## Dutch Caribbean genetics

*Diagnostics of congenital anomalies and genetic disorders in small island communities*

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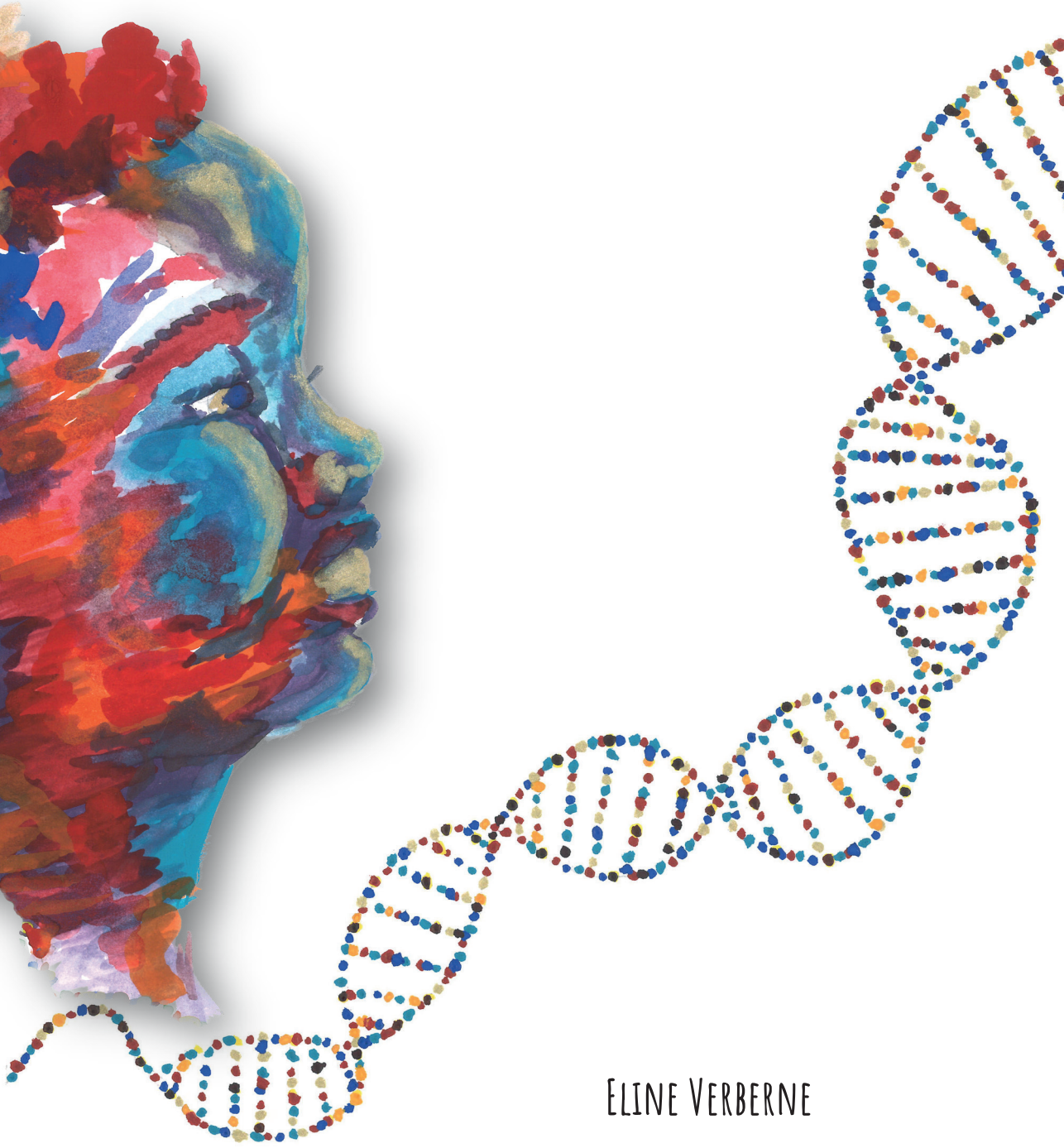

Eline Verberne

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Diagnostics of congenital anomalies and genetic disorders
in small island communities

**Eline Verberne**

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# Dutch Caribbean Genetics

Diagnostics of congenital anomalies and genetic disorders
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# Contents

# Chapter 1

## General introduction and outline of the thesis

*Based on: Verberne EA, Ecury-Goossen GM, Manshande ME, Ponson-Wever M, de Vroomen M, Tilanus M, et al. Clinical and community genetics services in the Dutch Caribbean. J Community Genet. 2021;12(3):497-501.*

# General introduction

### Congenital anomalies

Congenital anomalies can be defined as structural or functional anomalies that occur during intrauterine life and are present at birth. They may be identified prenatally, at birth or later in infancy (1). They are also known as birth defects, congenital disorders or congenital malformations. In spite of the official definition, these terms – in particular congenital malformations – are often used to refer to structural defects.

Congenital anomalies have emerged as an important global health problem, as many countries underwent an epidemiological transition by controlling other causes of infant mortality such as infectious diseases and malnutrition (1-3). The World Health Organization (WHO) estimates that 6% of neonates worldwide are born with a congenital anomaly (4). Low- and middle-income countries are disproportionally affected, as both the proportion of births with congenital anomalies as well as the absolute number of births is much higher in these countries (5). In addition, the burden of congenital anomalies in low- and middle-income countries is amplified by a lack of treatment and support services.

There are several known causes of congenital anomalies and they can be roughly categorized into two groups: genetic and non-genetic causes. Genetic causes include chromosomal disorders, such as Down syndrome, and single gene defects, such as sickle cell disease. Genetic causes of congenital anomalies may be influenced by other factors. For example, it is well known that advanced maternal age increases the risk of aneuploidy (6). Another factor is consanguinity, which increases the risk of congenital anomalies, mostly attributable to a higher risk of autosomal recessive disorders (7-9). Non-genetic causes and risk factors include certain maternal diseases (e.g. maternal diabetes, Zika virus infection), maternal nutritional deficiencies (e.g. folate deficiency), maternal use of certain drugs (e.g. alcohol, methotrexate) and exposure to radiation and certain pollutants (1). Finally, many congenital anomalies cannot be linked to a specific cause and are assumed to result from complex interactions between genetic predisposition and environmental factors. Some of the aforementioned risk factors are modifiable and certain congenital anomalies may thus be prevented by public health measures. These include the removal of risk factors, for example reducing or eliminating exposure to teratogens such as alcohol and pesticides, and the reinforcement of protective factors, for example ensuring adequate intake of folic acid before and during pregnancy (1).

### Genetic disorders

Genetic disorders are congenital anomalies caused by one or more abnormalities in the genome. They can be categorized into monogenic disorders (involving a single gene),

polygenic disorders (involving multiple genes) and chromosomal abnormalities. Although polygenic disorders are the most common, the term genetic disorder is at present mostly used to refer to a condition with a single genetic cause. A few examples of relatively common genetic disorders include Down syndrome, Turner syndrome, Fragile-X syndrome, Marfan syndrome, cystic fibrosis, sickle cell anemia and Duchenne muscular dystrophy. Most genetic disorders however are rare. Currently there are over 4,000 known rare genetic disorders, comprising 71.9% of the total number of rare diseases (10). It is estimated that at least 3.5–5.9% of the world population is affected by a rare disease (10).

Recent advances in genomic technologies and their clinical application have greatly increased the probability of obtaining a diagnosis for patients with suspected genetic disorders (11-13). A genetic diagnosis enables a better understanding of prognosis, more tailored management and improved surveillance (14). Moreover, a genetic diagnosis provides information about recurrence risk and enables patients and parents to make informed reproductive choices (14, 15). It may end a long 'diagnostic odyssey' and facilitate access to patient support groups, education, health and social care (14). In addition, for more than 600 genetic disorders a treatment based on the underlying pathogenesis is currently available, including for example dietary management, enzyme replacement therapy and medication (16).

However, there are many populations worldwide that do not yet benefit from these genomic advances, including ethnic minorities, indigenous populations, underserved and marginalized populations in urban and rural areas and those living in developing countries throughout the world (17). Evident barriers to delivering genetic services include a lack of adequately equipped diagnostic laboratories and a shortage of clinical geneticists and genetic counsellors (18-20), but also a lack of knowledge about genetic disorders amongst healthcare providers, and logistic, financial and knowledge barriers for patients (21, 22). In addition, genetic research has mainly focused on individuals of European ancestry. This results not only in increased health disparities, but also limits our understanding of how genetics influence disease (23). Nevertheless, efforts are being made to improve genetic service delivery worldwide and to include diverse populations in genetic research (24-28).

**Small island developing states**

Small island developing states (SIDS) were first recognized as a special group at the United Nations Conference on Environment and Development in 1992 (29). A total of 58 SIDS are currently recognized by the United Nations, located in three geographical regions: (1) the Caribbean, (2) the Pacific and (3) the Atlantic, Indian Ocean and South China Sea (30). Irrespective of income grouping or geographical location, SIDS share similar and unique social, environmental and economic vulnerabilities, related to their remote geography,

**Box 1.** Summary of relevant concepts and names

**Congenital anomalies** are structural or functional anomalies that occur during intrauterine life and are present at birth. Synonyms include: **birth defects**, **congenital disorders** and **congenital malformations**. These terms are also used to refer to structural defects, especially the term congenital malformations.

**Genetic disorders** are congenital anomalies caused by one or more abnormalities in the genome.

**Rare diseases** are health conditions that affect only a small proportion of the population, although there is no single, universally accepted definition. Most rare diseases have a genetic cause.

**Clinical genetics** is a medical specialty which involves diagnosis and counseling of individuals and families with, or at risk of, a genetic disorder.

**Small island developing states** are a group of small island countries that share similar challenges and vulnerabilities related to their small size, remoteness and fragile environment.

**Dutch Caribbean**: six islands located in the Caribbean Sea that are part of the Kingdom of the Netherlands: Aruba, Curaçao and St. Maarten are constituent countries within the Kingdom of the Netherlands, while Bonaire, St. Eustatius and Saba are special municipalities of the Netherlands.

- **BES islands:** Bonaire, St. Eustatius and Saba, special municipalities of the Netherlands.
- **ABC islands:** Aruba, Bonaire and Curaçao, located in the same archipelago.
- **SSS islands:** St. Maarten, St. Eustatius and Saba, are located in the same archipelago.

small size and exposure to natural hazards. They are extremely vulnerable to the effects of climate change, such as sea level rise and an increasing frequency of cyclones, storms and hurricanes (29). They strongly depend on external markets because of a lack of natural and human resources, while they face high import and export costs, placing them at a disadvantage economically and preventing economies of scale (29). Health care organization is also hampered by scale issues, including a shortage of adequately skilled human resources due to small population sizes and disproportionate costs of purchasing supplies in small quantities (31). In addition, a 'brain drain' of health professionals away from these islands exists (32). To increase access to health services that are otherwise not locally available, several SIDS have adopted schemes to provide overseas medical treatment (31) and have organized collaborations with visiting medical specialists.

Because SIDS have relatively small and isolated populations, founder effects may result in a high prevalence of certain rare monogenic disorders. For example, in the Cuban province Holguin, the highest global prevalence of spinocerebellar ataxia type 2 – a rare autosomal dominant neurodegenerative disorder – has been reported (~40 per 100,000 inhabitants),

resulting from a putative founder effect (33). Another interesting example is the Bahamas, where a remarkably high percentage of 27% of unselected breast cancer patients was found to carry a *BRCA1* or *BRCA2* pathogenic variant, of which 92% carried one of seven founder variants (34). Knowledge of founder variants and common genetic diseases in a certain population provides opportunities for better care, for example through targeted preconception carrier screening. In addition, genetically isolated populations provide a unique opportunity to discover new genes that underlie rare genetic disorders. For instance, a new gene associated with a unique form of Hermansky–Pudlak syndrome was discovered in a genetic isolate of central Puerto Rico (35).

**The Dutch Caribbean**

The Dutch Caribbean consists of six islands located in the Caribbean Sea that are part of the Kingdom of the Netherlands. In 1954, after being part of the Dutch colony of Curaçao and Dependencies, these islands were united into a single country – the Netherlands Antilles – within the Kingdom of the Netherlands. The island of Aruba seceded from the Netherlands Antilles in 1986 and became a separate constituent country of the Kingdom of the Netherlands. In 2010, the Netherlands Antilles were dissolved and Curaçao and St. Maarten became constituent countries within the Kingdom of the Netherlands as well, while Bonaire, St. Eustatius and Saba (BES islands) became special municipalities of the Netherlands (Figure 1). Together, these six islands are still commonly referred to as the Dutch Caribbean. The constituent countries of Aruba, Curaçao and St. Maarten are officially recognized by the United Nations as SIDS (30). Aruba, Bonaire and Curaçao are the three westernmost islands of the Dutch Caribbean. They are located close to Venezuela and are collectively known as the ABC islands. St. Maarten, St. Eustatius and Saba are located in the northeast Caribbean Sea near Puerto Rico and are sometimes referred to as the SSS islands (Figure 2).

Curaçao is the largest of the six islands, with a population of 153,671 (36). The smallest island, Saba, has a population of only 1,918 (37). The population of the Dutch Caribbean is characterized by mixed ancestry and high ethnic diversity. However, the majority of the population is of African descent, with the exception of Aruba, where the population is of predominantly Amerindian origin (38). The Dutch Caribbean populations are characterized by high migration rates in the last decades, with many immigrants from the Netherlands and Latin American countries, including Venezuela, the Dominican Republic and Colombia (39-41). The official languages of the ABC islands are Papiamentu and Dutch, with English as a third official language in Curaçao. Papiamentu is the most widely spoken language on these islands. English and Dutch are the official languages of the SSS islands, with English being most commonly spoken. The majority of the Dutch Caribbean population is religious, with Roman Catholicism as the main religion. The economies of the Dutch Caribbean islands

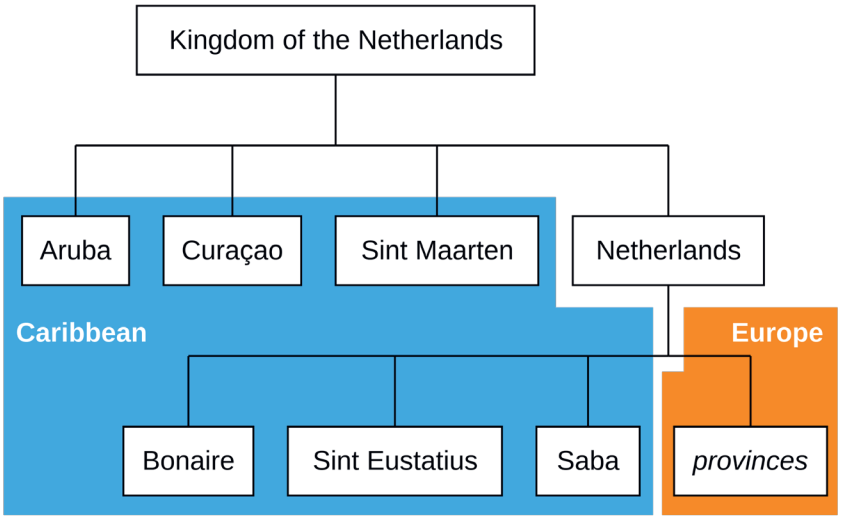


**Figure 1.** Organization of Kingdom of the Netherlands. Aruba, Curaçao, St. Maarten and the Netherlands are constituent countries of the Kingdom of the Netherlands. Bonaire, St. Eustatius, and Saba (BES islands) are special municipalities of the Netherlands. *By Thayts - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=20361998*


**Figure 2.** Geography of the Kingdom of the Netherlands. *Source: Ministry of Foreign Affairs, The Hague.*

are all defined as high-income according to the World Bank Classification. However, gross domestic product (GDP) per capita of each of the six Dutch Caribbean islands is much lower compared to the Netherlands and income inequality, as measured by the Gini coefficient, is higher (Table 1). In addition, the economies of the Dutch Caribbean islands are fragile, with a high dependence on tourism, which has resulted in a severe economic impact of the recent coronavirus disease 2019 (COVID-19) pandemic (42-44). Compared to the Netherlands, life expectancies in Aruba and Curaçao are shorter and infant and maternal mortality rates are higher (45). Average life expectancy in the BES islands estimated for the year 2013 was however approximately the same as in the Netherlands (46).

**Table 1.** General characteristics of the Kingdom of the Netherlands

| | Constituent countries | | | | Special municipalities of the Netherlands | | |
|---|---|---|---|---|---|---|---|
| | The Netherlands | Aruba | Curaçao | St. Maarten | Bonaire | St. Eustatius | Saba |
| Population[a] | 17,475,415 | 107,932 | 153,671 | 42,577 | 21,745 | 3,142 | 1,918 |
| Area (km²) | 33,720 | 180 | 444 | 34 | 288 | 21 | 13 |
| Population density (per km²) | 518 | 600 | 346 | 1,252 | 76 | 150 | 148 |
| GDP per capita (US$) | 53,044 (2018) | 30,253 (2018) | 19,630 (2018) | 28,988 (2018) | 25,400 (2018) | 33,300 (2018) | 23,500 (2018) |
| World Bank Classification | High income | High income | High income | High income | - | - | - |
| Gini coefficient | 0.30 | 0.44 | 0.42 | N/A | 0.39 | 0.43 | 0.38 |
| Unemployment rate (%) | 3.8 (2018) | 7.3 (2018) | 13.4 (2018) | 9.9 (2018) | 3.2 (2018) | 4.3 (2018) | 2.4 (2018) |
| Life expectancy at birth (years) | 82 (2019) | 76 (2019) | 79 (2019) | 78 (2016) | | 80 (2013) | |
| Infant mortality rate (per 1,000 live births) | 3 (2015–2020) | 14 (2015–2020) | 9 (2015–2020) | N/A | N/A | N/A | N/A |

[a] On January 1st, 2021.
*Abbreviations:* GDP: gross domestic product, N/A: not available.

*Health care*

The health care systems of the Dutch Caribbean largely mirror that of the Netherlands, with a general practitioner as the first point of contact. Secondary care is provided at hospitals and private clinics. All legal residents of Aruba, Curaçao and the BES islands are entitled to a basic health insurance, which is paid through income tax. As yet, there is no universal health coverage in St. Maarten. There are two general hospitals in Curaçao. The main hospital, Curaçao Medical Center, offers the most specialized medical care of the six islands, including pediatric cardiology and a neonatal intensive care unit. Aruba and St. Maarten each have

one hospital, that offers all major medical specialties. In Bonaire, secondary care is provided at the hospital Fundashon Mariadal. In St. Eustatius and Saba there are no hospitals that provide secondary care, as both are very small islands, but each island has a medical center. Secondary care is provided by visiting medical specialists and through medical transfers to St. Maarten, St. Martin, Colombia and Guadeloupe. On all six islands, certain specialized care that is not locally available, is provided through medical transfers to neighboring islands, Colombia or the Netherlands. Visiting medical specialists from the Netherlands provide additional (specialized) care on a regular basis.

*Genetic diseases*

With a large part of the population being of African descent, sickle cell disease, thalassemia and glucose-6-phosphate dehydrogenase (G6PD) deficiency are relatively common monogenic disorders in the Dutch Caribbean (47-49). Although epidemiological data are scarce, it has been estimated that the incidence of sickle cell disease is 0.05% in Aruba, 0.31% in St Maarten and 0.25% in Curaçao (49). The results from neonatal blood spot screening in the BES islands indicate that approximately 4-7% of newborns is a carrier of sickle cell disease, although the yearly rate fluctuates in Saba and St-Eustatius because of the low birth number (50). Another genetic disease that is prevalent in the Dutch Caribbean is Hereditary Hemorrhagic Telangiectasia (HHT), or Rendu-Osler-Weber disease. This is a rare autosomal dominant disorder characterized by the presence of multiple arteriovenous malformations. The point prevalence of HHT in Bonaire and Curaçao was estimated to be at least 1 in 1,331 inhabitants above the age of 12 years, which is the highest in the world (51). This high prevalence is most likely due to a founder effect. Indeed, two common pathogenic variants in the ENG gene have been identified in families with HHT in the former Netherlands Antilles and one of these variants was also found in a Dutch family with the same disease haplotype (52). Thus, it appears that at least one ENG pathogenic variant has been introduced into the populations of Bonaire and Curaçao by a Dutch colonist (52).

*Prenatal screening and testing*

Congenital anomalies can be detected early in pregnancy through prenatal screening and diagnostic testing. Early detection allows potential interventions, such as termination of pregnancy, or, when the pregnancy is continued, tailored pregnancy management and delivery planning, while also enabling parents to prepare for taking care of an affected child (53). In the Dutch Caribbean, prenatal ultrasonography to detect congenital anomalies in the second trimester is offered to all pregnant women. Screening for Down's syndrome, Edwards' syndrome and Patau's syndrome through the "combined test" or non-invasive prenatal testing (NIPT) is available, although the indications, reimbursements and uptake differ between the islands. Invasive prenatal testing (chorionic villus sampling or

amniocentesis) is only available in Aruba; women on the other islands have to go abroad for these prenatal diagnostic tests. Termination of pregnancy is not allowed by law in Aruba, Curaçao and St. Maarten, although in Curaçao there has been an 'institutionalized tolerance' policy since 1999 (54). Under this policy, termination of pregnancy for fetal defects with a great probability of causing death within the first year of life is tolerated after approval of the hospital's ethical board. On the BES islands, termination of pregnancy was legalized after they became special municipalities of the Netherlands in 2010.

*Neonatal screening*

The objective of neonatal screening is to identify newborns with treatable conditions that are not clinically evident shortly after birth, thus enabling early treatment to reduce the impact of these disorders. In 2013, it was decided by the Dutch Minister for Health, Welfare and Sport to introduce blood spot screening in the BES islands. Neonatal blood spot screening started in Bonaire on 1 January 2015, and in St. Eustatius and Saba in October of that same year. The screening program is coordinated by the local Public Health Services under the direction of the Dutch National Institute for Public Health and the Environment. Blood samples are sent to the Netherlands once per week and screened for a number of disorders, including congenital hypothyroidism, adrenogenital syndrome, hemoglobinopathies, cystic fibrosis and several metabolic disorders (equal to the program of the Netherlands). Uptake of this neonatal screening (NBS) program is high: > 90% in Saba and St Eustatius and ≥ 99% in Bonaire (in some years, uptake was > 100% in Bonaire because of high maternal mobility around childbirth, resulting in a higher number of screened newborns than registered live births). Sickle cell disease was the most frequently diagnosed condition in the first six years of this NBS program (50, 55).

In contrast to the BES islands, there is no national NBS program in Aruba, Curaçao and St. Maarten. However, there are hospital-based initiatives that offer NBS. Screening for congenital hypothyroidism is offered to newborns admitted to the pediatric department of the Dr. Horacio E. Oduber Hospital in Aruba and to all newborns delivered at Sint Maarten Medical Center. In Curaçao, screening for hemoglobinopathies in umbilical cord blood is offered to all newborns delivered at the Curaçao Medical Center, as well as screening for congenital hypothyroidism. In addition, screening for phenylketonuria is offered to newborns that are not (or only partly) Afro-Caribbean, as phenylketonuria is less prevalent in populations with African ancestry. The percentage of newborns screened through these hospital-based programs is unknown.

*Clinical genetics*

Until 2011, there was no local clinical genetics service in the Dutch Caribbean. In order to provide the pediatric population of these islands the same genetic care that is provided

for other citizens of the Kingdom of the Netherlands, a collaboration between the local pediatricians and a clinical geneticist from the Netherlands has been established, resulting in a bi-annual joint pediatric-genetics clinic. The Dutch clinical geneticist visits the pediatric departments of the local hospitals in Curaçao (since 2011), Aruba (since 2012), and Bonaire (since 2013) twice a year to evaluate patients suspected of having a genetic disorder. Pediatric patients from St. Maarten, Saba, and St. Eustatius are referred to the joint pediatric-genetics clinic at St. Maarten Medical Hospital since 2014. The total number of patients seen per year by the visiting clinical geneticist on each island is shown in Figure 3. During the genetic consultations, medical and family histories are obtained, followed by a full dysmorphology examination. If indicated, blood samples are sent to the Netherlands for genetic testing to establish or confirm a diagnosis. The costs of genetic testing are covered by the local health insurances, although there are limitations to the number and costs of genetic tests that can be requested on an annual basis. Because of these financial restrictions, gene panels based on next generation sequencing (NGS) are initially only performed in the proband. Subsequently, segregation analysis is performed in the family of the affected individual if a variant of unknown significance (VUS) is identified. Trio whole exome sequencing is not routinely offered because of the high associated costs. With this approach an effort is made to keep costs of genetic testing to a minimum, while preserving diagnostic capacity as far as possible. If a genetic diagnosis is established, patients or their caregivers receive genetic counseling during a follow-up visit with the clinical geneticist. During this visit, the genetic cause and implications of the diagnosis are explained and, if applicable, recurrence risk and risks for family members are discussed. Since the clinical geneticist visits only two times a year, results are sometimes already communicated by the local pediatrician and additional counseling is provided during the next visit of the clinical geneticist. Continuity of the service throughout the year is realized through electronic consultations between pediatricians and the clinical geneticist.

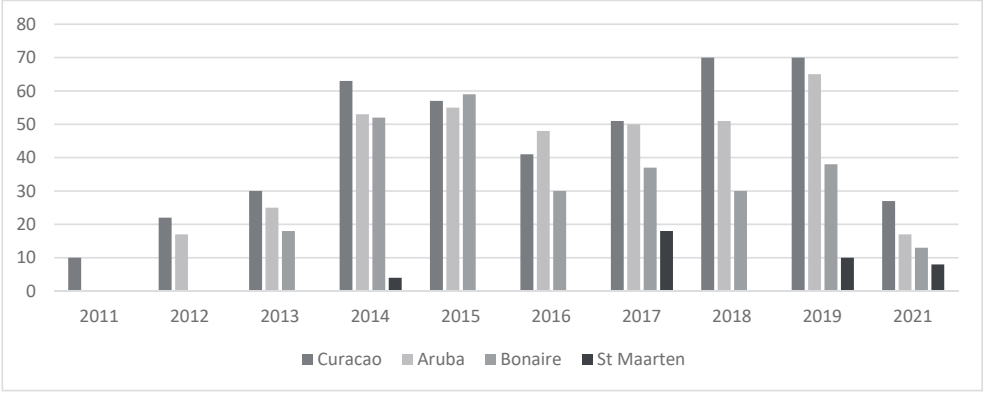


**Figure 3.** Number of patients seen by the visiting clinical geneticist per year on each island. Both new referrals as well as follow-up visits are included. Because of the COVID-19 pandemic, the outpatient clinics were cancelled in 2020 and in 2021 the clinical geneticist visited only once instead of twice.

## Objectives and outline of the thesis

The main objectives of this thesis are: (1) to determine if certain congenital anomalies are more prevalent in the relatively isolated small island populations of the Dutch Caribbean, (2) to describe and evaluate the delivery of a genetics service with a visiting clinical geneticist in the Dutch Caribbean, as an example for other small and isolated communities and (3) to provide examples of how genetic research in traditionally under-investigated populations can contribute to global scientific knowledge.

**Chapter 1** provides a general introduction to the topics of this thesis and presents the relevant background on the Dutch Caribbean. In **Chapter 2** we describe the birth prevalence and pattern of structural congenital anomalies in Aruba, Bonaire and Curaçao (ABC islands). This is the first study to describe these prevalence rates in the Dutch Caribbean. In **Chapter 3** we evaluate the delivery of clinical genetics services with a visiting clinical geneticist in the Dutch Caribbean. We investigate the diagnostic yield and the impact of a genetic diagnosis on clinical management in this resource-limited setting. The impact of a genetic diagnosis as experienced by parents of patients and their views on the provided clinical genetics service are described in **Chapter 4** in a qualitative study. In **Chapter 5, 6, 7 and 8** we illustrate how our research in the Dutch Caribbean has contributed to knowledge on genetic disorders and congenital anomalies, which is relevant for patients and clinicians worldwide.

## References


1. World Health Organization. Birth defects [Internet]. 2022 Feb 28 [cited 2022 Mar 28]. Available from: https://www.who.int/news-room/fact-sheets/detail/birth-defects.
2. Christianson A, Modell B. Medical genetics in developing countries. Annu Rev Genomics Hum Genet. 2004;5:219-65.
3. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet. 2016;388(10063):3027-35.
4. World Health Organisation. Congenital anomalies: overview [Internet]. 2022 [cited 2022 Mar 28]. Available from: https://www.who.int/health-topics/congenital-anomalies#tab=tab_1.
5. Christianson A, Howson CP, Modell B. March of Dimes global report on birth defects: the hidden toll of dying and disabled children. White Plains, New York: March of Dimes Birth Defects Foundation; 2006.
6. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet. 2001;2(4):280-91.
7. Sheridan E, Wright J, Small N, Corry PC, Oddie S, Whibley C, et al. Risk factors for congenital anomaly in a multiethnic birth cohort: an analysis of the Born in Bradford study. Lancet. 2013; 382(9901):1350-9.
8. Bittles AH, Black ML. The impact of consanguinity on neonatal and infant health. Early Hum Dev. 2010;86(11):737-41.
9. Oniya O, Neves K, Ahmed B, Konje JC. A review of the reproductive consequences of consanguinity. Eur J Obstet Gynecol Reprod Biol. 2019;232:87-96.
10. Nguengang Wakap S, Lambert DM, Olry A, Rodwell C, Gueydan C, Lanneau V, et al. Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. Eur J Hum Genet. 2020;28(2):165-73.
11. Adams DR, Eng CM. Next-Generation Sequencing to Diagnose Suspected Genetic Disorders. N Engl J Med. 2018;379(14):1353-62.
12. Miller DE, Sulovari A, Wang T, Loucks H, Hoekzema K, Munson KM, et al. Targeted long-read sequencing identifies missing disease-causing variation. Am J Hum Genet. 2021;108(8):1436-49.
13. Sadikovic B, Levy MA, Kerkhof J, Aref-Eshghi E, Schenkel L, Stuart A, et al. Clinical epigenomics: genome-wide DNA methylation analysis for the diagnosis of Mendelian disorders. Genet Med. 2021;23(6):1065-74.
14. Wright CF, FitzPatrick DR, Firth HV. Paediatric genomics: diagnosing rare disease in children. Nat Rev Genet. 2018;19(5):253-68.
15. Lim Q, McGill BC, Quinn VF, Tucker KM, Mizrahi D, Patenaude AF, et al. Parents' attitudes toward genetic testing of children for health conditions: A systematic review. Clin Genet. 2017;92(6):569-78.
16. Bick D, Bick SL, Dimmock DP, Fowler TA, Caulfield MJ, Scott RH. An online compendium of treatable genetic disorders. Am J Med Genet C Semin Med Genet. 2021;187(1):48-54.
17. Groft SC, Posada M, Taruscio D. Progress, challenges and global approaches to rare diseases. Acta Paediatr. 2021;110(10):2711-6.
18. Tekola-Ayele F, Rotimi CN. Translational Genomics in Low- and Middle-Income Countries: Opportunities and Challenges. Public Health Genomics. 2015;18(4):242-7.
19. Christianson A, Zimmern R, Kristoffersson U, Schmidtke J, Kent A, Raouf R, et al. Health needs assessment for medical genetic services for congenital disorders in middle- and low-income nations. J Community Genet. 2013;4(3):297-308.

20. Kamp M, Krause A, Ramsay M. Has translational genomics come of age in Africa? Hum Mol Genet. 2021;30(20):R164-r73.
21. Suther S, Kiros GE. Barriers to the use of genetic testing: a study of racial and ethnic disparities. Genet Med. 2009;11(9):655-62.
22. Zhong A, Darren B, Loiseau B, He LQB, Chang T, Hill J, et al. Ethical, social, and cultural issues related to clinical genetic testing and counseling in low- and middle-income countries: a systematic review. Genet Med. 2021;23(12):2270-80.
23. Genetics for all. Nat Genet. 2019;51(4):579.
24. Thong MK, See-Toh Y, Hassan J, Ali J. Medical genetics in developing countries in the Asia-Pacific region: challenges and opportunities. Genet Med. 2018;20(10):1114-21.
25. Prada CE, Cavalcanti D, Schwartz IVD, Zarate YA. Introduction to the special issue on Clinical Genetics in Latin America. Am J Med Genet C Semin Med Genet. 2020;184(4):873-5.
26. Choudhury A, Aron S, Botigué LR, Sengupta D, Botha G, Bensellak T, et al. High-depth African genomes inform human migration and health. Nature. 2020;586(7831):741-8.
27. Moreno-Estrada A, Gignoux CR, Fernández-López JC, Zakharia F, Sikora M, Contreras AV, et al. Human genetics. The genetics of Mexico recapitulates Native American substructure and affects biomedical traits. Science. 2014;344(6189):1280-5.
28. Wonkam A, Tekendo CN, Sama DJ, Zambo H, Dahoun S, Béna F, et al. Initiation of a medical genetics service in sub-Saharan Africa: experience of prenatal diagnosis in Cameroon. Eur J Med Genet. 2011;54(4):e399-404.
29. United Nations. United Nations Conference on Environment & Development Rio de Janerio, Brazil, 3 to 14 June 1992 - AGENDA 21. 1992.
30. United Nations. Small Island Developing States [Internet]. 2022 [cited 2022 Apr 8]. Available from: https://sustainabledevelopment.un.org/topics/sids/list.
31. WHO country presence in small island developing states (SIDS). Geneva: World Health Organization; 2021.
32. World Health Organization. Small island developing states: health and WHO: country presence profile. Geneva: World Health Organization; 2017. Contract No.: WHO/CCU/17.08.
33. Velázquez Pérez L, Cruz GS, Santos Falcón N, Enrique Almaguer Mederos L, Escalona Batallan K, Rodríguez Labrada R, et al. Molecular epidemiology of spinocerebellar ataxias in Cuba: insights into SCA2 founder effect in Holguin. Neurosci Lett. 2009;454(2):157-60.
34. Akbari MR, Donenberg T, Lunn J, Curling D, Turnquest T, Krill-Jackson E, et al. The spectrum of BRCA1 and BRCA2 mutations in breast cancer patients in the Bahamas. Clin Genet. 2014;85(1):64-7.
35. Anikster Y, Huizing M, White J, Shevchenko YO, Fitzpatrick DL, Touchman JW, et al. Mutation of a new gene causes a unique form of Hermansky-Pudlak syndrome in a genetic isolate of central Puerto Rico. Nat Genet. 2001;28(4):376-80.
36. Central Bureau of Statistics Curaçao. Population Tables [Internet]. 2020 [cited 2022 Apr 11]. Available from: https://www.cbs.cw/population-tables.
37. Statistics Netherlands. Caribbean Netherlands; population, country of birth, nationality [Internet]. 2021 [cited 2022 Apr 11]. Available from: https://opendata.cbs.nl/statline/#/CBS/en/dataset/84757ENG/table?ts=1649672230340.
38. Toro-Labrador G, Wever R, Martínez-Cruzado J. Mitochondrial DNA Analysis in Aruba: Strong Maternal Ancestry of Closely Related Amerindians and Implications for the Peopling of Northwestern Venezuela. Carib J Sci. 2003;39.
39. Central Bureau of Statistics Curaçao. Migration Tables [Internet]. 2020 [cited 2022 Apr 15]. Available from: https://www.cbs.cw/migration-tables.

40. Central Bureau of Statistics Aruba. Recent Migrants in our society. Aruba; 2018.
41. Department of Statistics Sint Maarten. Statistical Yearbook 2017. St. Maarten; 2017.
42. Centrale Bank Curaçao & Sint Maarten. Investing in education and labor market skills required for sustainable and inclusive economic recovery [Internet]. Willemstad; 2021 Jul 30 [cited 2022 May 11]. Available from: https://www.centralbank.cw/publications/annual-reports-quarterly-bulletins/2021/pb2021-010-investing-in-education-and-labor-market-skills-required-for-sustainable-and-inclusive-economic-recovery.
43. International Monetary Fund. Kingdom of the Netherlands—Aruba: 2021 Article IV Consultation Discussions-Press Release; Staff Report; and Staff Supplement. 2021.
44. Statistics Netherlands. Trends in the Caribbean Netherlands 2021 [Internet]. 2021 Dec 6 [cited 2022 May 11]. Available from: https://www.cbs.nl/nl-nl/publicatie/2021/49/trends-in-the-caribbean-netherlands-2021.
45. Verstraeten SPA. Population health in the Dutch Caribbean. A comparative study of political context and health policy performance. The Netherlands: Erasmus University Rotterdam; 2020.
46. Statistics Netherlands. Levensverwachting in Caribisch Nederland verschilt weinig met Nederland [Internet]. 2015 Jan 22 [cited 2022 May 12]. Available from: https://www.cbs.nl/nl-nl/nieuws/2015/04/levensverwachting-in-caribisch-nederland-verschilt-weinig-met-nederland#:~:text=De%20levensverwachting%20bij%20geboorte%20in,uit%20op%2080%2C2%20jaar.
47. Rijksinstituut voor Volksgezondheid en Milieu (RIVM). Neonatale hielprikscreening in Caribisch Nederland. Uitvoeringstoets 2013. 2013.
48. van der Dijs FP, van den Berg GA, Schermer JG, Muskiet FD, Landman H, Muskiet FA. Screening cord blood for hemoglobinopathies and thalassemia by HPLC. Clinical chemistry. 1992;38(9):1864-9.
49. van Heyningen AM, Levenston MJ, Tamminga N, Scoop-Martijn EG, Wever RM, Verhagen AA, et al. Estimated incidence of sickle-cell disease in Aruba and St. Maarten suggests cost-effectiveness of a universal screening programme for St. Maarten. The West Indian medical journal. 2009;58(4):301-4.
50. Wins S, Verkerk PH, van der Ploeg K. Neonatale hielprikscreening in Caribisch Nederland. Monitor over 2020. 2021.
51. Westermann CJ, Rosina AF, De Vries V, de Coteau PA. The prevalence and manifestations of hereditary hemorrhagic telangiectasia in the Afro-Caribbean population of the Netherlands Antilles: a family screening. American journal of medical genetics Part A. 2003;116a(4):324-8.
52. Gallione CJ, Scheessele EA, Reinhardt D, Duits AJ, Berg JN, Westermann CJ, et al. Two common endoglin mutations in families with hereditary hemorrhagic telangiectasia in the Netherlands Antilles: evidence for a founder effect. Human genetics. 2000;107(1):40-4.
53. Krstić N, Običan SG. Current landscape of prenatal genetic screening and testing. Birth Defects Res. 2020;112(4):321-31.
54. Boersma A, Alberts J, Bruijn JD, Meyboom BD, Kleiverda G. Termination of pregnancy in Curaçao: need for improvement of sexual and reproductive healthcare. Global journal of health science. 2012;4(3):30-8.
55. Wins S, Verkerk PH, van der Ploeg K. Neonatale hielprikscreening in Caribisch Nederland. Monitor over 2019. 2020.

# Part I

## Congenital anomalies in the Dutch Caribbean

# Chapter 2

## Prevalence of congenital anomalies in the Dutch Caribbean islands of Aruba, Bonaire, and Curaçao

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## Abstract

**Background:** Congenital anomalies represent an important global health issue. Data on the prevalence and pattern of congenital anomalies in the Caribbean region are scarce and lacking altogether in Aruba, Bonaire and Curaçao (ABC islands).

**Methods:** We performed a population-based surveillance study to determine the prevalence of structural congenital anomalies in the ABC islands, including all live births and stillbirths between 1-1-2008 and 31-12-2017 with major congenital anomalies according to EUROCAT guide 1.5. Terminations of pregnancy for fetal anomaly were included as well. Cases were identified by active case ascertainment, using multiple sources including pediatric patient files and discharge letters, delivery records and clinical genetic patient files. Total and subgroup prevalence rates were compared between the three islands and to the French West Indies and Northern Netherlands.

**Results:** Total prevalence of congenital anomalies on the ABC islands was 242.97 per 10,000 births. Total prevalence of congenital anomalies in Bonaire (325.15 per 10,000 births) was higher compared to Aruba (233.29 per 10,000 births) and Curaçao (238.58 per 10,000 births), which was mainly attributable to a higher prevalence of limb anomalies, in particular polydactyly, in Bonaire. Total prevalence of congenital anomalies on the ABC islands was comparable to the French West Indies (248.69 per 10,000 births) but significantly lower compared to the Northern Netherlands (298.98 per 10,000 births). In the subgroup prevalence analysis, the prevalence of polydactyly and atrial septal defect on the ABC islands was significantly higher compared with the French West Indies and the Northern Netherlands, while the prevalence of congenital anomalies of the kidney and urinary tract and genetic disorders was significantly lower.

**Conclusions:** This is the first study to establish the prevalence and pattern of congenital anomalies on the ABC islands, which is important to inform healthcare managers and policy makers and to provide a basis for continuous surveillance of congenital anomalies.

## Introduction

Congenital anomalies, also known as birth defects or congenital malformations, can be defined as structural or functional anomalies that are present at birth. They may be caused by genetic, maternal and environmental factors, although in most cases the exact cause remains unknown (1). The worldwide live birth prevalence of congenital anomalies is estimated to be 3–6% (2, 3). However, estimates vary widely across registries, which is to a large extent attributable to differences in surveillance methods and inclusion and exclusion criteria, but may also reflect true differences in prevalence rates, related for example to genetic or environmental factors (2). Congenital anomalies represent a major global health issue and are an important cause of perinatal, neonatal, infant and child morbidity and mortality, as well as long-term disability (4, 5). The proportion of congenital anomalies contributing to overall child mortality has increased in particular in regions where under-five mortality rates declined because other causes of child mortality such as infectious diseases and malnutrition were controlled (1, 4, 6).

Accurate local prevalence data on congenital anomalies are important to better understand the extent of the problem and to guide health care policies that aim to prevent congenital anomalies and to provide care and support to affected individuals (7). In 2010, the World Health Assembly published a resolution urging member states to develop and strengthen registration and surveillance systems for birth defects (8). However, there is still a paucity of prevalence data on congenital anomalies in many parts of the world, especially in resource-limited areas. A more recent consensus statement on congenital anomalies in Latin America and the Caribbean listed improved surveillance and epidemiologic research as key action points to improve birth defects prevention and care (9).

In this study, we aim to estimate the prevalence and pattern, that is the prevalence of different subgroups, of structural congenital anomalies on the Dutch Caribbean islands of Aruba, Bonaire and Curaçao (ABC islands). These islands are part of the Kingdom of the Netherlands and are located in the Caribbean Sea, near the coast of Venezuela. Besides establishing baseline prevalence rates, we will compare the prevalence data of the ABC islands with those of two EUROCAT registries: the French West Indies (Guadeloupe and Martinique) and Northern Netherlands. The French West Indies are comparable to the ABC islands in terms of geographical location, as these islands are also located in the Caribbean Sea, and in terms of ancestral background, as their population is mainly of African descent (10). The rationale behind comparison with the Northern Netherlands is that the ABC islands are part of the Kingdom of the Netherlands. Comparison with these two EUROCAT registries will allow better interpretation of the prevalence and pattern of congenital anomalies on the ABC islands.

## Methods

**Study setting**

The ABC islands are part of the Kingdom of the Netherlands, although their legal status differs: Aruba and Curaçao are constituent countries, while Bonaire is a special municipality within the country of the Netherlands.

The healthcare systems of ABC islands largely mirror that of the Netherlands, with a general practitioner as the first point of contact and secondary care being provided at general hospitals. Certain specialized care that is not locally available is provided through medical transfers to overseas hospitals and by medical specialists visiting from abroad. All legal residents are entitled to a basic health insurance, which is paid through income tax.

Curaçao is the largest island with a population of 153,671 (11) and approximately 1,500 to 2,000 births per year. There are two hospitals, of which Curaçao Medical Center (CMC) is the largest hospital. CMC opened in 2019 and replaced the Sint-Elisabeth Hospital (SEHOS). It provides all major medical specialties and is the only hospital in Curaçao that provides obstetric and pediatric care, including pediatric cardiology and a neonatal intensive care unit (NICU). Outpatient pediatric services are also offered at a number of private clinics. Approximately 80% of deliveries in Curaçao take place at the CMC (H. Holtsema, personal communication). Prenatal ultrasound to detect structural anomalies in the second trimester is offered to all pregnant women. Screening for Down's syndrome, Edwards' syndrome and Patau's syndrome through non-invasive prenatal testing (NIPT) or the "combined test" is performed only sporadically. NIPT is covered by health insurance for women ≥ 40 years old and/or with a medical indication. Invasive prenatal testing is not available, but can be performed in Aruba or Colombia. Termination of pregnancy (TOP) is prohibited by law, although there has been an "institutionalized tolerance" policy since 1999. Under this policy, TOP for fetal defects with a great likelihood of causing death within one year after birth is tolerated after approval of the hospital's ethical board (12).

Aruba has a population of 107,457 (13), with approximately 1,200 to 1,300 births per year. There is one general hospital, the Dr. Horacio E. Oduber Hospital (HOH), where all major medical specialties are provided, including obstetric and pediatric care. The pediatric cardiologist of the CMC visits the HOH on a regular basis to evaluate patients with (suspected) heart disease. The majority of deliveries take place at the HOH. Prenatal ultrasound to detect structural anomalies in the second trimester is offered to all pregnant women. Screening for Down's syndrome, Edwards' syndrome and Patau's syndrome is performed through the "combined test" or NIPT, with an uptake of approximately 41%. NIPT was paid out-of-pocket up until October 2021, but due to unavailability of the "combined test" it is now offered

as part of the standard care to all pregnant women after counseling and covered by the national health insurance. Invasive prenatal testing is performed at the HOH and is covered by health insurance. TOP for medical indication is discussed on a case basis before it can be offered and has to be carried out before 24 weeks of gestation.



Bonaire is the smallest of the three islands, with a population of 21,745 (14) and approximately 250 births per year. Secondary care, including obstetric and pediatric care, is provided at the hospital Fundashon Mariadal (FM), where all deliveries on the island take place. All pregnant women are offered prenatal ultrasound to detect structural anomalies in the second trimester. NIPT for fetal aneuploidies is offered to pregnant women of 36 years and above and covered by health insurance. NIPT for women below the age of 36 years is only performed on request and has to be paid out-of-pocket. Women are referred to the HOH on Aruba if there is an indication for invasive prenatal testing. TOP was legalized after October 10, 2010, when Bonaire became a special municipality of the Netherlands.

On all three islands, a lack of certain (highly) specialized care leads to a relatively high mobility around birth, particularly in Bonaire where pediatric care is least advanced. For example, pregnant women from Aruba and Bonaire may be transferred to Curaçao or Colombia when a (very) preterm birth is expected and pregnant women from Curaçao may be transferred to Colombia when neonatal surgery is required.

Autopsy after fetal or neonatal death is very rarely performed on the ABC islands.

**Study design and population**

We performed a population-based surveillance study. All children born between 1-1-2008 and 31-12-2017 with at least one structural congenital anomaly, whose mother was living on the ABC islands at the time of delivery, were included in the study. Stillbirths with a gestational age ≥ 20 weeks and/or birthweight > 500 grams were also included, as were terminations of pregnancy for fetal anomaly (TOPFA). To enable comparison with other registries, we only included cases with major structural congenital anomalies according to EUROCAT, a European network of population-based registries for the epidemiological surveillance of congenital anomalies, guide 1.5 (15). Major congenital anomalies are defined as structural changes that have significant medical, social or cosmetic consequences for the affected individual, and typically require medical intervention.

**Outcomes**

We calculated the total and subgroup prevalence rates on the ABC islands and compared these between the three islands. In addition, total and subgroup prevalence rates of the ABC

islands were compared to EUROCAT prevalence data of the French West Indies (Guadeloupe and Martinique) and the Northern Netherlands. Congenital anomalies were classified in subgroups and clustered into 12 categories in accordance with EUROCAT guide 1.5, Chapter 3.3. These 12 categories are: 1) nervous system anomalies, 2) eye anomalies, 3) ear, face and neck anomalies, 4) congenital heart defects, 5) respiratory anomalies, 6) oro-facial clefts, 7) gastro-intestinal anomalies, 8) abdominal wall defects, 9) congenital anomalies of kidney and urinary tract, 10) genital anomalies, 11) limb anomalies, and 12) genetic disorders. In contrast to the EUROCAT guide, we did not further classify the category oro-facial clefts into the subgroups "cleft lip with or without cleft palate" and "cleft palate", as this information was often not recorded in the medical files, resulting in a total of 102 anomaly groups instead of 104.

**Data collection**

Cases were identified by active case ascertainment (2018–2020), using multiple sources including pediatric patient files, pediatric discharge letters and delivery records (in which all live births as well as stillbirths and TOP are registered) from the HOH, FM, CMC and SEHOS. Additionally, NICU records from the CMC, outpatient medical records from private pediatric clinics in Curaçao and patient files from the bi-annual joint pediatric-genetics clinics on the ABC islands were searched. Detailed information on each congenital anomaly was collected, as well as additional data, including sex, birth plurality, year of birth, place of birth, type of birth, birth weight and maternal age.

Livebirth statistics were obtained from the Central Bureau of Statistics (CBS) of Aruba (13), Curaçao (16) (Central Bureau of Statistics Curaçao, personal communication) and Caribbean Netherlands (17, 18). The CBS corrects for the high mobility around birth in their livebirth statistics by registering live born children by the municipality registration of the mother, even if the child was born somewhere else. However, this only applies to children who arrive in Bonaire within the first 45 days of their life. After 45 days, the child is counted as an immigrant. There are no official data on the number of stillbirths on the ABC islands. Therefore, the delivery records of the HOH, FM and SEHOS were searched to determine the total number of stillbirths during the study period.

Data on prevalence of congenital anomalies in the French West Indies (Guadeloupe and Martinique) from 2009–2018 (data not available for 2008) and the Northern Netherlands from 2008–2017 were derived from the online EUROCAT data registry (https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-data/prevalence/export/, accessed on 25/7/2022).

