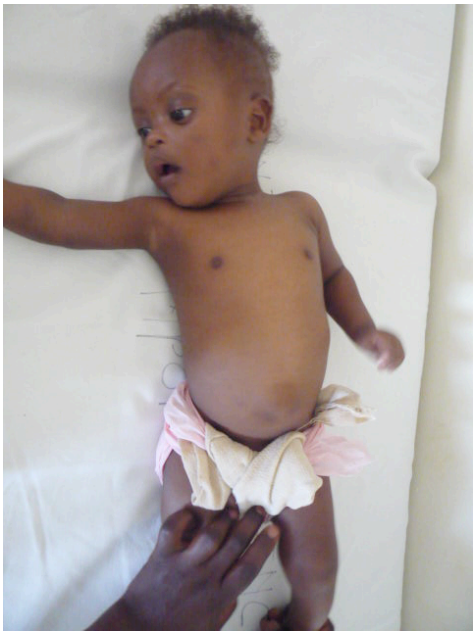
RESULTS AND DISCUSSION

The clinical presentation of all the 5 patients was



• Suggestive facies

• Hypertorolism



• Large Philtrum

• Wide spaced nipples

Figure 4: Photograph of patient 5 with suggestive Noonan Syndrome, 8 month-old

consistent with NS. Four of them were classified highly diagnostic for this condition according to classical van der burgt et al. criteria (Table1). Patient 3 had the best outcome due to the early and well conducted and coordinated follow up. Though she continued to fail to thrive, this being associated with her NS, her cardio-respiratory system was otherwise better off compared to other patients. Patient 2 had the poorest outcome, her hospital stay has been the longest due to the severity of her pulmonary stenosis. Patient 1 and 4 have had the same poor outcome as management has not been early and their complications are directly linked to their cardiologic more or less severe presentation. About patient 5, due to delayed consultation, he developed pulmonary hypertension as a complication to his cardiac defect (AVSD). Poor social economic status of the families and limited hospital settings account for most of the challenges met in the management of the condition and hence affect the short and long term outcome of our NS patients.

Echocardiography investigations performed in these patients (as stated in the clinical cases) confirmed the heterogeneity of cardiac defect in patients with Noonan phenotype. However, the cardio respiratory component of the clinical pictures of these children being directly linked to their morbidity as well as their length of stay in the hospital, all these patients are being followed in the pediatric cardiology unit with regular heart check up and surgical interventions done by visiting heart teams whenever possible.

We performed karyotype studies for all the patients and this cytogenetic study was normal in all of them. So, we excluded aberrant chromosomal disorders like Turner syndrome which can mimic the same clinical presentation as NS in female patients. However, there are still many similar conditions that have been reported so far to cause problems in the differentials of NS. These have been identified as the Neuro-cardio-facio-cuteneous syndrome [4].

For all the patients, DNA extraction has been performed for the search of possible mutations in the PTPN11 gene, as well as in SOS1, KRAS, RAF1, MEK1 all reported to cause this genetic condition [8, 11]. The PTPN11 missense mutation has been indentified as the cause of NS in 50% of cases. The other mutations account for 10-20% of the reminder of cases [11]. This means that there is 30% of NS cases with as yet unidentified mutations.

Additionally, mutations in PTPN11 gene have been reported in Leopard syndrome and Noonan like Multiple giant cell lesion syndrome [11].

We maintain that the PTPN11 mutation search test is not enough sensitive nor specific. We therefore support the importance of clinical diagnosis using the Van der Burgt et al criteria in confirming the diagnosis and managing NS patients, especially in low income settings. Research has shown that there is no difference in the clinical presentation in Noonan patients who harbor the PTPN11 gene mutation and those one without identifiable mutation [11]. Hence, we maintain that management of patients with characteristic clinical features for NS should be done as early as possible and should not wait confirmation by mutation studies. To the best of our knowledge, this is a first report of Rwandan patients with clinical phenotype of Noonan syndrome.

Table 1. Clinical signs associated with NS, as described at clinical synopsis available at the Online Mendelian Inheritance in Man website (OMIM 163950)

Characteristics	Sign
Inheritance	Autosomal dominant
Growth	Short stature (postnatal onset)
	Failure to thrive in infancy
Head and neck	Triangular face with age
	Low-set posteriorly rotated ears
	Ptosis
	Hypertelorism
	Down-slanting palpebral fissures
	Epicanthal folds
	Myopia
	Deeply grooved philtrum
	High peaks of upper lip vermilion border
	High arched palate
	Micrognathia
	Dental malocclusion
	Low posterior hairline
	Webbed neck
Cardiovascular	Congenital heart defect
	Atrial septal defects
	Ventricular septal defects
	Pulmonic stenosis
Chest	Shield chest
	Pectus carinatum superiorly
	Pectus excavatum inferiorly
Genitourinary	Cryptorchidism
Skeletal	Vertebral abnormalities
	Cubitus valgus
	Clinodactyly
	Brachydactyly
	Blunt fingertips
Neurological	Articulation difficulties
	Mental retardation

Table 2. Van der Burgt et al criteria for Noonan Syndrome clinical diagnosis

Clinical characteristics	***Major***	***Minor***
Facial	Typical face	Suggestive face
Cardiac	Pulmonary stenosis and/or suggestive ECG	Other defects
Height	Less than 5th percentile	Less than 10th percentile
Chest wall	Pectus carinatum/excavatum	Wide thorax
Family history	First degree relative with definite diagnosis	First degree relative with suggestive diagnosis
Others: mental retardation, cryptorchidism, lymphatic dysplasia.	All three	Any of the three

Definite NS: typical face with one major or two minor clinical characteristics, or suggestive face plus three minor clinical characteristics [11].

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