From genotype to phenotype: clinical syndrome delineation in the era of genomics

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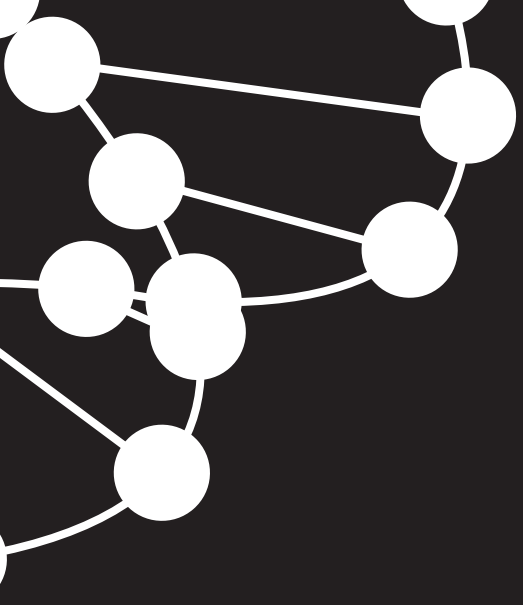
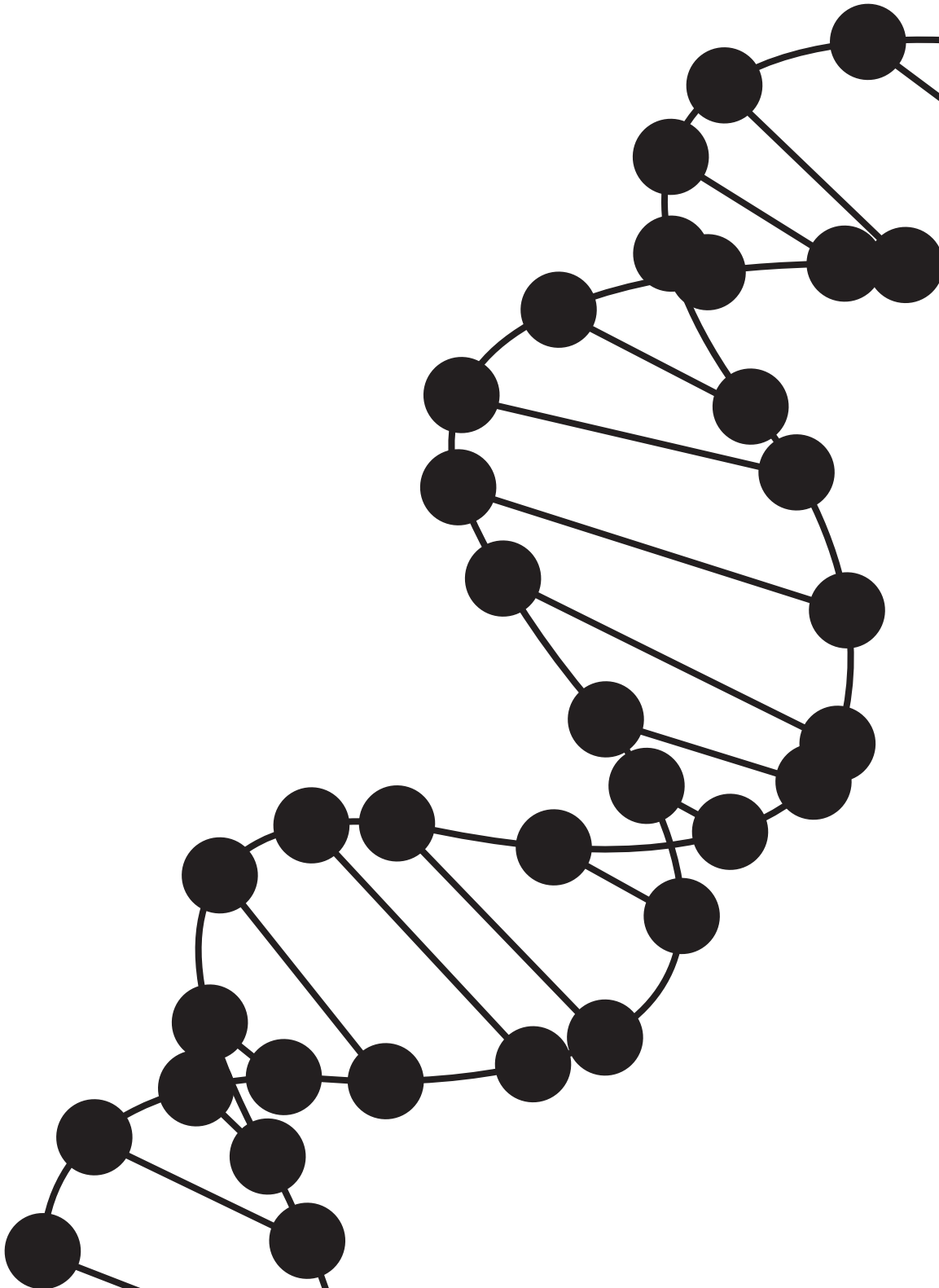
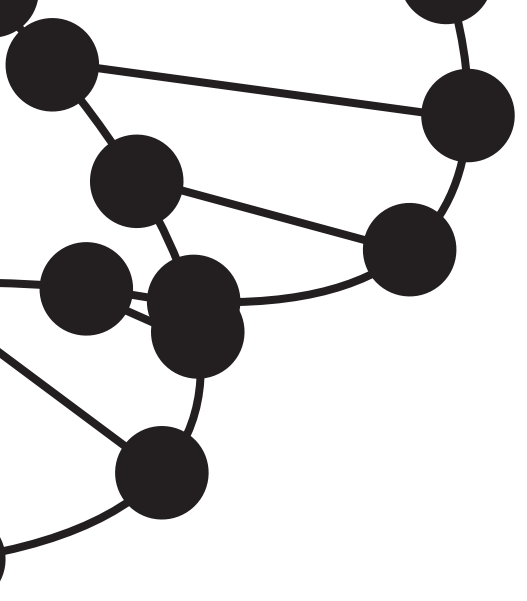