**Data analysis**

Total and subgroup prevalence of congenital anomalies was calculated as described in EUROCAT guide 1.5 (15):

$$\text{Prevalence} = \frac{\text{Number of cases (live births + stillbirths + TOPFA)}}{\text{Number of births (live births + stillbirths)}} \times 10{,}000$$

The 95% confidence intervals (CI) were calculated using the Poisson distribution. A child/fetus with several major anomalies was counted once within each anomaly group. Thus, the number of cases in various anomaly groups cannot be added to reach a total number. In any given prevalence, a child/fetus was counted only once. For example, a child with Down syndrome and atrioventricular septal defect (AVSD) is counted once in the overall prevalence rate of congenital anomalies, once in the category congenital heart defects (CHD), once in the subgroup severe CHD, once in the subgroup AVSD, once in the category genetic disorders and once in the subgroup Down syndrome / trisomy 21.

Chi-square test was used to test differences in baseline characteristics and prevalence rates. Two-tailed Fisher's exact test or Fisher-Freeman-Halton exact test was used if more than 20% of cells of the contingency table had an expected value of < 5. A p-value of $< 0.05$ was considered to be statistically significant. A Bonferroni correction for multiple comparisons was applied for comparing prevalence rates of each of the 102 anomaly groups ($p = 0.05 / 102 = 0.00049$). Data were analyzed using SPSS version 26 and Microsoft Excel 2016. An online statistics tool was used to calculate Fisher's exact tests (https://www.socscistatistics.com/tests/fisher/default2.aspx).

**Ethical statement**

The study was approved by the Medical Ethical Committee and/or Board of Directors of the HOH, FM and CMC. Verbal consent for the study was obtained from the children's caregivers by telephone. This was followed by an email with a summary of the information that was discussed and written information about the study, as well as instructions on how to revoke consent. If we were unable to reach caregivers, all personal data of the child were deleted and the case was included anonymously.

## Results

A total of 34,367 births (live and stillbirths) were recorded during the study period. There were 873 children/fetuses who met the inclusion criteria. Caregivers of 38 children (4.4%) declined participation in the study or revoked their consent and these children were thus

not included (2.6% in Aruba, 13.9% in Bonaire and 3.9% in Curaçao). A total of 835 children/fetuses were included in the study: 303 from Aruba, 68 from Bonaire and 464 from Curaçao. Baseline characteristics are shown in Table 1. Mean birth weight and maternal age were not calculated because of too many missing data.

**Table 1.** Baseline characteristics of children/fetuses with congenital anomalies

| | Aruba n = 303 | Bonaire n = 68 | Curaçao n = 464 | p-value | Total n = 835 |
|---|---|---|---|---|---|
| Sex | | | | | |
| Male (%) | 165 (56) | 41 (62) | 258 (56) | 0.68 | 464 (56) |
| Female (%) | 131 (44) | 25 (48) | 204 (44) | | 360 (44) |
| Place of birth | | | | | |
| Same as residence mother (%) | 289 (95) | 54 (79) | 457 (98) | < 0.00 | 800 (96) |
| Plurality | | | | | |
| Singleton (%) | 299 (99) | 61 (90) | 444 (96) | < 0.01 | 804 (96) |
| Twins (%) | 4 (1) | 7 (10) | 20 (4) | | 31 (4) |

Total prevalence of congenital anomalies in the ABC islands was 242.97 per 10,000 births (95% CI: 226.77–260.01). In Bonaire the total prevalence was higher compared to Aruba and Curacao (p = 0.0018 and p = 0.0022, respectively) (Table 2). Total prevalence of congenital anomalies did not differ significantly between Aruba and Curaçao.

**Table 2.** Total prevalence of congenital anomalies in Aruba, Bonaire and Curaçao

| | Aruba | Bonaire | Curaçao | Total |
|---|---|---|---|---|
| Number of cases | 303 | 68 | 464 | 835 |
| Total prevalence per 10,000 births (95% CI) | 233.29 (207.78–261.08) | 352.15 (273.51–446.38) | 238.58 (217.38–261.30) | 242.97 (226.77–260.01) |

*Note:* Prevalence was calculated as follows: number of cases (live births + stillbirths + TOPFA) / number of births (live births + stillbirths) * 10,000.
*Abbreviations:* CI: confidence interval, TOPFA: termination of pregnancy for fetal anomaly.

Prevalence per congenital anomaly category for each island is shown in Figure 1. The prevalence of limb anomalies was significantly higher in Bonaire compared with Aruba (119.11 vs 49.28, p = 0.0002) and Curaçao (119.11 vs 37.54, p < 0.0001). This was mainly attributable to a higher prevalence of polydactyly in Bonaire (82.86 per 10,000 births) compared to Aruba (33.11 per 10,000 births) and Curaçao (22.11 per 10,000 births) (Supplementary Table 1). There was no statistically significant difference between the three islands for any of the other categories. An overview of prevalence rates for all 102 anomaly subgroups for each island can be found in Supplementary Table 1.

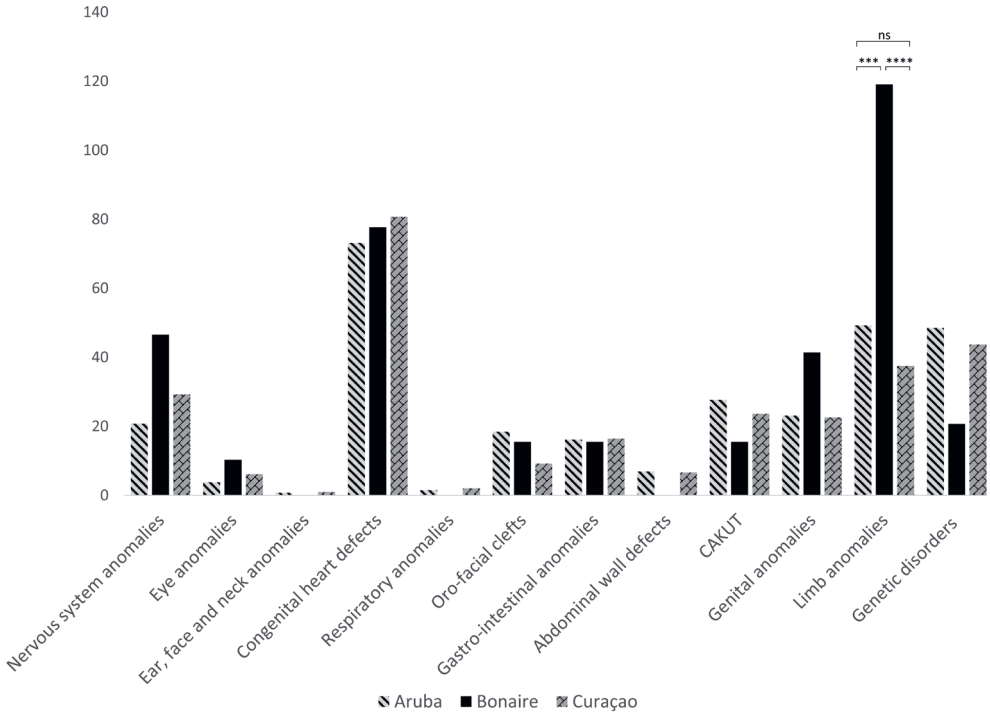


**Figure 1.** Prevalence of congenital anomalies per main subgroup in Aruba, Bonaire and Curaçao (per 10,000 births). CAKUT: congenital anomalies of the kidney and urinary tract, ns: not significant, *** p-value < 0.001, **** p-value < 0.0001.

Total and subgroup prevalence of congenital anomalies in the ABC islands were compared with those in the French West Indies and the Northern Netherlands (Table 3, Supplementary Table 2). Total prevalence of congenital anomalies did not differ significantly between the ABC islands and the French West Indies (242.97 versus 248.69 per 10,000 births, p-value 0.87), but it was significantly lower in the ABC islands compared to the Northern Netherlands (242.97 versus 298.98 per 10,000 births, p-value < 0.0001). In the subgroup prevalence analysis, four out of the 102 anomaly groups showed a statistically significant difference in prevalence rate between the ABC islands and the French West Indies as well as the Northern Netherlands (Supplementary Table 2). The prevalence of atrial septal defect was 23.28 per 10,000 births in the ABC islands, compared with 7.59 in the French West Indies (p-value < 0.0001) and 9.84 in the Northern Netherlands (p-value < 0.0001). The prevalence of polydactyly was also significantly higher in the ABC islands compared with the French West Indies and the Northern Netherlands (29.68 versus respectively 10.47 and 13.34 per 10,000 births, both p-values < 0.0001). Congenital anomalies of the kidney and urinary tract (CAKUT) were less prevalent in the ABC islands (24.73 per 10,000 births) compared to the French West Indies (43.92 per 10,000 births, p-value < 0.0001) and the Northern Netherlands

(45.94 per 10,000 births, p-value < 0.0001). Finally, the prevalence of genetic disorders was lower in the ABC islands (44.23 per 10,000 births) compared with the French West Indies (76.09 per 10,000 births, p-value < 0.0001) and the Northern Netherlands (67.37 per 10,000 births, p-value < 0.0001).

**Table 3.** Prevalence of congenital anomalies on the ABC islands, French West Indies and Northern Netherlands (total and per category)

| | Prevalence per 10,000 births (95% CI) | | |
|---|---|---|---|
| | ABC islands n = 835 | French West Indies n = 2,327 | Northern Netherlands n = 4,953 |
| All anomalies | 242.97 (226.77–260.01) | 248.69 (238.69–259.00) | 298.98 (290.71–307.43) |
| Nervous system anomalies | 27.06 (21.85–33.15) | 39.12 (35.21–43.33) | 26.86 (24.43–29.48) |
| Eye anomalies | 5.53 (3.33–8.64) | 5.66 (4.24–7.41) | 8.09 (6.78–9.58) |
| Ear, face and neck anomalies | 0.87 (0.17–2.57) | 4.27 (3.05–5.82) | 3.20 (2.40–4.18) |
| CHD | 77.69 (68.66–87.59) | 59.63 (54.79–64.79) | 84.93 (80.55–89.49) |
| Respiratory anomalies | 1.75 (0.63–3.81) | 4.81 (3.51–6.43) | 4.29 (3.35–5.41) |
| Oro-facial clefts | 13.09 (9.55–17.52) | 8.12 (6.40–10.17) | 20.70 (18.57–23.01) |
| Gastro-intestinal anomalies | 16.29 (12.31–21.16) | 17.10 (14.55–19.96) | 19.07 (17.03–21.30) |
| Abdominal wall defects | 6.40 (4.01–9.69) | 10.26 (8.31–12.53) | 6.58 (5.40–7.94) |
| CAKUT | 24.73 (19.76–30.58) | 43.92 (39.78–48.38) | 45.94 (42.73–49.32) |
| Genital anomalies | 23.86 (18.98–29.61) | 25.54 (22.41–28.99) | 28.85 (26.33–31.56) |
| Limb anomalies | 46.56 (39.63–54.35) | 38.79 (34.91–43.00) | 60.24 (56.56–64.10) |
| Genetic disorders | 44.23 (37.48–51.84) | 76.09 (70.61–81.89) | 67.37 (63.47–71.44) |

*Note:* Prevalence was calculated as follows: number of cases (live births + stillbirths + TOPFA) / number of births (live births + stillbirths) * 10,000. The complete list of congenital anomalies in the ABC islands, French West Indies and Northern Netherlands including p-values can be found in Supplementary Table 2.
*Abbreviations:* ABC: Aruba, Bonaire, Curaçao, CAKUT: congenital anomalies of the kidney and urinary tract, CHD: congenital heart defects, CI: confidence interval, TOPFA: termination of pregnancy for fetal anomaly.

## Discussion

In this study we established a total prevalence of congenital anomalies on the ABC islands of 242.97 per 10,000 births (95% CI: 226.77–260.01) in the period 2008 to 2017. The prevalence in Bonaire (325.15 per 10,000 births) was higher compared to Aruba (233.29 per 10,000 births) and Curaçao (238.58 per 10,000 births). The total prevalence of congenital anomalies on the ABC islands was comparable to the prevalence in the French West Indies, although it was significantly lower than the prevalence in the Northern Netherlands during the same period.

The strength of our study is that we performed a population-based study including all types of births and followed recent EUROCAT guidelines on in- and exclusion criteria for congenital anomalies. This allowed for more unbiased comparison with other EUROCAT registries,

like the population-based EUROCAT registries of the French West Indies and the Northern Netherlands, which also include all types of births (19, 20).

A limitation of our study is that insufficient medical information prompted us to exclude some children/fetuses, which may have led to an underestimation of the prevalence rate of congenital anomalies. For example, atrial septal defect type II is only included in the EUROCAT registry if there is still flow across the defect six months after birth (corrected for gestational age) and patent ductus arteriosus only in term babies (gestational age ≥ 37 weeks) after surgery/catheter closure or if still present six months after birth. Children were not included in our study if follow-up and/or necessary medical information was not documented.



When comparing the ABC islands to each other, we found a higher prevalence of congenital anomalies in Bonaire compared to Aruba and Curaçao. This may be explained by the higher percentage of twins in Bonaire, as these pregnancies are associated with a higher risk of congenital anomalies (21), but methodological differences may also play an important role. The very small size of Bonaire, with only ~250 births per year, allowed a more thorough search of the medical files and thus it is likely that ascertainment for less severe congenital anomalies was better. Indeed, we found a significantly higher prevalence of polydactyly in Bonaire compared to Aruba and Curacao. In addition, mobility around birth is higher in Bonaire compared to Aruba and Curacao, and determination of the number of livebirths by the CBS is different. Children who are born abroad are registered by the municipality registration of the mother, but only if the child arrives in Bonaire within the first 45 days of life. The total number of live births as registered by the CBS – which was used to calculate the denominator data in this study – will thus underestimate the true number of live births, leading to an overestimation of the prevalence rate of congenital anomalies. On the other hand, the percentage of children's caregivers who declined participation was highest in Bonaire (13.9% compared to 2.6% and 3.9% in Aruba and Curaçao, respectively), which may lead to a slight underestimation of the prevalence in Bonaire. We believe this difference might be (partially) explained by privacy concerns that caregivers may have had related to the very small size of Bonaire.

The total prevalence of congenital anomalies on the ABC islands was significantly lower compared to the Northern Netherlands, which might be explained by the availability of more advanced diagnostic technologies. When comparing the different subgroups of congenital anomalies between the ABC islands and the French West Indies and Northern Netherlands, we found that the prevalence of polydactyly (29.68 per 10,000 births) and atrial septal defect (23.28 per 10,000 births) was significantly higher in the ABC islands. The high prevalence of polydactyly can be explained by the African ancestry of a large part

of the population of the ABC islands, especially in Curaçao and Bonaire. It has long been known that postaxial polydactyly is common in individuals of African ancestry (22, 23), with an apparently autosomal dominant inheritance pattern with incomplete penetrance (24). Although the type of polydactyly was not registered in this study, there is anecdotal evidence that most cases of polydactyly in the ABC islands are postaxial. The high prevalence of atrial septal defect might be explained by the availability of a pediatric cardiologist on Aruba and Curaçao, resulting in a low threshold for referral and overdiagnosis of relatively mild CHD. However, it is also possible that there is a truly high prevalence of atrial septal defect on the ABC islands, related to genetic and/or environmental factors. The prevalence of CAKUT (24.73 per 10,000 births) and genetic disorders (44.23 per 10,000 births) was significantly lower on the ABC islands compared with the French West Indies and Northern Netherlands. We hypothesize that the lower prevalence of genetic disorders is mainly attributable to differences in diagnostic opportunities, as there are no local clinical genetics services in the ABC islands. The lower prevalence of CAKUT might also be explained by underdiagnosis, since the prevalence of CAKUT is influenced by the availability of prenatal ultrasound screening (25). Possibly, uptake of prenatal ultrasound screening is lower in the ABC island than in the French West Indies and Northern Netherlands, although this cannot be confirmed as data on uptake are not available for the ABC islands.

**Future directions**

Although data on maternal health in the ABC islands are scarce, some risk factors for congenital anomalies are known to be prevalent among the population of the ABC islands. For example, obesity (BMI ≥ 30.0 kg/m²), a risk factor for congenital anomalies (26), is known to be twice as prevalent in Curaçao compared with the Netherlands (27). We suggest to study women's health in the preconception period and during pregnancy on the ABC islands, with the aim of identifying certain risk factors, such as low uptake of preconceptional folic acid supplementation, for congenital anomalies that may be addressed in prevention programs. Moreover, further research may be aimed at identifying local risk factors for congenital anomalies. In Curacao, for example, there are many concerns about the health effects of an oil refinery that is located in the capital, Willemstad. Future studies may investigate if congenital anomalies are more prevalent in the surroundings of this oil refinery compared to other parts of Curaçao. In addition, the results of this study may be used for capacity planning of prenatal screening tests. For instance, the provided data on the prevalence of Down's syndrome, Edwards' syndrome and Patau's syndrome support further implementation of NIPT to become available for all pregnant women in the Dutch Caribbean.

**Conclusion**

This is the first study to report the prevalence and pattern of congenital anomalies in the ABC islands. The results of this study can be used to inform health care policies and may form an incentive to organize continuous surveillance programs in the Dutch Caribbean, in order to facilitate research, prevention and care for individuals with congenital anomalies. In Curaçao, a first step has been made with the establishment of CaribCAT, a prospective surveillance program for congenital anomalies in the Dutch Caribbean.

## References


1. World Health Organization. Birth defects [Internet]. 2022 Feb 28 [cited 2022 Mar 18]. Available from: https://www.who.int/news-room/fact-sheets/detail/birth-defects.
2. Kirby RS. The prevalence of selected major birth defects in the United States. Semin Perinatol. 2017;41(6):338-44.
3. World Health Organization. Congenital anomalies: overview [Internet]. 2022 [cited 2022 May 27]. Available from: https://www.who.int/health-topics/congenital-anomalies#tab=tab_1.
4. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. Lancet. 2016;388(10063):3027-35.
5. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2197-223.
6. Christianson A, Howson CP, Modell B. March of Dimes global report on birth defects: the hidden toll of dying and disabled children. White Plains, New York: March of Dimes Birth Defects Foundation; 2006.
7. Melo DG, Sanseverino MTV, Schmalfuss TO, Larrandaburu M. Why are Birth Defects Surveillance Programs Important? Front Public Health. 2021;9:753342.
8. World Health Assembly; 63. Birth defects: report by the Secretariat. World Health Organization. 2010.
9. Zarante I, Hurtado-Villa P, Walani SR, Kancherla V, López Camelo J, Giugliani R, et al. A consensus statement on birth defects surveillance, prevention, and care in Latin America and the Caribbean. Rev Panam Salud Publica. 2019;43:e2.
10. Mendisco F, Pemonge MH, Romon T, Lafleur G, Richard G, Courtaud P, et al. Tracing the genetic legacy in the French Caribbean islands: A study of mitochondrial and Y-chromosome lineages in the Guadeloupe archipelago. Am J Phys Anthropol. 2019;170(4):507-18.
11. Central Bureau of Statistics Curaçao. Population Tables [Internet]. 2022 [cited 2022 Mar 23]. Available from: https://www.cbs.cw/population-tables.
12. Boersma A, Alberts J, Bruijn JD, Meyboom BD, Kleiverda G. Termination of pregnancy in Curaçao: need for improvement of sexual and reproductive healthcare. Global journal of health science. 2012;4(3):30-8.
13. Central Bureau of Statistics Aruba. Quarterly Demographic Bulletin 2021 [Internet]. 2022 [cited 2022 Jul 18]. Available from: https://cbs.aw/wp/index.php/2022/03/07/quarterly-demographic-bulletin-2021/.
14. Statistics Netherlands. Caribbean Netherlands; population, sex, age and country of birth [Internet]. 2021 [cited 2022 Mar 23]. Available from: https://opendata.cbs.nl/statline/#/CBS/en/dataset/84712ENG/table?ts=1606311418329.
15. EUROCAT. EUROCAT Guide 1.5 [Internet]. 2022 [cited 2022 Oct 26]. Available from: https://eu-rd-platform.jrc.ec.europa.eu/eurocat/data-collection/guidelines-for-data-registration_en.
16. Central Bureau of Statistics Curaçao. Births by month 2011-2019 [Internet]. 2022 [cited 2022 Jul 18]. Available from: https://www.cbs.cw/population.
17. Statistics Netherlands. Caribisch Nederland; bevolkingsontwikkeling 2007-2015 [Internet]. 2016 [cited 2022 Jul 18]. Available from: https://opendata.cbs.nl/#/CBS/nl/dataset/80539ned/table.
18. Statistics Netherlands. Caribbean Netherlands; population, births, deaths, migration [Internet]. 2022 [cited 2022 Jul 18]. Available from: https://opendata.cbs.nl/statline/#/CBS/en/dataset/83774ENG/table?ts=1666789246153.

19. European Platform on Rare Disease Registration. EUROCAT members: French West Indies [Internet]. 2022 [cited 2022 Aug 22]. Available from: https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-members/registries/French-West-Indies_en.
20. European Platform on Rare Disease Registration. EUROCAT members: Northern Netherlands [Internet]. 2022 [cited 2022 Aug 22]. Available from: https://eu-rd-platform.jrc.ec.europa.eu/eurocat/eurocat-members/registries/Northern-Netherlands_en.
21. Dawson AL, Tinker SC, Jamieson DJ, Hobbs CA, Berry RJ, Rasmussen SA, et al. Twinning and major birth defects, National Birth Defects Prevention Study, 1997-2007. J Epidemiol Community Health. 2016;70(11):1114-21.
22. Scott-Emuakpor AB, Madueke ED. The study of genetic variation in Nigeria. II. The genetics of polydactyly. Hum Hered. 1976;26(3):198-202.
23. Woolf CM, Myrianthopoulos NC. Polydactyly in American negroes and whites. Am J Hum Genet. 1973;25(4):397-404.
24. Holmes LB, Nasri H, Hunt AT, Toufaily MH, Westgate MN. Polydactyly, postaxial, type B. Birth Defects Res. 2018;110(2):134-41.
25. Bakker MK, Bergman JEH, Fleurke-Rozema H, Streefland E, Gracchi V, Bilardo CM, et al. Prenatal diagnosis of urinary tract anomalies, a cohort study in the Northern Netherlands. Prenat Diagn. 2018;38(2):130-4.
26. Harris BS, Bishop KC, Kemeny HR, Walker JS, Rhee E, Kuller JA. Risk Factors for Birth Defects. Obstet Gynecol Surv. 2017;72(2):123-35.
27. Verstraeten S, Griffith M, Pin R. De nationale gezondheidsenquete Curacao 2017. Willemstad: Volksgezondheid Instituut Curaçao; 2018.

# Supplementary material

**Supplementary Table 1.** Prevalence of congenital anomalies in Aruba, Bonaire and Curaçao

| Anomaly group | Prevalence per 10,000 births | | | p-value |
|---|---|---|---|---|
| | Aruba | Bonaire | Curaçao | |
| All anomalies | 233.29 | 352.15 | 238.58 | 0.0056 |
| Nervous system anomalies | 20.79 | 46.61 | 29.31 | 0.0824 |
| Neural Tube Defects | 13.86 | 15.54 | 8.74 | 0.3299 |
| Anencephaly and similar | 1.54 | 5.18 | 4.11 | 0.3080 |
| Encephalocele and meningocele | 0.77 | - | 2.57 | 0.5850 |
| Spina Bifida | 11.55 | 10.36 | 2.06 | 0.0024 |
| Hydrocephaly | 1.54 | 20.71 | 10.80 | 0.0017 |
| Severe microcephaly | 1.54 | 10.36 | 3.09 | 0.0820 |
| Arhinencephaly / holoprosencephaly | 3.08 | - | 2.06 | 0.8250 |
| Agenesis of corpus callosum | 0.77 | 5.18 | 3.60 | 0.1580 |
| Eye anomalies | 3.85 | 10.36 | 6.17 | 0.4443 |
| Anophthalmos / microphthalmos | 0.77 | - | 1.54 | 0.7260 |
| Anophthalmos | - | - | - | - |
| Congenital cataract | 2.31 | 5.18 | 3.09 | 0.5420 |
| Congenital glaucoma | - | - | 1.03 | 0.5720 |
| Ear, face and neck anomalies | 0.77 | - | 1.03 | 1.0000 |
| Anotia and atresia / stenosis / stricture of external auditory canal | 0.77 | - | 0.51 | 1.0000 |
| Congenital Heart Defects | 73.14 | 77.68 | 80.73 | 0.7479 |
| Severe congenital heart defects | 29.26 | 10.36 | 28.28 | 0.3255 |
| Common arterial truncus | 0.77 | - | 2.06 | 0.7410 |
| Double outlet right ventricle | 1.54 | - | 0.51 | 0.6370 |
| Double outlet left ventricle | - | - | - | - |
| Complete transposition of great arteries | 3.08 | - | 2.06 | 0.8250 |
| Single ventricle | 0.77 | - | - | 0.4340 |
| Corrected transposition of great arteries | - | - | - | - |
| Ventricular septal defect | 25.41 | 41.43 | 43.19 | 0.0311 |
| Atrial septal defect | 30.03 | 15.54 | 19.54 | 0.1214 |
| Atrioventricular septal defect | 6.93 | - | 6.17 | 0.5157 |
| Tetralogy and pentatology of Fallot | 5.39 | - | 6.68 | 0.4927 |
| Triscuspid atresia and stenosis | - | - | 1.54 | 0.3940 |
| Ebstein's anomaly | 0.77 | - | 1.03 | 1.0000 |
| Pulmonary valve stenosis | 1.54 | 15.54 | 8.23 | 0.0130 |
| Pulmonary valve atresia | - | - | 2.06 | 0.3290 |
| Aortic valve atresia/stenosis | 2.31 | - | 0.51 | 0.2600 |
| Mitral valve atresia/stenosis | 1.54 | - | 1.03 | 1.0000 |
| Hypoplastic left heart | 3.08 | - | 4.63 | 0.5305 |
| Hypoplastic right heart | - | - | 0.51 | 1.0000 |
| Coarctation of aorta | 2.31 | 10.36 | 2.57 | 0.2190 |
| Aortic atresia / interrupted aortic arch | - | - | 0.51 | 1.0000 |

*Supplementary Table 1 continues on next page.*

**Supplementary Table 1.** *Continued*

| Anomaly group | Prevalence per 10,000 births | | | |
|---|---|---|---|---|
| | Aruba | Bonaire | Curaçao | p-value |
| Total anomalous pulmonary venous return | 0.77 | - | 2.06 | 0.7410 |
| PDA as only CHD in term infants | - | - | 1.03 | 0.5720 |
| Respiratory anomalies | 1.54 | - | 2.06 | 1.0000 |
| Choanal stenosis or atresia | 0.77 | - | 1.54 | 0.7260 |
| Congenital pulmonary airway malformations | - | - | - | - |
| Oro-facial clefts* | 18.48 | 15.54 | 9.26 | 0.0758 |
| Gastro-intestinal anomalies | 16.17 | 15.54 | 16.45 | 0.9944 |
| Oesophageal atresia with or without trachea-oesophageal fistula | 0.77 | - | 1.03 | 1.0000 |
| Duodenal atresia or stenosis | 2.31 | - | 1.54 | 0.7800 |
| Atresia or stenosis of other parts of small intestine | 1.54 | - | 1.54 | 1.0000 |
| Ano-rectal atresia or / and stenosis | 3.08 | - | 3.60 | 1.0000 |
| Hirschsprung's disease | - | 10.36 | 2.57 | 0.0160 |
| Atresia of bile ducts | 2.31 | - | - | 0.0870 |
| Annular pancreas | 0.77 | - | 1.54 | 0.7260 |
| Anomalies of intestinal fixation | 4.62 | - | 2.06 | 0.3910 |
| Diaphragmatic hernia | 2.31 | 5.18 | 2.57 | 0.5070 |
| Abdominal wall defects | 6.93 | - | 6.68 | 0.5174 |
| Gastroschisis | 6.16 | - | 2.57 | 0.2880 |
| Omphalocele | 0.77 | - | 4.11 | 0.1940 |
| Congenital anomalies of kidney and urinary tract | 27.72 | 15.54 | 23.65 | 0.5425 |
| Unilateral renal agenesis | 2.31 | - | 1.54 | 0.7800 |
| Bilateral renal agenesis including Potter sequence | 2.31 | 5.18 | 1.54 | 0.3300 |
| Multicystic renal dysplasia | 0.77 | - | 2.06 | 0.7410 |
| Congenital hydronephrosis including ureter obstruction | 18.48 | 5.18 | 10.80 | 0.1071 |
| Lobulated, fused and horseshoe kidney and ectopic kidney | 0.77 | - | 1.54 | 0.7260 |
| Bladder exstrophy and / or epispadia | - | - | - | - |
| Posterior urethral valve | 1.54 | - | 1.54 | 1.0000 |
| Prune belly syndrome | 0.77 | - | 0.51 | 1.0000 |
| Genital anomalies | 23.10 | 41.43 | 22.62 | 0.2644 |
| Hypospadias | 22.33 | 36.25 | 20.57 | 0.3749 |
| Indeterminate sex | - | 5.18 | - | 0.0560 |
| Limb anomalies | 49.28 | 119.11 | 37.54 | **0.0000** |
| Limb reduction defects | 2.31 | - | 2.57 | 1.0000 |
| Transverse LRD | 0.77 | - | - | 0.4340 |
| Longitudinal preaxial LRD | 0.77 | - | 2.06 | 0.7410 |
| Longitudinal postaxial LRD | - | - | - | - |
| Longitudinal central LRD | - | - | - | - |
| Intercalary LRD | - | - | - | - |
| Club foot | 6.16 | 36.25 | 14.40 | 0.0012 |
| Hip dislocation | 0.77 | - | - | 0.4340 |
| Polydactyly | 33.11 | 82.86 | 22.11 | **0.0000** |
| Syndactyly | 6.16 | - | 3.09 | 0.2670 |

*Supplementary Table 1 continues on next page.*

**Supplementary Table 1.** *Continued*

| Anomaly group | Prevalence per 10,000 births | | | p-value |
|---|---|---|---|---|
| | Aruba | Bonaire | Curaçao | |
| Other anomalies/syndromes | | | | |
| Craniosynostosis | 3.85 | 10.36 | 1.03 | 0.0310 |
| Congenital constriction bands / amniotic band sequence resulting in major malformations | - | - | - | - |
| Situs inversus | 0.77 | - | - | 0.4340 |
| Conjoined twins | - | - | 0.51 | 1.0000 |
| VATER / VACTERL association | 1.54 | - | 0.51 | 0.6370 |
| Pierre Robin sequence | 1.54 | - | - | 0.2520 |
| Caudal regression sequence | - | - | - | - |
| Sirenomelia | - | - | 0.51 | 1.0000 |
| Septo-optic dysplasia | - | - | - | - |
| Vascular disruption anomalies | 8.47 | - | 4.11 | 0.1491 |
| Laterality anomalies | 1.54 | - | 2.06 | 1.0000 |
| Teratogenic syndromes resulting in major malformations | 0.77 | - | 3.09 | 0.5030 |
| Valproate syndrome | - | - | - | - |
| Maternal infections resulting in major malformations | 0.77 | - | 2.06 | 0.7410 |
| Genetic disorders | 48.51 | 20.71 | 43.71 | 0.2258 |
| Skeletal dysplasias | 2.31 | 5.18 | 3.09 | 0.5420 |
| Down syndrome / trisomy 21 | 20.02 | - | 17.48 | 0.1447 |
| Patau syndrome / trisomy 13 | 0.77 | - | 3.09 | 0.5030 |
| Edwards syndrome / trisomy 18 | 4.62 | - | 6.17 | 0.4892 |
| Turner syndrome | 1.54 | - | 1.03 | 1.0000 |
| Triploidy and polyploidy | - | - | - | - |

* Type not further specified, as this was often not recorded in the medical files.
P-values in bold are statistically significant after Bonferroni correction ($p < 0.00049$ [0.05/102]).
PDA, patent ductus arteriosus; CHD, congenital heart defect; LRD, limb reduction defect
A child/fetus with several anomalies is counted once within each subgroup. Thus, the prevalence of different subgroups cannot be added to reach a total prevalence. A child/fetus is counted only once in any given prevalence.

**Supplementary Table 2.** Prevalence of congenital anomalies on the ABC islands, French West Indies and Northern Netherlands

| | Prevalence per 10,000 births | | | p-value | |
|---|---|---|---|---|---|
| Anomaly group | ABC islands[§] | French West Indies[†] | Northern Netherlands[§] | ABC vs FWI | ABC vs NNL |
| All anomalies | 242.97 | 248.69 | 298.98 | 0.8662 | **0.0000** |
| Nervous system anomalies | 27.06 | 39.12 | 26.86 | 0.0023 | 0.8334 |
| Neural Tube Defects | 11.06 | 10.15 | 10.5 | 0.5951 | 0.7041 |
| Anencephaly and similar | 3.20 | 4.81 | 4.59 | 0.2425 | 0.2846 |
| Encephalocele and meningocele | 1.75 | 1.82 | 1.09 | 0.9609 | 0.2790 |
| Spina Bifida | 6.11 | 3.53 | 4.83 | 0.0398 | 0.3028 |
| Hydrocephaly | 7.86 | 8.66 | 4.59 | 0.7172 | 0.0122 |
| Severe microcephaly | 2.91 | 3.95 | 3.2 | 0.4139 | 0.8203 |
| Arhinencephaly / holoprosencephaly | 2.33 | 2.78 | 1.03 | 0.6911 | 0.0584 |
| Agenesis of corpus callosum | 2.62 | 4.17 | 2.35 | 0.2216 | 0.7390 |
| Eye anomalies | 5.53 | 5.66 | 8.09 | 0.9771 | 0.1353 |
| Anophthalmos / microphthalmos | 1.16 | 2.46 | 1.33 | 0.3517 | 1.0000 |
| Anophthalmos | - | 0.64 | 0.18 | 0.1409 | 1.0000 |
| Congenital cataract | 2.91 | 1.39 | 3.02 | 0.0657 | 0.9539 |
| Congenital glaucoma | 0.58 | 0.85 | 0.48 | 1.0000 | 0.6814 |
| Ear, face and neck anomalies | 0.87 | 4.27 | 3.2 | 0.0037 | 0.0208 |
| Anotia and atresia / stenosis / stricture of external auditory canal | 0.58 | 0.43 | 1.51 | 0.6593 | 0.3008 |
| Congenital Heart Defects | 77.69 | 59.63 | 84.93 | **0.0001** | 0.2751 |
| Severe congenital heart defects | 27.64 | 28.32 | 30.18 | 0.9488 | 0.5234 |
| Common arterial truncus | 1.45 | 1.07 | 0.72 | 0.5622 | 0.1898 |
| Double outlet right ventricle | 0.87 | 2.35 | 2.35 | 0.0997 | 0.0903 |
| Double outlet left ventricle | - | 0.32 | - | 0.5703 | - |
| Complete transposition of great arteries | 2.33 | 3.53 | 4.35 | 0.3082 | 0.0976 |
| Single ventricle | 0.29 | 1.92 | 0.97 | 0.0357 | 0.3374 |
| Corrected transposition of great arteries | - | 0.21 | 0.91 | 1.0000 | 0.0912 |
| Ventricular septal defect | 36.37 | 23.4 | 40.75 | **0.0000** | 0.3182 |
| Atrial septal defect | 23.28 | 7.59 | 9.84 | **0.0000** | **0.0000** |
| Atrioventricular septal defect | 6.11 | 9.4 | 6.64 | 0.0854 | 0.7799 |
| Tetralogy and pentatology of Fallot | 5.82 | 2.99 | 2.96 | 0.0176 | 0.0077 |
| Triscuspid atresia and stenosis | 0.87 | 1.07 | 1.27 | 1.0000 | 0.7863 |
| Ebstein's anomaly | 0.87 | 0.32 | 0.91 | 0.1945 | 1.0000 |
| Pulmonary valve stenosis | 6.11 | 2.03 | 7.73 | **0.0002** | 0.3532 |
| Pulmonary valve atresia | 1.16 | 1.28 | 1.87 | 1.0000 | 0.3842 |
| Aortic valve atresia/stenosis | 1.16 | 0.43 | 2.41 | 0.2210 | 0.1648 |
| Mitral valve atresia/stenosis | 1.16 | 0.75 | 0.66 | 0.4971 | 0.3028 |
| Hypoplastic left heart | 3.78 | 2.99 | 3.62 | 0.4534 | 0.8444 |
| Hypoplastic right heart | 0.29 | 1.71 | 0.72 | 0.0562 | 0.7095 |
| Coarctation of aorta | 2.91 | 3.1 | 3.56 | 0.8990 | 0.5869 |
| Aortic atresia / interrupted aortic arch | 0.29 | 0.32 | 0.66 | 1.0000 | 0.7040 |
| Total anomalous pulmonary venous return | 0.29 | 0.43 | 1.03 | 1.0000 | 0.3422 |
| PDA as only CHD in term infants | 0.58 | 1.18 | 1.93 | 0.5343 | 0.0855 |

*Supplementary Table 2 continues on next page.*

**Supplementary Table 2.** *Continued*

| Anomaly group | Prevalence per 10,000 births | | | p-value | |
|---|---|---|---|---|---|
| | ABC islands[§] | French West Indies[†] | Northern Netherlands[§] | ABC vs FWI | ABC vs NNL |
| Respiratory anomalies | 1.75 | 4.81 | 4.29 | 0.0169 | 0.0322 |
| Choanal stenosis or atresia | 1.16 | 1.39 | 0.72 | 1.0000 | 0.3328 |
| Congenital pulmonary airway malformations | - | 0.53 | 0.72 | 0.3343 | 0.2389 |
| Oro-facial clefts* | 13.09 | 8.12 | 20.7 | 0.0079 | 0.0049 |
| Gastro-intestinal anomalies | 16.29 | 17.1 | 19.07 | 0.8384 | 0.3301 |
| Oesophageal atresia with or without trachea-oesophageal fistula | 0.87 | 2.89 | 2.41 | 0.0402 | 0.0814 |
| Duodenal atresia or stenosis | 1.75 | 1.82 | 0.78 | 0.9609 | 0.1174 |
| Atresia or stenosis of other parts of small intestine | 1.45 | 0.64 | 0.78 | 0.1744 | 0.2123 |
| Ano-rectal atresia or / and stenosis | 3.20 | 2.46 | 5.49 | 0.4425 | 0.0971 |
| Hirschsprung's disease | 2.04 | 1.28 | 1.45 | 0.3090 | 0.4030 |
| Atresia of bile ducts | 0.87 | 0.64 | 0.54 | 0.7073 | 0.4402 |
| Annular pancreas | 1.16 | 0.32 | 0.97 | 0.0856 | 0.7638 |
| Anomalies of intestinal fixation | 2.91 | 1.71 | 2.78 | 0.1685 | 0.8558 |
| Diaphragmatic hernia | 2.62 | 1.92 | 3.08 | 0.4238 | 0.6877 |
| Abdominal wall defects | 6.40 | 10.26 | 6.58 | 0.0520 | 0.9629 |
| Gastroschisis | 3.78 | 2.89 | 2.23 | 0.3930 | 0.0879 |
| Omphalocele | 2.62 | 6.73 | 3.74 | 0.0070 | 0.3374 |
| Congenital anomalies of kidney and urinary tract | 24.73 | 43.92 | 45.94 | **0.0000** | **0.0000** |
| Unilateral renal agenesis | 1.75 | 3.95 | 4.23 | 0.0616 | 0.0354 |
| Bilateral renal agenesis including Potter sequence | 2.04 | 1.18 | 1.45 | 0.2834 | 0.4030 |
| Multicystic renal dysplasia | 1.45 | 5.34 | 5.67 | 0.0034 | 0.0016 |
| Congenital hydronephrosis including ureter obstruction | 13.38 | 18.17 | 19.26 | 0.0808 | 0.0268 |
| Lobulated, fused and horseshoe kidney and ectopic kidney | 1.16 | 6.41 | 2.54 | **0.0002** | 0.1358 |
| Bladder exstrophy and / or epispadia | - | 1.28 | 1.33 | 0.0446 | 0.0398 |
| Posterior urethral valve | 1.45 | 2.46 | 1.57 | 0.2981 | 0.9034 |
| Prune belly syndrome | 0.58 | - | 0.12 | 0.0704 | 0.1358 |
| Genital anomalies | 23.86 | 25.54 | 28.85 | 0.6867 | 0.1456 |
| Hypospadias | 22.11 | 16.99 | 25.84 | 0.0438 | 0.2633 |
| Indeterminate sex | 0.29 | 0.43 | 0.54 | 1.0000 | 1.0000 |
| Limb anomalies | 46.56 | 38.79 | 60.24 | 0.0353 | 0.0044 |
| Limb reduction defects | 2.33 | 8.55 | 6.46 | **0.0002** | 0.0043 |
| Transverse LRD | 0.29 | 1.6 | 0.91 | 0.0869 | 0.5001 |
| Longitudinal preaxial LRD | 1.45 | 2.03 | 1.69 | 0.5267 | 0.7834 |
| Longitudinal postaxial LRD | - | 0.53 | 0.48 | 0.3343 | 0.3665 |
| Longitudinal central LRD | - | 0.21 | 0.91 | 1.0000 | 0.0912 |
| Intercalary LRD | - | 0.43 | 0.24 | 0.5792 | 1.0000 |

*Supplementary Table 2 continues on next page.*

**Supplementary Table 2.** *Continued*

| Anomaly group | Prevalence per 10,000 births | | | p-value | |
|---|---|---|---|---|---|
| | ABC islands[§] | French West Indies[†] | Northern Netherlands[§] | ABC vs FWI | ABC vs NNL |
| Club foot | 12.51 | 11.54 | 12.86 | 0.5893 | 0.9491 |
| Hip dislocation | 0.29 | 0.32 | 18.11 | 1.0000 | **0.0000** |
| Polydactyly | 29.68 | 10.47 | 13.34 | **0.0000** | **0.0000** |
| Syndactyly | 4.07 | 3.74 | 6.58 | 0.7471 | 0.0999 |
| Craniosynostosis | 2.62 | 0.53 | 3.2 | 0.0034 | 0.6099 |
| Congenital constriction bands / amniotic band sequence resulting in major malformations | - | 1.07 | 0.72 | 0.0722 | 0.2389 |
| Situs inversus | 0.29 | 1.71 | 1.15 | 0.0562 | 0.2324 |
| Conjoined twins | 0.29 | 0.43 | 0.06 | 1.0000 | 0.3102 |
| VATER / VACTERL association | 0.87 | 0.32 | 1.27 | 0.1945 | 0.7863 |
| Pierre Robin sequence | 0.58 | 0.64 | 0.97 | 1.0000 | 0.7549 |
| Caudal regression sequence | - | - | 0.12 | - | 1.0000 |
| Sirenomelia | 0.58 | 0.32 | - | 0.6135 | 0.0287 |
| Septo-optic dysplasia | - | 0.43 | - | 0.5792 | - |
| Vascular disruption anomalies | 5.53 | 5.98 | 5.25 | 0.8132 | 0.7883 |
| Laterality anomalies | 1.75 | 2.67 | 3.14 | 0.3650 | 0.1801 |
| Teratogenic syndromes resulting in major malformations | 2.04 | 4.38 | 0.78 | 0.0606 | 0.0646 |
| Valproate syndrome | - | - | 0.18 | - | 1.0000 |
| Maternal infections resulting in major malformations | 1.45 | 3.95 | 0.54 | 0.0317 | 0.0732 |
| Genetic disorders | 44.23 | 76.09 | 67.37 | **0.0000** | **0.0000** |
| Skeletal dysplasias | 2.91 | 2.46 | 3.38 | 0.6242 | 0.6975 |
| Down syndrome / trisomy 21 | 17.46 | 33.88 | 19.14 | **0.0000** | 0.5932 |
| Patau syndrome / trisomy 13 | 2.04 | 3.53 | 2.05 | 0.1954 | 0.9827 |
| Edwards syndrome / trisomy 18 | 5.24 | 11.86 | 7.12 | 0.0012 | 0.2485 |
| Turner syndrome | 1.16 | 4.6 | 3.68 | 0.0051 | 0.0204 |
| Triploidy and polyploidy | - | 0.53 | 1.15 | 0.3343 | 0.0599 |

* Type not further specified, as this was often not recorded in the medical files.
[§] From 2008–2017.
[†] From 2009–2018.
P-values in bold are statistically significant after Bonferroni correction ($p < 0.00049$ [0.05/102]).
FWI, French West Indies; NNL, Northern Netherlands; PDA, patent ductus arteriosus; CHD, congenital heart defect; LRD, limb reduction defect
A child/fetus with several anomalies is counted once within each subgroup. Thus, the prevalence of different subgroups cannot be added to reach a total prevalence. A child/fetus is counted only once in any given prevalence.

# Part II

## Delivering genetic services in the small island communities of the Dutch Caribbean

# Chapter 3

## Genetic care in geographically isolated small island communities: 8 years of experience in the Dutch Caribbean

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## Abstract

Worldwide, there are large inequalities in genetic service delivery. In 2011, we established a bi-annual joint pediatric-genetics clinic with a visiting clinical geneticist in the Dutch Caribbean. This retrospective study evaluates the yield of diagnostic testing and the clinical utility of a diagnosis for patients with rare diseases on these relatively isolated, resource-limited islands. A total of 331 patients that were referred to the clinical geneticist between November 2011 and November 2019 and had genetic testing were included in this study. A total of 508 genetic tests were performed on these patients. Microarray, next-generation sequencing gene panels, and single-gene analyses were the most frequently performed genetic tests. A molecularly confirmed diagnosis was established in 33% of patients (n = 108). Most diagnosed patients had single nucleotide variants or small insertions and/or deletions (48%) or copy number variants (34%). Molecular diagnostic yield was highest in patients referred for seizures and developmental delay/intellectual disability. The genetic diagnosis had an impact on clinical management in 52% of patients. Referrals to other health professionals and changes in therapy were the most frequently reported clinical consequences. In conclusion, despite limited financial resources, our genetics service resulted in a reasonably high molecular diagnostic yield. Even in this resource-limited setting, a genetic diagnosis had an impact on clinical management for the majority of patients. Our approach with a visiting clinical geneticist may be an example for others who are developing genetic services in similar settings.

## Introduction

Rare diseases are estimated to affect at least 3.5–5.9% of the global population (1). Most rare diseases have a genetic basis (71.9%) and have an exclusively pediatric-onset (69.9%) (1). Recent genetic technological advances including exome and genome sequencing result in an increased diagnostic yield for patients with suspected genetic disorders (2-6) and improve clinical management and reproductive decision making (7). However, due to global inequalities in genetic service delivery, patients with rare diseases in lower-resource countries have limited access to genetic testing and counseling and thus remain undiagnosed. Barriers to delivering genetic services in resource-limited areas include a lack of adequately equipped diagnostic laboratories, a shortage of clinical geneticists and genetic counselors, logistic and financial barriers for patients, and a lack of knowledge about genetic disorders among healthcare providers (8-11).



For small islands like those of the Dutch Caribbean, the delivery of genetic services is further complicated by their remote geography and small population sizes. The Dutch Caribbean consists of six islands located in the Caribbean sea: Aruba, Curacao, and St. Maarten (constituent countries within the Kingdom of the Netherlands) and Bonaire, St. Eustatius, and Saba (special municipalities of the Netherlands). Although these islands are high-income economies, they face several unique challenges due to their small size and relative remoteness, including healthcare, infrastructure, and environmental challenges (12). The largest island, Curaçao, has a population of only 156,223 (13). Providing highly specialized medical care for a small population means low demand and therefore disproportionally high costs (12). In addition, with low patient volumes, medical specialists may not be able to maintain and improve the knowledge and skills needed for a high degree of specialization (14).

Although available resources differ per island until recently there was no local clinical genetics service on any of the six Dutch Caribbean islands. Local pediatricians occasionally sent blood samples to diagnostic laboratories abroad, for example, to confirm a clinical diagnosis of Down syndrome (usually by karyotyping). Alternatively, patients were sent abroad to a tertiary hospital (in the Netherlands or Colombia) for diagnostic evaluation, including genetic testing. This was, however, only possible on the strict indication because of the high associated costs (including transportation, hospital admission, and accommodation for the accompanying family members). To increase access to genetic testing and counseling for the pediatric population of the Dutch Caribbean, a bi-annual joint pediatric-genetics clinic with a visiting clinical geneticist was established in 2011. Here, we report the outcomes of this clinical genetics service, including the diagnostic yield as well as the impact of a genetic diagnosis on clinical management in this resource-limited setting.

## Methods

### Setting

In 2011, a bi-annual joint pediatric-genetics clinic with a visiting Dutch clinical geneticist (MvH) was established in the Dutch Caribbean. Patients were referred by the local pediatrician for a clinical genetic evaluation at the outpatient pediatric clinics of the Curacao Medical Center (previously Sint-Elisabeth Hospital), Dr. Horacio E. Oduber Hospital in Aruba, Fundashon Mariadal in Bonaire, and St. Maarten Medical Center. Patients from the two smallest islands (Saba and St. Eustatius) were referred to the pediatric-genetics clinics at St. Maarten Medical Center. During the genetic consultations, medical and family histories were obtained, followed by a detailed (dysmorphologic) physical examination. If genetic testing was indicated, biological samples were shipped to one of the accredited university laboratories in the Netherlands where DNA was extracted from peripheral blood or buccal cells for genetic testing. Occasionally, genetic testing (mainly microarray analysis) was requested by the local pediatrician prior to the visit of the clinical geneticist to speed up the diagnostic process. In addition, genetic testing could be requested prior to the visit of the clinical geneticist after consultation between the pediatrician and the clinical geneticist by telephone or email, for example, when a neonate or child was critically ill.

