Genetic Investigation of South Africans with the Noonan Syndrome Phenotype Using Targeted Next Generation Sequencing

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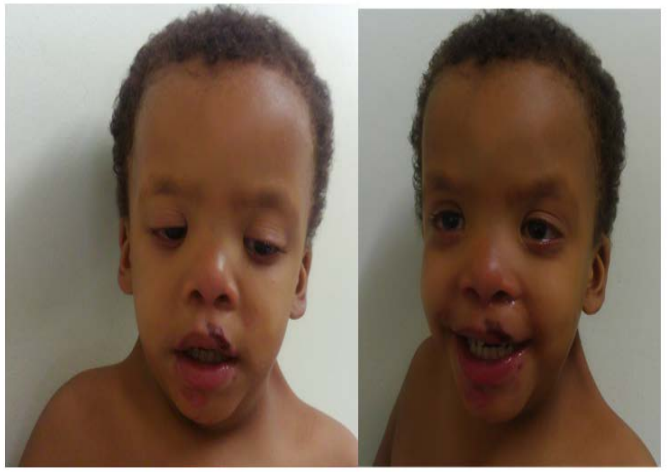
Background

Noonan Syndrome (NS)

- Common autosomal dominant condition, 1:1000 to 1:2500 live births



- Clinical characteristics
  - short stature
  - distinctive facial dysmorphism
  - cardiovascular anomalies (50-80%)
  - Other (bleeding diathesis, skeletal, Neurology…)

(Adapted from Bhambhani & Muenke, 2014; Kruszka *et al*, 2017)

Background (2)

Noonan Syndrome

- NS is caused by germline variants in more than 10 genes, components or regulators of the Ras/MAPK signaling pathway [9]
- known mutations account for ~80% of all cases, resulting in gain-of-function within the Ras/MAPK pathway [10]
- The majority of mutations are missense
- Missense mutations in *PTPN11* alone are found in ~ 50% of affected individuals[11]
- Mutations occur *de novo* in ~ 40% of cases[9]

(9) *Aoki et al*, 2016; (10) Cordeddu *et al*, 2015; (11) Tartaglia & Gelb, 2005

Aim of the study

To investigate selected genes within a group of paediatric and adult patients with a clinical diagnosis of NS

- To identify, both retrospectively and prospectively, familial and simplex cases of NS
- To undertake a thorough phenotyping of each selected proband
- To perform molecular analysis on a genetic sample from each proband
- To establish Genotype-Phenotype correlations of NS within the study population

Material & Methods

- Cross-sectional & descriptive study
- Retrospective & prospective recruitment (Jan 2015-Jan 2017)
- Adult and paediatric patients of both genders meeting the *Van der Burgt* criteria for NS involved
- **Total patients included: 26**
- Ethical approval granted by the HREC of the University of Cape Town

Van der Burgt, 2007

Material & Methods(2)

- ***Phenotyping***
  - Families' pedigree
  - Antenatal features
  - NS dysmorphology assessment
  - Cardiac evaluation
  - Biological investigations
- ***Molecular analysis***
  - Blood sample collection & DNA extraction
  - Targeted NGS on Ion PGM™
    - Panel of 14 genes (*A2ML1, BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2, SOS1* & *SPRED1*)
  - **Total of 16 DNA samples sequenced**
  - Pathogenicity assessed using variant prediction tools

Results & Discussion

- Socio-demographics
  - Children (n=20; 77%) in majority
  - Median age at clinical diagnosis: 4.5 years (range: 1month-51years)
  - Slight predominance of males (n=15;57.7%) vs (n=11;42.3%) females, (sex ratio: 1.36)