Chapter 1

General introduction, aims and outline of this thesis





Chapter 1.1

General introduction

Genomics driven diagnosis: a new era

Next generation sequencing (NGS) has altered genetic practice in a revolutionary way, by enabling rapid genome wide sequencing to identify a single gene defect for a fraction of the costs of traditional Sanger sequencing.[1-4]

In 2003, 50 years after the description of the chemical structure of DNA by Watson and Crick and 13 years after its initiation, the Human Genome Project was successfully completed: scientists had succeeded in completing our full DNA-structure, the human genome sequence.[5,6]

Within five subsequent years, the introduction of next generation sequencing facilitated DNA-sequencing of the complete genome of an individual by massively parallel DNA sequencing in just two months.[7]

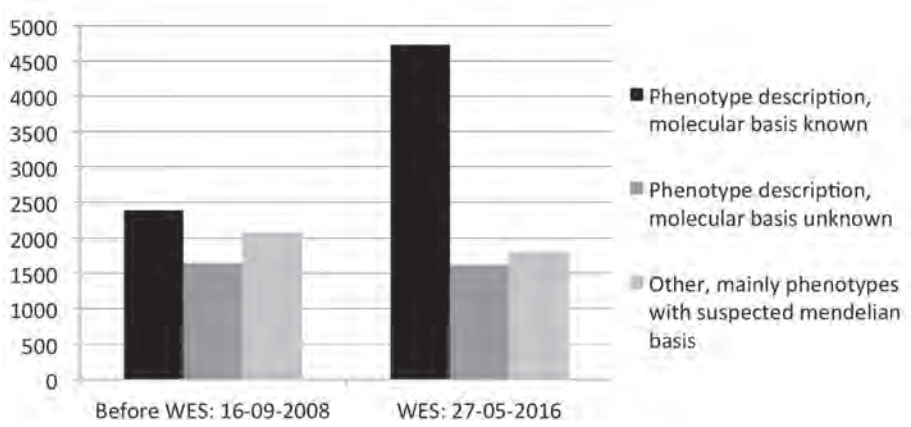
The development of next generation sequencing brought about a staggering cost reduction: the Human Genome Project cost 2.7 billion dollars, while the first massively parallel sequenced genome of a single individual cost 1.5 million dollars. However, since these costs were still high and only ~1-2% of our genome encodes for proteins, researchers developed 'whole exome sequencing' (WES): a technique for targeted sequencing of all protein-coding exons of the genome of one individual through massively parallel sequencing in a single test.[8]

In 2015, an individual genome could be sequenced for approximately 1750 dollars, bringing the targeted price of 1000 dollar per genome within reach. However, even though the costs of the actual sequencing have been reduced, the costs of the sequencing facilities and storage and analysis of data are still relatively high, hampering its unrestricted worldwide use in a diagnostic setting.[9] Still, in prosperous countries, whole exome sequencing is slowly replacing Sanger sequencing and chromosomal micro-arrays as a first tier diagnostic tool.