Genetic testing was performed at the departments of Genome Diagnostics of the Amsterdam University Medical Centers (Amsterdam UMC) and the Utrecht University Medical Center (UMC Utrecht). Genetic tests that were not available at these laboratories (specific genes or gene panels) were performed at one of the other five accredited university laboratories in the Netherlands. Costs of genetic testing were reimbursed by the local health insurance, although financial restrictions had to be taken into account. Trio exome sequencing (ES) was not routinely offered because of the high associated costs. To further keep costs at a minimum, all next-generation sequencing (NGS) gene panels were initially only performed in the index patient. Subsequently, segregation analysis in the parents/family of the affected individual was performed if variants of unknown significance (VUS) were identified. However, this was not always possible because of financial restrictions from local health insurance or because parental samples were not available. Abnormal genetic test results were communicated to the caregivers and/or patients by the clinical geneticist upon a follow-up visit. As the clinical geneticist visits only two times a year, the results were sometimes already communicated by the local pediatrician and discussed again during the next visit of the clinical geneticist.

### Study design and patient selection

We performed a retrospective cohort study of patients in the Dutch Caribbean referred to the visiting Dutch clinical geneticist between November 2011 and November 2019. A

total of 48 clinics were held on the four different islands during this period (Table 1). All children (age < 18 years) that were referred to the genetics clinic and had genetic testing were consecutively included in this study. We also included patients that were ≥18 years if they were referred by their pediatrician. In addition, we included critically ill neonates who deceased before they could be evaluated by the clinical geneticist. In those cases, genetic testing was advised by the clinical geneticist during electronic consultation and requested by the pediatrician shortly after birth (or in one case performed in both parents). Caregivers subsequently had a consultation with the clinical geneticist to discuss the results. To avoid overestimation of the diagnostic yield, we did not include siblings with the same molecularly confirmed diagnosis as the proband. Over the years, a few adults had been referred to the clinical geneticist (mostly oncogenetic and cardiogenetic referrals). These patients were, however, excluded from the present study. Since presymptomatic genetic testing is not (yet) covered by local insurance companies, we also excluded healthy children that were referred for genetic testing because of their family history. Previously diagnosed patients who were referred for additional counseling were also excluded.



**Table 1.** General characteristics of the Kingdom of the Netherlands

| | Constituent countries | | | | Special municipalities of the Netherlands | | |
|---|---|---|---|---|---|---|---|
| | The Netherlands | Aruba | Curaçao | St. Maarten | Bonaire | St. Eustatius | Saba |
| Population | 17,475,415[a] | 112,190[b] | 153,671[c] | 42,577[d] | 21,745[e] | 3,142[e] | 1,918[e] |
| Area[f] | 41,543 km² | 180 km² | 444 km² | 34 km² | 288 km² | 21 km² | 13 km² |
| GNI per capita (US$)[g] | 51,060 (2020) | 27,120 (2017) | 17,140 (2020) | 27,680 (2018) | - | - | - |
| Number of clinical genetics visits (2011–2019) | - | 15 | 16 | 3 | 14 | - | - |

[a] On January 1st, 2021. Statistics Netherlands, Population; key figures. https://opendata.cbs.nl/statline/#/CBS/en/dataset/37296eng/table?ts=1640263768026. Accessed 23 December 2021.

[b] On January 1st, 2020. Central Bureau of Statistics Aruba, Quarterly Demographic Bulletin 2020. https://cbs.aw/wp/index.php/2020/12/17/quarterly-demographic-bulletin-2019-2/. Accessed 23 December 2021.

[c] On January 1st, 2021. Central Bureau of Statistics Curaçao, Population Tables. https://www.cbs.cw/population-tables. Accessed 23 December 2021.

[d] On January 1st, 2021. Department of Statistics Sint Maarten, Population Estimates and Vital Statistics 2021. http://stats.sintmaartengov.org/. Accessed 23 December 2021.

[e] On January 1st, 2021. Statistics Netherlands, Caribbean Netherlands; population, sex, age and country of birth. https://opendata.cbs.nl/statline/#/CBS/en/dataset/84712ENG/table?ts=1606311418329. Accessed 23 December 2021.

[f] Government of the Netherlands, What are the different parts of the Kingdom of the Netherlands? https://www.government.nl/topics/caribbean-parts-of-the-kingdom/question-and-answer/what-are-the-different-parts-of-the-kingdom-of-the-netherlands. Accessed 23 December 2021.

[g] The World Bank, GNI per capita, Atlas method (current US$). https://data.worldbank.org/indicator/ny.gnp.pcap.cd?year_high_desc=truel. Accessed 23 December 2021.

Informed consent to publish medical data was obtained from the caregivers of patients with a diagnosis. The caregivers of 12 patients did not give permission and these patients were, therefore, not included in this study. If the caregivers of a patient could not be contacted, we included only general data about the diagnosis (type, inheritance pattern, etc.) and we did not include these patients in Tables 2–4.

### Data collection and analysis

Clinical data and results of genetic testing were abstracted from the medical records. All variants reported as a variant of unknown significance were reviewed and, if applicable, reclassified according to current guidelines (15-17). We considered a diagnosis to be established if a likely pathogenic or pathogenic variant was identified that explained the phenotype. Diagnostic yield was determined for all patients that received genetic testing. In addition, diagnostic yield per type of genetic test and per reason for referral was calculated. We only calculated diagnostic yield for subgroups with n > 10. VUS rate was calculated per type of genetic test. To assess the clinical utility of the diagnosis, the referring physicians were asked to report if the diagnosis had led to changes in clinical management and if so, what the changes were. The answers were subsequently categorized into different subgroups. The answer 'no further diagnostics' was not included as a clinical consequence, as this would apply to all patients who received a genetic diagnosis. In addition, "special education" was excluded since this is also available without a genetic diagnosis. All descriptive statistics were performed using SPSS version 26.0 and Excel.

## Results

### Patient demographics

A total of 331 patients were included in this study. The median age at the time of the first genetic consultation was 3.95 years (range 0–18.7), excluding the 9 children that deceased before they could be seen by the clinical geneticist. The most common reasons for referral were developmental delay (DD) and/or intellectual disability (ID) (39%), with or without other anomalies, and congenital anomalies (24%). Other reasons for referral to the visiting clinical geneticist included short stature (8%), suspected connective tissue disorder (5%), obesity (5%), and seizures (4%).

### Genetic testing

A total of 508 genetic tests were performed (average of 1.5 tests per patient). One genetic test was performed in 60% of patients, two genetic tests in 29%, three genetic tests in 8%,

four genetic tests in 3%, and five genetic tests in <1%. Microarray was the most frequently requested test (n = 247; 49%), followed by (NGS) gene panels (targeted or exome based) (n = 123; 24%), single-gene analysis (n = 86; 17%), methylation studies (n = 21; 4%), FMR1 repeat expansion analysis (n = 12; 2%), and karyotyping (n = 10; 2%). Trio ES, fluorescence in situ hybridization (FISH), and X-exome each comprised < 1% of the total amount of genetic tests. Previous genetic testing had been performed in only 11 (3%) of the patients, with normal or inconclusive results.



**Diagnostic yield**

A molecularly confirmed diagnosis was established in 108 patients (33%). In 52 patients (48%) single nucleotide variants (SNVs) or small insertions and/or deletions (indels) were detected. Copy number variants (CNVs) were identified in 37 patients (34%). Other variant types included aneuploidies (n = 9; 8%), derivative chromosomes (n = 3; 3%), aberrant methylation (n = 3; 3%), repeat expansions (2; 2%), loss of heterozygosity consistent with uniparental disomy (UPD) (n = 1; 1%), and multiple variant types (n = 1; 1%) (Figure 1A). Of the 52 patients with small variants (SNVs/indels), 39 (75%) had a variant associated with an autosomal dominant disorder: six of these variants were de novo, two were inherited from an affected parent, and for 31 variants inheritance was unknown. Small variants associated with autosomal recessive disorders were identified in nine patients (17%): three patients with homozygous variants and six with compound heterozygous variants (Figure 1B). The molecular diagnostic results and associated conditions of patients for whom informed consent was obtained are shown in Table 2–4. Recurrent molecular diagnoses included: Down syndrome (n = 4), Marfan syndrome (n = 2), Sotos syndrome (n = 2), Tuberous Sclerosis type 2 (n = 2), Neurofibromatosis type 1 (n = 2), and Fragile X syndrome (n = 2). Patients with the same diagnosis were not related. No recurrent variants were detected in this cohort.

Molecular diagnostic yield was highest in patients referred for seizures (7/14; 50%) and in patients referred because of DD/ID (47/130; 36%). The lowest molecular diagnostic yield was found in patients that received genetic testing for obesity (1/18; 6%) (Figure 1C). The diagnostic yield per type of genetic test was highest for single gene testing (24/86; 28%), followed by NGS gene panels (29/123; 24%) and microarray (49/247; 20%) (Figure 1D).

In addition to the 108 molecular diagnoses, 7 clinical diagnoses were established in this cohort. These included amniotic band syndrome, fetal methotrexate syndrome (18), VACTERL association, and oculo-auriculo-vertebral spectrum (OAVS), for which there is no known genetic cause. For the other clinical diagnoses (Apert syndrome, Tuberous Sclerosis, and oculoectodermal syndrome [OES]), genetic testing was negative or not yet performed.

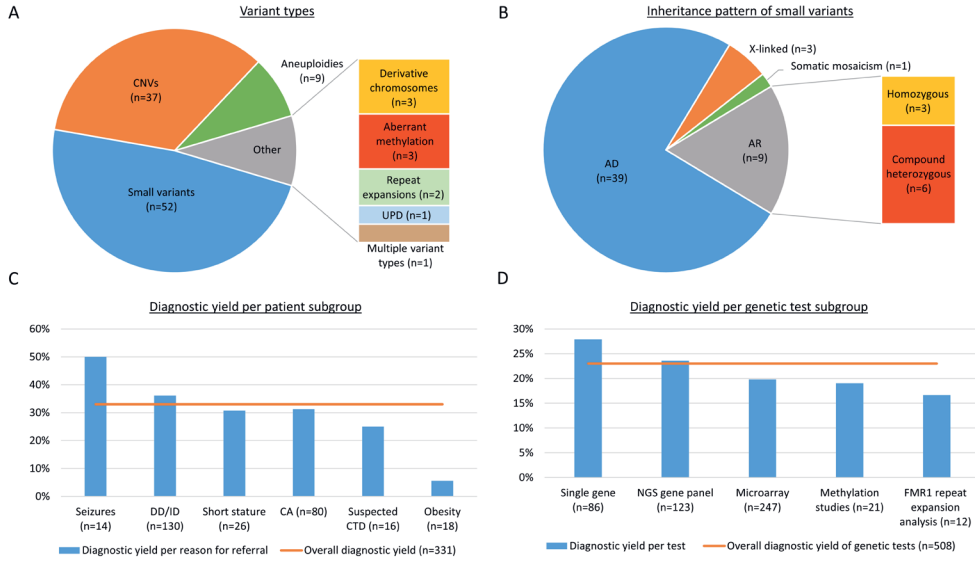


**Figure 1.** Molecular diagnostic results. **A:** Variant types. **B:** Mode of inheritance in patients with small variants (single nucleotide variants or small insertions and/or deletions). **C:** Molecular diagnostic yield per primary reason for referral (reason for referral only included if n > 10). **D:** Molecular diagnostic yield per type of genetic test (genetic test only included if n > 10). AD: autosomal dominant, AR: autosomal recessive, CA: congenital anomalies, CNVs: copy number variants, CTD: connective tissue disorder, DD/ID: developmental delay and/or intellectual disability (with or without other anomalies), NGS: next generation sequencing, UPD: uniparental disomy.

### Variants of unknown significance and incidental findings

One or more VUS were identified in 44/247 (18%) microarrays that were performed, in 34/123 (28%) NGS gene panels, in 1/86 (1%) single-gene tests, and in 2/3 (67%) trio ES (excluding heterozygous VUS in genes associated with autosomal recessive conditions). In total, 110 VUS were identified in 78 patients (24% of the cohort), including 59 CNVs and 51 small variants. Segregation analysis was performed for 49 variants and resulted in the reclassification of 23/33 (70%) CNVs as rare familial polymorphisms and 5/16 (31%) small variants as likely benign. Three VUS were identified more than once: a ~122 kb deletion in 5p12 that was identified in five individuals and a ~393 kb 9q22.1 duplication coupled with a ~538 kb 9q22.31 duplication in four individuals. Two of the individuals with a 5p12 deletion were halfsiblings, but all other individuals were apparently unrelated. There was no common phenotype between individuals with the same VUS. Segregation analysis was performed for three individuals with the 5p12 deletion and one individual with the 9q22.1 and 9q22.31 duplications: these VUS were all inherited from a healthy parent.

Finally, two incidental findings were detected by microarray, both susceptibility loci for neurodevelopmental disorders that did not explain the phenotype for which the patient was referred.

**Table 2.** Molecular diagnoses: single nucleotide variants and small insertions and/or deletions

| # | Sex | Phenotype | Variant | Zygosity; inheritance | Transcript | Cl. | Associated condition, inheritance pattern [MIM number] |
|---|---|---|---|---|---|---|---|
| Diagnostic test: targeted or exome based NGS gene panels | | | | | | | |
| 1 | M | ID, hyperthyroidism | *THRB* c.1286G>A, p.(Arg429Gln) | het; unk | NM_001252634.1 | LP | Thyroid hormone resistance, AD [188570] |
| 50 | F | ID, microcephaly, epilepsy, strabismus, facial dysmorphism | *DYRK1A* c.1399C>T, p.(Arg467*) | het; unk | NM_001396.4 | P | Autosomal dominant mental retardation 7, AD [614104] |
| 51 | F | Short stature, ASD, pulmonary stenosis, webbed neck, facial dysmorphism | *SOS1* c.512T>G, p.(Val171Gly) | het; unk | NM_005633.3 | LP | Noonan syndrome 4, AD [610733] |
| 65 | M | ID, microcephaly, short stature, facial dysmorphism | *ASXL3* c.3039+1G>T | het; unk | NM_030632.3 | P | Bainbridge-Ropers syndrome, AD [615485] |
| 74 | F | ID, DD, microcephaly, agenesis of the corpus callosum | *SOX11* c.877dup, p.(Leu293Profs*105) (novel) | het; unk | NM_003108.3 | LP | Coffin-Siris syndrome 9, AD [615866] |
| 127 | F | Cleft palate, cataract | *BCOR* c.254del, p.(Pro85Argfs*25) (novel) | het; unk | NM_017745.5 | P | Oculofaciocardiodental syndrome, XLD [300166] |
| 131[a] | F | ID, DD, short stature, ataxia, nystagmus, hypodontia | *POLR3B* c.1568T>A, p.(Val523Glu) | hmz; mat/pat | NM_018082.5 | P | Hypomyelinating leukodystrophy 8, AR [614381] |
| 133[b] | M | ID, facial dysmorphism | *USP7* c.3202+1G>T (novel[h]) | het; dn | NM_003470.2 | LP[i] | Hao-Fountain syndrome, AD [616863] |
| 164 | M | ID, facial dysmorphism, sialorrhea | *ATRX* c.7367_7371del, p.(Met2456Argfs*40) (novel) | hem; dn | NM_000489.4 | LP | X-linked mental retardation-hypotonic facies syndrome, XLR [309580] |
| 166 | M | ID, speech and language delay, cryptorchidism, hirsutism, facial dysmorphism | *ARID1B* c.6700_6701del, p.(Leu2234Glyfs*7) | het; dn | NM_020732.3 | P | Coffin-Siris syndrome 1, AD [135900] |
| 176 | M | Short stature, pseudomuscular build | *FBN1* c.5284G>A, p.(Gly1762Ser) | het; unk | NM_000138.5 | P | Acromicric dysplasia, AD [102370] |
| 243 | F | Obesity | *MC4R* c.240C>A, p.(Tyr80*) | het; unk | NM_005912.2 | P | Obesity, AD [618406] |
| 251 | M | Bone fractures, blue sclerae | *COL1A1* c.1126_1127del, p.(Pro376Trpfs*15) (novel) | het; unk | NM_000088.3 | P | Osteogenesis imperfecta type I, AD [166200] |

*Table 2 continues on next page.*

**Table 2.** *Continued*

| # | Sex | Phenotype | Variant | Zygosity; inheritance | Transcript | Cl. | Associated condition, inheritance pattern [MIM number] |
|---|---|---|---|---|---|---|---|
| 303 | F | Neonatal seizures | *KCNQ2* c.620G>A, p.(Arg207Gln) | het; unk | NM_172107.2 | P | Benign familial neonatal seizures 1, AD [121200] |
| 307 | M | PDA, PFO, hypospadias, cryptorchidism, short stature, facial dysmorphism, hemangioma | *BRAF* c.1741A>G, p.(Asn581Asp) | het; unk | NM_004333.4 | LP | Cardiofaciocutaneous syndrome, AD [115150] |
| 349 | F | Myasthenic syndrome | *CHAT* c.1165T>C, p.(Cys389Arg) (novel)<br>*CHAT* c.1493C>T, p.(Ser498Leu) | het; pat<br>het; mat | NM_020549.5 | VUS; LP | Presynaptic congenital myasthenic syndrome 6, AR [254210] |
| 395 | M | ID, short stature, facial dysmorphism | *KMT2A* c.6665dup, p.(Tyr2222*) (novel) | het; dn | NM_001197104.1 | P | Wiedemann-Steiner syndrome, AD [605130] |
| 436 | M | ID, DD, hypotonia, vision problems, unilateral hearing loss | *GNB1* c.233A>G, p.(Lys78Arg) | het; dn | NM_002074.4 | LP | Autosomal dominant mental retardation 42, AD [616973] |
| 458 | M | Short stature, scoliosis, carpal synostosis | *FLNB* c.7029T>G, p.(Tyr2343*) | hmz, unk | NM_001457.3 | P | Spondylocarpotarsal synostosis syndrome, AR [272460] |
| 464 | F | ID, neonatal diabetes, annular pancreas, duodenal malrotation | *ABCC8* c.638T>C, p.(Leu213Pro) | het; unk | NM_001287174.1 | LP | Permanent neonatal diabetes mellitus 3, AD [618857] |
| 466 | F | Excess skin folds neck, pulmonary artery stenosis, low-set ears | *LZTR1* c.848G>A, p.(Arg283Gln) | het; unk | NM_006767.4 | LP | Noonan syndrome 10, AD [616564] |
| 507 | F | Hemihypertrophy | *PIK3CA* c.1133G>A, p.(Cys378Tyr) | somatic mosaicism | NM_006218.2 | P | Low-level PIK3CA mosaicism associated with hemihypertrophy |
| 511 | F | Long QT syndrome | *KCNH2* c.2453C>T, p.(Ser818Leu) | het; pat | NM_000238.3 | P | Long QT syndrome 2, AD [613688] |
| 525 | M | ASD, cryptorchidism, short stature, facial dysmorphism | *PTPN11* c.417G>C, p.(Glu139Asp) | het; unk | NM_002834.4 | P | Noonan syndrome 1, AD [163950] |
| 567 | F | Seizures | *SCN1A* c.2134C>T, p.(Arg712*) | het; unk | NM_001165963.1 | P | Generalized epilepsy with febrile seizures plus (GEFS+) type 2, AD [604403] |

**Table 2.** *Continued*

| # | Sex | Phenotype | Variant | Zygosity; inheritance | Transcript | Cl. | Associated condition, inheritance pattern [MIM number] |
|---|---|---|---|---|---|---|---|
| 568 | M | Seizures | *NPRL2* c.883C>T, p.(Arg295*) | het; unk | NM_006545.4 | P | Familial focal epilepsy with variable foci 2, AD [617116] |
| 574 | M | Seizures, cortical tubers on brain MRI | *TSC1* c.2509_2512del, p.(Asn837Valfs*11) | het; unk | NM_000368.4 | P | Tuberous sclerosis 1, AD [191100] |
| 621 | F | Focal segmental glomerulosclerosis | *NPHS2* c.686G>A, p.(Arg229Gln)<br>*NPHS2* c.851C>T, p.(Ala284Val) | het; unk<br>het; unk | NM_014625.3 | P; P | Nephrotic syndrome type 2, AR [600995] |
| Diagnostic test: single gene analysis | | | | | | | |
| 15 | M | Multiple café au lait spots | *NF1* c.4563delT, p.(Ala1523Hisfs*30) (novel) | het; unk | NM_000267.1 | P | Neurofibromatosis type 1, AD [162200] |
| 40 | M | ID, tall stature, macrocephaly, PDA, facial dysmorphism | *NSD1* c.6049C>T, p.(Arg2017Trp) | het; unk | NM_022455.3 | P | Sotos syndrome, AD [117550] |
| 46 | M | Hypotonia, loss of motor skills, areflexia | *SMN1* c.835-?_*262+?del | hmz; unk | NM_000344.3 | P | Spinal muscular atrophy type I, AR [253300] |
| 66 | F | Short stature, short arms and legs, macrocephaly | *FGFR3* c.1620C>A, p.(Asn540Lys) | het; unk | NM_000142.4 | P | Hypochondroplasia, AD [146000] |
| 69[c] | F | Insensitivity to pain | *SCN9A* c.5085_5091dup, p.(Asn1698Tyrfs*3) (novel[h])<br>*SCN9A* c.688+131_901+2760del, p.(Gly230_Lys301delinsGlu) (novel[h]) | het; mat<br>het; pat | NM_002977.3 | P; P | Congenital insensitivity to pain, AR [243000] |
| 80 | F | Developmental regression, growth retardation | *MECP2* c.91del, p.(Val31*) (novel) | het; unk | NM_004992.3 | P | Rett syndrome, XLR [312750] |
| 106 | M | ID, microcephaly, hypospadias, feeding difficulties, facial dysmorphism | *NIPBL* c.5778_5808+2dup, p.(Val1936_Val1937ins11) (novel) | het; unk | NM_133433.3 | P | Cornelia de Lange syndrome 1, AD [122470] |

*Table 2 continues on next page.*

**Table 2.** *Continued*

| # | Sex | Phenotype | Variant | Zygosity; inheritance | Transcript | Cl. | Associated condition, inheritance pattern [MIM number] |
|---|---|---|---|---|---|---|---|
| 114 | F | DD, macrocephaly, facial dysmorphism | *PTEN* c.1003C>T, p.(Arg335*) | het; unk | NM_000314.4 | P | Cowden syndrome, AD [158350] |
| 150[d] | F | Hypertension, brachydactyly | *PDE3A* c.1333_1335del, p.(Thr445del) (novel[h]) | het; mat | NM_000921.4 | P | Hypertension and brachydactyly syndrome, AD [112410] |
| 162 | M | Macrocephaly, facial dysmorphism, deep plantar creases | *FGFR2* c.799T>C, p.(Ser267Pro) | het; unk | NM_000141.4 | P | Crouzon syndrome, AD [123500] |
| 178 | F | Seizures, cortical tubers on brain MRI | *TSC2* c.1443+1G>A (novel) | het; unk | NM_000548.3 | P | Tuberous sclerosis 2, AD [613254] |
| 244 | M | DD, behavioral problems, facial dysmorphism | *NSD1* c.6152-14G>A (novel) | het; dn | NM_022455.3 | P | Sotos syndrome, AD [117550] |
| 263[e] | M | Lung hypoplasia, enlarged kidneys | *PKHD1* c.5485C>T, p.(Gln1829*)<br>*PKHD1* c.11284C>A, p.(Pro3762Thr) | het; mat<br>het; pat | NM_138694.3 | P; LP | Polycystic kidney disease 4, AR [263200] |
| 294 | F | Short stature, short limbs, macrocephaly | *FGFR3* c.1138G>A, p.(Gly380Arg) | het; unk | NM_000142.4 | P | Achondroplasia, AD [100800] |
| 302 | F | Seizures, cortical and subcortical tubers on brain MRI , hypopigmented macules | *TSC2* c.4183C>T, p.(Gln1395*) | het; unk | NM_000548.3 | P | Tuberous sclerosis 2, AD [613254] |
| 304[f] | M | Camptodactyly of the fingers, bowed lower limbs, club feet, facial dysmorphism | *LIFR* c.1252C>T, p.(Arg418*)<br>*LIFR* c.1789C>T, p.(Arg597*) | het; unk<br>het; unk | NM_002310.6 | P; P | Stuve-Wiedemann syndrome, AR [601559] |
| 338 | F | Tall stature, increased arm span-to-height ratio, myopia | *FBN1* c.5839T>A, p.(Cys1947Ser) (novel) | het; unk | NM_000138.3 | P | Marfan syndrome, AD [154700] |
| 411 | M | Capillary malformations | *RASA1* c.655del, p.(Ser219Hisfs*6) (novel) | het; unk | NM_002890.2 | P | Capillary malformation-arteriovenous malformation 1, AD [608354] |
| 439 | F | Tall stature, increased arm span-to-height ratio | *FBN1* c.1496G>C, p.(Cys499Ser) | het; unk | NM_000138.3 | P | Marfan syndrome, AD [154700] |
| 488 | F | DD, multiple café au lait spots | *NF1* c.4267A>G, p.(Lys1423Glu) | het; unk | NM_000267.3 | P | Neurofibromatosis type 1, AD [162200] |

**Table 2.** *Continued*

| # | Sex | Phenotype | Variant | Zygosity; inheritance | Transcript | Cl. | Associated condition, inheritance pattern [MIM number] |
|---|---|---|---|---|---|---|---|
| Diagnostic test: trio ES | | | | | | | |
| 16[g] | F | ID, short stature, GH deficiency, congenital hypothyroidism, congenital cataract | *RNPC3* c.259C>T, p.(Gln87*) (novel[h])<br>*RNPC3* c.443G>C, p.(Gly148Ala) (novel[h]) | het; mat<br>het; pat | NM_017619.3 | P; LP[j] | Pituitary hormone deficiency, combined or isolated, 7 [618160] |

*Note:* Only patients for whom informed consent was obtained are included in this table.
*Abbreviations:* AD, autosomal dominant; AR, autosomal recessive; ASD, atrial septal defect; Cl., classification; dn, de novo; ES, exome sequencing; F, female; GH, growth hormone; hem, hemizygous; het, heterozygous; hmz, homozygous; ID, intellectual disability; LP, likely pathogenic; M, male; mat, maternal; NGS, next generation sequencing; P, pathogenic; pat, paternal; PDA, patent ductus arteriosus; PFO, patent foramen ovale; unk, unknown; VUS, variant of unknown significance
[a] Previously published (19).
[b] Previously published (20). Affected twin with the same molecular diagnosis not included in this cohort.
[c] Previously published (21).
[d] Previously published (22).
[e] Genetic testing was performed in both parents based on the results of the autopsy (clinical diagnosis of autosomal recessive polycystic kidney disease).
[f] Previously published (23).
[g] Previously published (24). Affected siblings with the same molecular diagnosis not included in this cohort.
[h] Novel variant, but genetic details regarding this individual have been previously published.
[i] Variant classification different from original report. *USP7* c.3202+1G>T had been reported as VUS but reclassified as likely pathogenic after more patients with variants in this gene were identified and the phenotype had been described (criteria according to Richards et al., 2015: PVS1, PS2, PM2).
[j] Variant classification different from original report. The *RNPC3* c.443G>C p.(Gly148Ala) variant was reclassified from VUS to likely pathogenic after segregation analysis in siblings (criteria according to Richards et al., 2015: PM2, PM3, PP1, PP3).

**Table 3.** Molecular diagnoses: copy number variants

| # | Sex | Phenotype | Variant(s) | Class | Associated condition |
|---|---|---|---|---|---|
| 35 | M | ID, speech and language delay, *pectus excavatum*, widely spaced eyes | arr[GRCh37] 15q11.2(22750305_23272733)x1 | P (sl) | 15q11.2 deletion syndrome |
| 45 | F | DD, hypotonia, clubfoot, facial dysmorphism | arr[GRCh37] 19p13.3(275925_2286201)x3 dn | P | 19p13.3 duplication (~2 Mb) |
| 83 | M | ID, seizures, microcephaly, clinodactyly | arr[GRCh37] 15q11.2q13.2(22299434_30657952)x3,15q13.2q13.3(30936285_32514341)x3 | P; P (sl) | 15q11.2q13.2 duplication/triplication (~8.4 Mb; overlapping PWS/AS deletion syndrome region) and 15q13.2q13.3 duplication ( ~1.6 Mb; including *CHRNA7*) |
| 84 | M | ID, hirsutism, ptosis, downslanted palpebral fissures, high forehead, uplifted earlobe | arr[GRCh37] 19q13.4 2q13.43(55447595_59097160)x3,22q11.1q11.21(16197021_20733495)x3 | P; P | 19q13.42q13.43 terminal duplication (~3.6 Mb) and 22q11.1q11.21 duplication ( ~4.5 Mb; overlapping with cat eye syndrome region and part of DiGeorge syndrome critical region) |
| 104 | F | Short stature, failure to thrive, Dandy-Walker variant, patent foramen ovale | arr[GRCh37] 17q12(34815551_36450598)x3 dn | P | 17q12 duplication syndrome |
| 122[a] | F | ID, facial dysmorphism | arr[GRCh37] 6p22.3(15374392_15405436)x1 dn | LP | 6p22.3 deletion (~31 kb; including exon 2 of *JARID2*) |
| 128 | M | DD, cryptorchidism, hypotonia, obesity, partial empty sella | arr[GRCh37] 15q11.2q13.1(23656946_28535266)x1 dn MS-MLPA: deletion of paternal 15q11q13 allele | P | Prader-Willi syndrome |
| 129 | M | Agenesis of the corpus callosum, hypotonia, facial dysmorphism | arr[GRCh37] 8p23.3p23.1(164984_7007415)x1 ,8p23.1p11.1(12494265_43674370)x3,8q23.3q24.3(115320834_146293414)x3 dn | P | Suggestive of 8p inverted duplication/deletion syndrome |
| 132 | M | DD, microcephaly, lissencephaly, agenesis of the corpus callosum, short stature, facial dysmorphism | arr[GRCh37] 10q23.31q24.1(91959144_99204791)x1 dn | P | 10q23.31q24.1 deletion ( ~7.2 Mb; including *KIF11* and *LGI1*) |
| 154 | M | Supravalvular aortic stenosis, supravalvular and peripheral pulmonary stenosis | arr[GRCh37] 7q11.23(72994476_74000679)x1 | P | 7q11.23 deletion ( ~1.0 Mb; including *ELN*) |

**Table 3.** *Continued*

| # | Sex | Phenotype | Variant(s) | Class | Associated condition |
|---|---|---|---|---|---|
| 163 | F | ID, hypotonia, severe scoliosis, facial dysmorphism | arr[GRCh37] 10p14p12.33(8926204_18332112) x1 | P | 10p14p12.33 deletion (~9.4 Mb) |
| 169 | M | DD, hypotonia, facial dysmorphism | arr[GRCh37] 14q32.3 2q32.33(103306215_106199579)x1 dn | P | 14q32.32q32.33 deletion (~2.9 Mb) |
| 174 | M | ID, facial dysmorphism | arr[GRCh37] 3q24q26.1(144955389_161517861)x1 | P | 3q24q26.1 deletion (~16.5 Mb) |
| 249 | F | Lissencephaly, facial dysmorphism | arr[GRCh37] 17p13.3(2540998_2563261)x1 | P | 17p13.3 deletion (~22 kb; including exon 2 of *PAFAH1B1*) |
| 252 | M | DD, macrocephaly, facial dysmorphism | arr[GRCh37] 16p12.2(21839340_22409463)x1 | P | 16p12.2 deletion syndrome |
| 271 | M | DD, microcephaly, cryptorchism, synophrys, hirsutism | arr[GRCh37] 6q25.3q26(156833278_161216608)x1 | P | 6q25.3q26 deletion (~4.4 Mb; including *ARID1B*) |
| 300 | F | Speech and language delay, short stature, obesity, facial dysmorphism | arr[GRCh37] 9q31.1q31.3(105904199_114366248)x1 | P | 9q31 deletion syndrome |
| 310 | M | DD, VSD, facial dysmorphism | arr[GRCh37] 1q21.1q21.2(146089268_149376652)x1 dn | P | 1q21.1 deletion syndrome |
| 340 | M | DD, microcephaly, failure to thrive, facial dysmorphism | arr[GRCh37] 7q21.13q21.3(89613897-94411523)x1 dn | P | 7q21.13q21.3 deletion (~4.8 Mb) |
| 352 | F | Facial dysmorphism | arr[GRCh37] 9p24.3p22.3(203861_16031471)x1 | P | 9p24.3p22.3 deletion (~16 Mb) |
| 375 | F | Mayer-Rokitansky-Küster-Hauser syndrome, obesity | arr[GRCh37] 16p11.2(28825605_29043450)x1 | P | 16p11.2 microdeletion syndrome |
| 380 | F | Short stature, obesity, irregular menstruation | arr[GRCh37] 15q11.2(25068609_25084319)x1 | P | 15q11.2 deletion ( ~15.7 kb; including exon 1-3 of *SNRPN*) |
| 433 | M | DD, hypotonia, peripheral pulmonary stenosis, microcephaly, facial dysmorphism | arr[GRCh37] 7q11.23(72718277_74142190) x1,16p13.11(15058820_16330672)x3 | P; P (sl) | Williams syndrome; 16p13.11 deletion syndrome |

*Table 3 continues on next page.*

**Table 3.** *Continued*

| # | Sex | Phenotype | Variant(s) | Class | Associated condition |
|---|---|---|---|---|---|
| 494 | F | Club feet, agenesis of the corpus callosum, hydronephrosis, hypotonia, pulmonary hypertension, facial dysmorphism | arr[GRCh37] 3q11.1q21.3(93878600_126099226)x1 | P | 3q11.1q21.3 deletion (~32 Mb) |
| 510 | F | Scimitar syndrome, facial dysmorphism | arr[GRCh37] 6q27(166502703_170919470)x1 | P | 6q27 deletion (~4.4 Mb) |
| 518 | F | PKD, subcortical tubers, hypertrophic cardiomyopathy, hypopigmentation | arr[GRCh37] 16p13.3(2106894_2131457)x1 dn | P | 16p13.3 deletion (~25 kb; including several exons of *TSC2* and possibly *PKD1*) |
| 538 | M | Cleft lip and palate, mild ID, tubular nose, low-set ears, long fingers | 46,XY,del(21)(q22.3).arr[GRCh37] 21q22.3(43229099_46312018) x1,21q22.3(46337565_48100155)x1 dn | P; P | 21q22.3 deletion ( ~3.1 Mb) and 21q22.3 terminal deletion (~1.8 Mb) |
| 539 | M | DD, facial dysmorphism | 46,XY.nuc ish 22q13.2(RP11-101F24x3).arr[GRCh37] 22q13.1q13.2(39606071_43462451)x3 dn | P | 22q13.1q13.2 duplication (~3.9 Mb) |
| 570 | F | DD, seizures, autism | arr[GRCh37] 15q11.2(22669052-23217514)x3 | P (sl) | 15q11.2 duplication syndrome |
| 571 | M | Seizures, microcephaly | arr[GRCh37] 20q13.33(61987414_62147896)x1 | P | 20q13.33 deletion ( ~160 kb; including *CHRNA4*, *KCNQ2* and *EEF1A2*) |
| 616 | M | Cleft lip and palate, hearing loss, tubular nose, long fingers | arr[GRCh37] 22q11.2 1q11.22(21808750_22955072)x1 dn | P | Distal 22q11.2 deletion syndrome |

*Note:* Only patients for whom informed consent was obtained are included in this table.
*Abbreviations:* Class, classification; DD, developmental delay; dn, de novo; ID, intellectual disability; LP, likely pathogenic; MS-MLPA, methylation-specific multiplex ligation-dependent probe amplification; P, pathogenic; PKD, polycystic kidney disease; sl, susceptibility locus; VSD, ventricular septal defect.
a Previously published (25).

**Table 4.** Molecular diagnoses: other variant types

| # | Sex | Phenotype | Variant | Associated condition |
|---|---|---|---|---|
| Aneuploidies | | | | |
| 109 | F | Severe midline defect | 47,XX,+13.arr(13)x3 | Patau syndrome |
| 165 | F | ID, facial dysmorphism | arr(21)x3 | Down syndrome |
| 218 | F | ID, hypotonia, facial dysmorphism | arr(21)x3 | Down syndrome |
| 278 | F | DD, scoliosis, facial dysmorphism | arr(13)x3[0.15] | Mosaic trisomy 13 |
| 401 | M | Speech and language delay, tall stature | arr(X)x1,(Y)x2 | XYY syndrome |
| 544 | M | PDA, facial dysmorphism | arr(X)x2,(Y)x1 | Klinefelter syndrome |
| 550 | F | PDA, facial dysmorphism, sandal gap | 47,XX,+21.arr(21)x3 | Down syndrome |
| 610 | F | Hypotonia, facial dysmorphism, sandal gap | arr(21)x3 | Down syndrome |
| Derivative chromosomes | | | | |
| 7 | F | Hypotonia, short stature, hypopigmentation, sparse hair, frontal bossing, thick eyebrows, widely spaced eyes | nuc ish(ETV6x4,RUNX1x2)[24/100] | Pallister-Killian syndrome |
| 115[a] | F | DD, hypotonia, microcephaly, facial dysmorphism | mos 47,XX,+der(1)(::q10->q23.3::)[4]/46,XX[12].arr[GRCh37] 1q21.1q23.3(144854574_162843606)x2~3 | Mosaic trisomy 1q10q23.3 |
| 161 | F | Agenesis of the corpus callosum, HLHS, truncus arteriosus, TAPVD, hirsutism | 46,XX,inv(12)(p?11.2q?14),der(13)t(8;13)(p11.1;p11.1).arr[GRCh37] 8p23.3p11.1(164984_43674370)x3 | Trisomy 8p |
| Aberrant methylation | | | | |
| 324 | F | Hemihyperplasia | Hypomethylation *KCNQ1OT1* (*LIT1*) | Isolated hemihyperplasia |
| 447 | M | Short stature, relative macrocephaly, frontal bossing, clinodactyly of the 5th fingers | Hypomethylation *H19* | Silver-Russell syndrome |
| 471 | M | Omphalocele, ear lobe creases | Hypomethylation *KCNQ1OT1* (*LIT1*) | Beckwith-Wiedemann syndrome |
| Repeat expansions | | | | |
| 48 | M | ID, long face, hand flapping | *FMR1*: CGG repeats in premutation (n = ~100 and n= ~200) and mutation range (n>200) | Fragile X syndrome |
| 492 | M | ID, DD, epilepsy, hand biting, large testes | *FMR1*: CGG repeats in premutation (n = ~78 and n= ~142) and mutation range (n>200) | Fragile X syndrome |

*Table 4 continues on next page.*

**Table 4.** *Continued*

| # | Sex | Phenotype | Variant | Associated condition |
|---|---|---|---|---|
| UPD | | | | |
| 449 | M | SGA, short stature, relative macrocephaly, facial dysmorphism | arr[GRCh37] 7p15.3q21.11(24803592_81535350) x2 hmz,7q3 4q36.3(138746752_159126310)x2 hmz | UPD7, clinically suggestive of Silver-Russell syndrome (maternal UPD) |
| Multiple variant types | | | | |
| 88 | M | Bilateral radial aplasia, bilateral ulnar hypoplasia, thrombocytopenia | arr[GRCh37] 1.q21.1(145395440_145762959) (paternal)<br>*RBM8A* c.-21G>A, p.(?), hemizygous (maternal) | Thrombocytopenia-absent radius (TAR) syndrome |

*Note:* Only patients for whom informed consent was obtained are included in this table.
*Abbreviations:* DD, developmental delay; HLHS, hypoplastic left heart syndrome; ID, intellectual disability; PDA, patent ductus arteriosus; SGA, small for gestational age; TAPVD, total anomalous pulmonary venous drainage; UPD, uniparental disomy.
[a] Previously published (26).

**Impact on clinical management**

Information on the clinical consequences of the molecularly confirmed diagnosis was available for 88 patients (81%). The genetic diagnosis had an impact on clinical management in 46 (52%) of these patients. The reported clinical consequences are summarized in Table 5. The most frequently reported consequences were referrals to health professionals. These were mainly referrals to other medical specialists for screening for associated risks and/or therapeutic advice, but also included referrals to a physiotherapist, speech therapist, and dietician. Changes in therapy or medication included, for example, a change in anti-epileptic medication in patient 567 with GEFS+ and patient 574 with Tuberous Sclerosis and indication for growth hormone therapy in two patients with Silver-Russel syndrome, but also discontinuing corticosteroid treatment in patient 621 with steroid-resistant nephrotic syndrome. Standardized follow-up care according to the protocol of a specific disorder, such as Down syndrome, Marfan syndrome, and Noonan syndrome, was started in nine individuals. Additional diagnostics were reported in five patients and include, for example, magnetic resonance imaging (MRI) in patient 131 with hypomyelinating leukodystrophy 8 and in patient 411 with capillary malformation-arteriovenous malformation 1. In some patients, the genetic diagnosis guided clinical decision making. For instance, the decision was made to continue anti-epileptic medication in patient 571 after he was diagnosed with a 20q13.33 deletion encompassing several epilepsy-associated genes. Consequences for surveillance include tumor screening in patient 114 with Cowden syndrome and patient 471 with Beckwith-Wiedemann syndrome, but also discontinuing tumor screening in patient

324 with isolated hemihyperplasia related to KCNQ1OT1 hypomethylation. Finally, other examples of clinical consequences include the referral of patient 574 with Tuberous Sclerosis to an expertise center in the Netherlands, but also prevention of an overseas referral to Colombia for diagnostic work-up in patient 567 with GEFS+.

**Table 5.** Impact on clinical management of molecularly confirmed diagnosis

| Consequence in management | n= |
|---|---|
| Referral to health professional(s) | 13 |
| Change in therapy/medication | 11 |
| Standardized follow-up care | 9 |
| Additional diagnostics | 5 |
| Guided clinical decision making | 4 |
| Change in surveillance | 3 |
| Treatment limitations | 3 |
| (Avoid) overseas referral | 2 |
| Tailored advice | 2 |
| Access to support services | 2 |

*Note:* Total does not add up to 46, as there were several different clinical consequences for some patients.



## Discussion

In this retrospective cohort study, we demonstrate that our genetic service with a visiting clinical geneticist in the Dutch Caribbean results in a molecularly confirmed diagnosis in 33% of patients with a suspected genetic disorder. Since ES is not (yet) part of standard care in the Dutch Caribbean and financial restrictions prompt a more targeted and proband-only approach, we believe this is a reasonably high diagnostic yield. In addition, in 52% of patients, the established diagnosis had an impact on clinical management.

Over the past few years, several other efforts have been made to improve access to genetic services in the Caribbean. Recently, Sobering et al. described their experiences with offering genetic services with a visiting clinical geneticist on several resource-limited Caribbean islands. They present the results of genetic testing in more than 100 individuals with suspected genetic disorders and report a diagnostic yield of exome sequencing of ~50% (27). Another study reports on an international telemedicine program in the Dominican Republic, through which a genetic molecular diagnosis was obtained for 39/57 (68%) individuals that received genetic testing, mostly through exome sequencing (28). Finally, Scantlebury et al. describe their experience with performing ES for the first time in five patients on the Eastern Caribbean island of Barbados, identifying a diagnostic pathogenic variant in three patients and a VUS in one patient (29). Studies in other resource-limited areas show similar promising results, with a diagnostic yield ranging from 29% for proband-only exome sequencing of

known Mendelian disease genes in a Chinese study (30), to a yield of 68% in a Mexican study on clinical genome sequencing (31). Moreover, studies that also investigated clinical consequences of the genetic diagnosis report an impact on clinical management in 45–69% of patients (28, 30, 31). Thus, genetic services can significantly contribute to healthcare even in lower resource settings.

In the present study, we describe the outcomes of genetic testing in the largest Caribbean cohort so far. A possible limitation of our study is that selection bias due to financial restrictions could explain the relatively high diagnostic yield, as patients with a high suspicion of a genetic disorder were more likely to receive genetic testing. On the contrary, the percentage of patients with a genetic disorder in our cohort is probably an underestimation, as more extensive genetic testing such as trio ES was not performed in the majority of patients. In addition, 12 patients with a diagnosis were excluded from this study as informed consent was not provided, resulting in a slight underestimation of the diagnostic yield. The main strength of our study is the retrospective design, which allows evaluation of the outcomes of the actual decision making process in clinical practice. In addition, although the consultations and counseling are currently provided as a service from the Amsterdam UMC Human Genetics Department, all residents of the Dutch Caribbean (except for St. Maarten) are entitled to basic health insurance that should cover the costs of genetic testing. This increases the accessibility and sustainability of our genetic service.

In our cohort, the highest diagnostic yield was achieved in patients with seizures and patients with DD/ID (50% and 37%, respectively). These percentages reflect the combined yield of the different genetic tests that were performed. Diagnostic yield for DD/ID is comparable to previous studies on ES (32), but diagnostic yield in patients with seizures is high compared to previous reports (33, 34). However, this number may be biased as there were only 14 patients with epilepsy included in our study. The lowest molecular diagnostic yield in our cohort was found for patients with obesity (6%), which is comparable to previous studies (35, 36). The diagnostic yield of single-gene testing and NGS gene panels was relatively high in our cohort. This may be explained by the stringent selection of patients in clinical practice due to financial limitations. the diagnostic yield of microarray in our cohort was 20%, which is within the previously reported range of 15–20% (37).

Because the Dutch Caribbean islands have small populations, we expected to find a high rate of recessive disorders. This hypothesis was however not confirmed in our cohort: 9 (17%) of the 52 patients with small variants had an autosomal recessive disorder, of which only 3 patients had a homozygous variant. Nevertheless, autosomal recessive hemoglobinopathies are relatively common in the Dutch Caribbean population (38, 39). These patients are, however, generally not referred to the clinical geneticist and, therefore, not included in

our cohort. Furthermore, founder effects have been observed in (small) island populations for autosomal dominant disorders, including in the Dutch Caribbean islands of Bonaire and Curaçao, where the highest worldwide known prevalence of hereditary hemorrhagic telangiectasia (also known as Osler-Weber-Rendu disease) has been found (40). Although we identified a few recurrent molecular diagnoses in our cohort, we did not detect any recurrent (founder) variants. However, our study was not designed to detect founder variants.

One of the challenges of our proband-only approach was the interpretation of VUS. Ideally, segregation analysis in the family or functional testing is performed to further classify a VUS, but this was often not possible because of financial restrictions. In addition, the Dutch Caribbean population is predominantly of African and Latin-American descent. Although ethnic diversity in genome reference data is increasing, several populations of non-European ancestry, including African and Latin American, are still underrepresented in population-based genomic studies (41, 42). This may lead to racial/ethnic disparities in VUS rates, with higher proportions of VUS in individuals of non-European ancestry (43-45). In our cohort, three recurrent VUS were identified: a 5p12 deletion and 9q22.1 and 9q22.31 duplications. We argue that these are likely normal genetic variants in the Dutch Caribbean population. In view of this, our aim for the future is to establish a database of genomic variants for the (Dutch) Caribbean population.



Finally, there are several challenges in the organization and realization of this bi-annual pediatric-genetics clinic. For example, pediatricians have to be very selective in referring patients, as there are generally only two or 3 days of clinic per island. During these days, follow-up visits also have to be scheduled. Moreover, when genetic test results are known, patients may have to wait several weeks or even months before they can speak to the clinical geneticist again. In these instances, telemedicine may be useful to provide additional consultations between the live visits. In addition, telemedicine may provide a good alternative for live visits when travel restrictions because of the coronavirus disease 2019 (COVID-19) pandemic, apply.

In conclusion, we show that despite financial restrictions, a diagnostic yield of 33% can be reached with targeted genetic testing in patients with a high suspicion of a genetic disorder. Moreover, we show that even in this resource-limited setting, the genetic diagnosis had an impact on clinical management in 52% of patients. Our approach with a visiting clinical geneticist may be an example for other countries, in particular other small islands where clinical genetics services are not (yet) available.

## References


1. Nguengang Wakap S, Lambert DM, Olry A, Rodwell C, Gueydan C, Lanneau V, et al. Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. European journal of human genetics : EJHG. 2020;28(2):165-73.
2. Gilissen C, Hehir-Kwa JY, Thung DT, van de Vorst M, van Bon BW, Willemsen MH, et al. Genome sequencing identifies major causes of severe intellectual disability. Nature. 2014;511(7509):344-7.
3. Lionel AC, Costain G, Monfared N, Walker S, Reuter MS, Hosseini SM, et al. Improved diagnostic yield compared with targeted gene sequencing panels suggests a role for whole-genome sequencing as a first-tier genetic test. Genetics in medicine : official journal of the American College of Medical Genetics. 2018;20(4):435-43.
4. Stark Z, Tan TY, Chong B, Brett GR, Yap P, Walsh M, et al. A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. Genetics in medicine : official journal of the American College of Medical Genetics. 2016;18(11):1090-6.
5. Stavropoulos DJ, Merico D, Jobling R, Bowdin S, Monfared N, Thiruvahindrapuram B, et al. Whole Genome Sequencing Expands Diagnostic Utility and Improves Clinical Management in Pediatric Medicine. NPJ genomic medicine. 2016;1:15012-.
6. Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, et al. Molecular findings among patients referred for clinical whole-exome sequencing. Jama. 2014;312(18):1870-9.
7. Malinowski J, Miller DT, Demmer L, Gannon J, Pereira EM, Schroeder MC, et al. Systematic evidence-based review: outcomes from exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability. Genetics in medicine : official journal of the American College of Medical Genetics. 2020;22(6):986-1004.
8. Angural A, Spolia A, Mahajan A, Verma V, Sharma A, Kumar P, et al. Review: Understanding Rare Genetic Diseases in Low Resource Regions Like Jammu and Kashmir - India. Frontiers in genetics. 2020;11:415.
9. Tekola-Ayele F, Rotimi CN. Translational Genomics in Low- and Middle-Income Countries: Opportunities and Challenges. Public health genomics. 2015;18(4):242-7.
10. Yip CH, Evans DG, Agarwal G, Buccimazza I, Kwong A, Morant R, et al. Global Disparities in Breast Cancer Genetics Testing, Counselling and Management. World journal of surgery. 2019; 43(5):1264-70.
11. Zhong A, Darren B, Loiseau B, He LQB, Chang T, Hill J, et al. Ethical, social, and cultural issues related to clinical genetic testing and counseling in low- and middle-income countries: a systematic review. Genetics in medicine : official journal of the American College of Medical Genetics. 2018.
12. Maria P, Jeung L, Duits A, Busari J. SARS-CoV-2 outbreak on the Caribbean islands of the Dutch Kingdom: a unique challenge. Rev Panam Salud Publica. 2020;44:e91.
13. Central Bureau of Statistics Curaçao. Population Tables [Internet]. 2020 [cited 2021 Mar 31]. Available from: https://www.cbs.cw/population-tables.
14. Croes M. Financiële relaties in het Koninkrijk. Na tien jaar: de reality check [Internet]. 2015 [cited 2021 Mar 16]. Available from: https://www.comitekoninkrijksrelaties.org/financiele-relaties-in-het-koninkrijk-na-tien-jaar-de-reality-check/.
15. Association for Clinical Cytogenetics. Professional guidelines for clinical cytogenetics: General best practice guidelines. Version 1.04. 2007.

16. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine : official journal of the American College of Medical Genetics. 2015;17(5):405-24.
17. Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genetics in medicine : official journal of the American College of Medical Genetics. 2020;22(2):245-57.
18. Verberne EA, Manshande ME, Wagner-Buitenweg NF, Elhage W, Holtsema H, van Haelst MM. Limb anomalies, microcephaly, dysmorphic facial features and fibroma of the tongue after failed abortion with methotrexate and misoprostol. Clinical dysmorphology. 2020;29(4):182-5.
19. Verberne EA, Dalen Meurs L, Wolf NI, van Haelst MM. 4H leukodystrophy caused by a homozygous POLR3B mutation: Further delineation of the phenotype. American journal of medical genetics Part A. 2020;182(7):1776-9.
20. Fountain MD, Oleson DS, Rech ME, Segebrecht L, Hunter JV, McCarthy JM, et al. Pathogenic variants in USP7 cause a neurodevelopmental disorder with speech delays, altered behavior, and neurologic anomalies. Genetics in medicine : official journal of the American College of Medical Genetics. 2019;21(8):1797-807.
21. Stunnenberg B, Ponson-Wever M, Verberne E, Peters I, Gerrits M, Haaxma C, et al. Novel SCN9A Mutations in a Compound Heterozygous Girl with Congenital Insensitivity to Pain. J Pediatr Neurol 2021;19(03):189-92.
22. Renkema KY, Westermann JM, Nievelstein RAJ, Lo-A-Njoe SM, van der Zwaag B, Manshande ME, et al. PDE3A gene screening improves diagnostics for patients with Bilginturan syndrome (hypertension and brachydactyly syndrome). Hypertension research : official journal of the Japanese Society of Hypertension. 2018;41(11):981-8.
23. Van De Maele K, Smulders C, Ecury-Goossen G, Rosina-Angelista I, Redeker E, van Haelst M. Stüve-Wiedemann syndrome: recurrent neonatal infections caused by impairment of JAK/STAT 3 pathway. Clinical dysmorphology. 2019;28(2):57-62.
24. Verberne EA, Faries S, Mannens M, Postma AV, van Haelst MM. Expanding the phenotype of biallelic RNPC3 variants associated with growth hormone deficiency. American journal of medical genetics Part A. 2020;182(8):1952-6.
25. Verberne EA, Goh S, England J, van Ginkel M, Rafael-Croes L, Maas S, et al. JARID2 haploinsufficiency is associated with a clinically distinct neurodevelopmental syndrome. Genetics in medicine : official journal of the American College of Medical Genetics. 2021;23(2):374-83.
26. Lo-A-Njoe S, van der Veken LT, Vermont C, Rafael-Croes L, Keizer V, Hochstenbach R, et al. De Novo Trisomy 1q10q23.3 Mosaicism Causes Microcephaly, Severe Developmental Delay, and Facial Dysmorphic Features but No Cardiac Anomalies. Case reports in genetics. 2016;2016:2861653.
27. Sobering AK, Li D, Beighley JS, Carey JC, Donald T, Elsea SH, et al. Experiences with offering pro bono medical genetics services in the West Indies: Benefits to patients, physicians, and the community. American journal of medical genetics Part C, Seminars in medical genetics. 2020; 184(4):1030-41.
28. Mena R, Mendoza E, Gomez Peña M, Valencia CA, Ullah E, Hufnagel RB, et al. An international telemedicine program for diagnosis of genetic disorders: Partnership of pediatrician and geneticist. American journal of medical genetics Part C, Seminars in medical genetics. 2020; 184(4):996-1008.

29. Scantlebury MH, Barrett KT, Jacinto S, Corbin DOC, Kerr M, Khan A. Cou Cou, flying fish and a whole exome please... lessons learned from genetic testing in Barbados. The Pan African medical journal. 2021;38:111.
30. Hu X, Li N, Xu Y, Li G, Yu T, Yao RE, et al. Proband-only medical exome sequencing as a cost-effective first-tier genetic diagnostic test for patients without prior molecular tests and clinical diagnosis in a developing country: the China experience. Genetics in medicine : official journal of the American College of Medical Genetics. 2018;20(9):1045-53.
31. Scocchia A, Wigby KM, Masser-Frye D, Del Campo M, Galarreta CI, Thorpe E, et al. Clinical whole genome sequencing as a first-tier test at a resource-limited dysmorphology clinic in Mexico. NPJ genomic medicine. 2019;4:5.
32. Srivastava S, Love-Nichols JA, Dies KA, Ledbetter DH, Martin CL, Chung WK, et al. Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders. Genetics in medicine : official journal of the American College of Medical Genetics. 2019;21(11):2413-21.
33. Sánchez Fernández I, Loddenkemper T, Gaínza-Lein M, Sheidley BR, Poduri A. Diagnostic yield of genetic tests in epilepsy: A meta-analysis and cost-effectiveness study. Neurology. 2019; 92(5):e418-28.
34. Symonds JD, McTague A. Epilepsy and developmental disorders: Next generation sequencing in the clinic. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2020;24:15-23.
35. Kleinendorst L, Massink MPG, Cooiman MI, Savas M, van der Baan-Slootweg OH, Roelants RJ, et al. Genetic obesity: next-generation sequencing results of 1230 patients with obesity. Journal of medical genetics. 2018;55(9):578-86.
36. Nordang GBN, Busk Ø L, Tveten K, Hanevik HI, Fell AKM, Hjelmesæth J, et al. Next-generation sequencing of the monogenic obesity genes LEP, LEPR, MC4R, PCSK1 and POMC in a Norwegian cohort of patients with morbid obesity and normal weight controls. Molecular genetics and metabolism. 2017;121(1):51-6.
37. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. American journal of human genetics. 2010;86(5):749-64.
38. van der Dijs FP, van den Berg GA, Schermer JG, Muskiet FD, Landman H, Muskiet FA. Screening cord blood for hemoglobinopathies and thalassemia by HPLC. Clinical chemistry. 1992;38(9):1864-9.
39. van Heyningen AM, Levenston MJ, Tamminga N, Scoop-Martijn EG, Wever RM, Verhagen AA, et al. Estimated incidence of sickle-cell disease in Aruba and St. Maarten suggests cost-effectiveness of a universal screening programme for St. Maarten. The West Indian medical journal. 2009;58(4):301-4.
40. Gallione CJ, Scheessele EA, Reinhardt D, Duits AJ, Berg JN, Westermann CJ, et al. Two common endoglin mutations in families with hereditary hemorrhagic telangiectasia in the Netherlands Antilles: evidence for a founder effect. Human genetics. 2000;107(1):40-4.
41. Landry LG, Ali N, Williams DR, Rehm HL, Bonham VL. Lack Of Diversity In Genomic Databases Is A Barrier To Translating Precision Medicine Research Into Practice. Health affairs (Project Hope). 2018;37(5):780-5.
42. Pereira L, Mutesa L, Tindana P, Ramsay M. African genetic diversity and adaptation inform a precision medicine agenda. Nature reviews Genetics. 2021.
43. Caswell-Jin JL, Gupta T, Hall E, Petrovchich IM, Mills MA, Kingham KE, et al. Racial/ethnic differences in multiple-gene sequencing results for hereditary cancer risk. Genetics in medicine : official journal of the American College of Medical Genetics. 2018;20(2):234-9.

44. Kurian AW, Ward KC, Hamilton AS, Deapen DM, Abrahamse P, Bondarenko I, et al. Uptake, Results, and Outcomes of Germline Multiple-Gene Sequencing After Diagnosis of Breast Cancer. JAMA oncology. 2018;4(8):1066-72.
45. Pottinger TD, Puckelwartz MJ, Pesce LL, Robinson A, Kearns S, Pacheco JA, et al. Pathogenic and Uncertain Genetic Variants Have Clinical Cardiac Correlates in Diverse Biobank Participants. Journal of the American Heart Association. 2020;9(3):e013808.

# Chapter 4

## Genetic diagnosis for rare diseases in the Dutch Caribbean: a qualitative study on the experiences and associated needs of parents

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## Abstract

Research on the perspectives of patients and parents regarding genetic testing and its implications has been performed mostly in Europe, Canada, the United States, Australia and New Zealand, even though genetic testing is becoming increasingly available worldwide. We aimed to fill this knowledge gap by exploring the experiences and needs of parents in the Dutch Caribbean who received a genetic diagnosis for the rare disease of their child. We conducted 23 semi-structured interviews with 30 parents of children diagnosed with various rare genetic diseases in Aruba, Bonaire and Curaçao (ABC-islands). Two researchers independently analysed the interviews using a thematic approach. Main themes identified were: (1) getting a genetic diagnosis, (2) coping, support and perceived social stigma, (3) living on a small island, and (4) needs regarding genetic services. Our results indicate that, despite reported limitations regarding the availability of healthcare and support services, receiving a genetic diagnosis for their child was valuable for most participants. While some of the participants' experiences with and attitudes towards the genetic diagnosis of their child were similar to those reported in previous studies, we identified a number of aspects that are more specifically related to this Dutch Caribbean setting. These include coping through faith and religion, social stigma and being the only one on the island with a specific genetic disorder. The results of this study and the provided recommendations may be useful when developing genetic testing and counselling services in similar settings.

## Introduction

Recent advances in genomic technologies have greatly increased the probability of obtaining a genetic diagnosis for early onset rare diseases. A genetic diagnosis can have several benefits for children and their families: it may end a long lasting search for a diagnosis, enable tailored management and surveillance, provide information about prognosis and recurrence risk and facilitate access to patient support groups, education, health and social care (1). As the costs of genetic testing are decreasing rapidly, genetic services are becoming increasingly available worldwide (2). In Europe, Canada, the United States, Australia and New Zealand the perspectives and experiences of parents who received a genetic diagnosis for their child have been studied extensively (3-12). However, little is known about the views of patients and parents in other parts of the world, even though there may be major differences due to different healthcare systems and unique economic, religious and cultural contexts. For example, access to therapy and support services might be limited (13) and options for future pregnancies, such as preimplantation genetic diagnosis, invasive prenatal diagnosis and termination of pregnancy, might be unavailable, illegal or unaccepted (14). This could, in turn, negatively affect the value of receiving a genetic diagnosis. A recent systematic review on clinical genetic testing and counselling in low- and middle-income countries identified several ethical, social, and cultural issues that should be considered when (further) developing genetic services in these countries (2). However, the majority of the studies included in this review was of a quantitative nature and the authors addressed the need for more qualitative studies, in order to gain more insight into the psychosocial and behavioural issues that could influence implementation and uptake of genetic services (2).

In 2011, a joint pediatric-genetics clinic with a visiting Dutch clinical geneticist was established to improve diagnostic opportunities for children with undiagnosed rare diseases in the Dutch Caribbean. Although the islands of the Dutch Caribbean are high-income economies, as defined by the World Bank (15), they face specific economic and healthcare challenges, due to their small size and relative remoteness. Because of the novelty of the local genetic service established on these islands and the aforementioned knowledge gap, we conducted a qualitative study to explore parents' experiences with obtaining a genetic diagnosis for their child, their attitudes towards the genetic diagnosis and their needs regarding genetic services. The results of this study may provide useful insights that can contribute to improving genetic care for the Dutch Caribbean population. In addition, the findings can be used when establishing or improving genetic services in other countries.

## Methods

### Setting

The Dutch Caribbean consists of six islands that are part of the Kingdom of the Netherlands. Three of these islands (Aruba, Bonaire and Curaçao) are located in the southern Caribbean Sea just off the coast of Venezuela. Collectively, they are referred to as the ABC-islands. The population of the ABC-islands is of mixed ancestry and the majority of the population is religious (mainly Roman Catholic). Papiamento is the most widely spoken language, but most people speak Dutch, English and/or Spanish as well.

The health systems of the ABC-islands largely mirror that of the Netherlands, with a general practitioner as the first point of contact. Secondary care is provided at hospitals and private clinics. Residents are entitled to (basic) health insurance, which is paid through income tax. Highly specialized care that is not available on the island is provided through medical transfers to hospitals overseas. For example, there is no neonatal intensive care unit (NICU) in Bonaire and Aruba and patients from these islands are thus transferred by air ambulance to Curacao or Colombia. Visiting medical specialists provide additional specialized care, for example, a pediatric neurologist who visits Curaçao once a year to evaluate complex patients. Until 2011, there was no local clinical genetics service in the Dutch Caribbean, and because of this a joint pediatric-genetics clinic was established. Since then, a Dutch clinical geneticist (MvH) visits the pediatric departments of the local hospitals of the ABC-islands twice a year to evaluate patients suspected of having a genetic disorder. Patients are referred to the clinical geneticist by their pediatrician, who is usually present during the genetic consultation. Medical and family history are obtained and a dysmorphologic physical examination is performed. If indicated, blood samples are sent to the Netherlands for genetic testing to establish or confirm a diagnosis. If a genetic diagnosis is established, patients and their parents receive counselling during a follow-up visit with the clinical geneticist. During this visit the cause and implications of the genetic diagnosis are explained and, if applicable, recurrence risk and risks for family members are discussed. As the clinical geneticist visits only twice a year, the results of genetic testing are sometimes already communicated to parents by the pediatrician and parents receive additional counselling during the next visit of the clinical geneticist. A more extensive description of the Dutch Caribbean, its healthcare systems and the established clinical genetics service has been published elsewhere (16).

### Study design

A qualitative study with semi-structured interviews was conducted with parents living in Aruba, Bonaire or Curaçao, whose child was diagnosed with a rare genetic disease. The interviews took place at local hospitals on all three islands (Dr. Horacio E. Oduber Hospital,

Hospital San Francisco [Fundashon Mariadal] and Sint Elisabeth Hospital) in November 2018 and April/May 2019. Written informed consent for participating in the study was obtained from each participant.

**Participants**

From the start of the genetic service program (November 2011) until November 2018, a total of 113 children (age at first visit < 18 years) that were referred to the clinical genetics outpatient clinics in Aruba, Bonaire and Curaçao received a molecularly confirmed genetic diagnosis. A few of them had already received the genetic diagnosis elsewhere and were referred for (additional) genetic counselling. For this study, we included parents who (1) received a genetically confirmed diagnosis for the rare disease of their child at least six months ago, but no longer than five years ago, and (2) were able to speak Dutch and/or English. Initially, parents who spoke Spanish were also included. However, after the first interview in Spanish it became clear that a higher level of Spanish proficiency of the interviewer was needed to conduct an interview of good quality. Therefore, this interview was excluded and subsequently only parents who spoke Dutch and/or English were included.



Parents who met the inclusion criteria were invited at random for an interview. Participants were recruited until no new themes or perspectives arose during the interviews. The parents of 35 children had been invited by telephone to participate in the study, of which 11 families cancelled the interview appointment later or did not show up. A total of 30 parents of 24 children (including one twin) participated.

**Data collection**

A semi-structured interview guide was developed by a clinical researcher (EV), together with a health scientist (LH) and clinical geneticist (MvH). Topics that were addressed included: (1) impact and consequences of receiving a genetic diagnosis, (2) reproductive decisions/ intentions, (3) satisfaction with genetic counselling and services, and (4) (health)care needs and future expectations (see Supplementary 1 for the complete interview guide). At the end of the interview, additional questions were asked to capture the sociodemographic characteristics.

The interviews were conducted by a clinical researcher from the Netherlands (EV). She had met 11 of the 30 participants prior to the interviews, when attending the consultations of the clinical genetics outpatient clinic, in which she played an observational role. The interviews lasted between 16 and 69 minutes, with a median duration of 38 minutes. After the interview, participants received a financial compensation (the local equivalent of 10 euro) for their participation and travel costs.

**Data analysis**

All interviews were audio recorded, after which they were transcribed verbatim and anonymized. Thematic analysis was performed as described by Braun and Clarke (17). The software program MaxQDA 2020 was used to conduct thematic analysis. The transcripts were read repeatedly and coded independently by two researchers (EV and LvdH). Any discrepancies between the two researchers were discussed until consensus was reached. Based on coding analysis, main and subthemes were identified. Final themes were discussed with three researchers (EV, LvdH and LH). Exemplar quotes were translated into English and presented in the results section.

## Results

A total of 23 interviews including 30 participants were conducted. Table 1 shows characteristics of the participants and their children. Seven interviews took place with both parents and 16 with one parent. The median age of the participants was 39 years (range 28–46 years) and 70% was female. Children had a median age of seven years (range 11 months – 20 years) at the moment the interviews were conducted, with a median age at genetic diagnosis of six years (range 2 months – 17 years). Eleven out of the 24 children (46%) had intellectual disability (ID). Monogenic ID syndromes were the most frequently established diagnoses. Most disorders were autosomal dominant and occurred *de novo* or inheritance was not determined because of financial restrictions or unavailable parental samples. To protect the privacy of the participants we do not include the specific diagnoses in this paper.

Four main themes were identified: (1) Getting a genetic diagnosis, (2) Coping, support and perceived social stigma, (3) Living on a small island, and (4) Needs regarding genetic services. Illustrative quotations from the interviews are presented in Table 2.

### Theme 1: Getting a genetic diagnosis

*Need for a diagnosis*

Most participants reported that after realizing their child had ‘something’, they wanted to find out what it was and where it came from. Some of them already visited various healthcare professionals for this reason and were actively looking for (more) help (Table 2, quote 1.1). Participants especially wanted to know what they could expect for the future and whether they could do anything to improve the health and/or development of their child. A few participants, however, did not think their child had (many) health problems and agreed to genetic testing because it was advised by the pediatrician. One participant even mentioned he was not aware that genetic testing had been requested.

**Table 1.** Sociodemographic and clinical characteristics of participants and their children

| Participants N = 30 | N (%) |
|---|---|
| Gender | |
| Male | 9 (30) |
| Female | 21 (70) |
| Relation to the patient | |
| Biological parent | 29 (97) |
| Foster parent | 1 (3) |
| Age | |
| 20–30 years | 3 (10) |
| 30–40 years | 15 (50) |
| 40–50 years | 12 (40) |
| Education level | |
| Primary school | 1 (3) |
| High school | 7 (23) |
| Secondary vocational education | 14 (47) |
| Higher education[a] | 7 (23) |
| Unknown | 1 (3) |
| Religion | |
| Christian | 26 (87) |
| Islamic | 2 (7) |
| No religion | 2 (7) |
| Island | |
| Aruba | 8 (27) |
| Bonaire | 4 (13) |
| Curaçao | 18 (60) |
| Language spoken during interview | |
| Dutch | 27 (90) |
| English | 3 (10) |
| **Participants' children N = 24** | **N (%)** |
| Gender | |
| Male | 13 (54) |
| Female | 11 (46) |
| Age | |
| 0–4 years | 6 (25) |
| 4–8 years | 7 (29) |
| 8–12 years | 5 (21) |
| 12–16 years | 5 (21) |
| ≥ 16 years | 1 (4) |
| Relation of parents | |
| Married/relationship | 14 (58) |
| Divorced/separated | 10 (42) |
| Intellectual disability | |
| Yes | 11 (46) |
| No | 13 (54) |

*Table 1 continues on next page.*

**Table 1.** *Continued*

| Participants' children N = 24 | N (%) |
|---|---|
| Genetic tests, total | |
| 1 | 12 (50) |
| 2 | 10 (42) |
| 3 | 2 (8) |
| Genetic test, diagnostic | |
| NGS gene panel | 10 (42) |
| Single gene | 7 (29) |
| Microarray | 3 (13) |
| Methylation analysis | 2 (8) |
| Multiple diagnostic tests | 2 (8) |
| Genetic diagnosis | |
| Monogenic ID syndrome | 6 (25) |
| Microdeletion syndrome | 3 (13) |
| Overgrowth syndrome | 3 (13) |
| Connective tissue disorder | 3 (13) |
| Congenital malformation syndrome | 3 (13) |
| Genetic obesity | 2 (8) |
| Other | 4 (17) |
| Inheritance | |
| Autosomal dominant/X-linked | |
| *de novo* | 5 (21) |
| inherited from affected parent | 2 (8) |
| suspected *de novo*[b] | 10 (42) |
| Autosomal recessive | 5 (21) |
| Methylation defect | 2 (8) |
| Age at genetic diagnosis | |
| < 1 year | 4 (17) |
| 1–4 years | 6 (25) |
| 4–8 years | 5 (21) |
| 8–12 years | 8 (33) |
| ≥ 12 years | 1 (4) |

*Abbreviations:* ID: intellectual disability, NGS: next-generation sequencing.
[a] Higher professional education and university education.
[b] Because of financial restrictions inheritance is not determined if parents are healthy and segregation is not necessary to establish the diagnosis.

*Impact of the genetic diagnosis*

Despite initial feelings of shock, worry and disappointment after receiving the genetic diagnosis, many participants were relieved to get an explanation for the problems of their child. It brought them closure and acceptance (Table 2, quote 1.2). Other positive aspects that participants reported were feeling prepared for the future and being able to get in contact with other (parents of) patients with the same disorder. Additionally, some participants

mentioned that the genetic diagnosis enabled them to make informed reproductive choices. For example, one participant could finally pursue her wish to have another child after hearing that the recurrence risk was negligible. Other participants decided not to have another child or were still contemplating it because of the recurrence risk (Table 2, quote 1.3). Some participants reported changes in clinical management through screening for additional medical problems related to the condition or through support services. Finally, a few participants reported that the diagnosis did not change anything, mainly because they were already doing as much as possible to guide and stimulate the development of their child.

While many participants believed the genetic diagnosis was beneficial, the diagnosis also caused participants to worry about possible future problems that might arise as part of the diagnosed genetic syndrome. Although for some participants it was a relief to know that the condition was genetic and not caused by something they did (Table 2, quote 1.4), others felt guilty because it was genetic. For example, one participant felt guilty about being a carrier of the autosomal recessive disorder that her child was diagnosed with (Table 2, quote 1.5).



**Theme 2: Coping, support and perceived social stigma**

*Acceptance, positive reframing and a focus on being normal*

Many participants expressed that the genetic diagnosis and the associated health problems were just something they had to accept and live with. Some participants said they already accepted that they had a 'special' child before the genetic diagnosis (Table 2, quote 2.1). However, other participants found it hard to accept that their child had a genetic syndrome, mainly because their other children were healthy and/or no one in the family had the same disorder. One participant also mentioned that it was difficult to accept the diagnosis, because she did not see anything abnormal in the appearance of her child (Table 2, quote 2.2). Several participants coped with the genetic diagnosis and the problems of their child by focusing on the positive sides and putting things in perspective (Table 2, quote 2.3). For some participants it was important to treat their child as normal as possible and let them live a normal life. One couple even trivialized the medical problems of their child, as well as the genetic diagnosis, and said their child was healthy (Table 2, quote 2.4).

*Coping through faith and religion*

A coping mechanism for several participants was their faith in God. It helped them to accept the genetic disorder of their child, because they believed it was something given to them by God, and it brought them strength and hope for the future (Table 2, quote 2.5). Also, some participants felt emotionally supported by their church community. At the same time, one

participant felt conflicted between science and religion in her hope for her child to be cured (Table 2, quote 2.6). Another participant did not believe his child had a genetic syndrome, as he felt that this was something that was in the hands of God (Table 2, quote 2.7).

*Family and peer support*

Besides faith and religion, another source of support for some participants was their family. Participants received emotional support from their family members, mostly parents, as well as help with childcare. Furthermore, a few participants connected online with other parents of a child with the same disorder: this made them feel supported because these parents understood what they were going through (Table 2, quote 2.8).

*Perceived social stigma*

Several participants stated that in general, children with disabilities are not fully accepted by their society. They described that these children are not really part of the local community and not visible in everyday life (Table 2, quote 2.9). One participant mentioned that even her own husband never accepted the disorder of their child (Table 2, quote 2.10). A few participants discussed the possible reasons for this stigma. They explained that since the communities on these islands are relatively small, there is a lot of gossip which might lead to feelings of shame and fear of getting stigmatized. Two participants felt that in the Netherlands, where one of them had lived, people are more accepting towards people with disabilities. For one participant fear of stigma was a reason not to tell anyone besides her close family about her child's genetic diagnosis (Table 2, quote 2.11). Another participant only recently told her mother about the genetic diagnosis, because she did not want her child to be treated differently. However, some participants tried to oppose the stigma: they described that they did not hide their child (with a visible disorder), but instead took him/her outside of the house as much as possible. Some participants expressed their worries about the limited opportunities for their child to find a future internship or job because of this stigma (Table 2, quote 2.12).

A few participants felt upset or irritated by certain beliefs of other people regarding the cause or cure of the disease of their child. For example, one participant got advicefrom other people about how to cure her child, including praying to God, and giving cannabis oil and a certain type of milk to her child. Another couple mentioned that people believe that kidney diseases are caused by fright (Table 2, quote 2.13).

**Table 2.** Illustrative quotes per theme

| Theme | Representative quote | Quote # |
|---|---|---|
| *Theme 1: Getting a genetic diagnosis* | | |
| Need for a diagnosis | "When she was 4 and a half years old we were walking around like crazy here on the island, my mother and I, to see if we could get help to send her abroad maybe or… move forward a bit." [Child aged ≥ 12 years at diagnose, ID+, #15] | 1.1 |
| Impact of the genetic diagnosis | "I think it was very good to do it, because then you know and… then you know also that it is something genetic… and you have to deal with it, learn how to deal with it. There is nothing now in the world that… turns… removes a gene and… makes everything okay again. (…) No, he won't be cured, he is just like this, accept it and… yeah. Deal with the problem." [Child aged < 1 year at diagnose, ID-, #18] | 1.2 |
| | "If I eh… was younger and would have wanted another child after all then… it's up to me… the choice remains mine. I think that's a good thing. So if I still want to engage in that… that battle.. with another child… or I choose not to." [Child aged < 1 year at diagnose, ID-, #18] | 1.3 |
| | "So… during the pregnancy… I didn't have a nice pregnancy. So those were my feelings of guilt. Maybe… that's why the child is… like that. But yeah, it was a relieve to hear that it was a fault of nature… that… yeah, from conception it was like that." [Child aged 8–12 years at diagnose, ID+, #4] | 1.4 |
| | "Sometimes I think, yeah, then… it is our… fault that [daughter] is like that, because it was the… DNA of… of him and me, that something went wrong there, right? Then… I feel guilty that [daughter] is like that. Some days."[Child aged 4–8 years at diagnose, ID-, #12a] | 1.5 |
| *Theme 2: Coping, support and perceived social stigma* | | |
| Acceptance, positive reframing and a focus on being normal | "Yeah it was just nor… yeah I just accepted it, because in the Netherlands they also explained that I have to… accept the children how they are, really. Because… you cannot do anything about it, because if you… where the children are going to live they have to get the same guidance, it is just intensive guidance." [Child aged 8–12 years at diagnose, ID+, #13] | 2.1 |
| | "As a parent… you don't accept it so… so fast, because you think: all my children are healthy and now you have a syndrome? And I looked immediately at my son: what are they looking at, what do they see in him that they say he has [name syndrome]? (…) They said, no we see it in his eyes, we see it in… in the eyebrow. But I see nothing." [Child aged 1–4 years at diagnose, ID+, #22] | 2.2 |
| | "With my daughter I have something like, until now eh… she is a very strong child, eh… and that won't change. Eh… and whatever she has.. she will go through with it, with life, with grace and strength." [Child aged 1–4 years at diagnose, ID-, #20] | 2.3 |
| | Interviewer: "And how was that? To hear it, about the [name syndrome]."<br>"That was something new for me, but… I said… my child has nothing so… And he is healthy so… I am not going to eh… worry about it." [Child aged < 1 year at diagnose, ID-, #3a] | 2.4 |

*Table 2 continues on next page.*

**Table 2.** *Continued*

| Theme | Representative quote | Quote # |
|---|---|---|
| Coping through faith and religion | "I believe in God and I also think that… he doesn't give you something that you cannot handle, so. (…) If He… wants it that way… everything will be okay." [Child aged 1–4 years at diagnosis, ID-, #21] | 2.5 |
| | "You keep hoping that the child… gets cured. But it is… it is hard for someone, I have to say, working in healthcare, that you know eh… how the… things work. That you say, okay eh… being able to be cured is really… a miracle. Because yeah… books say this and… and faith says that." [Child aged < 1 year at diagnosis, ID+, #8a] | 2.6 |
| | "No because I think that my… my daughter has… no [name syndrome]. (…)"<br>Interviewer: "And why do you think that?"<br>"Just positive. Because I… I… also believe in God… God… does everything. God does… things that we cannot… not do." [Child aged < 1 year at diagnose, ID-, #17] | 2.7 |
| Family and peer support | "We share a lot of information together and eh… it is… it is pretty different… if you talk with other parents. It's more like, they understand it better and they come eh… eh… they don't give you this… you get more… their solutions, their ideas are more workable than others, you know. That's it. And it is… and… it doesn't sound like nonsense." [Child aged < 1 year at diagnose, ID-, #18] | 2.8 |
| Perceived social stigma | "Because here on [name island] the people are… they are ashamed or they have eh… they hide children with special… I walk with [name son], I walk everywhere. For here it is a bit of a… taboo. (…) You don't see eh… children on the street. Only at the pediatrician." [Child aged 8–12 years at diagnose, ID+, #5] | 2.9 |
| | "Here it is… here it is a taboo. Many people here – it begins especially with the parents – they don't accept that they have a special child. To… seek proper help. My husband never accepted that [name son] is special. He always used to say, the child has nothing, the child just needs to get a good beating." [Child aged 8–12 years at diagnose, ID+, #4] | 2.10 |
| | "Here we have a culture… a very different culture, let's say than in the Netherlands. Here if someone says, for example, I am talking with you now, I tell you that my daughter has this disease, after… after a few days, the whole neighbourhood knows it. (…) It's better to keep it a secret, a family secret, than telling someone else." [Child aged 8–12 years at diagnose, ID-, #10] | 2.11 |
| | "If I… if I compare it for example with the Netherlands… people with an intellectual disability they get… guidance in terms of housing, employment, but here on [name island] we are not… open… for that. People with a… with a… disability they don't get a job. And I don't want that for my son." [Child aged 8–12 years at diagnose, ID+, #4] | 2.12 |
| | "Because they say that kidneys are a… a… a disease of the eh (…) a disease of eh… being frightened. (…) They said that kidney diseases are a disease of fear. I don't know if that is true." [Child aged 4–8 years at diagnose, ID-, #12b] | 2.13 |

**Table 2.** *Continued*

| Theme | Representative quote | Quote # |
|---|---|---|
| *Theme 3: Living on an island* | | |
| Availability and quality of healthcare, support services and education | "For special children there is not… enough guidance. At his school, at the school of [name son] there is no speech therapy. No physio[therapy]. Eh… every time there is a vacancy… vacancy… or… And I have to get speech therapy outside school. I have to eh… physiotherapy I have to look for myself. So I am on the street often with eh… outside school. For [name son]." [Child aged 8–12 years at diagnose, ID+, #5] | 3.1 |
| | "Eh… yes sometimes… sometimes, not always but sometimes you feel that eh… you want to do a lot of things with your child but yes… it is not easy because eh… that… here on [name island] there are not so many things for a special child and yes… sometimes you really want to do more things with your child, but… there is not that much." [Child aged 8–12 years at diagnose, ID+, #16] | 3.2 |
| Being the only one on the island | "You know what… you feel lonely… I know… there is no one here I can go to, because he… he is not Down syndrome, he doesn't have… if he would have had Down syndrome, we would have had a lot on [name island]. Then I could have told people, just: hey, how is it going with the care, how… But… [name syndrome] is alone." [Child aged < 1 year at diagnose, ID+, #8a] | 3.3 |
| *Theme 4: Needs regarding genetic services* | | |
| Satisfaction with genetic services | "Because we live here and that… that hospital or laboratory is in the Netherlands, so… for me, on that basis, it was still good. That we didn't have to go back and forth with… with all those things." [Child aged 8–12 years at diagnose, ID-, #10] | 4.1 |
| | "Imagine that I knew I was a carrier, that he was a carrier, you know… then we might have eh… yeah… looked for help to… I don't know… to have a healthy child, together, you know, if we might have had to go to the Netherlands, I… I don't know, but we didn't have that option. I didn't have an option." [Child aged < 1 year at diagnose, ID+, #8a] | 4.2 |
| Information needs | "I… don't know if a person with [name syndrome] if he… when she gets children… that information I don't have… I don't have it clear you know. (…) Sometimes I think that maybe… if it… it… it depends on with whom she gets a eh… child. (…) I have to get more information about that" [Child aged 4–8 years at diagnose, ID-, #11] | 4.3 |

*Abbreviations:* ID: intellectual disability, +: present, -: absent.

**Theme 3: Living on a small island**

*Availability and quality of healthcare, support services and education*

All participants indicated that they had health insurance and that almost all medical expenses were covered. Many participants said that they received sufficient care and were satisfied with the quality of their healthcare providers. However, some participants indicated that certain care is missing or not easily accessible on their island, such as subspecialized pediatric care. Several participants had to go abroad to receive specialized medical care and a few participants went abroad on their own initiative, for example to get a second opinion. Services such as physical and speech therapy are available, but some participants indicated that a lot of self-initiative was needed to obtain these services and would have liked them to be provided by, for example, school (Table 2, quote 3.1). Apart from this, some participants who had a child with intellectual disability found it difficult to get appropriate education for their child and were not satisfied with the availability and quality of special education. They experienced a lack of opportunities and facilities to support their child in general (Table 2, quote 3.2). A few children were living in a (day)care institution. Their parents had different feelings about that: One couple was very negative about the circumstances in the care institution, while a participant from another island was satisfied with the provided care.

*Being the only one on the island*

Some participants expressed that they would like to get in touch with other parents who have a child with the same genetic disorder: they wanted to share experiences and get information and advice. However, because of the small size of the islands and the rareness of the disorder it was difficult to find these parents (Table 2, quote 3.3). Consequently, the only option for most participants was to digitally connect with other parents. Although this worked for a few participants, for others it created a barrier: they did not know where to start, tried but did not succeed or preferred meeting other parents in person.

**Theme 4: Needs regarding genetic services**

*Satisfaction with genetic services*

Most participants were satisfied with the provided genetic services, although a few participants felt that it took too long before they received the genetic test results. One participant mentioned she was glad this service was available on the island, instead of having to go abroad for this (Table 2, quote 4.1). If they could go back in time, almost everyone would choose again to do genetic testing. Many participants would have wanted to get their child's genetic diagnosis at a younger age. Participants expected that this would have had several consequences, such as getting appropriate help sooner, taking preventive measures

and spending less time in uncertainty. In addition, one couple mentioned that if they would have known they were both carriers of a genetic condition, they could have searched for a way to have a healthy child (Table 2, quote 4.2). On the other hand, some participants felt they received the diagnosis at the right moment and others were unsure about the timing or felt that it did not really matter. Only one participant indicated that he would have liked to wait with genetic testing until his daughter was a bit older.

*Information needs*

In most cases, the genetic diagnosis had been disclosed by the clinical geneticist; in some cases this was done by the pediatrician. Several participants indicated they were satisfied with the genetic counselling they received. They felt that the explanation was clear and that they had enough possibilities to ask questions. However, a few indicated they were too shocked to understand all the information and to ask questions. Others felt that too much medical jargon was used, making it difficult to understand the information. One participant mentioned that her Dutch was not that good and that she would have liked to have someone to translate during the consultation. When asked about it, many participants said they searched the internet for more information, including two participants who specifically mentioned that they did this because the information they received during counselling was incomprehensible or insufficient.

Topics that participants would have liked to get more information on include recurrence risk and reproductive options (for themselves or their child) (Table 2, quote 4.3). A few participants still had questions regarding the genetic diagnosis: they did not fully comprehend why their child had this genetic disorder or did not completely understand the result of the genetic test. One participant even did not know her child had a genetic diagnosis. One participant, who received the diagnosis several years ago, mentioned that she would like to get an update on what is known about the genetic disorder and if there are any new advices for disease management.

## Discussion

This is the first study in the Dutch Caribbean that explores the experiences of parents who received a genetic diagnosis for their child. The majority of the participants valued getting a genetic diagnosis and would, in retrospect, choose again to get genetic testing for their child. The consequences of a genetic diagnosis reported by our participants largely correspond with those reported by patients and parents in previous studies. These include benefits such as a sense of closure, reduced guilt, feeling prepared for the future, access to support groups and being able to make informed reproductive choices (4-10). Negative consequences

include worries about the future and feeling guilty because of passing on a disease/gene to their children (7, 11, 12). Interestingly, making an informed reproductive choice was mentioned as a benefit by our participants despite limited reproductive options. This suggests that even in situations where reproductive technologies, such as preimplantation genetic testing or invasive prenatal diagnosis are unavailable or difficult to access, parents still value information about recurrence risk and can still make an informed reproductive choice. Only some of our participants reported changes in clinical management following the diagnosis. This might be related to reported difficulties with accessing support services and lack of specialized medical care in the Dutch Caribbean. However, a lack of medical utility has been reported in previous studies as well (4).

Even though many of the experiences and views that our participants shared are similar to those reported previously in literature, some findings seem to be more specifically related to the Dutch Caribbean setting. First of all, apart from acceptance, positive reframing and a focus on being normal, finding comfort in faith and religion was an important coping mechanism for several participants. This is in line with qualitative research on sickle cell disease in Jamaica, another Caribbean island (18). A systematic review on genetic testing for cancer risk among ethnic minority groups described that spirituality and God were not a barrier to genetic testing, but a way of seeking guidance and support (19). This is in accordance with our findings, although for one participant religion played a role in being less accepting towards the genetic diagnosis. It should be noted that finding comfort in faith and religion is a well-known coping mechanism in response to crises (20) and not unique to this specific setting. However, it is likely to be a more prominent coping style in areas where a high percentage of the population is religious, such as the Dutch Caribbean.

Secondly, several participants described that they felt that children with disabilities are not fully accepted by society, not really part of the community and not visible in everyday life, indicating a social stigma. Some participants tried to protect their child from this stigma by not sharing the genetic diagnosis or only sharing it with close family and friends. Concerns about stigma associated with having a (genetic) disease and the related wish not to be treated differently have also been identified in literature reviews of genetic testing in ethnic minority groups (19) and low- and middle-income countries (2). Although social stigma associated with rare (genetic) diseases and health-related stigma in general are global phenomena (21-23), the burden of stigma may be higher for people in low-income and less developed settings (24, 25). A few participants in our study suggested that social stigma was related to the small size of their communities. Indeed, there is evidence that people living in small (rural) communities experience greater health-related stigma compared to those living in urban areas (26, 27).

Thirdly, participants' experience with receiving a genetic diagnosis was influenced by the relative isolation of living on a small island. Although participants were generally satisfied with the available healthcare, some indicated that certain specialized care was lacking on their island. In addition, patients reported that support services were not easily accessible and that there were insufficient opportunities and facilities for children with intellectual disability. Moreover, their child was (almost always) the only one on the island with a specific genetic condition. This complicated the possibility to find peer support. Although some participants managed to connect with other parents online, others did not succeed in this or preferred meeting face-to-face. Regardless of country, for patients with (very) rare genetic diseases it may always be difficult to connect with peers (10, 28). However, in many countries opportunities are created for (parents of) patients with rare diseases to connect with peers in person, in order to share experiences, learn from each other, and to give and receive emotional support (29, 30). In the Dutch Caribbean, given the small population sizes of these islands, even for more common genetic diseases there may be only one or two patients with the same syndrome. This decreases the possibility of finding peers and may increase feelings of isolation, which could be a problem in other small, isolated or rural communities as well (31).



Another finding of this study is the need of participants for more information regarding the genetic diagnosis. Consistent with previous studies (3), participants' understanding of the provided information was sometimes impaired by the use of too much medical jargon and feelings of shock after receiving the diagnosis. Culturally appropriate educational material explaining the diagnosis as well as general concepts of genetics and inheritance, using local language and illustrations may be a valuable instrument to improve patient knowledge (32-34). Additional follow-up visits with the clinical geneticist may be useful to further address any questions that patients may have and to review the provided information. In particular, telemedicine may improve availability of such follow-up visits in remote areas (35, 36). Furthermore, local clinicians should receive (additional) medical genetics education to address questions that patients may have during regular follow-up visits. Visiting medical specialists including clinical geneticists may contribute to medical genetics education through seminars and clinical teaching rounds (37).

One of the limitations of this study is that parents who did not speak English or Dutch were not included, possibly creating a selection bias. In addition, although all participants were proficient in Dutch or English, these languages were not the mother tongue of most participants and thus there was still a language barrier in some of the interviews. These participants may have misunderstood questions and may not have been able to express themselves fully. A recommendation for further research would be to have an interviewer that is also able to speak the local language (Papiamento). Furthermore, the interviewer had

previously met some of the participants, when attending the consultations of the clinical genetics outpatient clinic. Although she played only an observing role, participants who recognized her may have felt uncomfortable with fully disclosing their thoughts. Lastly, participants had received the genetic diagnosis up to five years ago, which may have resulted in recall bias regarding certain topics, such as the response to diagnosis and experiences with genetic services.

In conclusion, this study provides valuable insights into the experiences and needs of parents in the Dutch Caribbean who received a genetic diagnosis for their child. Some of the experiences and views reported by our participants, such as the benefits and drawbacks of a genetic diagnosis, are similar to those identified in previous studies. Aspects such as coping style and living with a child with a genetic disorder are more strongly influenced by the specific Dutch Caribbean context. The findings of this study can be used to improve the genetic service on these islands, but also to inform genetic services that are being developed in similar settings. Finally, although Aruba, Bonaire and Curaçao face several economic and healthcare challenges, these islands have relatively good economies and are classified as high-income countries. As genetic testing is becoming more widespread available, further research in low- and middle-income countries is required to assess the needs regarding genetic counselling and testing, in order to provide appropriate and culturally tailored genetic services.

## References


1. Wright CF, FitzPatrick DR, Firth HV. Paediatric genomics: diagnosing rare disease in children. Nat Rev Genet. 2018;19(5):253-68.
2. Zhong A, Darren B, Loiseau B, He LQB, Chang T, Hill J, et al. Ethical, social, and cultural issues related to clinical genetic testing and counseling in low- and middle-income countries: a systematic review. Genet Med. 2018.
3. Ashtiani S, Makela N, Carrion P, Austin J. Parents' experiences of receiving their child's genetic diagnosis: a qualitative study to inform clinical genetics practice. American journal of medical genetics Part A. 2014;164a(6):1496-502.
4. Lim Q, McGill BC, Quinn VF, Tucker KM, Mizrahi D, Patenaude AF, et al. Parents' attitudes toward genetic testing of children for health conditions: A systematic review. Clin Genet. 2017;92(6):569-78.
5. Carmichael N, Tsipis J, Windmueller G, Mandel L, Estrella E. "Is it going to hurt?": the impact of the diagnostic odyssey on children and their families. J Genet Couns. 2015;24(2):325-35.
6. Makela NL, Birch PH, Friedman JM, Marra CA. Parental perceived value of a diagnosis for intellectual disability (ID): a qualitative comparison of families with and without a diagnosis for their child's ID. American journal of medical genetics Part A. 2009;149a(11):2393-402.
7. Chassagne A, Pélissier A, Houdayer F, Cretin E, Gautier E, Salvi D, et al. Exome sequencing in clinical settings: preferences and experiences of parents of children with rare diseases (SEQUAPRE study). Eur J Hum Genet. 2019;27(5):701-10.
8. Halverson CM, Clift KE, McCormick JB. Was it worth it? Patients' perspectives on the perceived value of genomic-based individualized medicine. J Community Genet. 2016;7(2):145-52.
9. Esquivel-Sada D, Nguyen MT. Diagnosis of rare diseases under focus: impacts for Canadian patients. J Community Genet. 2018;9(1):37-50.
10. Rosell AM, Pena LD, Schoch K, Spillmann R, Sullivan J, Hooper SR, et al. Not the End of the Odyssey: Parental Perceptions of Whole Exome Sequencing (WES) in Pediatric Undiagnosed Disorders. J Genet Couns. 2016;25(5):1019-31.
11. Krabbenborg L, Vissers LE, Schieving J, Kleefstra T, Kamsteeg EJ, Veltman JA, et al. Understanding the Psychosocial Effects of WES Test Results on Parents of Children with Rare Diseases. J Genet Couns. 2016;25(6):1207-14.
12. McAllister M, Davies L, Payne K, Nicholls S, Donnai D, MacLeod R. The emotional effects of genetic diseases: implications for clinical genetics. American journal of medical genetics Part A. 2007;143a(22):2651-61.
13. Choudhury MC, Saberwal G. The role of patient organizations in the rare disease ecosystem in India: an interview based study. Orphanet journal of rare diseases. 2019;14(1):117.
14. Penchaszadeh VB. Ethical issues in genetics and public health in Latin America with a focus on Argentina. J Community Genet. 2015;6(3):223-30.
15. The World Bank. High income [Available from: https://data.worldbank.org/income-level/high-income?view=chart.
16. Verberne EA, Ecury-Goossen GM, Manshande ME, Ponson-Wever M, de Vroomen M, Tilanus M, et al. Clinical and community genetics services in the Dutch Caribbean. J Community Genet. 2021.
17. Braun V, Clarke V. Using thematic analysis in psychology. Qualitative Research in Psychology. 2006;3(2):77-101.
18. Anderson M, Asnani M. "You just have to live with it": coping with sickle cell disease in Jamaica. Qual Health Res. 2013;23(5):655-64.

19. Hann KEJ, Freeman M, Fraser L, Waller J, Sanderson SC, Rahman B, et al. Awareness, knowledge, perceptions, and attitudes towards genetic testing for cancer risk among ethnic minority groups: a systematic review. BMC Public Health. 2017;17(1):503.
20. Pargament KI. The psychology of religion and coping: Theory, research, practice. New York: Guilford Press; 1997.
21. Birbeck GL, Bond V, Earnshaw V, El-Nasoor ML. Advancing health equity through cross-cutting approaches to health-related stigma. BMC Med. 2019;17(1):40.
22. Rai SS, Syurina EV, Peters RMH, Putri AI, Zweekhorst MBM. Non-Communicable Diseases-Related Stigma: A Mixed-Methods Systematic Review. Int J Environ Res Public Health. 2020;17(18).
23. von der Lippe C, Diesen PS, Feragen KB. Living with a rare disorder: a systematic review of the qualitative literature. Mol Genet Genomic Med. 2017;5(6):758-73.
24. Epilepsy: a public health imperative. Geneva: World Health Organization; 2019.
25. Kemp CG, Jarrett BA, Kwon C-S, Song L, Jetté N, Sapag JC, et al. Implementation science and stigma reduction interventions in low- and middle-income countries: a systematic review. BMC Medicine. 2019;17(1):6.
26. Kalichman S, Katner H, Banas E, Kalichman M. Population Density and AIDS-Related Stigma in Large-Urban, Small-Urban, and Rural Communities of the Southeastern USA. Prev Sci. 2017; 18(5):517-25.
27. Elliot VL, Morgan D, Kosteniuk J, Froehlich Chow A, Bayly M. Health-related stigma of noncommunicable neurological disease in rural adult populations: A scoping review. Health Soc Care Community. 2019;27(4):e158-e88.
28. Germeni E, Vallini I, Bianchetti MG, Schulz PJ. Reconstructing normality following the diagnosis of a childhood chronic disease: does “rare” make a difference? Eur J Pediatr. 2018;177(4):489-95.
29. Delisle VC, Gumuchian ST, Rice DB, Levis AW, Kloda LA, Körner A, et al. Perceived Benefits and Factors that Influence the Ability to Establish and Maintain Patient Support Groups in Rare Diseases: A Scoping Review. Patient. 2017;10(3):283-93.
30. Rizzo R, Van den Bree M, Challenger A, Cuthbert A, Ayllon MA, Clarke A, et al. Co-creating a knowledge base in the “22q11.2 deletion syndrome” community. J Community Genet. 2020; 11(1):101-11.
31. Lauckner HM, Hutchinson SL. Peer support for people with chronic conditions in rural areas: a scoping review. Rural Remote Health. 2016;16(1):3601.
32. Lubitz RJ, Komaromy M, Crawford B, Beattie M, Lee R, Luce J, et al. Development and pilot evaluation of novel genetic educational materials designed for an underserved patient population. Genet Test. 2007;11(3):276-90.
33. Permuth-Wey J, Vadaparampil S, Rumphs A, Kinney AY, Pal T. Development of a culturally tailored genetic counseling booklet about hereditary breast and ovarian cancer for Black women. American journal of medical genetics Part A. 2010;152a(4):836-45.
34. Canedo JR, Miller ST, Myers HF, Sanderson M. Racial and ethnic differences in knowledge and attitudes about genetic testing in the US: Systematic review. J Genet Couns. 2019;28(3):587-601.
35. Hilgart JS, Hayward JA, Coles B, Iredale R. Telegenetics: a systematic review of telemedicine in genetics services. Genet Med. 2012;14(9):765-76.
36. Mena R, Mendoza E, Gomez Peña M, Valencia CA, Ullah E, Hufnagel RB, et al. An international telemedicine program for diagnosis of genetic disorders: Partnership of pediatrician and geneticist. Am J Med Genet C Semin Med Genet. 2020;184(4):996-1008.
37. Sobering AK, Li D, Beighley JS, Carey JC, Donald T, Elsea SH, et al. Experiences with offering pro bono medical genetics services in the West Indies: Benefits to patients, physicians, and the community. Am J Med Genet C Semin Med Genet. 2020;184(4):1030-41.