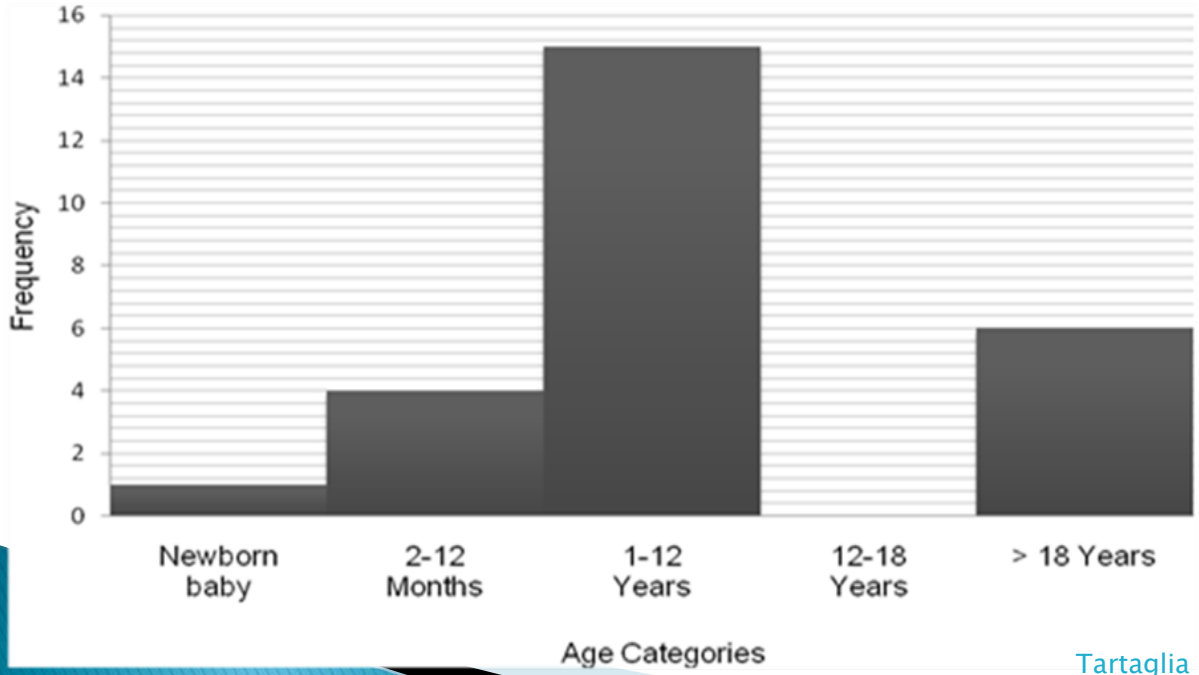


Figure 1: Age distribution in the cohort of 26 patients with a clinical diagnosis of NS

Tartaglia *et al*, 2004: Sex ratio bias in NS transmission (>1) favouring transmission to males

Results & Discussion (2)

- Individuals of mixed-ancestry background were the most represented group (53.8%), followed by black Africans (30.8%)