The use of whole exome sequencing has led to the identification of the underlying molecular cause of both well known and newly established syndromes.[10-13] This is reflected in the database of Online Mendelian Inheritance in Man (OMIM), where the number of phenotype descriptions with a known molecular basis has almost doubled from 2385 in September 2008 (just before the introduction of whole exome sequencing) to 4734 in May 2016, whilst the number of phenotype descriptions with an unknown molecular basis has remained relatively stable (1643 to 1622) and the number of other entries, mainly phenotypes with a suspected Mendelian basis has decreased only slightly (2080 to 1805) (Figure 1).[14]

Figure 1. The number of Entries in the database of Online Mendelian Inheritance in Man (OMIM).[14]

The number of phenotype descriptions with a known molecular basis has increased from 2385 in September 2008 (a few months before the introduction of whole exome sequencing in 2009) to 4734 in May 2016. The number of phenotype descriptions or loci without a known molecular basis though, remains relatively stable (1643 in September 2008 and 1622 in May 2016) and the number of other entries, mainly phenotypes with a suspected Mendelian base, has only slightly decreased (2080 in September 2008 to 1805 in May 2016). Possible explanations are that these phenotypes are not caused by an underlying (mono)genetic defect or that the genetic defect could not be detected by the current molecular techniques thus far. However, these numbers also include newly described phenotypes and new possible genetic abnormalities.



The introduction of whole exome sequencing subsequently brought about a new era of genomics driven diagnosis: the use of whole exome sequencing in a diagnostic setting has led to an expansion of the diagnostic range in genetics, providing patients with a diagnosis more often and in a shorter period of time. Before genome wide sequencing techniques were implemented in genetic practice, patients were often subjected to extensive and invasive investigations over the course of several years. After the introduction of whole exome sequencing, the duration of the diagnostic process is reduced and unnecessary investigations can be avoided, thereby reducing the diagnostic burden for patients as well as the financial burden for society.[15] Furthermore, an early diagnosis provides parents more often with reproductive options such as prenatal diagnosis (PND) or preimplantation genetic diagnosis (PGD).

Moreover, the unearthing of the genetic defect has led to increased knowledge of the phenotypic spectrum of many syndromes and has enabled fundamental research (e.g. through animal studies) to gain insights into the function of these genes and their role in developmental pathways.[11,16-19]

The specialty of clinical genetics and the role of clinical genetic phenotyping

What is the role of clinical genetics as a primary specialty in this new era of genomics driven diagnosis? Do we still need clinical geneticists?[20,21]

The UK Clinical Genetics Society states on their website (www.clingensoc.org/whatisclinicalgenetics):

'Clinical Genetics is the medical specialty which provides a diagnostic service and 'genetic counselling' for individuals or families with, or at risk of, conditions which may have a genetic basis. Genetic disorders can affect any body system and any age group. The aim of Genetic Services is to help those affected by, or at risk of, a genetic disorder to live and reproduce as normally as possible. Genetic disorders include:

- *Chromosomal abnormalities, which cause birth defects, mental retardation and/or reproductive problems.*
- *Single gene disorders such as cystic fibrosis, muscular dystrophy, Huntington's disease and sickle cell disease.*
- *Familial cancer and cancer-prone syndromes such as inherited breast or colorectal cancer and neurofibromatosis.*
- *Birth defects with a genetic component such as neural tube defects and cleft lip and palate.*

In addition, a large number of individuals with birth defects and/or learning disabilities are referred and investigated for genetic factors. Individuals identified through childhood or pregnancy screening programmes also require genetic services. In the future, as the genetic contributions to common later-onset disorders such as diabetes and coronary heart disease are identified, genetic services may be required for those at high risk. Testing for genetic factors that affect drug prescribing will also increasingly become an important activity.'

Clinical geneticists specialize in characterizing and distinguishing many rare genetic disorders. They aim to establish a clinical and molecular diagnosis in a patient, ensure proper clinical management and appropriate surveillance, resulting in earlier detection and treatment of associated disease manifestations. Furthermore, they inform patients and their parents about the prognosis, risks for other family-members, recurrence-risk in future pregnancies and reproductive options.