# Supplementary material

**Supplementary 1. Interview guide**

*Adjustments that were made after the first round of interviews are shown in red.*

General questions (1)

- How old is your son/daughter?
- Where was your son/daughter born?
- Do you have more children? How old are they?
- Do you have a relationship with the father/mother of your child? (Married, living together?)
- How is your child doing?

Experiences and needs – past

- When was the first time you noticed there were problems with the development/health of your child? What did you notice? How did you deal with that? How did your family/ partner deal with that?
- When was the first time you visited a doctor for these problems? Why did you decide to see a doctor at that moment? How did it go?
- What were your expectations of the doctor?
- Were you eager to know what the cause of the problems was? Why (not)? What did you do to find out the cause? (Visit doctors/other people)
- What did you think was the cause of the problems of your child?

Experiences and understanding – diagnosis

- Do you know now what the cause of the problems of your child is?
- What do you know about what your child has? How do you know this?
- Who told you what your child has and how did that go?
  - Who else was present at that moment? (Partner, children, intern, interpreter?) What did you think about them being there?
  - Were there any specific comments the doctor made that you remember?
- What was your first reaction when you found out what your child has? How is that now?
- Do you feel it took a long time before the doctors knew what your child has? How was that for you?
- ~~What does it mean for you~~ Was it useful for you to know what your child has?
  - What were the consequences for you or your child when you knew what your child has?

  - Did something change in the treatment after knowing what your child has? (For example: other support, more/less visits to the doctor, other school, referral to other specialist)
  - What kind of help/care does your child get now?
  - Are there negative sides about getting a genetic diagnosis?
- If you could go back in time, would you choose again to do the test?
  - Would you have liked to know the diagnosis earlier?
  - If you would have gotten the diagnosis earlier, what would have been different now?
- What do you expect for the future? And for your own role in it?
- Are there still questions that you have now?
- What are things that are difficult for you now?
- Do you know what the consequences are for your other children/if you want to have other children? Can you tell something about that?
  - Did it have any influence on your other children? (Worries about their health, genetic testing, but also impact on other children)
  - Did it influence your choice to have more children? Can you tell more about that?
  - Did you think about possible options if you want to get pregnant again? Can you tell more about that? What options are there? Why would/wouldn't you choose for that?
  - Do you know if there are consequences for your child if he/she wants to have children later?

Satisfaction with genetic services

- ~~Did you get sufficient information about what your child has? From whom did you get this information?~~ How was the consultation with the clinical geneticist?
  - What was good about it?
  - What could be improved?
  - Did the doctor use language that you could understand?
  - Were there enough possibilities to ask questions?
  - Did you get any written information to take with you? Or a referral to a website?
  - Did you have any unanswered questions?
- Did you look up information later on the internet? What did you think about this information?

Experiences and needs – surrounding

- Did you talk to people in your surrounding about what your child has? With whom? How did that go?
  - How do people in your family treat your child?

  - How do other people in your surrounding treat your child? (School, neighbors, friends, etc.)
  - Do people in your environment treat your child different now that they know what he/she has?
- Are there enough possibilities here to provide the care that your child needs? Why (not)? What is missing?
- Did you ever consider moving to the Netherlands for your child?
- Did you try to get in touch with other parents or an association of parents that have a child with the same condition? Is this something you would want? Did the pediatrician/ clinical geneticist point out any possibilities for this?
- To what extend can you pay all the cost for healthcare? Did that change after knowing what your child has? What is (not) covered by the healthcare insurance? Which healthcare insurance do you have?



General questions (2)

- How old are you?
- What is your job?
- What is the highest level of education that you finished?
- Where were you born?
- Where were your parents born?
- Are you religious? If yes: which religion and how active are you in this?

Final questions

- Is there anything you would like to add?
- Is there anything else you would like to say?
- How was it to participate in this interview?

# Part III

## Lessons learned from Dutch Caribbean patients

# Chapter 5

## 4H leukodystrophy caused by a homozygous *POLR3B* mutation: Further delineation of the phenotype

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## Abstract

4H leukodystrophy, also known as Pol III-related leukodystrophy, is a rare autosomal recessive neurodegenerative disorder characterized by hypomyelination, hypodontia and hypogonadotropic hypogonadism. It is caused by biallelic mutations in *POLR3A*, *POLR3B* or *POLR1C*. So far, only two patients have been described with homozygosity for the common c.1568T>A (p.Val523Glu) *POLR3B* mutation, both of them showing a remarkably mild clinical course. Here, we report another patient with homozygosity for the same mutation, but with a more severe phenotype including ataxia, developmental delay, and intellectual disability. This information is of importance for clinicians to provide comprehensive counseling to patients with 4H leukodystrophy and their families.

## Introduction

4H leukodystrophy, also known as Pol III-related leukodystrophy, is a rare autosomal recessive neurodegenerative disorder characterized by hypomyelination, hypodontia and hypogonadotropic hypogonadism. Age of onset is usually early childhood with a progression of motor dysfunction due to increasing ataxia (1). Other features include cognitive impairment, short stature and myopia. The clinical course of 4H leukodystrophy is highly variable, with some patients never being able to walk independently and having mild to moderate intellectual disability, while other reported cases present only in adolescence with idiopathic hypogonadotropic hypogonadism (1, 2).

4H leukodystrophy is caused by mutations in *POLR3A*, *POLR3B* or *POLR1C* (3-6). *POLR3A* and *POLR3B* encode the largest and second-largest subunits (RPC1 and RPC2, respectively) of RNA Polymerase III (Pol III). Together, RPC1 and RPC2 form the catalytic centre of Pol III. Pol III is an enzyme involved in the transcription of small non-coding RNAs (such as tRNAs, 5S RNA, 7SK RNA and U6 RNA) that play a role in processes like transcription regulation, RNA processing, ribosome assembly and translation, that ultimately lead to protein synthesis. The transcription of small non-codings RNAs by Pol III plays an essential role in cell growth and differentiation (7). Recently, it was discovered that 4H leukodystrophy can also be caused by biallelic pathogenic variants in POLR1C, another subunit of Pol III (6). It is hypothesized that mutations in *POLR3A*, *POLR3B* or *POLR1C* lead to a dysregulation of Pol III and thus to inadequate levels of certain tRNAs, which are needed for the synthesis of proteins essential for central nervous system myelination (3, 4, 6).

The most commonly encountered *POLR3B* mutation in 4H leukodystrophy is c.1568T>A (p.Val523Glu). The majority of patients are compound heterozygous and carry a second (different) mutation in addition to c.1568T>A. Only two patients have thus far been reported with homozygosity for this mutation, both of them showing a remarkably mild clinical course (1).

Here, we describe a third patient with 4H leukodystrophy due to homozygous c.1568T>A (p.Val523Glu) mutations in *POLR3B*. Our patient presents with ataxia, intellectual disability, developmental delay, hypogonadotropic hypogonadism, myopia, hypodontia and short stature, demonstrating that this genotype can also result in a more severe phenotype. This information is of importance for clinicians to provide comprehensive counseling including prenatal options to family members of patients with 4H leukodystrophy.

## Case report

The proband, a 21-year-old woman, was the first child of healthy non-consanguineous parents of Dutch Caribbean ancestry. She was born at term after an uncomplicated pregnancy and delivery, with a birth weight of 3.5 kg. At the age of 1½ years parents noticed a delay in her development, as she was not able to walk without support. When she was 2 years old she was evaluated by a pediatrician and a neurologist. Laboratory evaluation (blood cell count, electrolytes, renal function, liver enzymes, cholesterol, thyroid-stimulating hormone, free T4) showed no abnormalities. A computer tomography (CT) scan of the brain was performed and showed a wide 4th ventricle with a dilated cisterna magna and hypoplasia of the cerebellar vermis, which was interpreted as a Dandy Walker variant. She was diagnosed with infantile encephalopathy with ataxia. Since there was no permanent paediatric care on the island at that time, no follow-up took place.

At the age of 14 years she presented at the pediatric genetic clinic because her parents wanted to know the cause for her developmental delay. At that time she had two healthy younger brothers. She used a walker because of ataxia. She could only produce three-word sentences and there was dysarthria. Her IQ was estimated to be 40. On examination, her height was 143 cm (< -2 SD), weight: 66 kg (+4 SD) and head circumference: 54 cm (-0.5 SD). She was noted to have a short philtrum, thick everted lower lip, lateral flaring of the eyebrows, hypodontia and pes planus (Figure 1). There was cerebellar ataxia with problematic gait balance and an intention tremor. A gaze-evoked nystagmus was observed. She had bilateral myopia (-3.50/-5.50 dpt). Fundus examination revealed no abnormalities. Upon examination at the age of 15 years she had normal secondary sex characteristics (Tanner stage M4P4) but she did not yet have her menarche. Her plasma level of luteinizing hormone (LH) was 0.5 IU/L and the level of follicle-stimulating hormone (FSH) was 2.5 IU/L. With a luteinizing-hormone releasing hormone (LHRH) stimulation test, there was no significant LH or FSH response. Abdominal ultrasound showed no abnormalities.

Single nucleotide polymorphism (SNP) array showed a normal female profile with several large regions of homozygosity. Gene panel analysis of 761 genes associated with intellectual disability (virtual panel by whole exome analysis) revealed a homozygous pathogenic missense mutation in *POLR3B*, c.1568T>A p.(Val523Glu), establishing the diagnosis of 4H leukodystrophy. Both parents were carriers. In retrospect, one of the regions of homozygosity in the proband comprises the *POLR3B* gene. After this diagnosis a brain MRI was performed, which showed features consistent with 4H leukodystrophy (Figure 2).

At the age of 20 she was referred to the ophthalmologist because of a white glaze on her left pupil. She was diagnosed with mature cataract of the left lens for which subsequently a

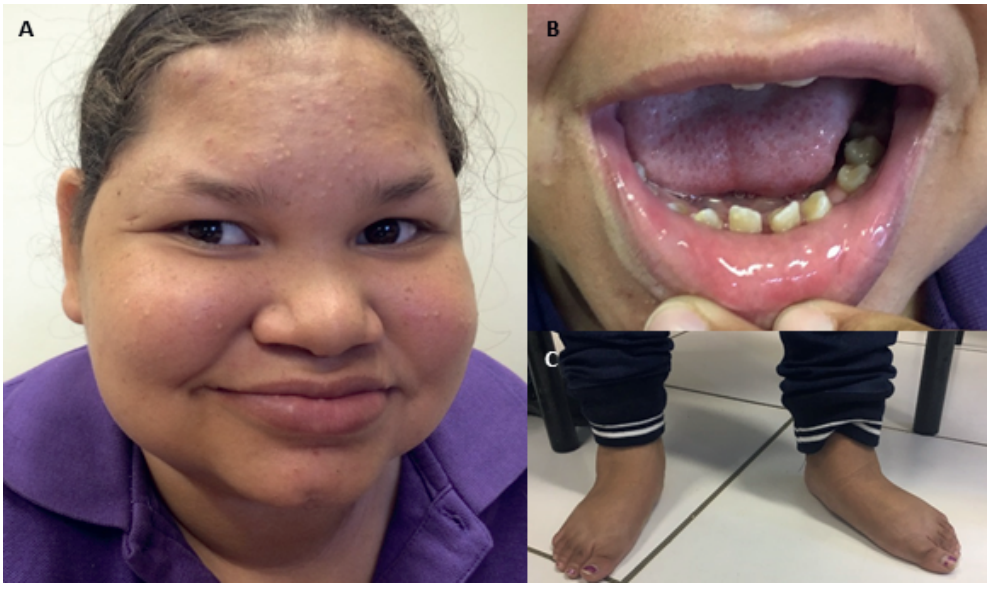


**Figure 1.** Patient at the age of 16. Dysmorphic features include: **A:** short philtrum, thick everted lower lip and lateral flaring of the eyebrows, **B:** hypodontia, and **C:** pes planus of both feet.



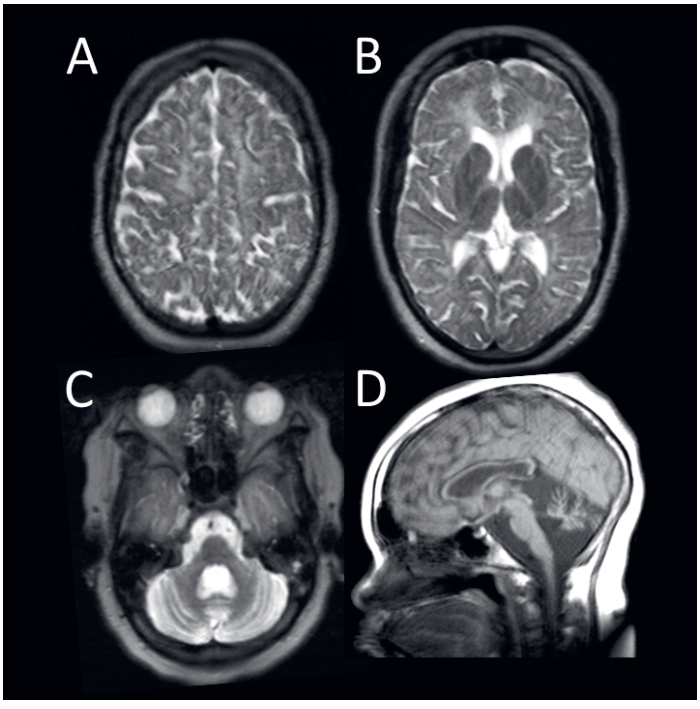


**Figure 2.** This brain MRI was made at age 17 years. **A-C** are T2-weighted axial images, demonstrating diffuse hyperintense signal of the white matter, mild supratentorial atrophy and severe cerebellar atrophy. The ventrolateral thalamus **(B)** and the medial lemniscus **(C)** are relatively hypointense, as often seen in 4H leukodystrophy. The sagittal T1-weighted image **(D)** demonstrates cerebellar atrophy and a thin corpus callosum.

cataract extraction was performed. A year later cataract of the right lens was diagnosed, for which an operation is planned.

## Discussion

The c.1568T>A substitution is the most commonly described *POLR3B* mutation in 4H leukodystrophy and is reported in the Genome Aggregation Database (gnomAD) with an allele frequency of 0.0003% (https://gnomad.broadinstitute.org/; accessed 28-2-2020). Almost all of these controls were from European descent and there were zero homozygotes. Daoud et al. showed that carriers of this mutation share a common haplotype, suggesting that this mutation derives from a single ancestor (8). Given the history of Spanish and Dutch colonization of the Caribbean island our patient was born, it could very well be that her parents have a shared European ancestor from which the mutation was inherited. In support of this, array analysis showed a region of homozygosity overlapping the *POLR3B* gene in our patient.

Homozygosity for this pathogenic variant was thus far reported in only two patients (a sibling pair) with 4H leukodystrophy. They were both mildly affected, with the older sister having no clinical symptoms of 4H leukodystrophy other than myopia at the age of 26. The younger brother was diagnosed with a learning disability at the age of 11 years and was referred to the neurology clinic at age 15 because of a tonic-clonic seizure. Neurological examination showed myopia and some stumbling on tandem gait testing. One year later he had abnormal upgaze saccades, hyperreflexia and mild dysmetria on examination. At the age of 23 years he did not have any new neurological deficits. Their brain MRIs showed diffuse hypomyelination with relative preservation of specific structures and significantly more residual myelin than typically seen in 4H leukodystrophy (9).

This is the first report showing that homozygosity for the c.1568T>A *POLR3B* mutation can have a typical 4H phenotype as well. Symptoms in our patient already started in early childhood with delayed motor development. She later developed cerebellar signs including nystagmus, intention tremor and ataxia, for which the use of a walker was required, and was found to have a severe intellectual disability. Other characteristic clinical features of 4H leukodystrophy are present as well, i.e. hypodontia, hypogonadotropic hypogonadism, short stature and myopia. There is no clinical suspicion of an additional syndrome causing her severe symptoms, as all clinical, radiologic and genetic features in our case are consistent with 4H leukodystrophy. Also, array results were normal and no other pathogenic variants were detected by intellectual disability gene panel analysis. It is known that the severity of 4H leukodystrophy can be highly variable, even within the same family, which is in line with our finding (1, 3).

Additionally, it is of interest that our patient developed cataract as an adolescent. In a cohort of 105 mutation-proven cases of 4H leukodystrophy, cataract was present in only three patients, including one sibling pair (1). Furthermore, three other cases of cataract in 4H leukodystrophy have been reported (10, 11). This additional case suggests that cataract is indeed a feature of 4H leukodystrophy, although its manifestation seems to be infrequent.

In conclusion, we demonstrate that homozygosity for the common c.1568T>A (p.Val523Glu) *POLR3B* mutation causing 4H leukodystrophy can have a severe clinical phenotype. This information is important for clinicians to provide adequate (prenatal) counseling of parents of patients with this genotype.

## References


1. Wolf NI, Vanderver A, van Spaendonk RM, Schiffmann R, Brais B, Bugiani M, et al. Clinical spectrum of 4H leukodystrophy caused by POLR3A and POLR3B mutations. Neurology. 2014;83(21):1898-905.
2. Richards MR, Plummer L, Chan YM, Lippincott MF, Quinton R, Kumanov P, et al. Phenotypic spectrum of POLR3B mutations: isolated hypogonadotropic hypogonadism without neurological or dental anomalies. Journal of medical genetics. 2017;54(1):19-25.
3. Bernard G, Chouery E, Putorti ML, Tetreault M, Takanohashi A, Carosso G, et al. Mutations of POLR3A encoding a catalytic subunit of RNA polymerase Pol III cause a recessive hypomyelinating leukodystrophy. American journal of human genetics. 2011;89(3):415-23.
4. Saitsu H, Osaka H, Sasaki M, Takanashi J, Hamada K, Yamashita A, et al. Mutations in POLR3A and POLR3B encoding RNA Polymerase III subunits cause an autosomal-recessive hypomyelinating leukoencephalopathy. American journal of human genetics. 2011;89(5):644-51.
5. Tetreault M, Choquet K, Orcesi S, Tonduti D, Balottin U, Teichmann M, et al. Recessive mutations in POLR3B, encoding the second largest subunit of Pol III, cause a rare hypomyelinating leukodystrophy. American journal of human genetics. 2011;89(5):652-5.
6. Thiffault I, Wolf NI, Forget D, Guerrero K, Tran LT, Choquet K, et al. Recessive mutations in POLR1C cause a leukodystrophy by impairing biogenesis of RNA polymerase III. Nature communications. 2015;6:7623.
7. Dumay-Odelot H, Durrieu-Gaillard S, Da Silva D, Roeder RG, Teichmann M. Cell growth- and differentiation-dependent regulation of RNA polymerase III transcription. Cell cycle (Georgetown, Tex). 2010;9(18):3687-99.
8. Daoud H, Tetreault M, Gibson W, Guerrero K, Cohen A, Gburek-Augustat J, et al. Mutations in POLR3A and POLR3B are a major cause of hypomyelinating leukodystrophies with or without dental abnormalities and/or hypogonadotropic hypogonadism. Journal of medical genetics. 2013;50(3):194-7.
9. DeGasperis SM, Bernard G, Wolf NI, Miller E, Pohl D. 4H leukodystrophy: Mild clinical phenotype and comorbidity with multiple sclerosis. Neurol Genet. 2020;6(2):e409.
10. Jurkiewicz E, Dunin-Wasowicz D, Gieruszczak-Bialek D, Malczyk K, Guerrero K, Gutierrez M, et al. Recessive Mutations in POLR3B Encoding RNA Polymerase III Subunit Causing Diffuse Hypomyelination in Patients with 4H Leukodystrophy with Polymicrogyria and Cataracts. Clinical neuroradiology. 2017;27(2):213-20.
11. Sato I, Onuma A, Goto N, Sakai F, Fujiwara I, Uematsu M, et al. A case with central and peripheral hypomyelination with hypogonadotropic hypogonadism and hypodontia (4H syndrome) plus cataract. Journal of the neurological sciences. 2011;300(1-2):179-81.

# Chapter 6

## Expanding the phenotype of biallelic *RNPC3* variants associated with growth hormone deficiency

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## Abstract

Pathogenic variants in components of the minor spliceosome have been associated with several human diseases. Recently, it was reported that biallelic *RNPC3* variants lead to severe isolated growth hormone deficiency and pituitary hypoplasia. The *RNPC3* gene codes for the U11/U12-65K protein, a component of the minor spliceosome. The minor spliceosome plays a role in the splicing of minor (U12-type) introns, which are present in approximately 700–800 genes in humans and represent about 0.35% of all introns. Here, we report a second family with biallelic *RNPC3* variants in three siblings with growth hormone deficiency, central congenital hypothyroidism, congenital cataract, developmental delay / intellectual deficiency and delayed puberty. These cases further confirm the association between biallelic *RNPC3* variants and severe postnatal growth retardation due to GH deficiency. Furthermore, these cases show that the phenotype of this minor spliceosome-related disease might be broader than previously described.

## Introduction

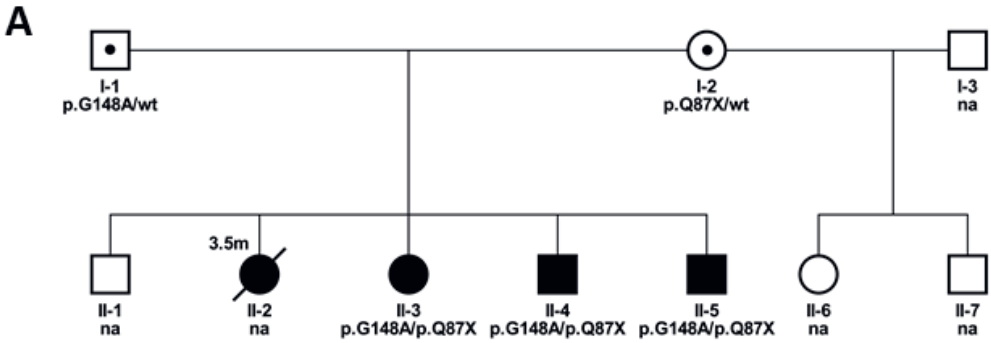
Pathogenic variants in components of the minor spliceosome have been associated with several human diseases (1, 2). One of these diseases is isolated growth hormone (GH) deficiency with pituitary hypoplasia, caused by biallelic *RNPC3* variants (3). The *RNPC3* gene codes for the U11/U12-65K protein, a component of the minor spliceosome. The minor spliceosome plays a role in the splicing of precursor mRNA, during which non-coding introns are recognized and removed. Most introns are removed by the major U2-dependent spliceosome, but a small subset of introns is removed by the minor U12-dependent spliceosome. These U12-type introns are present in approximately 700–800 genes in humans and represent about 0.35% of all introns (4).

After the publication by Argente et al. in 2014, no other cases of biallelic *RNPC3* variants have been reported in literature, apart from one conference abstract describing two siblings that had isolated GH deficiency and overlapping *RNPC3* variants (5). Here we report novel biallelic *RNPC3* variants in three siblings with GH deficiency, central congenital hypothyroidism, congenital cataract, developmental delay / intellectual deficiency and delayed puberty.

## Clinical report

Three affected siblings (Figure 1: II-3, II-4 and II-5) were born to healthy, non-consanguineous Caribbean parents. The mother has two healthy children from a previous relation. The parents have one healthy older son. Their second child (II-2) died at the age of 3.5 months, presumably due to aspiration during feeding. This girl was born at term with a normal birthweight and had bilateral congenital cataract, hypotonia, hyporeflexia and absence of sucking reflex, for which she received tube feeding.

The three siblings were born at term after an uncomplicated pregnancy and delivery with normal birth weights. They had congenital cataract, for which they were operated. They all had severe postnatal growth retardation with height ranging from -6.7 SD to -7.4 SD (Table 1). GH stimulation tests in patient II-3 and II-5 showed almost undetectable GH levels (patient II-4 not tested). Additionally, all three patients had almost undetectable levels of IGF-1, IGF-BP3 and prolactin. An X-ray of the hand was performed in patient II-3 and II-4, showing severely delayed bone age (bone age of 6 months at the age of 5 years and 8 months and bone age of 3 months at the age of 2 years and 2 months, respectively). They were diagnosed with central hypothyroidism and received replacement therapy with levothyroxine (patient II-3 at the age of seven months and patient II-4 and II-5 at the age of three months).





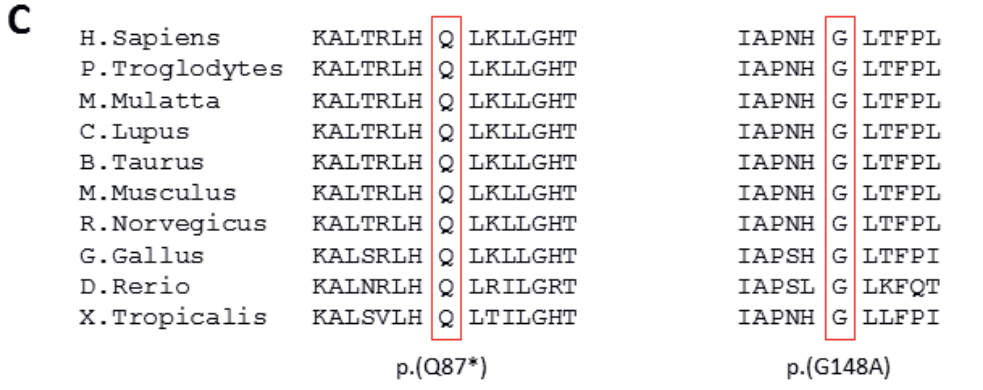



**Figure 1. A**: Pedigree showing that the three affected siblings (II-3, II-4 and II-5) are compound heterozygous for the *RNPC3* variants and that both parents are carrier. **B**: Patient II-3, II-4 and II-5 at the age of 25, 21 and 17, respectively. Note the short stature, central adiposity and facial features that are typical of growth hormone deficiency. **C**: Amino acid positions of both variants showing complete conservation across vertebrates.

In patient II-3 GH replacement therapy was started at the age of 1 year. After a short episode of treatment (with a small initial response), almost no effect on growth was noted. The patient did not attend regular follow-up visits and parents refrained from further use of GH. Because of this non-compliance and since GH therapy is an intensive therapy that requires daily injections and regular monitoring of (adverse) effects, it was decided not to start GH replacement therapy in patient II-4 and II-5.

All patients had a developmental delay / intellectual deficiency. At the age of 4, patient II-3 was able to sit but not stand without support. She had a speech delay, speaking only 2-word sentences at the age of 5. Neurocognitive examination at this age showed that her development was delayed by 3 years. Patient II-4 had a motor developmental delay, as he could walk only with support upon examination at the age of 2. During examination he was making sounds, although parents indicated that he was able to speak 2-word sentences. At the age of 11 his development was delayed by at least 6 years. Neurocognitive examination in patient II-5 at the age of 8 showed that his development was delayed by at least 4 years, with an IQ of <42.

Puberty was delayed and laboratory analysis in patient II-3 and II-4 was indicative of hypogonadotropic hypogonadism, patient II-5 was not tested (Table 1).

The patients were evaluated by the visiting clinical geneticist at the ages of 18, 14 and 10 years old, respectively. Height, weight and head circumference were all well below the 3rd percentile. Apart from short stature, they were noted to have a depressed nasal bridge, short philtrum and central adiposity (Figure 1).



At the moment, patient II-3 is 25 years old and she goes to a day care center on weekdays. Although her IQ has not been formally tested, she appears to have a more severe intellectual deficiency compared to her two younger brothers (patient II-4 and II-5). They are now 22 and 18 years old and attend special education.

**Genetic testing**

Trio whole exome sequencing was performed in patient II-3 in a diagnostic setting as described previously (6). Five variants in three genes were identified: a variant of unknown significance (VUS) in the *HGFAC* gene (c.1228G>C p.(Gly410Arg)), two variants of unknown significance in the *GIF* gene (c.183_186del p.(Met61fs) and c.379G>A p.(Ala127Thr)), a VUS in the *RNPC3* gene (c.443G>C p.(Gly148Ala) and a pathogenic *RNPC3* variant (c.259C>T p.(Gln87*)). All variants were verified by Sanger sequencing. The variant in the *HGFAC* gene was *de novo* and did not segregate with the disease, as it was not found in the two affected siblings. Parents were both carrier of one of the variants in the *GIF* gene, however, one of the two affected siblings carried

**Table 1.** Clinical features of previously reported cases and cases reported in this paper

| | *Previously reported* | | | | | *Reported in this paper* | | |
|---|---|---|---|---|---|---|---|---|
| Individual | 1† | 2† | 3† | 4‡ | 5‡ | 6 (II-3) | 7 (II-4) | 8 (II-5) |
| *RNPC3* variants | | c.1320C>A p.P474T; c.1504C>T p.R502X | | c.613C>T p.R205X; c.1420C>A p.P474T | | | c.259C>T p.(Q87X); c.443G>C p.(G148A) | |
| Height (SDS) | -5.9 | -5.0 | -6.7 | -5 | -9 | -7.4 (age 7 months) | -6.5 (age 10 months) | -6.7 (age 9 months) |
| GH deficiency | + | + | + | + | + | + | NA | + |
| Delayed bone maturation | + | + | + | + | + | + | + | NA |
| Prolactin level | Low normal | Low normal | Low normal | NR | NR | ↓↓ | ↓↓ | ↓↓ |
| Central hypothyroidism | - | - | - | - | - | + | + | + |
| Puberty | Normal | Delayed | Delayed | NR | NR | Delayed | Delayed | Delayed |
| DD and/or ID | - | - | - | NR | NR | + | + | + |
| Brain anomalies | Hypoplasia of AP | Hypoplasia of AP | Hypoplasia of AP | NR | NR | NA | NA | NA |
| Eye problems | NR | NR | NR | NR | NR | Congenital cataract | Congenital cataract | Congenital cataract |

† Argente et al. (2014)
‡ Guceva et al. (2015)
Abbreviations: ↓↓: almost undetectable, AP: anterior pituitary, DD: developmental delay, GH: growth hormone, ID: intellectual disability, NA: not assessed, NR: not reported, SDS: standard deviation score.

only one *GIF* variant. Thus, these variants were also discarded. The *RNPC3* variants were of particular interest, as biallelic variants in this gene were previously associated with growth hormone deficiency (3). Segregation analysis demonstrated that all three affected siblings had the compound heterozygous *RNPC3* variants and that parents were both carrier of one variant (Figure 1). This matches the autosomal recessive mode of inheritance that was expected based upon the pedigree (Figure 1). The pathogenic *RNPC3* variant c.259C>T p.(Gln87*) was present in the Genome Aggregation Database (gnomAD) with an allele frequency of 3.53E-5, all in the African population and with zero homozygotes, and has a Combined Annotation-Dependent Depletion (CADD) score of 38 (7). The c.443G>C p.(Gly148Ala) *RNPC3* variant was not present in gnomAD (https://gnomad.broadinstitute.org/; accessed 29-1-2020) and has a CADD score of 28. Both amino acid positions are completely conserved across vertebrates (Figure 1), indicating that they are likely important for the function of this gene. See also Supplementary Table 1 for bioinformatic prediction of the *RNPC3* variants in our patients and those previously reported.

No other variants (*de novo*, homozygous, hemizygous and/or compound heterozygous) that could be associated with the phenotype were detected. Comparative genomic hybridization (CGH) array analysis was performed in patient II-3, showing a normal (female) profile.

## Discussion

We here describe three siblings with a combination of growth hormone deficiency, central congenital hypothyroidism, congenital cataract, developmental delay / intellectual deficiency and delayed puberty with biallelic *RNPC3* variants. These cases further confirm the association between biallelic *RNPC3* variants and severe postnatal growth retardation due to GH deficiency, as previously described (3, 5). However, our patients show a more extensive phenotype (Table 1).

First of all, the previously described patients had normal levels of other pituitary hormones. This is in contrast to our patients, who had almost undetectable prolactin levels and central congenital hypothyroidism. As brain MRI scans in the patients reported by Argente et al. showed hypoplasia of the anterior pituitary, this could very likely be the cause of the pituitary hormone deficiencies in our patients as well. Unfortunately, this could not be assessed since brain MRI scans are unavailable for our patients. Additionally, our patients had delayed puberty with two of them showing hypogonadotropic hypogonadism upon laboratory analysis. Delayed puberty can be the result of GH deficiency. However, delayed puberty was noticed in two of the three patients initially reported by Argente et al., after treatment with GH for several years, indicating a possible relationship between *RNPC3* variants and impairment of the GnRH axis (8).

Secondly, Argente et al. reported normal development, while our patients have a developmental delay and intellectual deficiency. We consider it likely that this could be related to the untreated congenital hypothyroidism during the first months of live. When our patients were born there was no newborn screening for congenital hypothyroidism at the Caribbean island, which resulted in a delay in diagnosis and treatment. It is known that thyroid hormone is essential for normal brain development and that untreated congenital hypothyroidism leads to neurocognitive defects (9, 10). Also, patient II-3 was diagnosed with hypothyroidism only at the age of 7 months and has a more severe intellectual deficiency compared to her younger brothers (patient II-4 and II-5), who were diagnosed with hypothyroidism at the age of 3 months. Whole exome sequencing revealed no gene defects associated with intellectual deficiency. However, we cannot exclude that the *RNPC3* variants in our patients have contributed to the developmental delay / intellectual deficiency.

Lastly, all three patients were born with congenital cataract. No eye problems were described in the patients reported by Argente et al. and Guceva et al. There are several known environmental causes of congenital cataract. Genetic causes are found in approximately 10-29% of cases with congenital/infantile cataract (11). Since congenital cataract was present in all three affected patients and no known environmental causes were identified, a genetic cause is likely. Whole exome sequencing revealed no known gene defects associated with congenital cataract. Thus, the congenital cataract in our patients might be a result of the *RNPC3* variants.

The compound heterozygous *RNPC3* variants in the first reported family were functionally studied by Norppa et al. They showed that the nonsense R502X variant resulted in isoform-specific nonsense-mediated decay, while the missense P474T variant leads to misfolding and presumably increased decay of the U11/U12-65K protein. They propose that this causes defective recognition and missplicing of (a subset of) U12-type introns, leading to impaired pituitary gland development through (yet) unknown mechanisms (12).

We hypothesize that biallelic pathogenic variants in *RNPC3* can lead to a spectrum of disease, with patients on the severe end having not only GH deficiency, but deficiency of other anterior pituitary hormones as well. We further hypothesize that, on the severe end of the spectrum, *RNPC3* variants could lead to defective splicing of genes that play a role in the development of the eyes and possibly also the brain. In line with this, it has been found that pathogenic variants in *RNU4ATAC*, another gene that encodes a component of the minor spliceosome, are associated with three distinct clinical conditions (microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1), Roifman syndrome and Lowry Wood syndrome) that differ in severity but have overlapping features (1). There is some evidence for genotype-phenotype associations in these *RNU4ATAC*-associated disorders, which could

partially explain the clinical differences (13). Similarly, there might be a genotype-phenotype association in patients with biallelic variants in *RNPC3*, as the variants reported in our family differ from those reported before. However, not enough patients have yet been reported to evaluate if such an association truly exists. Additionally, modifier genes could (partly) explain the phenotypic variation between patients with *RNPC3* variants.

Of further interest, there have been reports of patients with MOPD1 that had bilateral cataract, which was a feature in our patients as well (14, 15).

In conclusion, we show that the phenotype associated with biallelic *RNPC3* variants is broader than previously described. The exact mechanisms through which pathogenic *RNPC3* variants cause different phenotypes still remain to be elucidated.

## References


1. Farach LS, Little ME, Duker AL, Logan CV, Jackson A, Hecht JT, et al. The expanding phenotype of RNU4ATAC pathogenic variants to Lowry Wood syndrome. American journal of medical genetics Part A. 2018;176(2):465-9.
2. Verma B, Akinyi MV, Norppa AJ, Frilander MJ. Minor spliceosome and disease. Seminars in cell & developmental biology. 2018;79:103-12.
3. Argente J, Flores R, Gutierrez-Arumi A, Verma B, Martos-Moreno GA, Cusco I, et al. Defective minor spliceosome mRNA processing results in isolated familial growth hormone deficiency. EMBO molecular medicine. 2014;6(3):299-306.
4. Turunen JJ, Niemela EH, Verma B, Frilander MJ. The significant other: splicing by the minor spliceosome. Wiley interdisciplinary reviews RNA. 2013;4(1):61-76.
5. Guceva Z, Polenakovicb M, Tasica V, LeBoucc Y, Klammtd J, Pfaeffled J, et al. Severe isolated growth hormone deficiency and myopathy in two brothers with RNPC3 mutation. Horm Res Paediatr. 2015;84 (Suppl 1):447.
6. Houweling AC, Beaman GM, Postma AV, Gainous TB, Lichtenbelt KD, Brancati F, et al. Loss-of-function variants in myocardin cause congenital megabladder in humans and mice. The Journal of clinical investigation. 2019;129(12):5374-80.
7. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic acids research. 2019;47(D1):D886-d94.
8. Martos-Moreno GA, Travieso-Suarez L, Pozo-Roman J, Munoz-Calvo MT, Chowen JA, Frilander MJ, et al. Response to growth hormone in patients with RNPC3 mutations. EMBO molecular medicine. 2018;10(7).
9. Grosse SD, Van Vliet G. Prevention of intellectual disability through screening for congenital hypothyroidism: how much and at what level? Archives of disease in childhood. 2011;96(4):374-9.
10. Kooistra L, Laane C, Vulsma T, Schellekens JM, van der Meere JJ, Kalverboer AF. Motor and cognitive development in children with congenital hypothyroidism: a long-term evaluation of the effects of neonatal treatment. The Journal of pediatrics. 1994;124(6):903-9.
11. Reis LM, Semina EV. Genetic landscape of isolated pediatric cataracts: extreme heterogeneity and variable inheritance patterns within genes. Human genetics. 2019;138(8-9):847-63.
12. Norppa AJ, Kauppala TM, Heikkinen HA, Verma B, Iwai H, Frilander MJ. Mutations in the U11/U12-65K protein associated with isolated growth hormone deficiency lead to structural destabilization and impaired binding of U12 snRNA. RNA (New York, NY). 2018;24(3):396-409.
13. Shelihan I, Ehresmann S, Magnani C, Forzano F, Baldo C, Brunetti-Pierri N, et al. Lowry-Wood syndrome: further evidence of association with RNU4ATAC, and correlation between genotype and phenotype. Human genetics. 2018;137(11-12):905-9.
14. Kilic E, Yigit G, Utine GE, Wollnik B, Mihci E, Nur BG, et al. A novel mutation in RNU4ATAC in a patient with microcephalic osteodysplastic primordial dwarfism type I. American journal of medical genetics Part A. 2015;167a(4):919-21.
15. Kroigard AB, Jackson AP, Bicknell LS, Baple E, Brusgaard K, Hansen LK, et al. Two novel mutations in RNU4ATAC in two siblings with an atypical mild phenotype of microcephalic osteodysplastic primordial dwarfism type 1. Clinical dysmorphology. 2016;25(2):68-72.

**Supplementary Table 1.** Bioinformatic predictions of *RNPC3* variants

| Source | Chr | Pos (hg38) | Ref | Alt | Consequence | oAA | nAA | protPos | SIFT | PolyPhen | mamPhCons | verPhCons | CADD score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reported in this paper | 1 | 103533757 | C | T | STOP_GAINED | Q | * | 87 | NA | NA | 1 | 1 | 38 |
| Reported in this paper | 1 | 103534857 | G | C | NON_SYNONYMOUS | G | A | 148 | delete-rious | probably_ damaging | 1 | 1 | 27.6 |
| Previously reported[1] | 1 | 103536183 | C | T | STOP_GAINED | R | * | 205 | NA | NA | 1 | 0.999 | 36 |
| Previously reported[1,2] | 1 | 103550999 | C | A | NON_SYNONYMOUS | P | T | 474 | delete-rious | probably_ damaging | 1 | 1 | 26.0 |
| Previously reported[2] | 1 | 103551730 | C | T | STOP_GAINED | R | * | 502 | NA | NA | 1 | 1 | 38 |

[1] Guceva Z, Polenakovicb M, Tasica V, LeBoucc Y, Klammtd J, Pfaeffled J, et al. Severe isolated growth hormone deficiency and myopathy in two brothers with RNPC3 mutation. Horm Res Paediatr. 2015;84 (Suppl 1):447.

[2] Argente J, Flores R, Gutierrez-Arumi A, Verma B, Martos-Moreno GA, Cusco I, et al. Defective minor spliceosome mRNA processing results in isolated familial growth hormone deficiency. EMBO molecular medicine. 2014;6(3):299-306.

# Chapter 7

## Limb anomalies, microcephaly, dysmorphic facial features and fibroma of the tongue after failed abortion with methotrexate and misoprostol

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## Introduction

Induced abortion is a common procedure worldwide. Although mifepristone and misoprostol is the preferred combination of drugs for medical abortion, methotrexate combined with misoprostol can be an alternative in countries where mifepristone is unavailable (1).

Methotrexate is a folic acid antagonist and inhibits the synthesis of DNA. It is commonly used in oncology, auto-immune diseases such as rheumatoid arthritis or psoriasis and for treatment of ectopic pregnancy. First trimester *in utero* exposure to methotrexate has been associated with multiple congenital anomalies, including microcephaly, craniosynostosis, limb anomalies, heart defects and dysmorphic facial features (2, 3). Misoprostol is a synthetic analog of prostaglandin E1. Although it is FDA approved for the prevention and treatment of gastric ulcers, it is mainly used for obstetrical and gynecological purposes. Congenital anomalies associated with misoprostol are thought to be related to vascular disruption caused by uterine contractions and include terminal transverse limb defects and Moebius sequence (4-6).

Here we present a case of a 2½-year-old girl with limb anomalies, microcephaly, dysmorphic facial features and fibroma of the tongue. She was born after a failed medical abortion with methotrexate and misoprostol. Fibroma of the tongue after *in utero* exposure to methotrexate and misoprostol has not been reported before. Although the occurrence of the fibroma of the tongue could be coincidental, we suggest that it might be an additional feature of the fetal methotrexate/misoprostol syndrome.

## Clinical report

The patient is the first child of healthy non-consanguineous Afro-Caribbean parents. At approximately 7 weeks' gestation (i.e. 5 weeks post-conception) the 21-year-old mother requested a medical abortion and received intramuscular methotrexate 1 mg/kg followed by 2x200 mcg oral misoprostol orally and 3x200 mcg misoprostol vaginally to induce abortion. She did not return for a regular follow-up control visit and presented again 2 months later. She reported that she had taken the medication, after which there was vaginal bleeding and possibly some loss of tissue. Ultrasound examination at that time showed a viable pregnancy of 16 weeks' gestation, indicating that the induced abortion had most likely been unsuccessful. A possible ventricular septal defect was detected, for which she was referred to a gynecologist. Advanced ultrasound imaging at 21 weeks of gestational age showed a structurally normal heart, but ectrodactyly of the feet, hypoplastic thumbs and mild brachycephaly. After counseling by the gynecologist, the mother chose to continue the pregnancy and she did not opt for invasive prenatal testing.

At a gestational age of 35 weeks and 1 day, labor was induced because of maternal pre-eclampsia and a girl was born with Apgar scores of 9 and 10 after respectively 1 and 5 minutes. Birthweight was 1490 gram, birth length 41 cm and occipital-frontal circumference (OFC) 28 cm, all below the 3rd percentile. On examination, she was noted to have small, low set ears, retrognathia, hypoplasia of the first digit of both hands and syndactyly of digit II–IV and absence of digit V of both feet (Figure 1 and 2, at the age of 2½ years). Cerebral and abdominal ultrasound revealed no abnormalities. A cardiac ultrasound showed a persistent foramen ovale without hemodynamic significance. The hypoplastic first digits of both hands were suture-ligated shortly after birth. At the age of 1½ years, X-rays of the hands showed a normal aspect of the distal radius and ulna, four digits on each hand and syndactyly of the soft tissue around the proximal phalanx on the left side (Figure 3A). X-rays of the feet showed only one metatarsal in each foot. This was most likely metatarsal 1, as only digit 1 had a normal aspect with a proximal and distal phalanx. At the lateral sides of the feet some ossification centers with syndactyly of the soft tissue were noted (Figure 3B). The distal tibia and fibula had a normal aspect. Her family history was negative for limb defects and physical examination of the mother revealed no (minor) limb defects. Unfortunately, the father was not available for examination.

**Figure 1.** Left hand (status after suture-ligation of hypoplastic thumb).

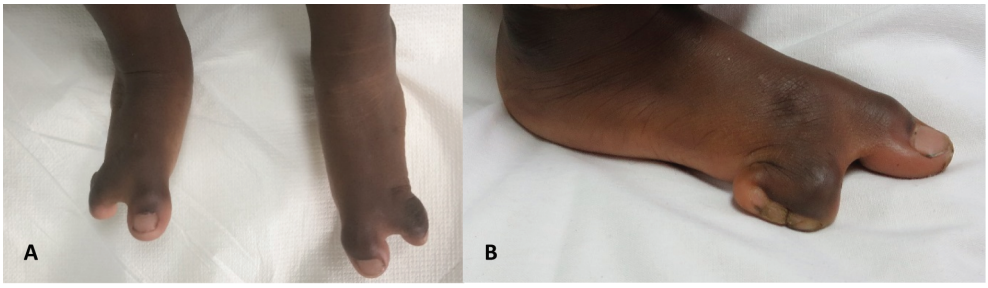


**Figure 2.** Feet **(A)** and lateral view of right foot **(B)**.

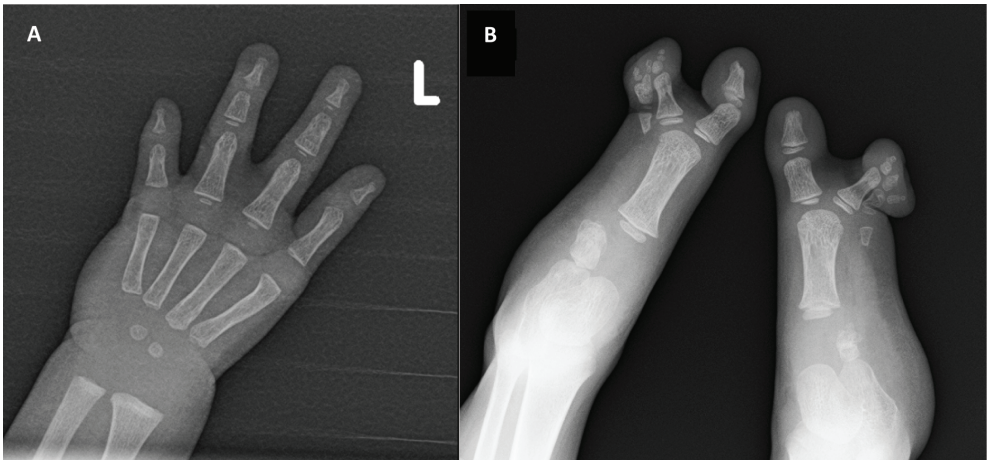


**Figure 3.** X-rays of left hand **(A)** and both feet **(B)**.

On physical examination at the age of 2½ years, the dysmorphic features were consistent with the neonatal observations. Weight at that time was 10 kg (-2.5 SD), height 91.8 cm (-2 SD) and OFC 44 cm (-3SD). Her speech and motor development were normal. A smooth round-shaped pedunculated tumor at the apex of the tongue was noticed, with a diameter of about 2 cm and a normal mucosal color (Figure 4). The mother of the patient reported that this had been present since birth, when it had the size of a pea. She was referred to the otorhinolaryngologist and the tumor was removed under general anesthesia. Microscopically, a spindle cell proliferation with myofibroblastic differentiation was found. No nuclear atypia or mitoses were seen. Based on morphology and localization, a fibroma or benign peripheral nerve sheath tumor were considered.

Immunohistochemical analysis showed focal positivity for smooth muscle actin, other markers were negative (desmin, Glut1, EMA, CD34, S100, beta-catenin, MUC4 and Myo-D1). There was no loss of H3K37 and the Ki67 proliferation index was very low. Thus, the lesion was classified as a fibroma of the oral mucosa.

Array comparative genomic hybridization (array-CGH) was performed, which showed a normal female profile.

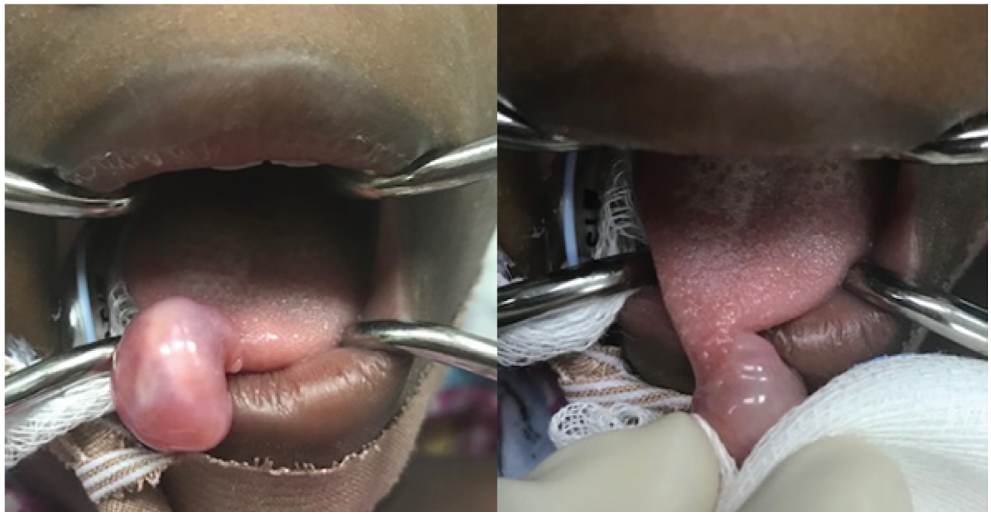

**Figure 4.** Fibroma of the tongue.

## Discussion

We present a patient with limb anomalies, microcephaly, dysmorphic facial features and fibroma of the tongue after a failed medical abortion with methotrexate and misoprostol. To our knowledge, this is the first report of a tongue fibroma after *in utero* exposure to methotrexate and/or misoprostol.