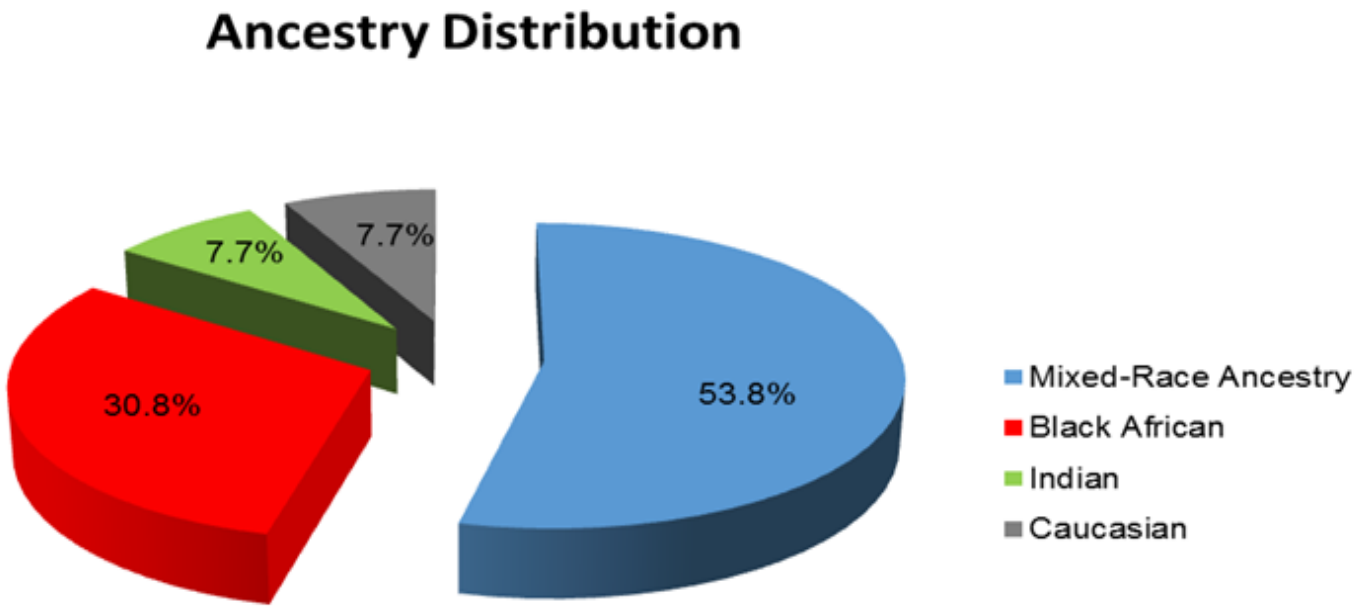


Figure 2: Ancestry distribution in the cohort of 26 patients with a clinical diagnosis of NS

Statistics South Africa, Census 2011: Western Cape Province, 48.7% of mixed-race ancestry and 32.8% of black Africans

Results & Discussion (3)

- Clinical Data
  - Family history
    - A family history suggestive of NS recorded in 50% (n=13) of cases; 42.3% (n=11) of patients were simplex cases
  - Developmental milestones
    - Gross motor milestones: ability to walk before the age of 18 months reported in 61.5% (n=16) of cases; Six (23.1%) patients were unable to walk after 24 months
    - By 24 months, 50% (n=13) of patients were able to speak simple two-word sentences

Allanson & Roberts, 2001: 30-75% of familial cases in NS

Sharland *et al*, 1992: ability to walk, average 21 months

Pierpont, 2016: average age of simple two-word sentences, 31-32 months

Results & Discussion (4)

- Craniofacial Features

Table 1: Comparison of craniofacial features between age groups

Features	New-born (n=1)	2-12 months (n=4)	1-12 years (n=15)	>18 years (n=6)
Macrocephaly	1(100%)	2(50%)	0	0
Tall and prominent forehead	1(100%)	2(50%)	8(53.3%)	1(16.7%)
Coarse face	0	1(25%)	2(13.3%)	5(83.3%)
Elongated face	1(100%)	1(25%)	6(40%)	4(66.7%)
Widely spaced eyes	0	3(75%)	5(33.3)	0
Epicanthic folds	1(100%)	3(75%)	9(60%)	3(50%)
Ptosis	0	3(75%)	10(66.7)	0
Low-set ears	1(100%)	2(50%)	9(60%)	3(50%)
Short, broad, depressed nasal root	0	3(75%)	12(80%)	3(50%)
Prominent naso-labial folds	0	0	2(13.3%)	2(33.3%)
High wide peaks of the vermilion	0	2(50%)	11(73.3)	2(33.3%)
Short neck	0	2(50%)	8(53.3%)	2(33.3%)
Webbed neck	0	1(25%)	3(20%)	1(16.7%)

- Features were more characteristic in infants (2–12 months)
- The most common features: widely spaced eyes, epicanthic folds, ptosis & broad nose with depressed nasal root

Roberts *et al*, 2013: NS features more distinctive in infancy

Results & Discussion (5)

- Craniofacial Features (2)
  - Most common features: epicanthic folds (65.4%), followed by low-set ears (57.7%). Widely spaced eyes present in only 30.8%, ptosis in 46.1%