'Phenotyping' plays a key role in clinical genetics. In the Oxford Desk Reference for Clinical Genetics, phenotype is defined as: '*the appearance or other characteristics of the organism, resulting from the interaction of its genetic constitution with the environment*'.[22]

Phenotyping in clinical genetics encompasses an assessment and description of the clinical characteristics of a patient through taking a medical history, family history and by performing a physical examination and, if necessary, additional clinical investigations (e.g. X-rays or ophthalmologic examination). Seemingly unimportant phenotypic details can be the clue to a specific diagnosis (e.g. hypoplastic or absent fifth nails of the fingers and toes in Coffin-Siris syndrome, or large upper central incisors in KBG syndrome (KBG are the initials of the surnames of the first patients described)[23,24]), so careful and thorough phenotyping is essential.

Clinical genetic practice in a historical perspective

The basis of clinical genetic practice and clinical genetic phenotyping was created in the nineteenth century, when Georg Mendel presented his work on what is now referred to as 'Mendelian inheritance' (1865; he described dominant and recessive inheritance, independent inheritance of separate genes and the passing of one randomly selected allele of a pair of alleles from each parent to their offspring) and Langdon Down identified Down's syndrome as a clinically distinct entity, based on overlapping facial features that were present in several patients with intellectual disability (ID; 1866).[25,26] Until then, no clear distinction was made between separate syndromes with ID, but afterwards, various other syndromes that involve ID (e.g. Tuberous Sclerosis Complex in 1880)[27] were categorized.

A similar development was seen in syndromes with short stature, where achondroplasia was identified as a separate clinical entity in several patients in 1878, based on the presence of a disproportionate short stature with rhizomelic shortening of limbs, short trident hands, lumbar lordosis, an enlarged head and a depressed nasal bridge.[28] This instigated the identification and description of numerous new syndromes with short stature (e.g. Morquio syndrome, now known as mucopolysaccharidosis type IV).[29]

At that time, every diagnosis was made clinically, since diagnostic cytogenetic, molecular genetic and biochemical techniques were not yet available.

Changes in clinical genetic practice were induced by the development of diagnostic cytogenetic, molecular genetic and biochemical techniques over the course of the nineteenth and twentieth century.

The evolution and widespread introduction of these techniques was complex and only slowly gained pace. It was predated by the first description of cells by Robert Hooke in 1665 and nuclei ('globules') by Antoni van Leeuwenhoek in 1674.[30,31] Their work eventually led to the extraction of DNA from white blood cells by Johann Friedrich Miescher in

1869, the description of chromosomes and the principle of mitosis in 1878 by Walther Flemming, the formation of the chromosome theory of heredity by Theodor Boveri and Walter Sutton in 1902, the introduction of the terms 'gene', 'genotype' and 'phenotype' by Wilhelm Johanssen between 1903 and 1909, the discovery that chromosomes carry genes by Thomas Hunt Morgan and the elucidation of the structure of DNA in 1953 by James Watson, Rosalind Franklin and Francis Crick.[5,32-39]

Genetic testing became a reality after the elucidation that the human karyotype includes 46 chromosomes in 1956 and the discovery that Down's syndrome is caused by an extra chromosome 21 in 1959.[40,41] Subsequent discoveries broadened the scope of genetic investigations: the development of in situ hybridization in 1969, chromosome banding in 1970, Sanger sequencing in 1977 and polymerase chain reaction (PCR) in 1985.[42-45] Genome wide microarray based studies (in which microscopic DNA spots are attached to a chip in order to genotype multiple regions of a genome or to measure expression levels of various genes) were the first high throughput techniques in 1995.[46,47]