Post-axial deformities of the distal limb, including hypodactyly and syndactyly of the finger and toes, are commonly found after first trimester exposure to both methotrexate and misoprostol (7). Studies comparing the malformation pattern of published methotrexate-exposed cases to the malformation pattern in surveillance programs for congenital anomalies showed that *in utero* exposure to methotrexate is associated with limb defects and this association has been found in animal studies as well. Additionally, the dysmorphic facial features and microcephaly in our patient are consistent with the teratogenic effects of methotrexate described in literature (2, 3).

Alternatively, a monogenetic syndrome diagnosis was considered. The differential diagnosis included a microdeletion or duplication, as these can be associated with congenital anomalies such as microcephaly, limb defects and dysmorphic facial features. In particular ectrodactyly can be associated with small deletions or duplications (e.g. 10q24 duplication, 17p13.3 duplication and 2q31 deletion) (8), but array analysis revealed no abnormalities. Interestingly,

another patient with very similar feet anomalies after failed medical abortion has been reported before. Although it was uncertain which medication was used, the malformation pattern was highly suggestive of fetal methotrexate/aminopterin syndrome (9). Additionally, our differential diagnosis includes Fanconi anemia, because of the combination of short stature, microcephaly and bilateral hypoplastic thumbs. Feet anomalies such as syndactyly or abnormal toes can be a feature of Fanconi anemia (10), however, we are unaware of any patients with Fanconi anemia that have a single metatarsal. All together, the malformation pattern is highly suggestive for fetal methotrexate/misoprostol syndrome.

Although it is unclear whether there is a causal relation between the fibroma of the tongue and the *in utero* methotrexate/misoprostol exposure, its neonatal presentation is remarkable and suggests a new feature associated with intrauterine methotrexate/misoprostol exposure.

Oral fibroma is a common benign tumor of the oral cavity and it is frequently found on the apex and dorsum of the tongue (11). Oral fibromas occur mostly in adults and in a study by Bouquot et al. their prevalence was estimated to be 12/1,000 in individuals above the age of 35 years (11, 12).

Tongue fibroma can result from chronic irritation, such as biting or dental prostheses (13). This diagnosis is however unlikely in our case since the lesion was already present at birth. Although neurofibroma was considered as a differential diagnosis, immunohistochemistry provided insufficient evidence for this diagnosis. Since it was a solitary lesion, rare conditions such as Cowden syndrome, tuberous sclerosis and familial fibromatosis are also unlikely. Moreover, family history was not suggestive for any of these conditions.



Although it is difficult to explain this fibroblastic proliferation from a teratogenic perspective, there are some possible theories that support a link between methotrexate and/or misoprostol exposure and congenital malformation of the tongue. The connective tissue of the tongue is derived from cranial neural crest cells and Hyoun et al. already suggested that neural crest malformations could be the result of *in utero* exposure to high dose levels of methotrexate before 6 weeks post-conception (2). Furthermore, the anterior two-third of the tongue is derived from the first pharyngeal arch (14). Vendramini et al. hypothesized that the vascular disruption mechanism of misoprostol could lead to classic first pharyngeal syndromes, such as such as oculoauriculovertebral spectrum (OAVS) with or without radial defects (6). However, clinical features associated with OAVS are different from the present case and fibroma of the tongue has never been reported in OAVS. Although the aforementioned theories support a link between methotrexate/misoprostol exposure and congenital malformation of the tongue, it still remains unclear how this could result in the development of a fibroma.

In conclusion, we found a new association between *in utero* methotrexate/misoprostol exposure and congenital fibroma of the tongue. However, as it could be a coincidental finding, further case reports are needed to confirm this association. Finally, with this case report we want to raise awareness on the devastating consequences of failed medical abortion with methotrexate and misoprostol. Failed abortion and the possible consequences of it should be discussed with patients requesting medical abortion and adequate follow-up should be performed.

## References


1. Jones HE, O'Connell White K, Norman WV, Guilbert E, Lichtenberg ES, Paul M. First trimester medication abortion practice in the United States and Canada. PloS one. 2017;12(10):e0186487.
2. Hyoun SC, Obican SG, Scialli AR. Teratogen update: methotrexate. Birth defects research Part A, Clinical and molecular teratology. 2012;94(4):187-207.
3. Verberne EA, de Haan E, van Tintelen JP, Lindhout D, van Haelst MM. Fetal methotrexate syndrome: A systematic review of case reports. Reproductive toxicology (Elmsford, NY). 2019.
4. da Silva Dal Pizzol T, Knop FP, Mengue SS. Prenatal exposure to misoprostol and congenital anomalies: systematic review and meta-analysis. Reproductive toxicology (Elmsford, NY). 2006; 22(4):666-71.
5. Vauzelle C, Beghin D, Cournot MP, Elefant E. Birth defects after exposure to misoprostol in the first trimester of pregnancy: prospective follow-up study. Reproductive toxicology (Elmsford, NY). 2013;36:98-103.
6. Vendramini-Pittoli S, Guion-Almeida ML, Richieri-Costa A, Santos JM, Kokitsu-Nakata NM. Clinical findings in children with congenital anomalies and misoprostol intrauterine exposure: a study of 38 cases. Journal of pediatric genetics. 2013;2(4):173-80.
7. Kozma C, Ramasethu J. Methotrexate and misoprostol teratogenicity: further expansion of the clinical manifestations. American journal of medical genetics Part A. 2011;155a(7):1723-8.
8. Sowinska-Seidler A, Socha M, Jamsheer A. Split-hand/foot malformation - molecular cause and implications in genetic counseling. Journal of applied genetics. 2014;55(1):105-15.
9. Aftimos S. Fetal methotrexate/aminopterin syndrome in an adult: a likely case with ectodermal abnormalities. Clinical dysmorphology. 2009;18(1):53-5.
10. Mehta PA, Tolar J. Fanconi Anemia. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. GeneReviews((R)). Seattle (WA): University of Washington, Seattle University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.; 1993.
11. Ono Y, Takahashi H, Inagi K, Nakayama M, Okamoto M. Clinical study of benign lesions in the oral cavity. Acta oto-laryngologica Supplementum. 2002(547):79-84.
12. Bouquot JE, Gundlach KK. Oral exophytic lesions in 23,616 white Americans over 35 years of age. Oral surgery, oral medicine, and oral pathology. 1986;62(3):284-91.
13. Henriques AC, Freitas RA, Pires BC, Gurgel CA, Santos JN. Histochemical and immunohistochemical differences between solitary oral fibroma and fibrous papule of the face. An Bras Dermatol. 2016;91(5):589-94.
14. Cobourne MT, Iseki S, Birjandi AA, Adel Al-Lami H, Thauvin-Robinet C, Xavier GM, et al. How to make a tongue: Cellular and molecular regulation of muscle and connective tissue formation during mammalian tongue development. Seminars in cell & developmental biology. 2019;91:45-54.

# Chapter 8

## *JARID2* haploinsufficiency is associated with a clinically distinct neurodevelopmental syndrome

Verberne EA*, Goh S*, England J*, van Ginkel M, Rafael-Croes L, Maas S, Polstra A, Zarate YA, Bosanko KA, Pechter KB, Bedoukian E, Izumi K, Chaudhry A, Robin NH, Boothe M, Lippa NC, Aggarwal V, De Vivo DC, Lehman A, Study C, Stockler S, Bruel AL, Isidor B, Lemons J, Rodriguez-Buritica DF, Richmond CM, Stark Z, Agrawal PB, Kooy RF, Meuwissen MEC, Koolen DA, Pfundt R, Lieden A, Anderlid BM, Glatz D, Mannens MMAM, Bakshi M, Mallette FA, van Haelst MM#, Campeau PM#

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# Equal last authors

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## Abstract

**Purpose:** *JARID2*, located on chromosome 6p22.3, is a regulator of histone methyltransferase complexes that is expressed in human neurons. So far, 13 individuals sharing clinical features including intellectual disability (ID) were reported with *de novo* heterozygous deletions in 6p22-p24 encompassing the full length *JARID2* gene (OMIM 601594). However, all published individuals to date have a deletion of at least one other adjoining gene, making it difficult to determine if *JARID2* is the critical gene responsible for the shared features. We aim to confirm *JARID2* as a human disease gene and further elucidate the associated clinical phenotype.

**Methods:** Chromosome microarray analysis, exome sequencing and an online matching platform (GeneMatcher) were used to identify individuals with single nucleotide variants or deletions involving *JARID2*.

**Results:** We report 16 individuals in 15 families with a deletion or single nucleotide variant in *JARID2*. Several of these variants are likely to result in haploinsufficiency due to nonsense-mediated mRNA decay. All individuals have developmental delay and/or intellectual disability and share some overlapping clinical characteristics such as facial features with those who have larger deletions involving *JARID2*.

**Conclusion:** We report that *JARID2* haploinsufficiency leads to a clinically distinct neurodevelopmental syndrome, thus establishing gene-disease validity for the purpose of diagnostic reporting.

## Introduction

The *JARID2* (jumonji, AT rich interactive domain 2; OMIM 601594) gene is located on chromosome 6p22.3 and encodes a protein that regulates the activity of various histone methyltransferase complexes (1-3). JARID2 forms a complex together with polycomb repressive complex 2 (PRC2) that is essential to recruit polycomb group proteins to its target genes. PRC2 can lower gene transcription by catalyzing the di- and tri- methylation of lysine 27 on histone H3 (H3K27me2/3). By the regulation of epigenetic changes, the JARID2-PCR2 complex is necessary to control development, differentiation and survival of embryonic cells (4, 5). *JARID2* also regulates pluripotency and embryonic stem cell differentiation through Nanog expression and β-catenin (6). In addition, *JARID2* has an important function in the Notch-1 pathway, which is essential for development of the central nervous system and other tissues (7). By the methylation of H3-K9 and repression of cyclin D1, *JARID2* also regulates cardiomyocytes proliferation and migration of neural progenitor cells (8).

*JARID2* is crucial in embryogenesis and morphogenesis, and multiple malformations can arise from its dysregulation in mice. In the mouse, *Jarid2* is involved in the development of the cardiovascular system, the liver, in hematopoiesis and in neural tube fusion (9). In human embryogenesis, *JARID2* is expressed in neurons, especially in the dorsal root ganglion, and in adults it is expressed in the neurons of the cerebral cortex (10).

*De novo* coding single nucleotide polymorphisms in *JARID2* have been found once per study in two autism studies (11, 12) (p.Arg827Gln and p.Met1181LeufsTer3) and once in a schizophrenia study (13) (p.Gly769Ser). However, these single findings did not reach significance in those large studies.

In another study, *JARID2* was found to be in linkage disequilibrium with non-syndromic cleft lip and/or palate. Mouse models showed that *Jarid2* is expressed in the merging palatal shelves at the time of fusion, supporting its involvement in palatal development (14). A more recent case-control study found that a deep-intronic *JARID2* single nucleotide variant was protective for non-syndromic cleft lip and/or palate in a Brazilian cohort (15).

Chromosomal deletions in 6p22-p24 involving *JARID2* have been identified by karyotype (16-19) and chromosome microarray analysis (20-22) in 15 individuals, of which 13 have a complete deletion of *JARID2*. These individuals have a common phenotype of borderline intellectual functioning to severe ID and share characteristic facial features. These features include prominent supraorbital ridges, deep set eyes, infraorbital dark circles and midface hypoplasia. Apart from *JARID2*, all of the reported deletions involve other neighboring genes as well (henceforth referred to as *JARID2*-plus deletions), which has complicated the identification of the critical gene(s). Based on the smallest region of overlap (involving the

genes *JARID2* and *DTNBP1*) in four individuals with de novo 6p22.3-24.1 deletions, it has been proposed that *JARID2* is a likely candidate gene contributing to the phenotype. This was supported by the finding that *JARID2* expression in leukocytes is significantly reduced in these individuals compared to controls (20). Because of the characteristic facial appearance in these individuals, Baroy et al. propose that *JARID2* haploinsufficiency may represent a clinically recognizable neurodevelopmental syndrome (20).

We describe 16 individuals with developmental delay and/or ID and overlapping clinical features with a deletion or single nucleotide variant of *JARID2*. Seven individuals have partial deletions of *JARID2* that are predicted to lead to nonsense-mediated mRNA decay and one individual has a complete deletion of *JARID2*. Five individuals have a single nucleotide variant in *JARID2* that leads to a frameshift, stop codon or splice site alteration, and three individuals have a missense variant. We thus confirm that *JARID2* haploinsufficiency leads to a clinically distinct neurodevelopmental syndrome.

## Materials and methods

### Subjects

Sixteen individuals from 15 unrelated families with a *JARID2* deletion or single nucleotide variant were identified in a diagnostic setting. A collaboration to further analyze and report these cases was established through GeneMatcher, an online platform that facilitates connections between clinicians and researchers who share an interest in the same gene (23). Clinical information was collected by reviewing the medical records. The characteristics of these individuals were compared to evaluate if there was a common phenotype.

### Ethics statement

Approval to share clinical and genetic information was received from local Institutional Review Boards (including the Institutional Review Board of CHU Sainte-Justine and Medical Research Ethics Committee of Amsterdam UMC). Informed consent to publish clinical data was obtained from all families. For individuals where pictures are shown, a signed consent for the publication of photographs was obtained.

### Microarray analysis

Comparative genomic hybridization (CGH) array and single nucleotide polymorphism (SNP) array were performed independently at different centers. CGH array was performed on an Agilent 180K oligo-array in individual 1 and her parents and on an Agilent 105K oligo-array in individual 2 and her parents. CGH-array was performed in individual 3 and his parents on

an Oxford Gene Technology (OGT) 180K oligonucleotide platform. For individual 4 and his parents, SNP array was performed using an Illumina HumanCytoSNP-12 (v2.1) BeadChip. Illumina CytoSNP-850k SNP array was performed in individual 5 and her father (individual 6), mother and brother. For individual 7, an oligo-SNP array was performed with Affymetrix CytoScan HD. SNP array with Illumina CytoSNP-12 (v2.1) was performed for individual 8, with parental microarrays performed on an Illumina Infinium Global Screening Array-24 (v2.0) kit.

**Exome sequencing**

Individual 9 had a commercial Autism/ID Xpanded Panel based on exome capture done at Gene Dx lab. This panel uses a trio approach and includes more than 2300 genes associated with autism spectrum disorder and/or ID. Individual 10 had proband-only exome sequencing performed through GeneDx. Individual 11 was enrolled through an institutional review board (IRB) approved research exome sequencing protocol. The process for variant filtering and variant prioritization has been previously described (24, 25). Trio-based exome sequencing was completed with clinical confirmation by Sanger sequencing of the *JARID2* variant. Individual 12 underwent trio-based exome sequencing as part of a research study (CAUSES Study, approved by University of British Columbia [REB#H15-00092]). Sequencing was performed at Ambry Genetics on an Illumina platform and analysis was performed by the research team at University of British Columbia. Individual 13 had solo exome sequencing performed with an in-house pipeline (26). Parental inheritance was assessed through Sanger sequencing. Individual 14 had trio exome sequencing performed clinically at the Children's Hospital of Philadelphia. Exons were captured with the Agilent SureSelect XT Clinical Research Exome Version 1 kit (per manufacturer's protocol) and sequenced on the Illumina HiSeq 2500 platform. Sequencing data were processed using an in-house custom-built bioinformatics pipeline (27-29). Individual 15 had a clinical diagnostic exome done with an in-house protocol (30) and her parents were assessed only for the variants identified. Individual 16 also had a clinical exome performed with the same protocol as individual 15 (30).



## Results

**Clinical characteristics**

We identified 4 females and 12 males with a median age of 9.5 years old (range 3.2 to 39 years) with a deletion or single nucleotide variant in *JARID2*.

*Development and behavior*

All individuals have various degrees of developmental delay. Mild to moderate intellectual disability was diagnosed in 11/15 (73%) of them. Three individuals had borderline intellectual functioning and one had learning difficulties. Features of autism are noted in more than half of the cohort (9/16 [56%]) and a formal diagnosis of autism spectrum disorder was established in three of these individuals. Behavior abnormalities are present in 7 out of 16 individuals (44%) and include an aggressive demeanor, tendency to obsessive/compulsive and perseverative behavior, attention deficit hyperactivity disorder (ADHD) and trouble with socialization. Rare manifestations that are only observed in one individual include: phonic processing disorder, speech sound disorder, motor dyspraxia, severe stutter and developmental coordination disorder. One individual also presents two psychotic episodes at the age of 16 years (Table 1, Supplementary Table 3).

*Neurologic manifestations*

Gait disturbance in individuals with *JARID2*-plus deletions was reported in the past by Di Benedetto and Baroy (20, 22), but we only identified one individual with a clumsy gait and frequent tripping in our cohort. Hypotonia is found in 5 out of 16 individuals (31%) and only one individual has bradykinesia and bradyphrenia. We identified epilepsy in 3 out of 16 individuals (19%) of the cohort. One individual developed acute epileptic encephalopathy at around the age of 2 years. Another individual has refractory focal epilepsy and absences. The third individual had epilepsy that resolved at 3 years of age. Nine individuals have been evaluated by brain MRI or CT-scan. Four individuals have various constitutional anomalies, including benign external hydrocephalus, posterior fossa cyst/mega cisterna magna, periventricular hyperintensities and arachnoid cyst, but no consistent finding is observed (Table 1, Supplementary Table 3).

*Dysmorphism*

Dysmorphic facial features are observed in 15 out of 16 individuals (94%) (Figure 1, Supplementary Table 3). Dysmorphisms that are observed in more than 2 individuals are presented in Table 1. The most common features are a high anterior hair line and deep set eyes (6 out of 16 individuals [38%]). Full lips are found in 5/16 (31%) individuals and a broad forehead, infraorbital dark circles, bulbous nasal tip or depressed nasal bridge in 4/16 individuals (25%). Other less frequently identified dysmorphisms include prominent supraorbital ridges, midface hypoplasia and a short philtrum (3 out of 16 individuals [19%]). Abnormalities involving hands or feet are found in 5 out of 16 individuals (31%) and include pes planus, clinodactyly of the 4$^{th}$ and 5$^{th}$ toes, persistent fetal pads, single palmar crease, camptodactyly of the 5$^{th}$ digit, syndactyly of the 2$^{nd}$ and 3$^{rd}$ toe and tapering of the fingers.

**Table 1.** Clinical summary

| Individual | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | nb male/total (%) |
| Gender | F | F | M | M | F | M | M | M | M | M | M | M | M | M | F | M | 12/16 (75%) |
| | | | | | | | | | | | | | | | | | median age (range) |
| Age (years) | 17 | 19 | 9 | 3.5 | 7 | 38 | 4 | 10 | 12.5 | 7.3 | 23 | 4 | 3.2 | 8 | 39 | 10.8 | 9.5 (3.2-39) |
| | | | | | | | | | | | | | | | | | |
| Type variant | Del | Del | Del | Del | Del | Del | Del | Del | FS | NS | FS | NS | SS | Mis | Mis | Mis | |
| Inheritance | dn | dn | dn | dn | P[a] | NA | NA | dn | dn | NA | dn | dn | dn | dn | dn | dn | |
| Clinical information | | | | | | | | | | | | | | | | | nb affected/nb assessed (%) |
| Growth | | | | | | | | | | | | | | | | | |
| Age at assessment (years) | 16 | 7 | 9 | 3.5 | 7 | 38 | 5 | 9.6 | 12.6 | 7.3 | 23 | 4 | 1.4 | 8 | 39 | 10.8 | |
| Height | N | ↑ | N | N | ↑ | NA | ↑ | N | ↑ | N | N | N | N | ↑ | N | N | |
| Weight | N | N | ↑ | N | N | NA | ↑ | N | N | N | N | N | N | ↑ | N | ↑ | |
| Head circumference | N | NA | NA | N | N | NA | ↑ | N | N | ↓ | NA | N | N | ↑ | N | N | |
| Microcephaly | - | NA | NA | - | - | NA | - | - | - | + | NA | - | - | - | - | - | 1/12 (8%) |
| Macrocephaly | - | NA | NA | - | - | NA | + | - | - | - | NA | - | - | + | - | - | 2/12 (17%) |
| Development/behavior | | | | | | | | | | | | | | | | | |
| Intellectual disability | + | - | + | - | + | + | + | - | + | + | + | + | NA | + | - | + | 11/15 (73%) |
| Developmental delay | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 16/16 (100%) |
| Behavior abnormalities | - | + | - | - | - | - | + | - | + | + | + | - | - | + | + | - | 7/16 (44%) |
| Autistic features | + | - | + | + | - | - | + | - | + | + | - | + | - | + | + | - | 9/16 (56%) |
| ASD diagnosis | - | - | - | - | - | - | - | - | + | - | - | - | - | + | + | - | 3/16 (19%) |

*Table 1 continues on next page.*

**Table 1.** *Continued*

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Neurologic | | | | | | | | | | | | | | | | | |
| Hypotonia | - | - | - | + | - | - | + | - | - | + | - | + | + | - | - | - | 5/16 (31%) |
| Gait disturbance | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | 1/16 (6%) |
| Epilepsy | - | - | - | - | - | + | + | - | - | - | + | - | - | - | - | - | 3/16 (19%) |
| MRI abnormalities | NA | NA | NA | - | - | NA | - | - | + | NA | - | + | + | + | NA | NA | 4/9 (44%) |
| Dysmorphisms | | | | | | | | | | | | | | | | | |
| Broad forehead | - | - | + | - | + | + | - | - | - | - | - | - | + | - | - | - | 4/16 (25%) |
| High anterior hair line | + | + | - | + | + | + | - | - | + | - | - | - | - | - | - | - | 6/16 (38%) |
| Prominent supraorbital ridges | - | - | - | - | + | + | - | - | - | - | + | - | - | - | - | - | 3/16 (19%) |
| Deep set eyes | + | + | - | - | + | + | - | - | - | - | - | + | + | - | - | - | 6/16 (38%) |
| Infraorbital dark circles | + | - | + | - | + | + | - | - | - | - | - | - | - | - | - | - | 4/16 (25%) |
| Midface hypoplasia | - | + | - | - | + | + | - | - | - | - | - | - | - | - | - | - | 3/16 (19%) |
| Depressed nasal bridge | + | - | - | + | + | + | - | - | - | - | - | - | - | - | - | - | 4/16 (25%) |
| Bulbous nasal tip | + | - | - | - | + | + | + | - | - | - | - | - | - | - | - | - | 4/16 (25%) |
| Short philtrum | + | + | - | - | - | - | - | - | + | - | - | - | - | - | - | - | 3/16 (19%) |
| Full lips | + | - | - | + | - | - | - | - | + | + | + | - | - | - | - | - | 5/16 (31%) |
| Hand/Foot abnormalities | + | - | + | - | + | - | + | - | - | - | - | - | - | - | + | - | 5/16 (31%) |

**Table 1.** *Continued*

| Other | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cardiac anomalies | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | 1/16 (6%) |
| Musculoskeletal anomalies | - | - | - | + | - | - | - | + | - | - | + | + | + | - | - | - | 5/16 (31%) |
| Dental anomalies | - | - | - | - | + | - | - | + | - | - | - | - | - | - | - | - | 2/16 (13%) |
| Cleft lip/palate | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1/16 (6%) |
| Eye/vision anomalies | - | - | + | - | - | - | - | - | - | + | - | - | - | + | + | - | 4/16 (25%) |
| Cutaneous anomalies | + | - | - | - | + | - | - | - | + | - | - | - | - | - | - | - | 3/16 (19%) |
| Perinatal complications | - | - | + | + | - | + | - | - | + | NA | - | + | - | + | + | - | 7/15 (47%) |

Abbreviations: ASD: autism spectrum disorder, nb: number, Del: deletion, dn: de novo, F: female, FS: frameshift, M: male, Mis: missense, MRI: magnetic resonance image, NA: not available,
NS: nonsense, p: paternal, SS: splice site, +: yes, −: no, ↑: over 2 SD, ↓: under 2 SD, N: between −2 SD and +2 SD.
[a] Individual 6 is father.


**Figure 1.** Facial appearance of patients with *JARID2* deletions and single-nucleotide variants. Individual 1 **(a)**, individual 2 **(b)**, individual 3 **(c)**, individual 5 **(d)**, individual 6 (who is the father of individual 5) **(e)**, individual 7 **(f)**, individual 11 **(g)**, individual 14 **(h)**, and individual 16 **(i)**. Some individuals share physical features similar to others in the literature with *JARID2*-plus deletions, including high anterior hairline, broad forehead, deep set eyes, infraorbital dark circles, depressed nasal bridge, bulbous nasal tip and full lips.

*Other*

Several individuals have had perinatal complications, such as neonatal hyperbilirubinemia (three individuals) and neonatal feeding problems (two individuals). There are five individuals that have a tall stature and four individuals are overweight. One individual has microcephaly, while two have macrocephaly. Only one individual has a cardiac anomaly (tricuspid regurgitation). Musculoskeletal anomalies are observed in five individuals: three individuals have joint hyperlaxity, one has scoliosis and one has congenital torticollis. Dental anomalies are seen in two individuals: one had hypodontia and the other prominent upper central incisors and irregularly spaced teeth. One individual has a bifid uvula and a submucous cleft palate. Cutaneous findings are inconsistent throughout the cohort. One individual has café au lait macules, one has acanthosis nigricans in the neck and axillae (secondary to obesity) with hirsutism and another has a patch of prominent capillaries on the upper back. Refractory errors and strabismus are noted in four individuals. There are no individuals with hearing impairment or inner ear anomalies (Table 1, Supplementary Table 3).

**Genetic variants**

*Deletions*

Microarray analysis revealed whole or partial deletions of *JARID2* in eight individuals (Figure 2 and 3, Table 2). All deletions occurred *de novo* or were inherited from an affected parent, although for two individuals inheritance was not determined. Two *de novo* deletions were identified that involve only exon 2 of *JARID2* (individual 1 and 3) and two that involve exon 2 and 3 (individual 2 and 4). Individual 5 was found to have a 140 kb deletion comprising exon 2–5 of *JARID2*. Her father (individual 6) has a similar deletion, with differences in breakpoints due to inherent measurement uncertainty of the array platform. The error margins of their breakpoints lie fully within the intronic region. The mother of individual 5 has a normal female microarray profile and the healthy brother of individual 5 has a normal targeted array for the familial deletion. Individual 7 has a deletion that includes exon 1 and 2 of *JARID2*. Individual 8 has a *de novo* deletion encompassing all of *JARID2* and the distal end of *DTNBP1* (involving the last three exons).

The intragenic *JARID2* deletions are likely to result in a frameshift that will lead to a premature stop codon. The predicted effect would be a loss of normal protein function through nonsense mediated mRNA decay. Complete deletion of *JARID2*, as identified in one individual, is predicted to be pathogenic.

*Single nucleotide variants*

We identified single nucleotide variants of *JARID2* in eight individuals (Figure 3 and Table 2). Two *de novo* frameshift variants were identified (individual 9 and 11). Two individuals have a nonsense variant, of which one is *de novo* (individual 12). For the other one inheritance could not be determined because of adoption (individual 10). One individual (individual 13) has a *de novo* variant c.2731+1G>C that is predicted to affect splicing since it affects a canonical splice site nucleotide. However, functional testing was not performed. These five variants are predicted to be pathogenic and lead to protein loss-of-function due to a splicing aberration or nonsense-mediated mRNA decay.

There are three individuals with a *de novo* missense variant (individual 14, 15 and 16). The missense variants affect highly conserved residues as shown in Figure 3. Pathogenicity predictions for missense and splice site variants are shown in Supplementary Table 1. Multiple pathogenicity prediction tools classified missense variants as pathogenic; all were considered pathogenic by DANN, FATHMM-MKL, MutationTaster and SIFT although other tools predicted they were benign. They all had CADD scores above 20 (26.5, 31 and 24.6, respectively), which means they are classified among the top 1% of variants in the genome with respect to pathogenicity probability.

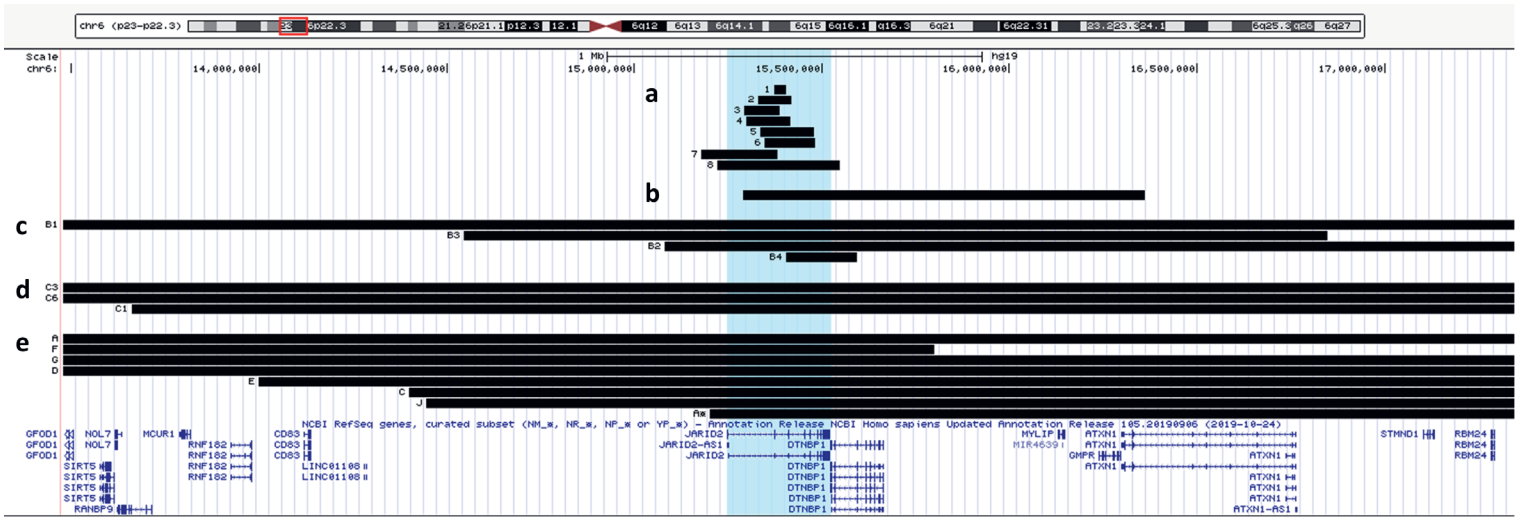


**Figure 2.** Localization of deletions reported in this study and previously reported *JARID2*-plus deletions. Deletions reported in this study **(a)** and *JARID2*-plus deletions previously reported by Di Benedetto et al. (22) **(b)**, Baroy et al. (20) **(c)**, Celestino-Soper et al. (21) **(d)** and deletions from Swaay et al. (19), Davies et al. (16), Davies et al. (17) and Zirn et al. (18) as labeled by Celestino-Soper et al. (21) **(e)**. Blue shade covers the *JARID2* gene region. For some of the reported individuals exact breakpoints were not given: the minimal size of the deleted region is presented in this figure. Data were uploaded into UCSC Genome Browser (genome.ucsc.edu).

**Table 2.** Variant summary

| Individual | Variant | Inheritance | Type | Position (Hg19) | Size (Mb) | Exon/intron |
|---|---|---|---|---|---|---|
| 1 | arr[GRCh37] 6p22.3(15374392_15405436)x1 | *De novo* | Deletion | 15374392-15405436 | 0.03 | Exon 2 |
| 2 | arr[GRCh37] 6p22.3(15330889_15419256)x1 | *De novo* | Deletion | 15330889- 15419256 | 0.09 | Exon 2-3 |
| 3 | arr[GRCh37] 6p22.3(15291644_15388348)x1 | *De novo* | Deletion | 15291644-15388348 | 0.1 | Exon 2 |
| 4 | arr[GRCh37] 6p22.3(15298601_15417235)x1 | *De novo* | Deletion | 15298601-15417235 | 0.12 | Exon 2-3 |
| 5 | arr[GRCh37] 6p22.3(15334789_15479224)x1 | Paternal (individual 6 is father) | Deletion | 15334789- 15479224 | 0.14 | Exon 2-5 |
| 6 | arr[GRCh37] 6p22.3(15346717_15481262)x1 | Unknown | Deletion | 15346717- 15481262 | 0.13 | Exon 2-5 |
| 7[a] | arr[GRCh37] 6p23p22.3(15177338_15382780)x1 | Unknown | Deletion | 15177338-15382780 | 0.205 | Exon 1-2 |
| 8 | arr[GRCh37] 6p22.3(15222515_15547476)x1 | *De novo* | Deletion | 15222515- 15547476 | 0.32 | All (exon 1-18) |
| 9[b] | c.2866dupG, p.(Glu956GlyfsTer72) | *De novo* | Frameshift | 15511547 | | Exon 13 |
| 10[c] | c.2341C>T, p.(Gln781Ter) | Unknown | Nonsense | 15501533 | | Exon 8 |
| 11 | c.3344dupG, p.(Ser1116GlnfsTer71) | *De novo* | Frameshift | 15513546 | | Exon 16 |
| 12[d] | c.3379C>T, p.(Arg1127Ter) | *De novo* | Nonsense | 15513582 | | Exon 16 |
| 13 | c.2731+1G>C | *De novo* | Splice site | 15507648 | | Intron 11 |
| 14 | c.351T>G, p.(Phe117Leu) | *De novo* | Missense | 15452264 | | Exon 4 |
| 15[e] | c.2363G>A, p.(Arg788Gln) | *De novo* | Missense | 15501555 | | Exon 8 |
| 16 | c.1930G>A, p.(Glu644Lys) | *De novo* | Missense | 15497386 | | Exon 7 |

*Note:* all variants based on NM_004973.4.

[a] Individual also has a maternally inherited pathogenic *PKP2* variant (associated with arrhythmogenic right ventricular cardiomyopathy (ARVC)).

[b] Individual also has a likely benign duplication of chromosome 15q13.3 (0.432 Mb).

[c] Individual also has a c.5046 G>C, p.(Leu1682Phe) variant in *ZNF292*.

[d] Individual also has compound heterozygous likely pathogenic variants in *MRFP* and a maternally inherited variant of unknown significance in *ZNF711* (c.1199G>A, p.(Arg400Lys)).

[e] Individual also has two *de novo TNRC18* variants: c.2291A>T, p.(His764Leu) and del(7)(p22.1p22.1).

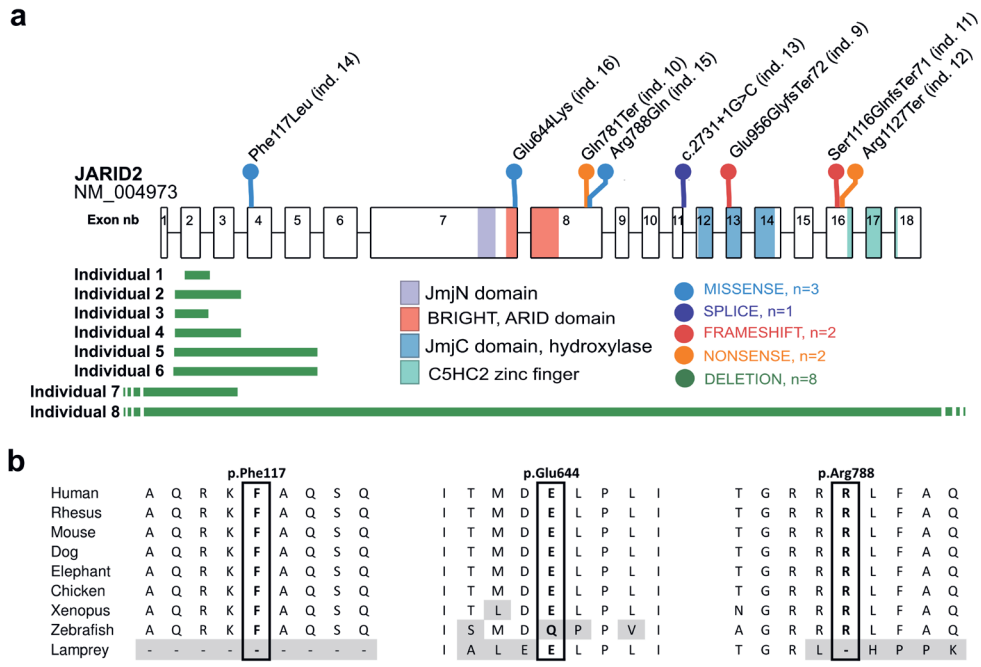


**Figure 3.** Graphical overview of the *JARID2* single-nucleotide variants and deletions. Location of the single nucleotide variants and deletions (introns not drawn to scale) **(a)** and conservation of the amino acids affected by missense variants **(b)**. All variants based on NM_004973.4.

### Individuals with a different phenotype or other explanatory variants

We identified two other individuals with deletion or single nucleotide variation in *JARID2* but they presented with a different phenotype or had other variations that could explain their phenotype. One individual with rhabdomyolysis had a *de novo* missense variant (c.3362A>G, p.(Asp1121Gly)) in *JARID2*. Another individual with a *de novo* missense variant in *JARID2* (c.2480G>A, p.(Arg827Gln)) was reported with a phenotype similar to our patients. The individual had ID, global developmental delay, autistic features, hypotonia, pes planus and delayed myelination on MRI. He also had short stature, dysplastic semicircular canals, cardiac anomalies, feeding and breathing difficulties at birth and some dysmorphisms that were not overlapping those of our patients (telecanthus, epicanthal folds, narrow palpebral fissures, broad nose and long philtrum). Trio exome sequencing showed another de novo variant in *TLK2* (c.887T>C, p.(Leu296Pro)). This variant was further reclassified as likely pathogenic by the diagnostic laboratory and is currently the main candidate to explain the clinical phenotype. We are uncertain if the *JARID2* variant contributes to or exacerbates the phenotype, so we did not include this individual in our previous analyses. Bioinformatic predictions of these variants, as well as variants that were previously reported in the literature, are presented in Supplementary Table 2.

## Discussion

We describe 16 individuals from 15 families with a deletion or single nucleotide variant of *JARID2*. All individuals described in this paper have developmental delay and the majority has ID. The four individuals without ID however have borderline intellectual functioning and/or learning difficulties. Other common characteristics include hypotonia, autistic features and behavior abnormalities, especially aggressive behavior. In some patients, we report similar physical features to previously reported cases in the literature with *JARID2*-plus deletions, including high anterior hairline, broad forehead, deep set eyes, infraorbital dark circles, depressed nasal bridge, bulbous nasal tip and full lips. Patients with deletions tend to have more overlapping facial features than individuals with missense variants. This may be because missense variants cause a more moderate loss-of-function effect on JARID2. Our cohort is not large enough to determine if this trend is significant.

The identified *JARID2* deletions are predicted to lead to a loss of normal protein function, as well as the frameshift, nonsense and splice site variants that were detected. Hence, these cases confirm the hypothesis by Barøy et al. that it is *JARID2* haploinsufficiency which leads to a clinically distinct neurodevelopmental syndrome.

It is noteworthy that in one case (individual 5) the *JARID2* deletion was inherited from an affected parent (individual 6). As some individuals only have a mild developmental delay or borderline intellectual functioning, we expect further patients to be identified with a pathogenic *JARID2* variant inherited from a mildly affected parent. There are no segmental duplications within *JARID2* that could explain the potentially recurrent breakpoint within intron 1, but there are Alu sequences that could potentially mediate Alu/Alu recombination.

Thus far, there has only been one other report of *de novo* intragenic *JARID2* deletions. That study described five individuals with ID and *de novo* intragenic *JARID2* deletions (as well as two duplications), all of them involving only exon 6 (exon 5 in NM_004973.4, 177 nucleotides) (31). Further *in silico* investigation showed that heterozygous loss or gain of *JARID2* exon 6 does not predict a frameshift and is likely to be tolerated. Additionally, they found a high frequency (> 14%) of *JARID2* exon 6 copy number variants (CNVs) in control populations (32). The authors therefore concluded that these CNVs are unlikely to be causative for ID, although they might have a contributory effect. The *JARID2* deletions in our patients, however, were predicted to lead to a frameshift and no comparable losses were found in control populations reported in the Database of Genomic Variants (DGV, http://dgv.tcag.ca, accessed May 26, 2020).

The DGV contains only one individual with a *JARID2*-plus deletion (deletion of exon 7-18 of *JARID2* and a partial deletion of the adjacent gene *DTNBP1*) (33). This partial deletion of

*JARID2* and *DTNBP1* is similar to the deletion identified in one of the patients reported by Baroy et al. (Figure 2, B4) (20) who had an IQ of 74. Possibly, healthy population databases might contain data on people with borderline intellectual functioning. One other exonic deletion is reported in the DGV, that encompasses only exon 1 of *JARID2* (34). There are no deletions that involve all of *JARID2* in the DGV. Finally, a small deletion (133kb) involving the first three exons of *JARID2* was previously reported in an individual with isolated talipes equinovarus and his unaffected father who were reported to be cognitively normal and without a history of developmental delay (Gurnett, personal communication) (35).

Interestingly, there is one previous report of an individual with a de novo probably pathogenic missense variant in *JARID2* (c.2255C>T, p.(Pro752Leu)) from a cohort of 92 patients with syndromic ID (36). Although no pathogenic *JARID2* single nucleotide variants were described before, pathogenic variants in other members of the JmjC-domain-containing family of proteins have been associated with human diseases, including neurodevelopmental disorders (37-40). Because JARID2 bears most resemblance to JARID1 proteins, pathogenic variants in *KDM5C* (*JARID1C*, OMIM 314690), associated with X-linked ID (OMIM 300534), and pathogenic variants in *KDM5B* (*JARID1B*, OMIM 605393), causing a form of autosomal recessive ID (OMIM 618109), are of most interest. In addition to the JmjC domain, these JARID1 proteins contain a Jumonji N (JmjN) domain, AT rich interaction domain (ARID) and a zinc finger (ZF) as well (6).

Furthermore, expected and observed counts of single nucleotide changes in the Genome Aggregation Database (gnomAD) show that *JARID2* is extremely intolerant to loss-of-function variants (probability of loss of function intolerance [pLI] score 1; observed/expected [o/e] ratio 0.09 [90% confidence interval: 0.05–0.19]). Also, fewer missense variants are observed than expected (o/e ratio 0.73 [90% confidence interval: 0.68–0.78] with a Z-score of 2.69) (https://gnomad.broadinstitute.org/, accessed May 20, 2020). Regarding further bioinformatic analysis of *JARID2* as a dominant disease gene, the %HI score (from DECIPHER) is 12.14%. High %HI ranks (e.g. 0–10%) indicate a gene is more likely to exhibit haploinsufficiency. The *JARID2* P(AD) score is 0.996 (from DOMINO, wwwfbm.unil.ch/domino, accessed May 20, 2020). A P(AD) score of ≥ 0.95 is highly associated with autosomal dominant inheritance through haploinsufficiency, gain-of-function or dominant-negative effects (41).

**Conclusion**

We propose that *JARID2* should be considered as a critical gene in the 6p22-p24 region with haploinsufficiency resulting in developmental delay and/or borderline intellectual functioning to severe intellectual disability. In addition to *JARID2* deletions, loss-of-function

single nucleotide variants in this gene result in a similar neurodevelopmental syndrome. Currently, there are only three tests available in the Genetic Testing Registry that offer *JARID2* sequencing (https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=jarid2, accessed May 14, 2020). Our data provide further evidence for establishing gene-disease validity for the purpose of diagnostic reporting and we suggest adding *JARID2* to ID gene panels.

In summary, we propose that haploinsufficiency of *JARID2* be considered as a new, clinically distinct neurodevelopmental syndrome.

## References


1. Li G, Margueron R, Ku M, Chambon P, Bernstein BE, Reinberg D. Jarid2 and PRC2, partners in regulating gene expression. Genes & development. 2010;24(4):368-80.
2. Mysliwiec MR, Carlson CD, Tietjen J, Hung H, Ansari AZ, Lee Y. Jarid2 (Jumonji, AT rich interactive domain 2) regulates NOTCH1 expression via histone modification in the developing heart. The Journal of biological chemistry. 2012;287(2):1235-41.
3. Pasini D, Cloos PA, Walfridsson J, Olsson L, Bukowski JP, Johansen JV, et al. JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. Nature. 2010;464(7286):306-10.
4. Shen X, Kim W, Fujiwara Y, Simon MD, Liu Y, Mysliwiec MR, et al. Jumonji Modulates Polycomb Activity and Self-Renewal versus Differentiation of Stem Cells. Cell. 2009.
5. Peng JC, Valouev A, Swigut T, Zhang J, Zhao Y, Sidow A, et al. Jarid2/Jumonji Coordinates Control of PRC2 Enzymatic Activity and Target Gene Occupancy in Pluripotent Cells. Cell. 2009.
6. Landeira D, Bagci H, Malinowski AR, Brown KE, Soza-Ried J, Feytout A, et al. Jarid2 Coordinates Nanog Expression and PCP/Wnt Signaling Required for Efficient ESC Differentiation and Early Embryo Development. Cell reports. 2015;12(4):573-86.
7. Yoon K, Gaiano N. Notch signaling in the mammalian central nervous system: insights from mouse mutants. Nature neuroscience. 2005;8(6):709-15.
8. Shirato H, Ogawa S, Nakajima K, Inagawa M, Kojima M, Tachibana M, et al. A jumonji (Jarid2) protein complex represses cyclin D1 expression by methylation of histone H3-K9. The Journal of biological chemistry. 2009;284(2):733-9.
9. Jung J, Mysliwiec MR, Lee Y. Roles of JUMONJI in mouse embryonic development. Developmental dynamics : an official publication of the American Association of Anatomists. 2005;232(1):21-32.
10. Berge-Lefranc JL, Jay P, Massacrier A, Cau P, Mattei MG, Bauer S, et al. Characterization of the human jumonji gene. Human molecular genetics. 1996;5(10):1637-41.
11. Yuen RK, Merico D, Cao H, Pellecchia G, Alipanahi B, Thiruvahindrapuram B, et al. Genome-wide characteristics of de novo mutations in autism. NPJ Genom Med. 2016;1:160271-1602710.
12. De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Cicek AE, et al. Synaptic, transcriptional and chromatin genes disrupted in autism. Nature. 2014;515(7526):209-15.
13. Fromer M, Pocklington AJ, Kavanagh DH, Williams HJ, Dwyer S, Gormley P, et al. De novo mutations in schizophrenia implicate synaptic networks. Nature. 2014;506(7487):179-84.
14. Scapoli L, Martinelli M, Pezzetti F, Palmieri A, Girardi A, Savoia A, et al. Expression and association data strongly support JARID2 involvement in nonsyndromic cleft lip with or without cleft palate. Human mutation. 2010;31(7):794-800.
15. Messetti AC, Machado RA, de Oliveira CE, Martelli-Junior H, de Almeida Reis SR, Moreira HS, et al. Brazilian multicenter study of association between polymorphisms in CRISPLD2 and JARID2 and non-syndromic oral clefts. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2017;46(3):232-9.
16. Davies AF, Olavesen MG, Stephens RJ, Davidson R, Delneste D, Van Regemorter N, et al. A detailed investigation of two cases exhibiting characteristics of the 6p deletion syndrome. Human genetics. 1996;98(4):454-9.
17. Davies AF, Mirza G, Sekhon G, Turnpenny P, Leroy F, Speleman F, et al. Delineation of two distinct 6p deletion syndromes. Human genetics. 1999;104(1):64-72.

18. Zirn B, Hempel M, Hahn A, Neubauer B, Wagenstaller J, Rivera-Bruguès N, et al. Polyneuropathy, scoliosis, tall stature, and oligodontia represent novel features of the interstitial 6p deletion phenotype. American Journal of Medical Genetics, Part A. 2008;146(22):2960-5.
19. Swaay E, Beverstock GC, Kamp JJP. A patient with an interstitial deletion of the short arm of chromosome 6. Clinical genetics. 2008;33(2):95-101.
20. Baroy T, Misceo D, Stromme P, Stray-Pedersen A, Holmgren A, Rodningen OK, et al. Haploinsufficiency of two histone modifier genes on 6p22.3, ATXN1 and JARID2, is associated with intellectual disability. Orphanet journal of rare diseases. 2013;8:3.
21. Celestino-Soper PB, Skinner C, Schroer R, Eng P, Shenai J, Nowaczyk MM, et al. Deletions in chromosome 6p22.3-p24.3, including ATXN1, are associated with developmental delay and autism spectrum disorders. Molecular cytogenetics. 2012;5:17.
22. Di Benedetto D, Di Vita G, Romano C, Giudice ML, Vitello GA, Zingale M, et al. 6p22.3 deletion: report of a patient with autism, severe intellectual disability and electroencephalographic anomalies. Molecular cytogenetics. 2013;6(1):4.
23. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Human mutation. 2015;36(10):928-30.
24. Petrovski S, Aggarwal V, Giordano JL, Stosic M, Wou K, Bier L, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet (London, England). 2019;393(10173):758-67.
25. Zhu X, Petrovski S, Xie P, Ruzzo EK, Lu YF, McSweeney KM, et al. Whole-exome sequencing in undiagnosed genetic diseases: interpreting 119 trios. Genetics in medicine : official journal of the American College of Medical Genetics. 2015;17(10):774-81.
26. Nambot S, Thevenon J, Kuentz P, Duffourd Y, Tisserant E, Bruel AL, et al. Clinical whole-exome sequencing for the diagnosis of rare disorders with congenital anomalies and/or intellectual disability: substantial interest of prospective annual reanalysis. Genetics in medicine : official journal of the American College of Medical Genetics. 2018;20(6):645-54.
27. Baker SW, Murrell JR, Nesbitt AI, Pechter KB, Balciuniene J, Zhao X, et al. Automated Clinical Exome Reanalysis Reveals Novel Diagnoses. The Journal of molecular diagnostics : JMD. 2019; 21(1):38-48.
28. Gibson KM, Nesbitt A, Cao K, Yu Z, Denenberg E, DeChene E, et al. Novel findings with reassessment of exome data: implications for validation testing and interpretation of genomic data. Genetics in medicine : official journal of the American College of Medical Genetics. 2018;20(3):329-36.
29. Wu C, Devkota B, Evans P, Zhao X, Baker SW, Niazi R, et al. Rapid and accurate interpretation of clinical exomes using Phenoxome: a computational phenotype-driven approach. European journal of human genetics : EJHG. 2019;27(4):612-20.
30. Lelieveld SH, Reijnders MR, Pfundt R, Yntema HG, Kamsteeg EJ, de Vries P, et al. Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability. Nature neuroscience. 2016;19(9):1194-6.
31. Tucker T, Zahir FR, Griffith M, Delaney A, Chai D, Tsang E, et al. Single exon-resolution targeted chromosomal microarray analysis of known and candidate intellectual disability genes. European journal of human genetics : EJHG. 2014;22(6):792-800.
32. Zahir FR, Tucker T, Mayo S, Brown CJ, Lim EL, Taylor J, et al. Intragenic CNVs for epigenetic regulatory genes in intellectual disability: Survey identifies pathogenic and benign single exon changes. American journal of medical genetics Part A. 2016;170(11):2916-26.
33. Shaikh TH, Conlin LK, Geiger EA, Haldeman-Englert C, Medne L, Spinner NB, et al. High-resolution mapping and analysis of copy number variations in the human genome: A data resource for clinical and research applications. Genome Research. 2009;19(9):1682-90.