Table 2: Comparison of key dysmorphic features between ethnic groups

Features	Black African (n=8)	Coloured (n=14)	Caucasian (n=2)	Indian (n=2)
Widely spaced eyes	2(25%)	5(35.7%)	0	1(50%)
Ptosis	6(75%)	7(50%)	0	0
Epicanthic folds	7(87.5%)	9(64.2%)	0	1(50%)
Low-set ears	6(75%)	7(50%)	1(50%)	1(50%)
Webbed neck	3(37.5%)	1(7%)	1(50%)	0
Short stature	7(87.5%)	12(87.5%)	0	2(100%)

- Black Africans presented with more typical dysmorphic features
- Epicanthic folds, ptosis & low-set ears were most common in Black Africans

Kruszka *et al*, 2017: 3 most common dysmorphic features present in >70% of individuals: widely spaced eyes (≥80%); low-set ears (>80%) & short stature (>70%)

Christianson *et al*, 1995: Epicanthic folds are very common in the general black South African population

Results & Discussion (6)

- Cardiovascular features
  - At least one cardiac abnormality was identified in 65.4% (n=17) of patients

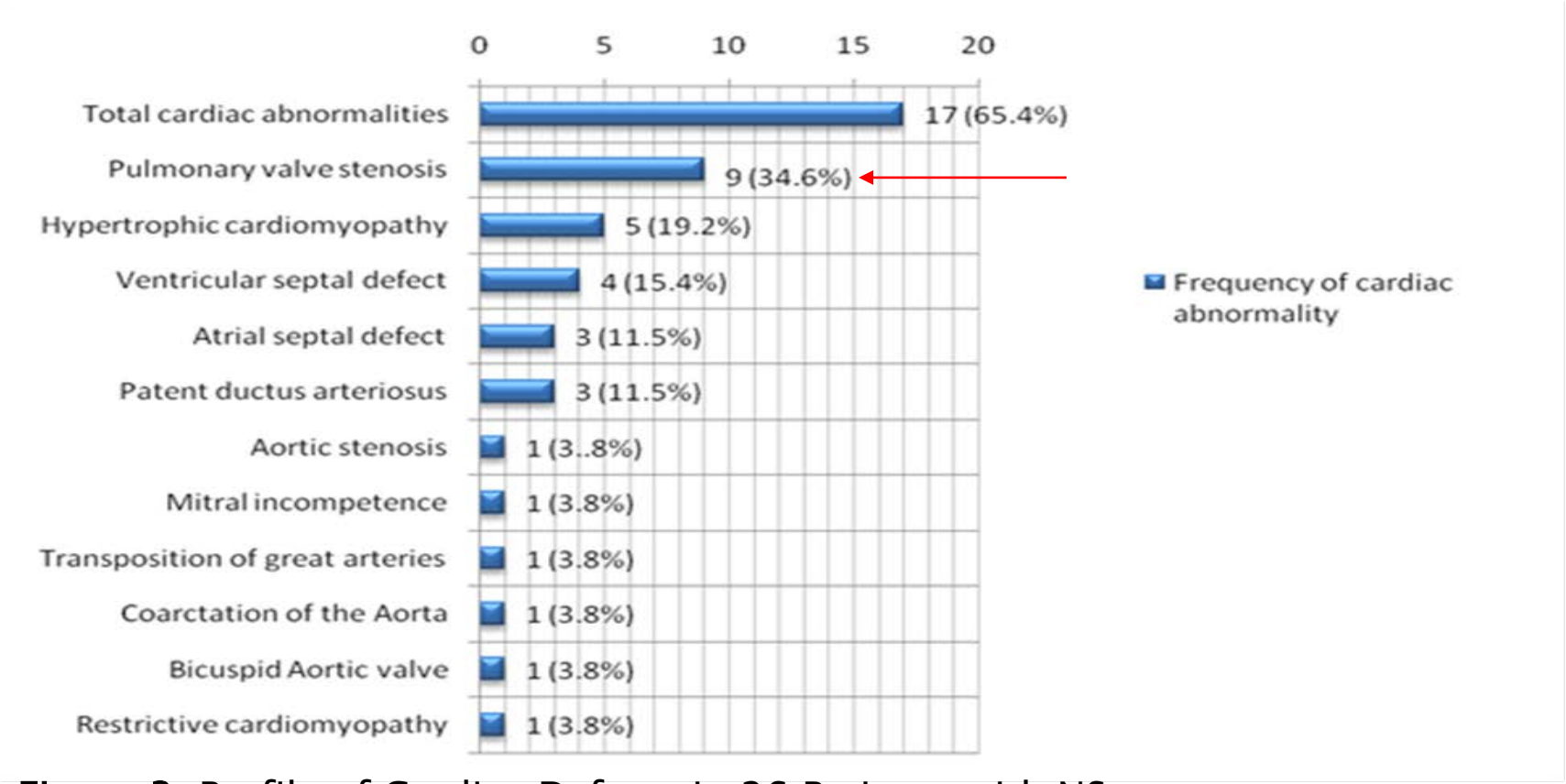


Figure 3: Profile of Cardiac Defects in 26 Patients with NS

Van der Burgt 2007; Prendiville *et al*, 2014: pulmonary stenosis 50-60% in NS

Results & Discussion (7)

- Molecular Genetic Data
  - Variants Profile
    - Variants predicted pathogenic were detected in 7 (43.7%) cases
    - Mutation-positive patients included 6 males (86%) and 1 female (14%)
    - Five (5/7; 71%) were detected in patients with family history suggestive of NS & 2(2/7; 29%) were apparently *de novo*
    - Three (42.8%) had a pathogenic variant in ***CBL***, 2(28.6%) in ***PTPN11*** & 2(28.6%) in ***MAP2K1***

Lepri et al. 2014: Detection rate of 47.5%

Aoki *et al*, 2016: Detection rate of >70% is anticipated when using a comprehensive multigene panel or WES

Results & Discussion (9)

- Genotype–phenotype Correlations

Table 4: Genotype-phenotype comparisons of the 3 genes identified

Characteristics	*PTPN11* (n=2)	*CBL* (n=3)	*MAP2K1* (n=2)
Mean age at Diagnosis (year)	3.3	11.1	1
Positive family history	2(100)	2(66.7)	1(50)
Antenatal features	0	0	2(100)
Short stature	2(100)	3(100)	2(100)