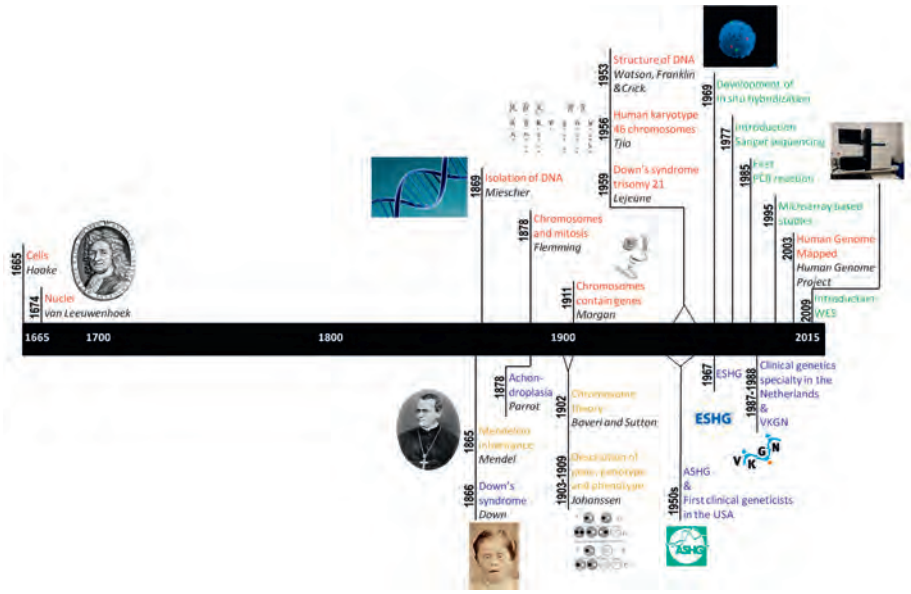
The development of cytogenetics as the first diagnostic tool coincided with the establishment of clinical genetics as a separate specialty in medicine. Before clinical genetics evolved into a separate medical specialty, pediatric departments were leading in the clinical description of genetic disorders, syndromes and malformations. In the 1950s, the first medical doctors started to work as clinical geneticists and founded the American Society of Human Genetics (ASHG; www.ashg.org). Among these pioneers were famous clinical geneticists, such as Robert J. Gorlin (a dentist and a father of syndromology), Victor A. McKusick (a cardiologist and the author of the Mendelian Inheritance in Man (MIM), a catalogue of human genes and genetic disorders)[48] and David W. Smith (a pediatrician). In Europe, Jan Mohr (a geneticist) founded the European Society of Human Genetics (ESHG; www.eshg.org) in 1967. In the Netherlands, clinical genetics was only officially recognized as a separate medical specialty in 1987, followed by the foundation of the Vereniging Klinische Genetica Nederland (VKGN; www.vkgn.org) in 1988.

A timeline of the history of genetics with an overview of the discoveries and developments of cyto-, molecular and clinical genetics is provided in Figure 2.

Figure 2. Timeline of the field of Genetics in a historic perspective.[5-8,25,26,28,30-42,44-47]

The discoveries and developments in the fields of cytogenetics and molecular genetics are listed above the black timeline, those in the field of clinical genetics are listed below the black timeline.

Important discoveries in molecular biology are represented in red, cytogenetic and molecular techniques in green. Important genetic theories are represented in brown and developments in clinical genetics in blues.



Clinical genetic phenotyping: developments over time

Before the introduction of genetic diagnostic laboratory techniques, a patient was clinically diagnosed with a syndrome when a specific combination of clinical features could be recognized. This was most evident in the field of dysmorphology (a term first introduced by David W. Smith in 1966).[49] Dysmorphology is a clinical genetic subspecialty that aims to diagnose patients with syndromes through the description of the morphology of body parts and the recognition of specific patterns of malformations. When Sanger sequencing was introduced, targeted molecular genetic testing ensued, confirming or discarding the clinical diagnosis/suspicion. However, physicians were often unable to establish a definitive diagnosis, since many syndromes are difficult to recognize clinically and can show considerable phenotypic variability and overlap with other syndromes. Also, the molecular cause of countless syndromes was unknown and the vagaries of clinical presentation of disorders resulted in misdiagnosis.

In many syndromes, the true phenotypic spectrum was only established after the identification of the molecular cause. This was the case for FG syndrome (FGS; FG

are the initials of the surnames of the first patients described).[50] In 2007, mutations in the X-linked gene *MED12* were identified as a cause of FGS. Around the same time, mutations in *FLNA*, *BRWD3* and *UPF3B* were associated with phenotypes overlapping with FGS.[51-54] Many patients previously diagnosed with FGS, however, did not have mutations in *MED12*. In one particular study, only one out of thirty clinically diagnosed patients had a mutation in this gene.[55] This patient exhibited the typical features associated with this mutation. No mutations in the *MED12* or *FLNA* genes could be detected in the other 29 patients (DNA analysis of the *BRWD3* and *UPF3B* genes was not performed). Only three of the 29 patients without mutations were thought to have clinical features falling within the FGS phenotypic spectrum. The phenotype of the remaining 26 varied widely. In seven of these patients an alternative diagnosis (ATRX syndrome, Rett syndrome and submicroscopic chromosomal aberrations) could subsequently be made. Clearly, the absence of molecular verification had allowed patients with a wide range of phenotypes to be erroneously grouped under a single unifying clinical diagnosis. After the elucidation of the underlying molecular defect, the phenotypic spectrum of FG syndrome was narrowed considerably.[56]