34. Wong KK, deLeeuw RJ, Dosanjh NS, Kimm LR, Cheng Z, Horsman DE, et al. A comprehensive analysis of common copy-number variations in the human genome. American journal of human genetics. 2007;80(1):91-104.
35. Alvarado DM, Buchan JG, Frick SL, Herzenberg JE, Dobbs MB, Gurnett CA. Copy number analysis of 413 isolated talipes equinovarus patients suggests role for transcriptional regulators of early limb development. European journal of human genetics : EJHG. 2013;21(4):373-80.
36. Martinez F, Caro-Llopis A, Rosello M, Oltra S, Mayo S, Monfort S, et al. High diagnostic yield of syndromic intellectual disability by targeted next-generation sequencing. Journal of medical genetics. 2017;54(2):87-92.
37. Abidi F, Miano M, Murray J, Schwartz C. A novel mutation in the PHF8 gene is associated with X-linked mental retardation with cleft lip/cleft palate. Clinical genetics. 2007;72(1):19-22.
38. Adam MP, Banka S, Bjornsson HT, Bodamer O, Chudley AE, Harris J, et al. Kabuki syndrome: international consensus diagnostic criteria. Journal of medical genetics. 2019;56(2):89-95.
39. Stolerman ES, Francisco E, Stallworth JL, Jones JR, Monaghan KG, Keller-Ramey J, et al. Genetic variants in the KDM6B gene are associated with neurodevelopmental delays and dysmorphic features. American journal of medical genetics Part A. 2019;179(7):1276-86.
40. Najmabadi H, Hu H, Garshasbi M, Zemojtel T, Abedini SS, Chen W, et al. Deep sequencing reveals 50 novel genes for recessive cognitive disorders. Nature. 2011;478(7367):57-63.
41. Quinodoz M, Royer-Bertrand B, Cisarova K, Di Gioia SA, Superti-Furga A, Rivolta C. DOMINO: Using Machine Learning to Predict Genes Associated with Dominant Disorders. American journal of human genetics. 2017;101(4):623-9.

# Supplementary material

**Supplementary Table 1.** Characteristics of missense and splice site variants (NM_004973.4)

| Individual | Variant | Domain | Present in gnomAD[1] v2.1.1 | Pathogenicity predictions from Varsome/dbNSFP | CADD score | Metadome[2] missense tolerance score | MTR[3] missense tolerance |
|---|---|---|---|---|---|---|---|
| 13 | c.2731+1G>C | | No | 4 pathogenic predictions from DANN, EIGEN, FATHMM-MKL and MutationTaster vs no benign predictions. | 35 | | |
| 14 | c.351T>G, (p.Phe117Leu) | | No | 6 pathogenic predictions from DANN, EIGEN, FATHMM-MKL, M-CAP, MutationTaster and SIFT vs 5 benign predictions from DEOGEN2, MVP, MutationAssessor, PrimateAI and REVEL. | 26.5 | 0.88 (slightly tolerant) | MTR 0.935, FDR 0.869 |
| 15 | c.2363G>A; (p.Arg788Gln) | | Once (het) | 9 pathogenic predictions from DANN, EIGEN, FATHMM-MKL, M-CAP, MutationAssessor, MutationTaster, PrimateAI, REVEL and SIFT vs 2 benign predictions from DEOGEN2 and MVP. | 31 | 0.77 (neutral) | MTR 0.915, FDR 0.805 |
| 16 | c.1930G>A, (p.Glu644Lys) | ARID | No | 4 pathogenic predictions from DANN, FATHMM-MKL, MutationTaster and SIFT and the position is not conserved (GERP++ rejected substitutions = 4.36 is less than 5.5) vs 7 benign predictions from DEOGEN2, EIGEN, M-CAP, MVP, MutationAssessor, PrimateAI. | 24.6 | 0.13 (highly intolerant) | MTR 0.468, FDR 0.05 |

*Note:* Regarding MTR score, an MTR (missense tolerance ratio) of 1 or above represents neutrality, and an FDR (false discovery rate) below 0.1 is considered significant.

1. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *bioRxiv*. 2020:531210.
2. Wiel L, Baakman C, Gilissen D, Veltman JA, Vriend G, Gilissen C. MetaDome: Pathogenicity analysis of genetic variants through aggregation of homologous human protein domains. *Human mutation*. 2019;40(8):1030-1038.
3. Traynelis J, Silk M, Wang Q, et al. Optimizing genomic medicine in epilepsy through a gene-customized approach to missense variant interpretation. *Genome Res*. 2017;27(10):1715-1729.

**Supplementary Table 2.** Characteristics of variants (NM_004973.4) of uncertain pathogenicity, or reported in the literature

| Source of variant | Variant | Domain | Present in gnomAD[1] v2.1.1 | Pathogenicity predictions from Varsome/dbNSFP | CADD score | Metadome[2] missense tolerance score | MTR[3] missense tolerance |
|---|---|---|---|---|---|---|---|
| Literature[4] | c.3541_3548delATGTACCG, (p.Met1181LeufsTer3) | C5HC2 Zinc finger | No | 1 pathogenic prediction from GERP vs no benign predictions | N/A | N/A | N/A |
| Literature[5] | c.2305G>A, (p.Gly769Ser) | | 5 times (het) | 9 pathogenic predictions from DANN, EIGEN, FATHMM-MKL, M-CAP, MutationAssessor, MutationTaster, PrimateAI, REVEL and SIFT vs 2 benign predictions from DEOGEN2 and MVP. | 29.2 | 0.82 (neutral) | MTR 0.921, FDR 0.81 |
| Literature[6] | c.2255C>T, (p.Pro752Leu) | | No | 10 pathogenic predictions from DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP, MutationAssessor, MutationTaster, PrimateAI, REVEL and SIFT vs 1 benign prediction from MVP | 28.7 | 0.44 (intolerant) | MTR 0.919, FDR 0.789 |
| Literature[7] and our study | c.2480G>A, (p.Arg827Gln) | | No | 11 pathogenic predictions from DANN, DEOGEN2, EIGEN, FATHMM-MKL, M-CAP, MVP, MutationAssessor, MutationTaster, PrimateAI, REVEL and SIFT vs no benign predictions | 32 | 0.14 (highly intolerant) | MTR 0.545, FDR 0.081 |

**Supplementary Table 2.** *Continued*

| Source of variant | Variant | Domain | Present in gnomAD[1] v2.1.1 | Pathogenicity predictions from Varsome/dbNSFP | CADD score | Metadome[2] missense tolerance score | MTR[3] missense tolerance |
|---|---|---|---|---|---|---|---|
| Our study | c.3362A>G, (p.Asp1121Gly) | | No | 7 benign predictions from DEOGEN2, EIGEN, MVP, MutationAssessor, PrimateAI, REVEL and SIFT vs 4 pathogenic predictions from DANN, FATHMM-MKL, M-CAP and MutationTaster and the position is not conserved (GERP++ rejected substitutions = 4.15 is less than 5.5) | N/A | 0.32 (intolerant) | MTR 0.322, FDR 0.004 |

*Note:* Regarding MTR score, an MTR (missense tolerance ratio) of 1 or above represents neutrality, and an FDR (false discovery rate) below 0.1 is considered significant.


1. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *bioRxiv*. 2020:531210.
2. Wiel L, Baakman C, Gilissen D, Veltman JA, Vriend G, Gilissen C. MetaDome: Pathogenicity analysis of genetic variants through aggregation of homologous human protein domains. *Human mutation*. 2019;40(8):1030-1038.
3. Traynelis J, Silk M, Wang Q, et al. Optimizing genomic medicine in epilepsy through a gene-customized approach to missense variant interpretation. *Genome Res*. 2017;27(10):1715-1729.
4. De Rubeis S, He X, Goldberg AP, et al. Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature*. 2014;515(7526):209-215.
5. Fromer M, Pocklington AJ, Kavanagh DH, et al. De novo mutations in schizophrenia implicate synaptic networks. *Nature*. 2014;506(7487):179-184.
6. Martinez F, Caro-Llopis A, Rosello M, et al. High diagnostic yield of syndromic intellectual disability by targeted next-generation sequencing. *Journal of medical genetics*. 2017;54(2):87-92.
7. Yuen RK, Merico D, Cao H, et al. Genome-wide characteristics of de novo mutations in autism. *NPJ Genom Med*. 2016;1:160271-1602710..

**Supplementary Table 3.** Clinical and genetic characteristics

| Individual | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Age | 17y | 19y | 9y | 3y6m | 7y | 38y | 4y | 10y | 12y6m |
| Gender | F | F | M | M | F | M | M | M | M |
| Genetic information | | | | | | | | | |
| Type of variant | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Deletion | Frameshift |
| Variant details (hg19) | 15374392-15405436 | 15330889-15419256 | 15291644-15388348 | 15298601-15417235 | 15334789-15479224 | 15346717-15481262 | 15177338-15382780 | 15222515-15547476 | c.2866dupG, p.(Glu956GlyfsTer72) |
| Size of deletion (Mb) | 0.03 | 0.09 | 0.1 | 0.12 | 0.14 | 0.13 | 0.205 | 0.32 | |
| Inheritance | *De novo* | *De novo* | *De novo* | *De novo* | Paternal (individual 6) | Unknown | Unknown | *De novo* | *De novo* |
| Other genetic findings | | Normal karyotype, normal FISH for 22q11.2 | Normal fragile X | | Normal fragile X, normal extended urine metabolic screen | | Maternally inherited pathogenic *PKP2* variant, associated with ARVC, on trio WES (GeneDx XomeDxPlus) | | Chromosome 15q13.3 duplication 0.432 Mb likely benign (identified on microarray Bluegnome Oligo platform (180k)) |
| **Growth** | | | | | | | | | |
| Age at assessment | 16y | 7y | 9y | 3y6m | 7y | 38y | 5y | 9y7m | 12y6m |
| Macrocephaly | - | NA | NA | - | - | NA | + | - | - |
| Microcephaly | - | NA | NA | - | - | NA | - | - | - |
| Height (cm) | 162.0 (-0.03 SD) | 135.0 (+2.3 SD) | 137.3 (+0.73 SD) | 104.5 (+1.41) | 134.2 (+2.19 SD) | NA | 123.0 (+3.08 SD) | 141.9 (+0.94 SD) | 167.0 (+2.03 SD) |
| Weight (kg) | 73.5 (+1.27 SD) | 27.6 (+0.82 SD) | 40.0 (+2.06 SD) | 16.4 (+0.56 SD) | 33.5 (+1.94 SD) | NA | 25.9 (+2.39 SD) | 31.5 (+0.17 SD) | 54.7 (+1.02 SD) |
| Head circumference (cm) | 56.0 (+1.52 SD) | NA | NA | 51.9 (+1.29 SD) | 52.1 (+0.55 SD) | NA | 55.1 (+2.86 SD) | 54.3 (+1.19 SD) | 54.0 (+0.05 SD) |
| Development | | | | | | | | | |
| Intellectual deficiency | Mild (IQ 50) | Borderline (IQ 82) | Mild (IQ 61-74 by WPPSY at 4y) | Borderline (tIQ 70, pIQ 72) | Mild | Mild to moderate | Mild | Normal intellect, but learning difficulties, decrement in working memory and processing speed | Moderate |
| Developmental delay | + | Yes, speech delay | + | Yes, motor delay and severe speech delay | + | + | + | Mild | + |
| Behavior abnormalities | - | Psychotic episodes | - | - | - | - | Social issues, aggressive behavior | - | Aggressive behavior |
| Autistic features | + | - | + | + | - | - | + | - | + |

*Supplementary Table 3 continues on next page.*

**Supplementary Table 3.** *Continued*

| Individual | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|
| Age | 7y4m | 23y | 4y | 3y2m | 8y | 39y | 10y9m |
| Gender | M | M | M | M | M | F | M |
| Genetic information | | | | | | | |
| Type of variant | Nonsense | Frameshift | Nonsense | Splice site | Missense | Missense | Missense |
| Variant details (hg19) | c.2341C>T, p.(Gln781Ter) | c.3344dupG, p.(Ser1116GlnfsTer71) | c.3379C>T, p.(Arg1127Ter) | c.2731+1G>C | c.351T>G, p.(Phe117Leu) | c.2363G>A, p.(Arg788Gln) | c.1930G>A, p.(Glu644Lys) |
| Size of deletion (Mb) | | | | | | | |
| Inheritance | Unknown | *De novo* | *De novo* | *De novo* | *De novo* | *De novo* | *De novo* |
| Other genetic findings | het *ZNF292* c.5046 G>C, p.(Leu1682Phe) on WES. Normal Agilent 4x180k aCGH+SNP array | | WES: compound het LP variants in *MRFP* (causes microphthalmia) and maternally inherited VUS in *ZNF711* (c.1199G>A, p.(Arg400Lys)). Normal Cytoscan HD array (Agilent) | | Normal SNP-array | *TNRC18* c.2291A>T, p.(His764Leu); *de novo* and *TNRC18* del(7)(p22.1p22.1) *de novo:* chr7:g.(5363986_5364726)_(5402456_5410005) | Normal CytoScan HD array |
| **Growth** | | | | | | | |
| Age at assessment | 7y4m | 23y | 4y for height/ weight, 2 years head circumference | 17 m | 8y | 39y | 10y9m |
| Macrocephaly | - | NA | - | - | + | - | - |
| Microcephaly | + | NA | - | - | - | - | - |
| Height (cm) | 123.5 (+0.06 SD) | 167.6 (-1.24 SD) | 102.0 (-0.05 SD) | 80.0 (-0.23 SD) | 150.5 (+3.85 SD) | 169.0 (+0.89 SD) | 152.0 (+1.50 SD) |
| Weight (kg) | 19.8 (-1.44 SD) | 61.7 (-0.87 SD) | 17.0 (+0.31 SD) | 10.7 (-0.70 SD) | 45.1 (+3.17 SD) | 51.5 (-0.88 SD) | 69.0 (+3.45 SD) |
| Head circumference (cm) | 49.0 (-2.29 SD) | NA | 49.0 (+0.24 SD) | 50.0 (+1.85 SD) | 60.0 (+5.79 SD) | 54.5 (+0.17 SD) | 53.0 (-0.03 SD) |
| Development | | | | | | | |
| Intellectual deficiency | Mild (IQ 62) | + | + | NA | Moderate | Borderline (IQ 79), learning difficulties | Mild (IQ 66) |
| Developmental delay | + | + | + | + | + | + | + (except motor normal) |
| Behavior abnormalities | Compulsive behavior | Aggressive, compulsive and perseverative behavior | - | - | ADHD, aggressive behavior | Social emotional difficulties, behavior problems, obsessive behavior | - |
| Autistic features | + | - | + | - | + | + | - |

*Supplementary Table 3 continues on next page.*

**Supplementary Table 3.** *Continued*

| Individual | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| ASD diagnosis | - | - | - | - | - | - | - | - | + |
| Other / details | - | - | Severe speech delay (bilingual), normal motor development | Stereotypic movements | - | Severe stutter | Speech sound disorder, phonic processing disorder | Motor delay and dyspraxia, delayed receptive langage skills, DCD | Non verbal, continence not attained, special education class |
| Nervous system | | | | | | | | | |
| Hypotonia | - | - | - | + | - | - | + | - | - |
| Gait disturbance | - | - | - | - | - | - | - | - | - |
| Seizures | - | - | - | - (Normal EEG) | - | Yes (until age 3y) | Refractory focal epilepsy and absence | - | - |
| MRI/CT-Scan | NA | NA | NA | Normal | Normal | NA | Normal | Normal | Posterior fossa cyst or mega cisterna magna |
| Other | - | - | - | - | Clumsy, falling over until 6y | - | - | - | - |
| Dysmorphic facial features | | | | | | | | | |
| Broad forehead | - | - | + | - | + | + | - | - | - |
| High anterior hair line | + | + | - | + | + | + | - | - | + |
| Prominent supraorbital ridges | - | - | - | - | + | + | - | - | - |
| Deep set eyes | + | + | - | - | + | + | - | - | - |
| Infraorbital dark circles | + | - | + | - | + | + | - | - | - |
| Midface hypoplasia | - | + | - | - | + | + | - | - | - |
| Depressed nasal bridge | + | - | - | + | + | + | - | - | - |
| Bulbous nasal tip | + | - | - | - | + | + | + | - | - |
| Short philtrum | + | + | - | - | - | - | - | - | + |
| Full lips | + | - | - | + | - | - | - | - | + |
| Hand/foot abnormalities | Fetal finger pads, tapering of 2nd and 5th digits | - | Pes planus | - | Pes planus | - | Bilateral 4th and 5th toe clinodactyly, fetal pads on toes, left single palmar crease | - | - |

*Supplementary Table 3 continues on next page.*

**Supplementary Table 3.** *Continued*

| Individual | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|
| ASD diagnosis | - | - | - | - | + | + | - |
| Other / details | - | Normal development until start of seizures at 2 years | - | Speech delay, walked at 24 m | Read simple words, count to 100, uses an augmentative communication device, 1:1 aide in school (for behavior issues) | Expressive language delay | Normal motor development (walk 12 m), expressive language delay |
| Nervous system | | | | | | | |
| Hypotonia | + | - | + | + | - | - | - |
| Gait disturbance | - | - | + | - | - | - | - |
| Seizures | - | Epileptic encephalopathy (begins at 2 years) | - | - | - | - | - |
| MRI/CT-Scan | NA | Changes secondary to craniotomy | Periventricular hyperintensity | Arachnoid cyst | EH | NA | NA |
| Other | - | Bradykinesia, bradyphrenia, vagal nerve stimulator | Clumsy gait, frequent tripping | - | - | | |
| Dysmorphic facial features | | | | | | | |
| Broad forehead | - | - | - | + | - | - | - |
| High anterior hair line | - | - | - | - | - | - | - |
| Prominent supraorbital ridges | - | + | - | - | - | - | - |
| Deep set eyes | - | - | + | + | - | - | - |
| Infraorbital dark circles | - | - | - | - | - | - | - |
| Midface hypoplasia | - | - | - | - | - | - | - |
| Depressed nasal bridge | - | - | - | - | - | - | - |
| Bulbous nasal tip | - | - | - | - | - | - | - |
| Short philtrum | - | - | - | - | - | - | - |
| Full lips | + | + | - | - | - | - | - |
| Hand/foot abnormalities | - | - | - | - | - | Camptodactyly 5$^{th}$ digit bilateral, syndactyly toes 2-3 | - |

*Supplementary Table 3 continues on next page.*

**Supplementary Table 3.** *Continued*

| Individual | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Other | Thick eyebrows, buffalo hump, central obesity | - | Prominent forehead, triangular face, marked cupid's bow | Mild frontal bossing, broad nasal bridge, upslanting palpebral fissures, epicanthic folds, low set ears | Sparse eyebrows, hypotelorism, deep philtrum groove, prominent cupid's bow, pointed chin, right accessory nipple | Sparse eyebrows, prominent/ pointed chin | Hypertelorism, bright blue iris, downslanting palpebral fissures, smooth philtrum | - | Heavy eyebrows, wide nasal tip |
| Other | | | | | | | | | |
| Cardiac anomalies | - | - | - | - | Resolved murmur | - | - | - | Hole in the heart (resolved) |
| Musculoskeletal anomalies | - | - | - | Joint hyperlaxity, spontaneous subluxation of thumbs | - | - | - | Joint hyperlaxity (hip) and subluxable shoulders | - |
| Dental anomalies | - | - | - | - | Irregularly spaced teeth (need braces), prominent upper central incisors, inability to establish overbite | - | - | Hypodontia | - |
| Cleft lip/ palate | - | Bifid uvula, submucous cleft palate | - | - | - | - | - | - | - |
| Eye/vision anomalies | - | - | Mild hyperopia | - | - | - | - | - | - |
| Inner ear/ hearing anomalies | - | - | - | - | - | - | - | No, audiology normal | - |
| Cutaneous | Acanthosis nigricans (neck and axillae), excess hair growth (back, arm, leg) | - | - | - | Patch of prominent capillaries on upper back | - | - | - | 2 café au lait macules |
| Perinatal complication | - | - | Neonatal hyperbilirubinemia and feeding problems | Maternal diabetes of pregnancy. Neonatal hyperbilirubinemia (phototherapy) | - | Placental insufficiency, maternal alcohol use | - | - | Neonatal hyperbilirubinemia |
| Other | Multiple urinary tract infections, kidney ultrasound normal | Frequent middle ear infections, four grommet insertions, adeno-tonsillectomy | Wakes up often during sleep | Unilateral cryptorchidism, umbilical hernia | Sleep apnea (resolved at 2y) | Testicular abscess, kidney infection in young age | Constipation, failure to thrive due to issue with solid and GERD | | |

**Supplementary Table 3.** *Continued*

| Individual | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|
| Other | Protruding ears, inverted nipples | Downslanting palpebral fissures, prominent nasal ridge, protruding ears (mild) | Triangular shaped face, microphtalmia, retrognathia | - | Frontal bossing | Upslanting palpebral fissures, ptosis, synophrys | Large ears |
| Other | | | | | | | |
| Cardiac anomalies | Tricuspid regurgitation | - | - | - | - | - | - |
| Musculoskeletal anomalies | - | Scoliosis | Joint hyperlaxity | Congenital torticollis | - | - | - |
| Dental anomalies | - | - | - | - | - | - | - |
| Cleft lip/palate | - | - | - | - | - | - | - |
| Eye/vision anomalies | Myopia, strabismus (s/p correction) | - | - | - | Hyperopia, esotropia (s/p correction) | Strabismus convergens, amblyopia | - |
| Inner ear/hearing anomalies | - | - | - | - | - | - | - |
| Cutaneous | - | - | - | - | - | - | - |
| Perinatal complication | Unknown (adopted) | - | Triple pregnancy (2 embryos absorbed), spotting 30w, born by C-section, respiratory insufficiency and intubation. 3 weeks in NICU | - | Prematurity (born 27 w) | Neonatal feeding problems | - |
| Other | | | | Right cryptorchidy | | Subclinical hypothyroidism | |

*Abbreviations:* ARVC: arrhythmogenic right ventricular cardiomyopathy, ASD: autism spectrum disorder, DCD: developmental coordination disorder, EH: External hydrocephalus, GERD: gastroesophageal reflux disease, het: heterozygous, NA: not available, LP: likely pathogenic, VUS: variant of unknown significance, WES: whole exome sequencing, +: yes, -: no.

# Chapter 9

General discussion and future perspectives

## General discussion

Research on congenital anomalies and genetic disorders has been mainly focused on western populations. However, as countries go through an epidemiologic transition, congenital anomalies and genetic disorders are becoming an increasingly important public health issue worldwide. This requires the development of healthcare services to diagnose, prevent and treat congenital anomalies and genetic disorders, accompanied by scientific research to ensure that these services are tailored to local needs. With this thesis we aim to contribute to better genetic care and research in the specific setting of the small island communities of the Dutch Caribbean. In this section we discuss the main findings of this thesis in the context of the scientific literature. In addition, we consider future directions for optimal delivery of genetic services in the Dutch Caribbean and provide suggestions for future research.

**Epidemiology of congenital anomalies in the Dutch Caribbean**

In **Chapter 2**, we describe the prevalence and pattern of structural congenital anomalies in Aruba, Bonaire and Curaçao (ABC islands). We found a total prevalence of congenital anomalies of 242.97 per 10,000 births (2.4%). Establishing the baseline prevalence rate of congenital anomalies is an important first step to enable organization of prevention programs and health care facilities for affected individuals (1). In addition, baseline prevalence data of congenital anomalies allow for the identification of changes in prevalence rates over time, thus facilitating the identification of potential new teratogenic exposures and evaluation of the effect of prevention programs (2, 3). Apart from describing baseline prevalence data of congenital anomalies on the ABC islands, we also compared these data to those of the French West Indies and the Northern Netherlands. In general, differences in prevalence rates between different countries or regions should be interpreted with caution, as they may be subjected to a number of biases. For example, prevalence rates may differ between registries depending on which congenital anomalies are in- and excluded, if they are hospital or population-based and whether or not stillbirths and terminations of pregnancy are included (2, 4). However, when these methodological differences are averted, variation in the prevalence of specific congenital anomalies between registries may yield clues to genetic and/or environmental factors risk factors. In our study, for example, the high prevalence of polydactyly on the ABC islands, which was 29.68 per 10,000 births (~0.3%), is likely explained by the African ancestry of a large part of the population of the ABC islands, since it is known that postaxial polydactyly is common in African populations (5, 6). Nonetheless, the estimated prevalence of congenital anomalies also depends upon available diagnostic technologies (2, 4). This probably explains why the prevalence of genetic disorders and kidney and urinary tract anomalies, as well as the total prevalence of congenital anomalies, was lower on the ABC islands compared to the Northern Netherlands.

Since we focused only on structural congenital anomalies, this study does not yield information about common genetic disorders without structural anomalies. However, genetic diagnoses that were established from the start of the joint pediatric-genetics clinic 2011 until 2019 are summarized in **Chapter 3**. We identified some recurrent genetic disorders, including Down syndrome, Marfan syndrome, Sotos syndrome, Tuberous Sclerosis type 2, Neurofibromatosis type 1, and Fragile X syndrome, which are all relatively common genetic disorders worldwide, but no recurrent (founder) variants were detected. A possible explanation for this is that the populations of the Dutch Caribbean islands are not as genetically isolated as we hypothesized beforehand. However, a founder effect has been previously observed in Curaçao and Bonaire for the autosomal dominant Rendu-Osler-Weber disease and we cannot exclude the possibility that there are indeed more founder variants in the Dutch Caribbean population which have not yet been discovered.

**Delivering genetic services in small island communities**

In an effort to provide the population of the Dutch Caribbean the same genetic care as provided for other citizens of the Kingdom of the Netherlands, a joint pediatric-genetics clinic was established in 2011. In the absence of a local genetic counselor in the Dutch Caribbean, a visiting clinical geneticist provides clinical diagnostic evaluations and counseling of patients and parents during bi-annual outpatient clinics, while blood samples are sent to diagnostic laboratories in the Netherlands for genetic testing. This approach with a visiting clinical geneticist has been described before as a method to provide clinical genetic services for small islands communities (7, 8). In fact, outreach services in general are a well-known strategy to improve access to specialized health care services in remote and rural areas (9). Especially in the case of small islands, where scale issues prohibit the local establishment of highly specialized healthcare, these outreach services may provide an important contribution to the local healthcare system. For example in Malta, a small island state in Europe, a wide array of highly specialized care is provided by visiting consultants from the United Kingdom (10). In the Dutch Caribbean, several of these visiting medical specialist services exist, including a visiting pediatric neurologist in Curaçao and a visiting Dutch medical team that provides surgical care for children with cleft lip and/or palate in Aruba. Compared to overseas referral of patients, visiting medical specialist services are less expensive and more patient-centered, as transportation and accommodation costs for patients and accompanying family members are avoided and patients stay in their familiar environment, near friends and family (11). The benefit of local availability of genetic services – as opposed to having to go abroad – was also specifically mentioned by one of the participants in our qualitative study (**Chapter 4**). One of the disadvantages of providing health care services with visiting medical specialists is the periodic availability. This may

however be addressed by providing additional telemedicine services, which is particularly suitable for clinical genetic services, since communication and counseling are main aspects of the consultation (12). Even dysmorphology examination may be performed successfully through video appointments (13). Finally, it is important to note that there is no 'one-fits-all' strategy for delivering clinical genetic services and other specialized health care services in small island communities. However, small island states may learn from each other as they plan the delivery of these services within the context of local possibilities, restrictions and needs.

**Delivering genetic services with limited financial resources**

Another important aspect of genetics service delivery in the Dutch Caribbean is that financial resources for genetic testing are limited in comparison to the European Netherlands. Although costs of genetic testing are covered by the local health insurances, there are limitations to the number and costs of genetic tests that can be requested on an annual basis, especially on the islands of Aruba, Curaçao and St. Maarten, which are not part of the Netherlands. We therefore applied several strategies to use scarce financial resources as effectively as possible.

Firstly, patients with the highest suspicion of a genetic disorder were selected for genetic testing. For example, patients with a clinical diagnosis of a specific genetic disorder and/or patients with a combination of two or more clinically suspect features, e.g. intellectual disability (ID), congenital anomalies and/or dysmorphic facial features. Although scarce financial resources are used as effectively as possible with this approach, it also leads to clinical challenges when trying to select patients with the highest suspicion of a genetic disorder, as well as ethical challenges, since some patients will miss the opportunity to receive a genetic diagnosis.

Secondly, more targeted genetic testing was performed. For instance, if there was a (strong) clinical suspicion of a certain genetic syndrome, single gene analysis was performed instead of a next generation sequencing (NGS) gene panel. In addition, NGS gene panels were chosen over whole exome sequencing (WES), a very comprehensive but also more expensive genetic test, which was performed only in a few individuals. A drawback of this approach however is that, even though individual targeted tests are less expensive, an additional genetic test has to be performed if the result of the first test is negative. This may altogether result in higher costs or less diagnoses when additional tests cannot be performed because of limited financial resources. Thus, it may ultimately be cheaper to perform one comprehensive genetic test, such as WES, instead of multiple targeted tests. Indeed, in several studies based in Europe and the United States it has been shown that WES

in patients with neurodevelopmental disorders and/or congenital anomalies is cost-effective compared to the traditional diagnostic trajectory of sequential testing (14-17), although cost-effectiveness depends on clinical context, patient population and other health system factors (18). Thus, the results of these studies cannot simply be generalized to resource-limited settings such as the Dutch Caribbean, where the costs of the traditional diagnostic trajectory may be lower.

Finally, we applied a "proband-first" or "proband-only" strategy for large gene panels which are usually performed in trio. For example, the ID panel available at Amsterdam UMC genome diagnostics, which currently includes 1537 genes (19), is usually offered as a trio test, but an exception is made for patients in the Dutch Caribbean. A "proband-only" analysis is approximately three times less expensive compared to trio analysis. If a pathogenic variant is identified, a substantial cost reduction is thus achieved. Even if a variant of unknown significance (VUS) is identified, the costs of segregation analysis of one variant in two parents will still be lower than the costs of trio analysis. However, disadvantages are that certain clinically relevant variants may be missed with "proband-only" analysis and that it entails significantly more labor for the laboratory specialist with regards to the interpretation of variants. In addition, if multiple VUS are identified, the price approximates or may even exceed that of the trio analysis. Nevertheless, previous studies reported that, although trio analysis improves diagnostic yield compared with "proband-only" testing (20), "proband-only" exome sequencing followed by parental testing of selective candidate variants may be a cost-effective alternative (21, 22).

Ultimately, the approaches described above all have certain (clinical) challenges and it is obvious that budgetary restrictions lead to a trade-off between diagnostic yield and financial costs. In addition, identifying different types of genetic variants requires different types of genetic testing, and there is currently no 'one-fits-all' genetic test. This is reflected in **Chapter 3**, where we found that, despite the attempted cost containment through targeted genetic testing, 40% of patients received more than one genetic test.

**Evaluation of clinical genetics services in the Dutch Caribbean**

In **Chapter 3**, we describe that, with the established clinical genetics service, a molecularly confirmed diagnosis was established in 33% (108/331) of pediatric patients with a suspected genetic disorder. This diagnostic yield can be considered high, given the targeted genetic testing and "proband-only" approach prompted by the financial restrictions. On the other hand, it can be considered low in view of the selection of patients with the highest suspicion of a genetic disorder, which was necessary because of these same financial restrictions. In general, diagnostic yield varies depending on the extent of genetic testing and the selection

of eligible patients, and its relevance thus has to be interpreted in consideration of the specific context. What is maybe more important is our finding that the genetic diagnosis had an impact on clinical management in 52% of patients, with the most frequent clinical consequences being referrals to other health professionals for screening and/or therapeutic advice, changes in therapy and follow-up according to standardized protocols. This percentage is similar to previous reports in other resource-limited areas (22-24) and we thus conclude that genetic services can significantly contribute to support and treatment of individuals with genetic disorders even in lower-resource settings.

In addition, given the paucity of qualitative studies on genetic testing and counseling in lower-resource settings, we set out to determine the significance of a genetic diagnosis to parents in the Dutch Caribbean and explored their opinions, experiences and needs regarding the clinical genetics service (**Chapter 4**). We found that most participants were satisfied with the provided genetic services and valued getting a genetic diagnosis for their child. The benefits of a genetic diagnosis as reported by the participants largely corresponded with those reported by patients and parents in previous studies and included a sense of closure, reduced guilt and feeling prepared for the future. In addition, participants were able to make informed reproductive choices based on the recurrence risk, even though certain reproductive options, such as preimplantation genetic testing or invasive prenatal diagnosis, were unavailable or difficult to access. Overall, these results demonstrate that the idea of genetic testing in lower-resource settings as unnecessary or unwanted by parents as they may have other more pressing concerns is not true, at least not for the population under study.

**Implications of this thesis for clinical genetics worldwide**

In **Chapter 5, 6, 7 and 8**, we describe three case reports of Dutch Caribbean patients and one case series including a Dutch Caribbean patient, expanding the knowledge on three rare genetic syndromes (4H leukodystrophy, *RNPC3*-related disorders and *JARID2*-neurodevelopmental syndrome) and one teratogenic syndrome (methotrexate/misoprostol syndrome). These papers illustrate that research in traditionally understudied populations may not only be beneficial to the population itself, but also to patients and clinicians worldwide. For example, we described for the first time that the phenotypic spectrum associated with biallelic *RNPC3* variants comprises not only severe growth hormone deficiency, but also deficiency of other anterior pituitary hormones (**Chapter 6**). Indeed, a recent study describing a cohort of 15 patients with biallelic pathogenic variants in *RNPC3* further confirmed that deficiency of other pituitary hormones, including thyrotropin and prolactin, is part of the spectrum of *RNPC3*-related disorders (25). Furthermore, ID was also part of the phenotype of the three Caribbean patients with biallelic *RNPC3* variants.

Although we hypothesized that this could be related to untreated congenital hypothyroidism during the first months of life, another patient with compound heterozygous variants in *RNPC3* was described later with severe proportional short stature and ID, leading the authors to conclude that ID is a key feature of the spectrum of *RNPC3*-related disorders (26). Furthermore, we initiated a cohort study on patients with *JARID2* variants, after identifying an intragenic *JARID2* deletion in a Dutch Caribbean patient with developmental delay, ID and dysmorphic facial features, and confirmed *JARID2* as a human disease gene associated with a neurodevelopmental syndrome (**Chapter 7**). *RNPC3* and *JARID2* have since been added to a number of ID gene panels which are provided by accredited genome laboratories in the Netherlands, expanding diagnostic opportunities for patients with neurodevelopmental disorders. In addition, we were able to identify a distinct DNA methylation signature (episignature) associated with *JARID2*-neurodevelopmental syndrome, which can be used as a biomarker for this syndrome (27).

With growing global migration rates (28), it is becoming increasingly important for western countries to gain knowledge on genetics and genomics in populations of diverse ancestry. In the Netherlands, 26% of the population has a non-Dutch Background (29). There are 185,000 people with a migration background from the Dutch Caribbean (born in the Dutch Caribbean or born in the Netherlands with one or two parents born in the Dutch Caribbean), which is approximately 1.1% of the total Dutch population (29). In some cities the percentage of people with a Dutch Caribbean background is particularly high, including Rotterdam (4.3%) and Den Haag (2.7%) (30). The findings of this thesis are thus also important for clinical geneticists and other healthcare providers in the Netherlands, especially for those in the above mentioned cities. For example, it is important to know – in particular in a prenatal setting – that polydactyly is a relatively common finding in individuals from the Dutch Caribbean and thus not necessarily part of a genetic syndrome.

## Future perspectives

**Diversity in genetic research**

Even though we make a small contribution to genetic research in non-western populations with this thesis, there are still important knowledge gaps in the field of genetic and genomic research in diverse populations.

First of all, non-European populations are underrepresented in reference databases of human genetic variation (31, 32). For example, in the most recent release of the genome aggregation database (gnomAD), despite an increase in ancestral diversity, approximately 45% of the genomes included are from individuals of (non-Finnish) European ancestry

(33). In contrast, only ~16% of the global population is of European descent (34). A lack of knowledge of genetic diversity across populations hampers clinical interpretation of genetic variants, because an important part of variant classification is assessing the frequency of a variant in the general population. Absence of a variant from the general population can contribute to establishing potential pathogenicity, while an allele frequency of > 5% in the general population is considered stand-alone evidence for benign interpretation (35). A lack of ancestral diversity in genetic reference databases may thus result in benign variants being misclassified as pathogenic. Several studies indeed found that certain variants previously classified as (likely) pathogenic were commonly observed in historically understudied non-European populations, including African, Latin American and South Asian populations, suggesting that these variants had been misclassified and that they are in fact benign (36-38). Moreover, several studies have shown that VUS rates are higher in individuals of non-European ancestry compared to those of European ancestry, which is also likely the result of an underrepresentation of non-European populations in genetic reference databases (39-45). In the population studied in this thesis, three recurrent copy number variants of unknown significance were identified, which likely represent normal genetic variation in the Dutch Caribbean population (**Chapter 3**). Thus, there is still a need to capture more of the genetic diversity in previously understudied populations, in particular African populations, where the greatest source of genetic variation lies (46), in order to advance our knowledge on the complete spectrum of human genetic variation and improve precision medicine for all (31).

Another important gap exists in our knowledge of dysmorphology in diverse populations. Phenotype images of genetic syndromes in literature and textbooks feature mainly individuals of European descent (47, 48). This hampers recognition of genetic syndromes in individuals from diverse ancestral backgrounds, since phenotypes may differ among various populations. It has for example been shown that Down syndrome and 22q11.2 deletion syndrome have a variable clinical presentation across different ethnicities (49, 50). Moreover, some features that are considered dysmorphic in European populations are normal findings in other populations, such as epicanthus in Asian populations and broad nasal bridge in African populations (47, 51). In addition, variation across populations is reflected not only in dysmorphic features of genetic disorders, but also in other phenotypic characteristics. For example, ethnic differences in the rate of atrioventricular septal defect (AVSD) have been found in individuals with Down syndrome (52). Knowledge of phenotypic presentation is especially important in countries where genetic testing is not widely available or accessible and diagnosis thus depends on recognition of clinical features. In recent years, some initiatives have emerged to improve knowledge of genetic syndromes in non-European populations, such as an electronic atlas of photographs of individuals with

human malformation syndromes from geographically diverse locations, including Africa, Asia and South America, (www.genome.gov/atlas) (48) and a new article type in the *American Journal of Medical Genetics Part A*: "Case Reports in Diverse Populations." The latter focuses on clinical reports of well-defined syndromes that demonstrate the phenotypic diversity in different genetic backgrounds (53). Moreover, it is important to consider ethnicity when developing and using facial analysis technology, which has been recently developed as a tool to assist clinical geneticists, as well as other clinicians, in establishing a (differential) diagnosis (54). It has been shown that test accuracy of this diagnostic tool increases significantly when algorithms are trained separately for different ethnic populations (50, 55-57). If properly trained, these facial analysis technologies may thus be particularly useful in regions where there is a lack of trained clinical geneticists and limited access to genetic testing.

**Future research and clinical perspectives for genetic services in the Dutch Caribbean**

Considering the above mentioned knowledge gap, an important recommendation for future research in the Dutch Caribbean is to create a reference database of human genetic variation in the (Dutch) Caribbean. Apart from facilitating better classification of (rare) variants in a clinical diagnostic setting, these data may also be used to study associations between genetic variants and common diseases, such as type 2 diabetes, which is needed since there is a lack of ancestral diversity in these kind of genome-wide association studies (GWAS) as well (58). In this context, it is interesting to highlight a recent initiative, the Human Heredity, Environment, and Health in the Caribbean (H3ECaribbean) project, which is modeled after the successful Human Heredity and Health in Africa (H3Africa) program, and aims to "*target issues of social justice by encouraging the inclusion of diverse Caribbean communities in genomics research*" (59).

Another research recommendation is to continue registration and surveillance of congenital anomalies in the Dutch Caribbean. This is essential for the identification of (new) teratogenic exposures and for assessing the impact of prevention programs (3). In this thesis we studied only the prevalence of congenital anomalies in Aruba and Curaçao, the two largest Dutch Caribbean islands, and Bonaire, but future studies may also include St. Maarten, St. Eustatius and Saba (SSS islands), although certain challenges will have to be taken into account. These include for example scale issues related to the very small size of Saba and St Eustatius, prompting the need for data collection over a large number of years in order to draw valid conclusions.

Currently, limited financial resources are the most important barrier towards further development of genetic services in the Dutch Caribbean. Advances in diagnostic technologies may however provide a solution for several issues related to these financial restrictions,

including technological developments towards more comprehensive 'one-test-fits-all' genetic testing, obviating the need for sequential testing. For example, it is now possible to reliably detect copy-number variants (CNVs) from WES data (60-62). This makes WES an even more attractive first-tier diagnostic test for a broad range of genetic disorders, as it enables simultaneous detection of both CNVs as well as single nucleotide variants (SNVs) and other small variants – avoiding the need to perform separate testing for CNV analysis. Although costs of WES may currently be a limiting factor, if (or when) these costs drop, it may increase diagnostic opportunities for individuals with genetic disorders in resource-limited areas worldwide. Another example of a new diagnostic technology is EpiSign, a clinical genome-wide DNA methylation test, which enables simultaneous assessment for imprinting disorders, fragile X syndrome and a rapidly expanding number of genetic disorders exhibiting DNA methylation episignatures (63). An important clinical use of EpiSign is the assessment and reclassification of VUS in genes with existing episignatures, which is particularly useful in situations where parental or family segregation studies are not available or inconclusive. Clinical utility of this test will increase as the number of genetic disorders associated with an episignature is expected to rise, and the benefits of EpiSign as a first-tier diagnostic tool are currently being investigated. An important recommendation for future research is to study cost-effectiveness of these different genetic tests in the local context of the Dutch Caribbean. In particular, it would be useful to perform a scenario analysis to determine if WES including CNV analysis would be a cost-effective first-tier test for patients with neurodevelopmental disorders and/or congenital anomalies.

In addition, several improvements can be made to the delivery of genetic services in the Dutch Caribbean. As described in **Chapter 4**, there is a need for better information provision regarding the established genetic diagnosis. This may be addressed by providing genetic educational materials in the local language, Papiamento, as well as other languages that are common in the Dutch Caribbean, such as Spanish. Ideally, the visiting genetic counselor would be able to speak one or more of these languages. More qualitative research on patient perspectives is warranted to improve genetic service delivery and ensure that local needs are being met. Some research questions that could be addressed are: Why do some people choose to refrain from genetic testing? What are the needs of women and their partners regarding genetic testing during pregnancy? Furthermore, the availability of genetic services, in particular genetic counseling, can be increased through additional telemedicine consultations. Finally, the provided genetic services have been mainly focused on pediatric patients with neurodevelopmental disorders and/or structural congenital anomalies. However, there has been an increasing demand for referral of patients with suspected hereditary cancer predisposition syndromes as well as inherited cardiac conditions. We thus suggest to develop appropriate genetic counseling and testing facilities for these types

of genetic referrals as well, taking into account local needs and possibilities. This should be accompanied by cost-effectiveness analyses, to ensure optimal use of limited financial resources.

Lastly, apart from clinical genetics services, there are several improvements to be made in the field of community genetic services. This includes the introduction of universal newborn screening in all Dutch Caribbean islands to detect treatable neonatal disorders, including congenital hypothyroidism and hemoglobinopathies, and further implementation of non-invasive prenatal testing to become available for all Dutch Caribbean women.

**Box 1.** Summary of recommendations for genetic research and services in the Dutch Caribbean

**Summary of recommendations**

1. Research recommendations:
   - Create a reference database of genetic variation in the Dutch Caribbean
   - Continue surveillance of congenital anomalies on all Dutch Caribbean islands
   - Evaluate cost-effectiveness of genetic testing strategies
   - Perform additional qualitative studies on patient perspectives
2. Clinical recommendations:
   - Improve information provision for patients by providing genetic educational material in Papiamento
   - Increase access to genetic services by providing telemedicine consultations
   - Develop cancer and cardio genetics services
   - Improve universal delivery of community genetic services, including newborn screening

## Conclusion

The results published in this thesis contribute to improving genetic service delivery in the Dutch Caribbean and demonstrate the importance of obtaining a genetic diagnosis even in a resource-limited setting. Our strategy with a visiting clinical geneticist may be used as an example for developing genetic services in other small and isolated communities. In addition, we reported for the first time the prevalence of structural congenital anomalies in three out of the six Dutch Caribbean islands, and described which congenital anomalies are more prevalent among these Dutch Caribbean populations. Finally, we have shown how genetic research in this traditionally understudied population can improve knowledge relevant for patients and clinicians worldwide. Future efforts may focus on improving and expanding genetic services in the Dutch Caribbean, while evaluating which genetic testing strategies are most cost-effective in this specific context.

## References


1. Melo DG, Sanseverino MTV, Schmalfuss TO, Larrandaburu M. Why are Birth Defects Surveillance Programs Important? Front Public Health. 2021;9:753342.
2. Birth defects surveillance: a manual for programme managers, second edition. Geneva: World Health Organization; 2020.
3. Dolk H. EUROCAT: 25 years of European surveillance of congenital anomalies. Arch Dis Child Fetal Neonatal Ed. 2005;90(5):F355-8.
4. Kirby RS. The prevalence of selected major birth defects in the United States. Semin Perinatol. 2017;41(6):338-44.
5. Scott-Emuakpor AB, Madueke ED. The study of genetic variation in Nigeria. II. The genetics of polydactyly. Hum Hered. 1976;26(3):198-202.
6. Woolf CM, Myrianthopoulos NC. Polydactyly in American negroes and whites. Am J Hum Genet. 1973;25(4):397-404.
7. Sobering AK, Li D, Beighley JS, Carey JC, Donald T, Elsea SH, et al. Experiences with offering pro bono medical genetics services in the West Indies: Benefits to patients, physicians, and the community. Am J Med Genet C Semin Med Genet. 2020;184(4):1030-41.
8. McWalter K, Hasegawa L, Au SM. Provision of genetics services on Guam. J Genet Couns. 2013; 22(6):885-9.
9. Roodenbeke ED, Lucas S, Rouzaut A, Bana F. WHO Guidelines Approved by the Guidelines Review Committee. Outreach Services as a Strategy to Increase Access to Health Workers in Remote and Rural Areas: Increasing Access to Health Workers in Rural and Remote Areas. Geneva: World Health Organization; 2011.
10. Gruen RL, Weeramanthri TS, Knight SE, Bailie RS. Specialist outreach clinics in primary care and rural hospital settings. Cochrane Database Syst Rev. 2004;2003(1):Cd003798.
11. Casha A, Casha R, Azzopardi Muscat N. Moving health professionals as an alternative to moving patients: The contribution of overseas visiting medical specialists to the health system in Malta. Health Policy. 2020;124(5):519-24.
12. Brown EG, Watts I, Beales ER, Maudhoo A, Hayward J, Sheridan E, et al. Videoconferencing to deliver genetics services: a systematic review of telegenetics in light of the COVID-19 pandemic. Genet Med. 2021;23(8):1438-49.
13. Szigety KM, Crowley TB, Gaiser KB, Chen EY, Priestley JRC, Williams LS, et al. Clinical Effectiveness of Telemedicine-Based Pediatric Genetics Care. Pediatrics. 2022;150(1).
14. Vrijenhoek T, Middelburg EM, Monroe GR, van Gassen KLI, Geenen JW, Hövels AM, et al. Whole-exome sequencing in intellectual disability; cost before and after a diagnosis. Eur J Hum Genet. 2018;26(11):1566-71.
15. Monroe GR, Frederix GW, Savelberg SM, de Vries TI, Duran KJ, van der Smagt JJ, et al. Effectiveness of whole-exome sequencing and costs of the traditional diagnostic trajectory in children with intellectual disability. Genet Med. 2016;18(9):949-56.
16. Vissers L, van Nimwegen KJM, Schieving JH, Kamsteeg EJ, Kleefstra T, Yntema HG, et al. A clinical utility study of exome sequencing versus conventional genetic testing in pediatric neurology. Genet Med. 2017;19(9):1055-63.
17. Stark Z, Schofield D, Alam K, Wilson W, Mupfeki N, Macciocca I, et al. Prospective comparison of the cost-effectiveness of clinical whole-exome sequencing with that of usual care overwhelmingly supports early use and reimbursement. Genet Med. 2017;19(8):867-74.