Typical dysmorphic features	2(100)	1(33.3)	2(100)
Webbed/Short neck	2(100)	3(100)	2(100)
Pectus deformity of the chest	0	2(66.7)	2(100)
Congenital Heart Defects	2(100)	1(33.3)	2(100)
Pulmonary valve stenosis	1(50)	0	1(50)
Hypertrophic cardiomyopathy	1(50)	1(33.3)	0
Coagulopathy	2(100)	1(33.3)	1(50)
Skin features	0	1(33.3)	2(100)
Intellectual disability	0	1(33.3)	2(100)

- Patients with variants in *MAP2K1* presented with more typical clinical features of NS, followed by patients with variants in *PTPN11*

Tartaglia *et al,* 2002; Yoshida *et al*, 2004a: positive association between short stature, pulmonary stenosis, coagulopathy, pectus deformities of the chest, and *PTPN11*; Negative association between HCM and *PTPN11*

Nava *et al,* 2007; Nyström *et al*, 2008: *MAP2K1* had typical facial dysmorphic features & skin manifestations

Martinelli *et al,* 2010: pectus deformities of the chest, stature >3rd c & short or webbed neck frequently present

Conclusion

- This study is, to the best of our knowledge, the first clinical and molecular research in SA individuals affected with NS
- These preliminary data suggest that the phenotype of affected individuals with NS in SA is globally similar to that reported in the literature
- The distribution of pathogenic variants in NS genes in SA may be different from that reported in other populations
- This study demonstrates that targeted NGS can be successfully applied to the molecular diagnosis of NS and related conditions in SA.

Perspectives

- In-depth bioinformatics analysis of the NGS files of individuals in whom no exonic variants were detected, with specific focus on the intron-exon boundaries
- Sanger sequencing validation of all putative pathogenic variants found
- Family segregation studies to establish causality of variants identified

Limitations

- The small size of our cohort
- Molecular analysis not offered to all the patients included
- Sanger sequencing validation of the detected pathogenic variants not performed

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