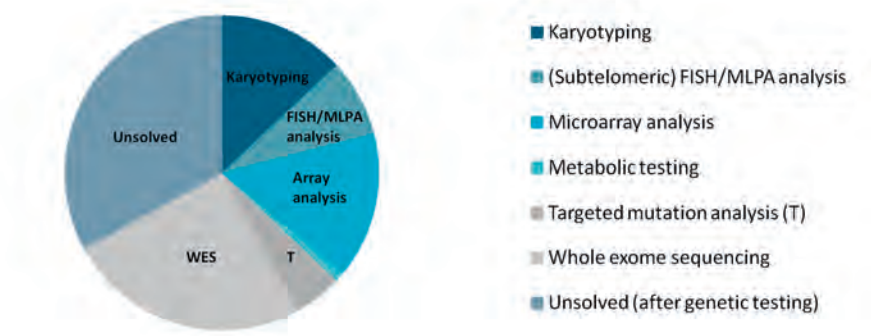
The development of genome wide techniques, such as microarrays and especially exome sequencing, has not only led to the discovery of the molecular origin of known syndromes and subsequent delineation of the phenotype of these syndromes, but also to the recognition of many new syndromes. These syndromes and their phenotypic spectrum could be distinguished only after the identification of their molecular cause. An example of such a syndrome is Koolen-de Vries syndrome, caused by a recurrent microdeletion of chromosome 17q21.31 or by a mutation in the *KANSL1* gene.[57-61]

Clinical genetic practice in the era of genomics: next generation phenotyping

The widespread use of whole exome sequencing has dramatically increased the diagnostic yield of genetic investigations in Mendelian disorders by approximately 25%, which is most apparent in the field of ID (Figure 3).[62,63] This means that the accuracy of the diagnostic process has roughly doubled by adding just this single test.

The introduction of new, genome-wide genetic techniques has given rise to the term 'next generation phenotyping', a process in which thorough clinical phenotyping remains of the utmost importance to make a correct diagnosis in patients with genetic disorders.[64]

Figure 3. Diagnostic yield of different cytogenetic and molecular techniques in intellectual disability (in percentages).[62,99-111]



Whole exome sequencing in ID, for instance, reveals from 0 to 7 (average 1.5) non-synonymous *de novo* mutations per person.[12] Careful clinical phenotyping is essential to interpret these results and determine which molecular finding(s) is (are) causative by comparing the phenotypic features of the patient to those of other humans (or animals) with mutations in the same gene.

Furthermore, many variants identified have not previously been detected in any laboratory or described in literature. It is impossible to diagnose a new syndrome in one individual with a mutation in a novel gene. An accurate description of the phenotype will contribute to matchmaking efforts in order to recognize other individuals with an overlapping phenotype and a mutation in the same gene and subsequently establish a new syndrome. Moreover, whole exome sequencing in two patients with the same clinical phenotype can be of great use in finding the molecular cause of this phenotype, by focusing on overlapping mutations in a single gene.

As phenotyping-experts, clinical geneticists continue to play a key role in the process of next generation phenotyping: dissecting the genetic, epigenetic and environmental influences that cause a phenotype necessitates 'ongoing and detailed phenotyping, refinement of clinical diagnostic assignments and iterative analyses of NGS data'.[64]

Genotype-phenotype studies

Genotype-phenotype studies remain essential in this era of next generation phenotyping, where the number of (rare) disorders with a known molecular genetic basis increases on a daily basis. For these studies, expert detailed phenotypic descriptions of patients are crucial.

Genotype-phenotype studies help us learn more about genetic disorders, through:

1. Delineation of the full phenotypic spectrum and prognosis of disorders. This knowledge enables us to provide tailor-made management advice for specific disorders.
2. Investigating the presence of clinical heterogeneity in patients with mutations in the same gene.

   Different types of mutations and mutations at different locations in the same gene can cause clinically separate disorders, as is the case in *FGFR3*-related syndromes, where specific mutations cause disorders such as achondroplasia, hypochondroplasia, thanatophoric dysplasia, SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans), Crouzon syndrome with acanthosis nigricans and Muenke syndrome.[65-71]
3. Determining whether variability in clinical expression of a disorder can be related to specific mutations and their effects on protein formation.