18. Schwarze K, Buchanan J, Taylor JC, Wordsworth S. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genet Med. 2018; 20(10):1122-30.
19. Amsterdam UMC Genome Diagnostics. AGDx NGS – Trio test Intellectual Disability panel v13 [Internet]. 2022 [cited 2022 Dec 5]. Available from: https://genomediagnostics.amsterdamumc.nl/product/ngs-panel-intellectual-disabilities-v10-1156-genes/.
20. Retterer K, Juusola J, Cho MT, Vitazka P, Millan F, Gibellini F, et al. Clinical application of whole-exome sequencing across clinical indications. Genet Med. 2016;18(7):696-704.
21. Kim SH, Kim B, Lee JS, Kim HD, Choi JR, Lee ST, et al. Proband-Only Clinical Exome Sequencing for Neurodevelopmental Disabilities. Pediatr Neurol. 2019;99:47-54.
22. Hu X, Li N, Xu Y, Li G, Yu T, Yao RE, et al. Proband-only medical exome sequencing as a cost-effective first-tier genetic diagnostic test for patients without prior molecular tests and clinical diagnosis in a developing country: the China experience. Genet Med. 2018;20(9):1045-53.
23. Mena R, Mendoza E, Gomez Peña M, Valencia CA, Ullah E, Hufnagel RB, et al. An international telemedicine program for diagnosis of genetic disorders: Partnership of pediatrician and geneticist. Am J Med Genet C Semin Med Genet. 2020;184(4):996-1008.
24. Scocchia A, Wigby KM, Masser-Frye D, Del Campo M, Galarreta CI, Thorpe E, et al. Clinical whole genome sequencing as a first-tier test at a resource-limited dysmorphology clinic in Mexico. NPJ genomic medicine. 2019;4:5.
25. Akin L, Rizzoti K, Gregory LC, Corredor B, Le Quesne Stabej P, Williams H, et al. Pathogenic variants in RNPC3 are associated with hypopituitarism and primary ovarian insufficiency. Genet Med. 2022;24(2):384-97.
26. Yamada M, Ono M, Ishii T, Suzuki H, Uehara T, Takenouchi T, et al. Establishing intellectual disability as the key feature of patients with biallelic RNPC3 variants. Am J Med Genet A. 2021; 185(6):1836-40.
27. Verberne EA, van der Laan L, Haghshenas S, Rooney K, Levy MA, Alders M, et al. DNA Methylation Signature for JARID2-Neurodevelopmental Syndrome. Int J Mol Sci. 2022;23(14).
28. McAuliffe M, Triandafyllidou A. World Migration Report 2022. International Organization for Migration (IOM), Geneva; 2021.
29. Centraal Bureau voor de Statistiek. Integratie en Samenleven 2022 [Internet]. 2022 [cited 2023 Jan 2]. Available from: https://longreads.cbs.nl/integratie-en-samenleven-2022/bevolking/.
30. Centraal Bureau voor de Statistiek. Bevolking; herkomstland, geboorteland, leeftijd, regio, 1 januari [Internet]. 2023 [cited 2023 Jan 16]. Available from: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/85458NED/table?ts=1673868209247.
31. Pereira L, Mutesa L, Tindana P, Ramsay M. African genetic diversity and adaptation inform a precision medicine agenda. Nat Rev Genet. 2021;22(5):284-306.
32. Landry LG, Ali N, Williams DR, Rehm HL, Bonham VL. Lack Of Diversity In Genomic Databases Is A Barrier To Translating Precision Medicine Research Into Practice. Health Aff (Millwood). 2018;37(5):780-5.
33. Tiao G, Goodrich J. gnomAD v3.1 New Content, Methods, Annotations, and Data Availability [Internet]. 2020 [updated 2021 Dec 17; cited 2022 May 18]. Available from: https://gnomad.broadinstitute.org/news/2020-10-gnomad-v3-1-new-content-methods-annotations-and-data-availability/.
34. Martin AR, Kanai M, Kamatani Y, Okada Y, Neale BM, Daly MJ. Clinical use of current polygenic risk scores may exacerbate health disparities. Nat Genet. 2019;51(4):584-91.

35. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.
36. Manrai AK, Funke BH, Rehm HL, Olesen MS, Maron BA, Szolovits P, et al. Genetic Misdiagnoses and the Potential for Health Disparities. N Engl J Med. 2016;375(7):655-65.
37. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285-91.
38. Choudhury A, Aron S, Botigué LR, Sengupta D, Botha G, Bensellak T, et al. High-depth African genomes inform human migration and health. Nature. 2020;586(7831):741-8.
39. Kurian AW, Ward KC, Hamilton AS, Deapen DM, Abrahamse P, Bondarenko I, et al. Uptake, Results, and Outcomes of Germline Multiple-Gene Sequencing After Diagnosis of Breast Cancer. JAMA Oncol. 2018;4(8):1066-72.
40. Caswell-Jin JL, Gupta T, Hall E, Petrovchich IM, Mills MA, Kingham KE, et al. Racial/ethnic differences in multiple-gene sequencing results for hereditary cancer risk. Genet Med. 2018;20(2):234-9.
41. Susswein LR, Marshall ML, Nusbaum R, Vogel Postula KJ, Weissman SM, Yackowski L, et al. Pathogenic and likely pathogenic variant prevalence among the first 10,000 patients referred for next-generation cancer panel testing. Genet Med. 2016;18(8):823-32.
42. Ricker C, Culver JO, Lowstuter K, Sturgeon D, Sturgeon JD, Chanock CR, et al. Increased yield of actionable mutations using multi-gene panels to assess hereditary cancer susceptibility in an ethnically diverse clinical cohort. Cancer Genet. 2016;209(4):130-7.
43. Tatineni S, Tarockoff M, Abdallah N, Purrington KS, Assad H, Reagle R, et al. Racial and ethnic variation in multigene panel testing in a cohort of BRCA1/2-negative individuals who had genetic testing in a large urban comprehensive cancer center. Cancer Med. 2022;11(6):1465-73.
44. Landry LG, Rehm HL. Association of Racial/Ethnic Categories With the Ability of Genetic Tests to Detect a Cause of Cardiomyopathy. JAMA Cardiol. 2018;3(4):341-5.
45. Pottinger TD, Puckelwartz MJ, Pesce LL, Robinson A, Kearns S, Pacheco JA, et al. Pathogenic and Uncertain Genetic Variants Have Clinical Cardiac Correlates in Diverse Biobank Participants. J Am Heart Assoc. 2020;9(3):e013808.
46. McClellan JM, Lehner T, King MC. Gene Discovery for Complex Traits: Lessons from Africa. Cell. 2017;171(2):261-4.
47. Koretzky M, Bonham VL, Berkman BE, Kruszka P, Adeyemo A, Muenke M, et al. Towards a more representative morphology: clinical and ethical considerations for including diverse populations in diagnostic genetic atlases. Genet Med. 2016;18(11):1069-74.
48. Muenke M, Adeyemo A, Kruszka P. An electronic atlas of human malformation syndromes in diverse populations. Genet Med. 2016;18(11):1085-7.
49. Kruszka P, Addissie YA, McGinn DE, Porras AR, Biggs E, Share M, et al. 22q11.2 deletion syndrome in diverse populations. Am J Med Genet A. 2017;173(4):879-88.
50. Kruszka P, Porras AR, Sobering AK, Ikolo FA, La Qua S, Shotelersuk V, et al. Down syndrome in diverse populations. Am J Med Genet A. 2017;173(1):42-53.
51. Lumaka A, Cosemans N, Lulebo Mampasi A, Mubungu G, Mvuama N, Lubala T, et al. Facial dysmorphism is influenced by ethnic background of the patient and of the evaluator. Clin Genet. 2017;92(2):166-71.
52. Freeman SB, Bean LH, Allen EG, Tinker SW, Locke AE, Druschel C, et al. Ethnicity, sex, and the incidence of congenital heart defects: a report from the National Down Syndrome Project. Genet Med. 2008;10(3):173-80.

53. Girisha KM, Wonkam A, Muenke M. Introducing in AJMG Part A: Case reports in diverse populations. Am J Med Genet A. 2018;176(7):1547-8.
54. Gurovich Y, Hanani Y, Bar O, Nadav G, Fleischer N, Gelbman D, et al. Identifying facial phenotypes of genetic disorders using deep learning. Nat Med. 2019;25(1):60-4.
55. Kruszka P, Porras AR, Addissie YA, Moresco A, Medrano S, Mok GTK, et al. Noonan syndrome in diverse populations. Am J Med Genet A. 2017;173(9):2323-34.
56. Kruszka P, Porras AR, de Souza DH, Moresco A, Huckstadt V, Gill AD, et al. Williams-Beuren syndrome in diverse populations. Am J Med Genet A. 2018;176(5):1128-36.
57. Dowsett L, Porras AR, Kruszka P, Davis B, Hu T, Honey E, et al. Cornelia de Lange syndrome in diverse populations. Am J Med Genet A. 2019;179(2):150-8.
58. Sirugo G, Williams SM, Tishkoff SA. The Missing Diversity in Human Genetic Studies. Cell. 2019; 177(4):1080.
59. Bolleddula J, Simeon D, Anderson SG, Shields L, Mullings J, Ossorio P, et al. No person left behind: Mapping the health policy landscape for genomics research in the Caribbean. The Lancet Regional Health – Americas. 2022;15.
60. Pfundt R, Del Rosario M, Vissers L, Kwint MP, Janssen IM, de Leeuw N, et al. Detection of clinically relevant copy-number variants by exome sequencing in a large cohort of genetic disorders. Genet Med. 2017;19(6):667-75.
61. Rajagopalan R, Murrell JR, Luo M, Conlin LK. A highly sensitive and specific workflow for detecting rare copy-number variants from exome sequencing data. Genome Med. 2020;12(1):14.
62. Zhai Y, Zhang Z, Shi P, Martin DM, Kong X. Incorporation of exome-based CNV analysis makes trio-WES a more powerful tool for clinical diagnosis in neurodevelopmental disorders: A retrospective study. Hum Mutat. 2021;42(8):990-1004.
63. Sadikovic B, Levy MA, Kerkhof J, Aref-Eshghi E, Schenkel L, Stuart A, et al. Clinical epigenomics: genome-wide DNA methylation analysis for the diagnosis of Mendelian disorders. Genet Med. 2021;23(6):1065-74.

# Appendix

# Summary

## Dutch Caribbean Genetics: diagnostics of congenital anomalies and genetic disorders in small island communities

Congenital anomalies and genetic disorders represent an important cause of neonatal, infant and child morbidity and mortality. They have emerged as a major global health problem, resulting from the epidemiological transition that many countries went through as they successfully reduced other causes of child mortality such as infectious diseases and malnutrition. However, healthcare and research programs to improve diagnosis, prevention and treatment of congenital anomalies and genetic disorders are still scarce in many regions, including small island developing states such as the Dutch Caribbean. With this thesis we aim to contribute to better healthcare for individuals with congenital anomalies and genetic disorders in the Dutch Caribbean.

In **Chapter 2**, we describe the prevalence and pattern of structural congenital anomalies in Aruba and Curaçao, the two largest Dutch Caribbean islands, and Bonaire. These three islands are located near to each other and are referred to as the ABC islands. We found a total prevalence of congenital anomalies on the ABC islands of 242.97 per 10,000 births (2.4%). The total prevalence of congenital anomalies was highest in Bonaire, although this is most likely explained by methodological differences related to the smaller size of Bonaire and the higher mobility around birth. To determine if certain congenital anomalies occur more frequently on the ABC islands, we compared the prevalence data of the ABC islands to those of the French West Indies, which are comparable to the ABC islands in terms of geographical location of ancestral background, and to the Northern Netherlands, which is part of the Kingdom of the Netherlands. We found that the total prevalence of congenital anomalies on the ABC islands was comparable to the French West Indies, but significantly lower compared to the Northern Netherlands, which might be explained by the availability of more advanced diagnostic technologies in the Northern Netherlands. The prevalence of polydactyly and atrial septal defect on the ABC islands was significantly higher compared to the French West Indies and Northern Netherlands, while the prevalence of congenital anomalies of the kidney and urinary tract and genetic disorders was significantly lower. The different prevalence rates of some of these anomaly subgroups may be explained by differences in diagnostic opportunities, but others, in particular the prevalence of polydactyly, may reflect true differences in prevalence rates related to genetic and/or environmental factors. The baseline prevalence data generated in this study allow for identification of changes in prevalence rates over time, which is important to identify potential new teratogenic exposures and to evaluate the effect of prevention programs.

To increase access to genetic services to diagnose and counsel individuals with genetic disorders, a joint pediatric-genetics clinic with a visiting clinical geneticist was established in 2011. These outpatient clinics are organized bi-annually and blood samples are sent to diagnostic laboratories in the Netherlands for genetic testing. In **Chapter 3 and 4**, we evaluate the delivery of these clinical genetics services in the Dutch Caribbean. We demonstrate in **Chapter 3** that a molecularly confirmed genetic diagnosis was established in 33% of patients. This is a reasonably high diagnostic yield, considering that whole exome sequencing is not (yet) part of standard genetic care in the Dutch Caribbean and that financial restrictions prompt a more targeted and a proband-only approach. In addition we found that, even in this lower-resource setting, the genetic diagnosis had an impact on clinical management in 52% of patients. This included referrals to other health professionals for screening and/or therapeutic advice, changes in therapy and follow-up according to standardized protocols. In **Chapter 4**, we explore the experiences and needs of parents in the Dutch Caribbean who received a genetic diagnosis for their child in a qualitative study. Most participants valued getting a diagnosis for their child, because it brought them closure and acceptance, it made them feel prepared for the future and enabled them to make informed reproductive choices. Specific challenges that parents faced in the context of small island communities were social stigma related to children with disabilities and a lack of local peer support because their child was the only one on the island with a specific genetic disorder. We found that most participants were satisfied with the provided genetic service, although it could be improved by more comprehensible as well as more extensive information provision on the genetic diagnosis, recurrence risks and reproductive options.

In **Chapter 5, 6, 7 and 8**, we illustrate how our research in the Dutch Caribbean has yielded insights relevant for patients and clinicians worldwide. We describe three case reports of patients in the Dutch Caribbean with genetic or teratogenic syndromes, as well as a case series including a Dutch Caribbean patient, thus expanding knowledge on the phenotypes of these rare syndromes.

The first case report describes a girl with 4H leukodystrophy, an autosomal recessive neurodegenerative disorder with a highly variable clinical course, characterized by hypomyelination, hypodontia, and hypogonadotropic hypogonadism (**Chapter 5**). It is caused by biallelic pathogenic variants in *POLR3A*, *POLR3B*, or *POLR1C*. We show that homozygosity for the c.1568T>A (p.Val523Glu) *POLR3B* variant – previously only reported in two individuals with a remarkably mild clinical course – can be associated with a severe 4H leukodystrophy phenotype as well. This is important prognostic information for (parents of) other patients with this genotype. In the second case report (**Chapter 6**), we describe three siblings with biallelic *RNPC3* variants. Only five individuals with biallelic *RNPC3* variants had been previously reported, all with severe isolated growth hormone deficiency. In addition to

severe growth hormone deficiency, the three Dutch Caribbean siblings had central congenital hypothyroidism, prolactin deficiency, delayed puberty, congenital cataract, developmental delay and intellectual deficiency, and we thus propose that the phenotypic spectrum associated with biallelic *RNPC3* variants is more extensive than previously reported. In the third case report (**Chapter 7**), we present a girl with fetal methotrexate/misoprostol syndrome and a fibroma of the tongue and suggest that this might be an additional feature of the fetal methotrexate/misoprostol syndrome. Finally, we studied a cohort of 16 patients, established through international collaboration, with developmental delay and a *JARID2* deletion or single-nucleotide variant, including one Dutch Caribbean patient (**Chapter 8**). This study confirmed *JARID2* as a human disease gene and further elucidated the associated clinical phenotype.

# Nederlandse samenvatting

## Genetica op de Nederlands-Caribische eilanden: diagnostiek van aangeboren en erfelijke aandoeningen in kleine eiland-gemeenschappen

Aangeboren en erfelijke aandoeningen vormen een belangrijke oorzaak van ziekte en sterfte op de zuigelingen- en kinderleeftijd. Doordat veel landen andere oorzaken van kindersterfte, zoals infectieziekten en ondervoeding, succesvol hebben beperkt, heeft er een epidemiologische transitie plaatsgevonden. Hierdoor zijn aangeboren en erfelijke aandoeningen wereldwijd een aanzienlijk gezondheidsprobleem geworden. Er is echter op veel plekken in de wereld een gebrek aan goede gezondheidszorg en onderzoeksprogramma's om deze aandoeningen te diagnosticeren, voorkomen en behandelen. Dit is onder andere het geval in veel kleine eilandstaten in ontwikkeling, zoals die van het Caribisch deel van het Koninkrijk der Nederlanden. Het doel van deze thesis is om bij te dragen aan betere gezondheidszorg voor mensen met aangeboren een erfelijke aandoeningen op de Nederlands-Caribische eilanden.

In **Hoofdstuk 2** beschrijven we de prevalentie en het patroon van structurele aangeboren aandoeningen in Aruba en Curaçao, de twee grootste Nederlands-Caribische eilanden, en Bonaire. Deze drie eilanden liggen dicht bij elkaar in de buurt en worden ook wel de ABC eilanden genoemd. De totale prevalentie van aangeboren aandoeningen op de ABC eilanden was 242,97 per 10.000 geboortes (2,4%). De totale prevalentie van aangeboren aandoeningen was het hoogst in Bonaire, alhoewel dit waarschijnlijk komt door methodologische verschillen die te maken hebben met de beperkte grootte van Bonaire en de hoge mobiliteit rondom geboortes. Om erachter te komen of bepaalde aangeboren aandoeningen vaker voorkomen op de ABC eilanden, hebben we de prevalentiedata van de ABC eilanden vergeleken met die van de Franse Antillen. De Franse Antillen zijn vergelijkbaar met de ABC eilanden wat betreft de geografische ligging en voorouderlijke achtergrond van de bevolking. Daarnaast hebben we een vergelijking getrokken met Noord-Nederland, dat net als de ABC eilanden onderdeel is van het Koninkrijk der Nederlanden. De totale prevalentie van aangeboren aandoeningen op de ABC eilanden was vergelijkbaar met de Franse Antillen, maar significant lager in vergelijking met Noord-Nederland. Dit laatste heeft waarschijnlijk te maken met de beschikbaarheid van meer geavanceerde diagnostische technologieën in Noord-Nederland. De prevalentie van polydactylie (extra vingers en/of tenen) en atrium septum defect (gaatje in het tussenschot van de boezems van het hart) op de ABC eilanden was significant hoger in vergelijking met zowel de Franse Antillen als Noord-Nederland. De prevalentie van aangeboren afwijkingen van de nieren en/of urinewegen en erfelijke aandoeningen was significant lager op de ABC eilanden. Een deel van deze verschillen in prevalentie kan waarschijnlijk worden verklaard door verschillen

in diagnostische mogelijkheden. Echter in sommige gevallen, bijvoorbeeld in het geval van polydactylie, vormt het waarschijnlijk een afspiegeling van daadwerkelijke verschillen in prevalentie. Dit wordt waarschijnlijk veroorzaakt door genetische en/of omgevingsfactoren. Nu de basisprevalentie van verschillende aangeboren aandoeningen op de ABC eilanden is vastgesteld, kunnen veranderingen hiervan in de toekomst worden opgemerkt. Dit is van belang om mogelijke nieuwe teratogene middelen te identificeren en om het effect van preventie programma's te monitoren.

De mogelijkheden voor diagnostiek en counseling van individuen met een erfelijke aandoening zijn vaak beperkt op kleine en relatief afgelegen eilanden, zoals de Nederlands-Caribische eilanden. Om de toegang tot genetische zorg op de Nederlands-Caribische eilanden te vergroten, werd in 2011 een polikliniek kindergenetica opgericht. De polikliniek vindt twee keer per jaar plaats, waarbij een klinisch geneticus uit Nederland de spreekuren verzorgt. De genetische diagnostiek wordt verricht in Nederlandse laboratoria, waar de bloedsamples van patiënten naartoe gestuurd worden. In **Hoofdstuk 3 en 4** evalueren we de uitkomsten van deze strategie om genetische zorg aan te bieden aan de inwoners van de Nederlands-Caribische eilanden. In **Hoofdstuk 3** laten we zien dat een moleculair bevestigde genetische diagnose werd gesteld bij 33% van de patiënten. Dit is een relatief hoge diagnostische opbrengst, aangezien uitgebreide genetische diagnostiek door middel van sequentie-analyse van het volledige exoom niet mogelijk is door financiële beperkingen. Ondanks beperkte middelen in de gezondheidszorg, had de genetische diagnose gevolgen voor het klinisch beleid bij 52% van de patiënten. Dit ging onder meer om verwijzingen naar andere specialisten voor screening en/of advies, aanpassingen in de behandeling en follow-up volgens standaard protocollen. In **Hoofdstuk 4** onderzoeken we in een kwalitatieve studie wat het betekent voor ouders op de Nederlands-Caribische eilanden om een genetische diagnose voor hun kind te krijgen. De meeste ouders die meededen aan het onderzoek vonden het waardevol dat er een diagnose werd gesteld bij hun kind. Ze benoemden onder andere dat de diagnose hen een stukje afsluiting en acceptatie bracht, dat ze zich nu beter voorbereid voelden op de toekomst en dat ze een geïnformeerde keuze voor een eventuele volgende zwangerschap konden maken. Door de kleine gemeenschappen op de eilanden kregen ouders te maken met specifieke uitdagen, zoals stigmatisering van mensen met een beperking en een gebrek aan contact met andere ouders, doordat er niemand op het eiland was met dezelfde aandoening als hun kind. De meeste ouders waren tevreden over de beschikbare genetische zorg, alhoewel er enkele verbeterpunten op het gebied van informatievoorziening werden genoemd. Dit ging met name om het krijgen van uitgebreidere en meer begrijpelijke informatie over de gestelde genetische diagnose, de bijbehorende herhalingsrisico's en reproductieve mogelijkheden.

In **Hoofdstuk 5, 6, 7 en 8** laten we zien hoe ons onderzoek in de Nederlands-Caribische eilanden heeft geleid tot inzichten die relevant zijn voor artsen en patiënten wereldwijd. We geven drie casusbeschrijvingen van patiënten uit de Nederlands-Caribische eilanden met genetische of teratogene syndromen. Daarnaast beschrijven we een groep van meerdere patiënten met dezelfde aandoening, waaronder één patiënt uit de Nederlands-Caribische eilanden. Hiermee leveren we een bijdrage aan de wereldwijde kennis over deze zeldzame syndromen.

De eerst casusbeschrijving gaat over een meisje met 4H leukodystrofie (**Hoofdstuk 5**). Dit is een autosomaal recessieve, neurodegeneratieve aandoening, die wordt veroorzaakt door biallelische (op beide allelen) pathogene varianten in het *POLR3A*, *POLR3B*, of *POLR1C* gen. Het wordt gekenmerkt door onder andere hypomyelinisatie (tekort aan myeline, het isolatielaagje rond zenuwvezels in de witte stof), hypodontie (aangeboren ontbreken van tanden) en hypogonadotroop hypogonadisme (niet spontaan op gang komen van de puberteitsontwikkeling doordat de aansturing vanuit de hypofyse ontbreekt). Het beloop van deze aandoening kan erg variabel zijn. Eerder werd homozygotie voor een bepaalde variant, c.1568T>A (p.Val523Glu), in het *POLR3B* gen beschreven in twee patiënten met een opvallend milde vorm van 4H leukodystrofie. We laten echter aan de hand van deze patiënt zien dat homozygotie voor deze specifieke variant ook geassocieerd kan zijn met een ernstigere vorm van deze aandoening. Dit is belangrijke prognostische informatie voor (ouders van) andere patiënten met deze mutatie.

De tweede casusbeschrijving gaat over twee broers en één zus met een biallelische varianten in het *RNPC3* gen (**Hoofdstuk 6**). Er waren eerder slechts vijf patiënten met biallelische varianten in *RNPC3* in de medisch wetenschappelijke literatuur gerapporteerd. Deze vijf patiënten hadden allemaal een ernstige, geïsoleerde groeihormoon deficiëntie. De drie patiënten uit de Nederlands-Caribische eilanden hadden echter naast een ernstige groeihormoon deficiëntie nog andere kenmerken, namelijk centrale aangeboren hypothyroïdie, prolactine deficiëntie, vertraagde puberteit, aangeboren staar, een ontwikkelingsachterstand en verstandelijke beperking. In dit artikel opperen we daarom dat het fenotypisch spectrum geassocieerd met biallelische *RNPC3* varianten breder is dan eerder werd beschreven.

De derde casusbeschrijving gaat over een meisje met het foetale methotrexaat/misoprostol syndroom (**Hoofdstuk 7**). Dit syndroom wordt veroorzaakt door blootstelling van de moeder aan methotrexaat/misoprostol tijdens de zwangerschap, wat leidt tot aangeboren aandoeningen bij het kind, waaronder afwijkingen van de ledematen en microcefalie. De patiënt die wij beschrijven heeft daarnaast een fibroom van de tong. Mogelijk is dit ook geassocieerd met het foetale methotrexaat/misoprostol syndroom.

Tot slot beschrijven we door middel van internationale samenwerking een groep van 16 patiënten, waaronder één patiënt uit de Nederlands-Caribische eilanden, met een ontwikkelingsachterstand en een variant in het *JARID2* gen (**Hoofdstuk 8**). Met deze studie bevestigen we dat pathogene varianten in het *JARID2* gen geassocieerd zijn met een neurologische ontwikkelingsstoornis en verduidelijken we de kenmerken van deze aandoening.

# Resúmen na Papiamentu

## Genétika na e islanan Karibe Hulandes: diagnósis di enfermedat kongénito i hereditario na komunidat insular chikitu

Enfermedatnan kongénito i hereditario ta forma un kousa importante di enfermedat i mortalidat durante infansia di mucha. Un transishon epidemiológiko a tuma lugá ya komo hopi pais a logra limitá otro kousa di mortalidat infantil, manera enfermedat infeksioso i desnutrishon. Komo resultado enfermedatnan kongénito i hereditario a bira un problema di salú importante ròmt mundu. Sinembargo na hopi parti di mundu ta falta programa di investigashon i atenshon médiko adekuá pa diagnostiká, prevení i trata e enfermedatnan akí. Esaki ta e kaso por ehèmpel, na hopi estado insular chikitu den desaroyo, manera na parti karibense di Reino Hulandes. Ophetivo di e tésis akí ta pa kontribuí na un mihó atenshon médiko pa e personanan ku enfermedatnan kongénito i hereditario na e islanan di Hulanda Karibense.

Na **Kapítulo 2** nos ta deskribí e prevalensia i e patronchi di e enfermedatnan hereditario na Boneiru, Aruba i Kòrsou. Aruba i Kòrsou ta e dos islanan di mas grandi di e parti karibense di Reino Hulandes. Aruba, Boneiru i Kòrsou ta banda di otro i tambe ta konosí komo e islanan ABC. E prevalensia total di enfermedat hereditario na e islanan ABC tabata 242,97 pa kada 10.000 nasementu (2,4%). E prevalensia total di enfermedat hereditario tabata di mas haltu na Boneiru. Ounke probablemente esaki ta pa motibu di diferensha metodológiko ku tin di aber ku e tamaño chikitu di Boneiru i e mobilidat haltu rondó di nasementu. Pa sa si sierto enfermedat hereditario ta mas komun na e islanan ABC, nos a kompará e datonan di prevalensia akí, ku esnan di e islanan franses. E islanan franses ta similar na e islanan ABC ora ta trata di ubikashon geográfiko i antesedente asendiente di e poblashon. Banda di esei nos a hasi un komparashon ku Noord-Nederland. E parti di Hulanda akí, méskos ku e islanan ABC, ta forma parti di Reino Hulandes. E prevalensia total di enfermedatnan hereditario na e islanan ABC tabata komparabel ku esun di Antias Franses, pero signifikativamente mas abou kompará ku esun di Noord-Nederland. Esaki probablemente tin di aber ku disponibilidat di e téknologianan mas avansá pa diagnostiká na Noord-Nederland. E prevalensia di polidactilia (dede èkstra na man i/òf pia) i e defekto atrium septum (un buraku den e partishon di e auríkulanan di e kurason) na e islanan ABC, tabata signifikante mas haltu, kompará ku tantu Antias Franses i Noord-Nederland. E prevalensia di anomalia hereditario di riñon i/òf via urinario, anto enfermedatnan hereditario tabata signifikante mas abou na e islanan ABC. Probablemente por splika parti di e diferensianan den e prevalensia akí, den e diferensianan di posibilidat pa diagnostiká. Sinembargo den algun kaso, por ehèmpel den kaso di polidaktilia, probablemente ta reflehá diferensia real den e prevalensia. Ta posibel ku faktor genétiko i/òf ambiental ta kousa esaki. Awor ku a establesé e prevalensia di referensia di

diferente enfermedat kongénito na e islanan ABC, por observá kambio den esakinan den futuro. Esaki ta importante pa identifiká posibel medio teratogéniko nobo i pa monitòr e efekto di e programanan di prevenshon.

E posibilidatnan pa diagnostiká i asesorá personanan ku un enfermedat kongénito, hopi biaha ta limitá na islanan chikitu i relativamente alehá, manera e islanan di Hulanda Karibense. Na aña 2011 a lanta un poliklínika pa genétika infantil pa oumentá akseso na kuido genétiko na e islanan di Hulanda Karibense. E polikínika ta habri su portanan dos biaha pa aña, anto na e momentu ei un genétiko klíniko di Hulanda ta tene konsulta. Ta realisá e diagnóstiko genétiko na laboratorionan hulandes, kaminda ta manda e muestra di sanger di e pashèntnan. Na **Kapítulonan 3 i 4** nos ta evaluá e resultadonan di e strategia pa ofresé kuido genétiko na habitantenan di e islanan hulandes karibense.

Na **Kapítulo 3** nos ta mustra ku serka 33% di e pashèntnan a realisá un diagnóstiko genétiko molekular konfirmá. Esaki ta un rindimentu diagnóstiko relativamente haltu, ya komo debí na e limitashonnan finansiero, no ta posibel pa realisá diagnóstiko genétiko médiko ekstenso pa medio di análisis di sekuensiashon di hinter e exoma. A pesar di e rekursonan limitá den kuido médiko, e diagnóstiko genétiko a afektá e maneho klíniko serka 52% di e pashèntnan. Esaki a trata entre otro di referensia na otro spesialista pa detekshon i/òf konseho, ahuste den tratamentu i follow-up sigun protokòl standart. Den un estudio kuantitativo na **Kapítulo 4**, nos ta investigá kiko ta nifiká pa mayornan na e islanan hulandes karibense pa haña un diagnóstiko genétiko pa nan yu. Mayoria di e mayornan ku a partisipá na e investigashon, a haña balioso ku a diagnostiká nan yu. Entre otro nan a menshoná e echo ku e diagnósis a duna nan chèns pa pone kosnan na nan lugá i a trese aseptashon. Nan a bisa ku nan ta sinti nan mes mihó prepará pa futuro i ku nan por tuma un desishon tokante un posibel próksimo embaraso. Debí na e komunidatnan chikitu na e islanan, mayornan a enfrentá desafionan espesífiko, manera stigmatisashon di personanan ku un limitashon i falta di kontakto ku otro mayornan, ya komo no tin otro hende den e komunidat ku e mésun kondishon ku nan yu. Mayoria di e mayornan tabata satisfecho ku e kuido genétiko optenibel. Ounke a menshoná algun punto di mehora den e área di suministro di informashon. Esei tabata trata mas tantu tokante optenshon di informashon mas ámplio i komprendibel tokante e diagnósis genétiko realisá, e riesgonan di reiterashon i posibilidatnan reproduktivo.

Na **Kapítulo 5, 6, 7 i 8** nos ta mustra kon nos investigashon na e islanan karibense di reino, a generá konosementu ku ta relevante pa dòkternan i pashèntnan mundialmente. Nos ta presentá tres kaso di pashèntnan di e islanan karibense di Reino Hulandes ku un síndrome genétiko òf teratogéniko. Ademas nos ta deskribí un grupo di mas pashènt ku e mésun enfermedat, bou di kua ún pashènt di Hulanda Karibense. Ku esaki nos ta kontribuí na e konosementu mundial tokante e síndromenan poko komun akí.

E deskripshon di e promé kaso ta trata tokante un mucha muhé ku leukodistrofia 4H (**Kapítulo 5**). Esaki ta un enfermedat neurodegenerativo outosómiko resesivo kousá pa variante patogéniko bialéliko (na ámbos alelo) den e gene *POLR3A*, *POLR3B*, òf *POLR1C*. E ta wòrdu karakterisá, entre otro, pa hipomielisashon (diferensha di mielina, e kapa aislante rondó di e fibranan di nèrvio den e sustansha blanku), hipodonsia (falta di djente kongénito) i hipogonadismo hipogonadotrópiko (inkapasidat pa insisiá desaroyo di pubertat debí na falta di kòntròl for di e hipófisis). Kurso di e enfermedat akí por varia masha. Antes a deskribí homosigosis pa un variante spesífiko, c.1568T>A (p.Val523Glu), den e gene *POLR3B* serka dos pashènt ku un forma notablemente leve di 4H leukodistrofia. Sinembargo a base di e pashènt akí nos ta mustra ku homosigosidat pa e variante spesífiko akí, tambe por ta asosiá ku un forma grave di e enfermedat akí. Esaki ta informashon pronóstiko importante pa (mayornan di) otro pashènt ku e mutashon akí.

E deskripshon di e di dos kaso ta trata dos ruman hòmber i un ruman muhé ku un variante bialéliko den e gene *RNPC3* (**Kapítulo 6**). Promé ta únikamente sinku pashènt ku variante bialéliko den e gene *RNPC3* a wòrdu raportá den literatura sientífiko. Tur e sinku pashèntnan akí tabatin un defisiensia grave di un hormona di kresementu. Sinembargo e tres pashèntnan di e parti aki di Hulanda Karibense, banda di un defisiensia grave di e hormona di kresementu tabatin otro karakterístika, esta hipotiroidismo kongénito sentral, defisiensia di prolactina, pubertat atrasá, katarata kongénito, retraso den desaroyo i deshabilidat mental. Pa e motibu ei den e artíkulo akí, nos ta trese dilanti ku e espektro fenotípiko asosiá ku e variantenan bialéliko di *RNPC3*, ta mas ámplio ku a wòrdu deskribí promé.

E deskripshon di e di tres kaso ta trata di un mucha muhé ku síndrome di metotrexato/misoprostol fetal (**Kapítulo 7**). E síndrome akí ta wòrdu okashoná na momentu ku durante e embaraso, e mama wòrdu eksponé na metotrexato/misoprostol. Esaki ta indusí na transtorno kongénito di e bebi, bou di kua anomalia di e ekstremidatnan i mikrosefalia. E pashènt ku nos ta deskribí, ademas tin un fibroma di su lenga. Esaki kisas tambe por wòrdu asosiá ku e síndrome di metotrexato/misoprostol fetal.

Finalmente, pa medio di un kolaborashon internashonal, nos ta deskribí un grupo di 16 pashènt, bou di nan tin un pashènt di e islanan di Hulanda Karibense, ku un retraso den desaroyo i un variante di e gene *JARID2* (**Kapítulo 8**). Ku e estudio akí nos ta konfirmá ku e variantenan patogéniko den e gene *JARID2*, ta asosiá ku un afektashon di e desaroyo neurológiko i nos ta aklará e karakterístikanan di e afektashon akí.

# List of publications

### Publications in this thesis

**Verberne EA**, Lo-A-Njoe SM, van Ginkel M, Zwolsman J, Nikkels S, Clement L, et al. Prevalence of congenital anomalies in the Dutch Caribbean islands of Aruba, Bonaire, and Curaçao. Birth Defects Res. 2023;115(6):595-604.

**Verberne EA**, Westermann JM, de Vries TI, Ecury-Goossen GM, Lo-A-Njoe SM, Manshande ME, et al. Genetic care in geographically isolated small island communities: 8 years of experience in the Dutch Caribbean. Am J Med Genet A. 2022;188(6):1777-91.

**Verberne EA**, van den Heuvel LM, Ponson-Wever M, de Vroomen M, Manshande ME, Faries S, et al. Genetic diagnosis for rare diseases in the Dutch Caribbean: a qualitative study on the experiences and associated needs of parents. Eur J Hum Genet. 2022;30(5):587-94.

**Verberne EA**, Dalen Meurs L, Wolf NI, van Haelst MM. 4H leukodystrophy caused by a homozygous *POLR3B* mutation: Further delineation of the phenotype. Am J Med Genet A. 2020;182(7):1776-9.

**Verberne EA**, Faries S, Mannens M, Postma AV, van Haelst MM. Expanding the phenotype of biallelic *RNPC3* variants associated with growth hormone deficiency. Am J Med Genet A. 2020;182(8):1952-6.

**Verberne EA**, Manshande ME, Wagner-Buitenweg NF, Elhage W, Holtsema H, van Haelst MM. Limb anomalies, microcephaly, dysmorphic facial features and fibroma of the tongue after failed abortion with methotrexate and misoprostol. Clin Dysmorphol. 2020;29(4):182-5.

**Verberne EA***, Goh S*, England J*, van Ginkel M, Rafael-Croes L, Maas S, et al. *JARID2* haploinsufficiency is associated with a clinically distinct neurodevelopmental syndrome. Genet Med. 2021;23(2):374-83. *Authors contributed equally to this work.

### Other publications

**Verberne EA***, van der Laan L*, Haghshenas S*, Rooney K, Levy MA, Alders M, et al. DNA Methylation Signature for *JARID2*-Neurodevelopmental Syndrome. Int J Mol Sci. 2022;23(14). *Authors contributed equally to this work.

Dingemans AJM, Truijen KMG, Kim JH, Alaçam Z, Faivre L, Collins KM, Gerkes EH, van Haelst M, van de Laar IMBH, Lindstrom K, Nizon M, Pauling J, Heropolitańska-Pliszka E, Plomp AS, Racine C, Sachdev R, Sinnema M, Skranes J, Veenstra-Knol HE, **Verberne EA**, Vulto-van Silfhout AT, Wilsterman MEF, Ahn EE, de Vries BBA, Vissers LELM. Establishing the phenotypic spectrum of ZTTK syndrome by analysis of 52 individuals with variants in *SON*. Eur J Hum Genet. 2022;30(3):271-81.

**Verberne EA**, Ecury-Goossen GM, Manshande ME, Ponson-Wever M, de Vroomen M, Tilanus M, et al. Clinical and community genetics services in the Dutch Caribbean. J Community Genet. 2021;12(3):497-501.

Stunnenberg B, Ponson-Wever M, **Verberne E**, Peters I, Gerrits M, Haaxma C, et al. Novel *SCN9A* Mutations in a Compound Heterozygous Girl with Congenital Insensitivity to Pain. Journal of Pediatric Neurology. 2021;19(3):189-92.

**Verberne EA**, de Haan E, van Tintelen JP, Lindhout D, van Haelst MM. Fetal methotrexate syndrome: A systematic review of case reports. Reprod Toxicol. 2019;87:125-39.

Van de Waarsenburg MK, **Verberne EA**, van der Vaart CH, Withagen MIJ. Recovery of puborectalis muscle after vaginal delivery: an ultrasound study. Ultrasound Obstet Gynecol. 2018;52(3):390-5.

## Author contributions

**Prevalence of congenital anomalies in the Dutch Caribbean islands of Aruba, Bonaire and Curaçao**

*E.A. Verberne, S.M. Lo-A-Njoe, M. van Ginkel, J. Zwolsman, S. Nikkels, L. Clement, M. de Vroomen, M.L.G. Wever, E. Arends, H. Holtsema, P.J. Hajenius, D. Moreta, G.M. Ecury-Goossen, M.M.A.M. Mannens, H.E.K. de Walle, J.E.H. Bergman, M.M. van Haelst.*

EAV and SMLAN designed the study, under supervision of HEKdW, JEHB and MMvH. EAV, SMLAN, MvG, JZ, SN and LC collected the data. EAV analyzed the data and drafted the manuscript. All authors contributed to the data interpretation, provided critical revisions of the manuscript and approved the final manuscript.

**Genetic care in geographically isolated small island communities: 8 years of experience in the Dutch Caribbean**

*E.A. Verberne, J.M. Westermann, T.I. de Vries, G.M. Ecury-Goossen, S.M. Lo-A-Njoe, M.E. Manshande, S. Faries, H.D. Veenhuis, P. Philippi, F.A. Falix, I. Rosina-Angelista, M. Ponson-Wever, L. Rafael-Croes, P. Thorsen, E. Arends, M. de Vroomen, S.Q. Nagelkerke, M. Tilanus, L.T. van der Veken, K. Huijsdens-van Amsterdam, A.M. van der Kevie-Kersemaekers, M. Alders M, M.M.A.M. Mannens, M.M. van Haelst.*

EAV and MMvH designed the study. EAV, JMW, and TIdV collected the data. LTvdV, KHvA, AMvdKK, and MA reviewed the results of genetic testing. EAV analyzed the data and drafted the manuscript. EAV, JMW, TIdV, GMEG, SMLAN, MEM, SF, HDV, PP, FAF, IRA, MPW, LRC, PT, EA, MdV, SQN, MT and MMvH performed the clinical evaluations of the patients. GMEG, MMAMM and MMvH supervised the project. All authors provided critical revisions of the manuscript and approved the final manuscript.

**Genetic diagnosis for rare diseases in the Dutch Caribbean: a qualitative study on the experiences and associated needs of parents**

*E.A. Verberne, L.M. van den Heuvel, M. Ponson-Wever, M. de Vroomen, M.E. Manshande, S. Faries, G.M. Ecury-Goossen, L. Henneman, M.M. van Haelst.*

EAV designed the study under supervision of LH and MMvH. The interviews were performed and transcribed by EAV. EAV and LMvdH analyzed the data under supervision of LH. EAV drafted the manuscript. All authors contributed to the data interpretation, provided critical revisions of the manuscript and approved the final manuscript.

**4H leukodystrophy caused by a homozygous *POLR3B* mutation: Further delineation of the phenotype**

*E.A. Verberne, L. Dalen Meurs, N.I. Wolf, M.M. van Haelst.*

EAV contributed to the concept, design and manuscript writing, under supervision of MMvH. LDM collected and interpreted patient data together with MMvH and contributed to the manuscript writing. NIW contributed to the interpretation of the patient data and helped supervising the project. All authors commented on the draft and approved the final manuscript.

**Expanding the phenotype of biallelic *RNPC3* variants associated with growth hormone deficiency**

*E.A. Verberne, S. Faries, M.M.A.M. Mannens, A.V. Postma, M.M. van Haelst.*

EAV contributed to the concept, design, interpretation of the data and manuscript writing, under the supervision of MMAMM and MMvH. SF was involved in the collection and interpretation of the clinical data. AVP contributed to the analysis and interpretation of the genetic data. All authors were involved in revising the manuscript and approved the final version.

**Limb anomalies, microcephaly, dysmorphic facial features and fibroma of the tongue after failed abortion with methotrexate and misoprostol**

*E.A. Verberne, M.E. Manshande, N.F. Wagner-Buitenweg, W. Elhage, H. Holtsema, M.M. van Haelst.*

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***JARID2* haploinsufficiency is associated with a clinically distinct neurodevelopmental syndrome**

*E.A. Verberne, S. Goh, J. England, M. van Ginkel, L. Rafael-Croes, S. Maas, A. Polstra, Y.A. Zarate, K.A. Bosanko, K.B. Pechter, E. Bedoukian, K. Izumi, A. Chaudhry, N.H. Robin, M. Boothe, N.C. Lippa, V. Aggarwal, D.C. De Vivo, A. Lehman, S. Stockler, A.L. Bruel, B. Isidor, J. Lemons, D.F. Rodriguez-Buritica, C.M. Richmond, Z. Stark, P.B. Agrawal, R.F. Kooy, M.E.C. Meuwissen, D.A. Koolen, R. Pfundt, A. Lieden, B.M. Anderlid, D. Glatz, M.M.A.M. Mannens, M. Bakshi, F.A. Mallette, M.M. van Haelst, P.M. Campeau.*

EAV, SG and JE contributed equally to this article. They designed the study, interpreted the data and drafted the manuscript, under the supervision of MMvH and PMC. All authors contributed to collection and interpretation of the clinical data. All authors provided critical revisions of the manuscript and approved the final manuscript.

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# PhD portfolio

Eline Verberne
PhD period 2018–2023
Supervisor: Prof. dr. Marcel Mannens
Co-supervisors: prof. dr. Mieke van Haelst, dr. Ginette Ecury

| 1. PhD training | Year | ECTS |
|---|---|---|
| **General courses** | | |
| - E-BROK | 2019 | 1.5 |
| - Practical Biostatistics (e-learning) | 2020 | 1.4 |
| - Teachers training VUmc | 2019 | 0.3 |
| **Specific courses** | | |
| - Genetic Epidemiology | 2020 | 1.1 |
| - Observational Epidemiology | 2020 | 0.6 |
| - Peer to Peer Group Coaching | 2021 | 0.5 |
| **Seminars, workshops and master classes** | | |
| - Young @ Heart Event, the Netherlands | 2018 | 0.3 |
| - Obesity and nutrition seminar, Aruba | 2018 | 0.1 |
| - Epilepsy seminar, Curaçao | 2018 | 0.1 |
| - Amsterdam Reproduction & Development (AR&D) Symposium | 2018 | 0.1 |
| - Science meeting Clinical Genetics Amsterdam UMC | 2019 | 0.3 |
| - Science meeting Clinical Genetics Amsterdam UMC | 2022 | 0.3 |
| **Presentations** | | |
| <u>Oral presentations</u> | | |
| - Caribbean Genetics – diagnosing syndromic disorders in diverse populations, LOG Dutch Clinical Genetics Society (VKGN) | 2020 | 0.5 |
| - Genetic service for birth defects at the Dutch Caribbean Islands, International Conference on Birth Defects and Disabilities in the Developing World, Sri Lanka | 2020 | 0.5 |
| - Episignatures, identification and diagnostic use (duo presentation), Science meeting Human Genetics Amsterdam UMC | 2022 | 0.5 |
| - Genetic care in geographically isolated small island communities: 8 years of experience in the Dutch Caribbean, Dutch Caribbean Research Week (online) | 2022 | 0.5 |
| <u>Poster presentations</u> | | |
| - Introducing Next Generation Sequencing in Curaçao: results of the first 11 intellectual disability (ID) panels (poster), Dutch Society for Human Genetics (NVHG) Annual Symposium | 2018 | 0.5 |
| - Genetic service for birth defects at the Dutch Caribbean Islands (poster), Dutch Society for Human Genetics (NVHG) & Belgian Society for Human Genetics (BeSHG) Annual Symposium | 2019 | 0.5 |

| 1. PhD training | Year | ECTS |
|---|---|---|
| **(Inter)national conferences** | | |
| - Dutch Society for Human Genetics (NVHG) Annual Symposium | 2018 | 0.6 |
| - Dutch Society for Human Genetics (NVHG) & Belgian Society for Human Genetics (BeSHG) Annual Symposium | 2019 | 0.6 |
| - LOG Dutch Clinical Genetics Society (VKGN) | 2020 | 0.3 |
| - International Conference on Birth Defects and Disabilities in the Developing World, Sri Lanka | 2020 | 1.0 |
| - Dutch Society for Human Genetics (NVHG) Annual Symposium (online) | 2020 | 0.3 |
| - LOG-LOD Dutch Clinical Genetics Society (VKGN) (online) | 2020 | 0.3 |
| - LOG Dutch Clinical Genetics Society (VKGN) | 2023 | 0.3 |
| **Other** | | |
| - Amsterdam Reproduction and Development (AR&D) Retreat | 2019 | 2.0 |
| - Patient care: outpatient clinical genetics clinics in the Dutch Caribbean (2x/year) | 2018–2019 | - |

| 2. Teaching | Year | ECTS |
|---|---|---|
| **Lecturing** | | |
| - Lectures on several clinical genetics topics for pediatricians and pediatric residents in Curaçao | 2018 | 0.5 |
| - Lecture on genetic testing for general practitioners and midwifes | 2018 | 0.2 |
| **Tutoring, Mentoring** | | |
| - Tutorship Bachelor Medicine (year 3) VUmc | 2019 | 2.2 |
| **Supervising** | | |
| - Supervision research student (Bachelor Medicine): *Birth prevalence of congenital heart defects in Aruba and Curaçao.* | 2018–2019 | 0.8 |
| - Supervision research student (Bachelor Medicine): *Birth prevalence of congenital anomalies in Curaçao.* | 2019 | 0.6 |
| - Supervision research student (Master Medicine): *Birth prevalence of congenital anomalies in Aruba.* | 2019–2020 | 1.0 |
| - Supervision research student (Bachelor Medicine): *Interstitial 3q24q26 deletions: four novel cases and further delineation of the genotype and phenotype.* | 2020–2021 | 0.6 |
| - Supervision research student (Bachelor Medicine): *Thrombocytopenia-absent radius (TAR) syndrome in the Dutch Caribbean: a case report and review of literature.* | 2020–2021 | 0.6 |

| 3. Parameters of Esteem | Year |
|---|---|
| **Grants** | |
| Simonsfonds travel grant | 2019 |
| Simonsfonds conference visit | 2020 |

## About the author

Eline Anne Verberne was born on the 5th of September 1992 in Amersfoort, the Netherlands. In 2010, she graduated from secondary school at the St.-Willibrord Gymnasium in Deurne (cum laude). In the same year she started her medical study at the UMC Utrecht. She attended internships in Nicaragua and Spain and followed the extracurricular Master Honors Program. She did an elective internship at the clinical genetics department at the VUmc, where prof. dr. Mieke van Haelst was her supervisor. After graduating medical school in 2017, she worked as a resident not in training at the obstetrics and gynecology department at the Spaarne Gasthuis in Hoofddorp. In 2018, she started her PhD study at the clinical genetics department at the Amsterdam University Medical Centers under supervision of prof. dr. Mieke van Haelst, prof. dr. Marcel Mannens and dr. Ginette Ecury. Her research focused on clinical and psychological impact of genetic services in the Dutch Caribbean and identification of genetic causes of congenital anomalies, which resulted in this thesis. She contributed to optimization of the outpatient genetics clinics in the Dutch Caribbean. She currently works as a resident not in training at the clinical genetics department at the UMC Utrecht.

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