   This has been shown in Duchenne and Becker muscular dystrophy, for instance.[72,73] The more severe phenotype of Duchenne muscular dystrophy is caused by pathogenic mutations in the *DMD* gene that typically disrupt the reading frame, leading to the production of a truncated dystrophin protein that is degraded. However, when some functional, but shortened dystrophin is left, patients develop the milder Becker muscular dystrophy. Becker patients generally have in-frame mutations in the *DMD* gene.
4. Establishing whether patients with a clinical diagnosis, but without a molecular diagnosis, really share a significant overlap in phenotypic features compared to patients in whom the diagnosis was molecularly confirmed. If this is not the case, the clinical diagnosis in these patients may be incorrect and other diagnoses (supported by additional genetic testing), should be (re-)considered.

   When the phenotype of a patient without a molecular diagnosis does significantly overlap with the original diagnosis, this suggests genetic heterogeneity. One of the well-known genetically heterogeneous disorders is Noonan syndrome, in which mutations in several genes cause the same or a highly similar disorder. Noonan syndrome can be caused by mutations in *PTPN11*, *SOS1*, *RAF1*, *KRAS*, *NRAS*, *BRAF*, *MAP2K1*, *RIT1*, SHOC2 and *CBL* and exhibits numerous overlapping phenotypic features with Noonan syndrome with multiple lentigines, Costello syndrome, cardio-facio-cutaneous syndrome, neurofibromatosis type 1, capillary malformation-arteriovenous malformation syndrome and Legius syndrome.[74-85] Together, these disorders constitute a group of clinically well described genetic disorders called 'Rasopathies' caused by mutations in genes encoding components of regulators of the RAS/MAPK signal transduction pathway.[86]

5. Determining which specific phenotypic features or patterns predict involvement of a specific syndrome of gene. For instance, the knowledge that certain specific features of that syndrome are only seen in patients with mutations in gene A and not in patients with mutations in gene B, can prompt the physician to perform targeted mutation analysis of gene A. This has been extensively studied in Noonan syndrome as well, where it is shown that patients with pulmonary stenosis most likely have a mutation in *PTPN11* whereas hypertrophic cardiomyopathy points to involvement of other Noonan genes.[74,87] This is important, since, at this moment, exome sequencing is still more expensive than Sanger sequencing of a single gene and exome sequencing is not yet available for everyone.

The expertise of clinical geneticists is a fundamental part of next-generation phenotyping in a time where the number of syndromes with a known molecular cause rapidly expands: in order to improve clinical patient care and enable genetic counselling, clinical geneticists distinguish and characterize individually rare syndromes and select homogeneous groups of patients for studies of the basic molecular genetic defect and pathophysiology of syndromes.[88]

Next-generation phenotyping of genetic disorders with a short stature

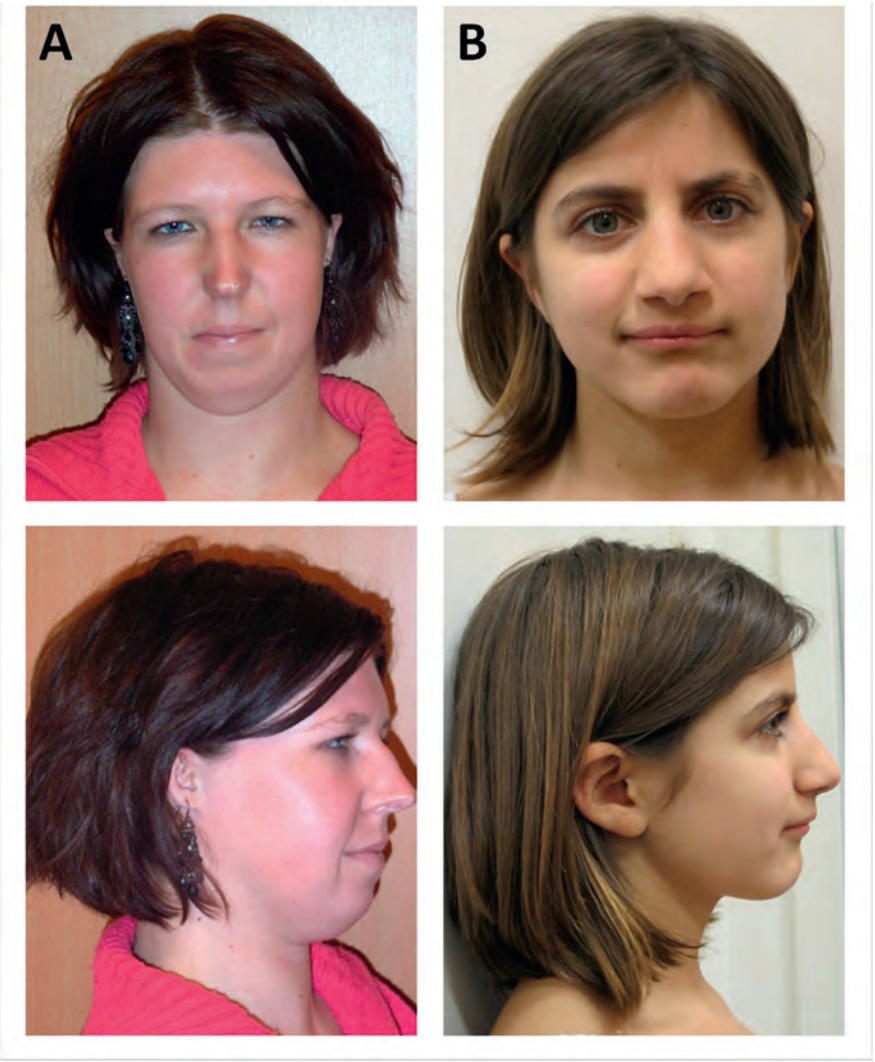
Next-generation phenotyping was performed in our cohorts of patients with (disorders resembling) Meier-Gorlin syndrome and Floating-Harbor syndrome, two rare syndromes with short stature in which the underlying molecular causes were identified by whole exome sequencing recently.

Meier-Gorlin syndrome (MGS) is a rare autosomal recessive disorder characterized by microtia, patellar a-/hypoplasia and pre- and postnatal growth retardation, first described by Meier *et al*. 56 years ago.[89] Associated features include specific facial features (a small mouth with full lips and micrognathia in infants, a narrow nose with broad nasal bridge in adults (Figure 4)), congenital pulmonary emphysema, feeding problems, mammary hypoplasia, genitourinary and skeletal anomalies.

Over the past 15 years, several patients with MGS were seen in the Radboud University Medical Centre Nijmegen in the Netherlands and clinical data and DNA of these patients and patients from Canada, France, India, New-Zealand, Saudi-Arabia, the UK and the USA were collected. In collaboration with the University of Edinburgh Western General Hospital in Scotland, biallelic mutations in *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6* were identified as the underlying genetic cause of MGS in 2011.[90,91] Simultaneously, Guernsey *et al*. identified *ORC4*, *ORC1* and *CDT1* mutations in eight MGS patients.[92] These genes encode proteins of the pre-replication complex, essential for the initiation of DNA replication.[93,94]

Floating-Harbor syndrome (FHS) was first described in 1973.[95] It is a rare autosomal dominant disorder defined by growth retardation, expressive language delays and distinctive facial features (a triangular shaped face, deep set eyes with long eye lashes, a narrow nose with broad nasal tip, a low columella, short philtrum and thin upper lip with everted lower lip) (Figure 4).

Figure 4. Facial features (frontal and lateral view) of two patients with Meier-Gorlin syndrome and Floating-Harbor syndrome. (**A**) Note microtia, the narrow nose with small profile, the small mouth and retrognathia in the individual with Meier-Gorlin syndrome. **(B)** In the individual with Floating-Harbor syndrome, deep-set eyes, a narrow root of the nose with a broad base, a short philtrum and low-set ears can be appreciated.



As part of the AnEUploidy project, the clinical data and DNA of 15 patients suspected to have FHS were collected over a time period of more than 10 years.

In 2011, we saw three additional patients who had been diagnosed clinically with FHS and received the medical data and DNA of two other patients by collaboration with clinical geneticists from Rotterdam, the Netherlands and Bilbao, Spain. That made for a total of 20 patients with a clinical diagnosis/suspicion of FHS. In 2012, monoallelic mutations in *SRCAP* were detected as the underlying cause of FHS.[96] *SRCAP* encodes a SNF2-related chromatin-remodeling ATPase, a coactivator for CREB-binding protein.[97,98]

We were able to perform molecular analysis of the *SRCAP* gene in our patients afterwards and identified *de novo SRCAP* mutations in seven patients out of our cohort of 20 patients.

Only two of the five patients we saw in 2011 could be diagnosed with FHS, based on the presence of an *SRCAP* mutation. In the third patient, we performed whole exome sequencing and we identified a *de novo* nonsense mutation in the *ZBTB18* gene.

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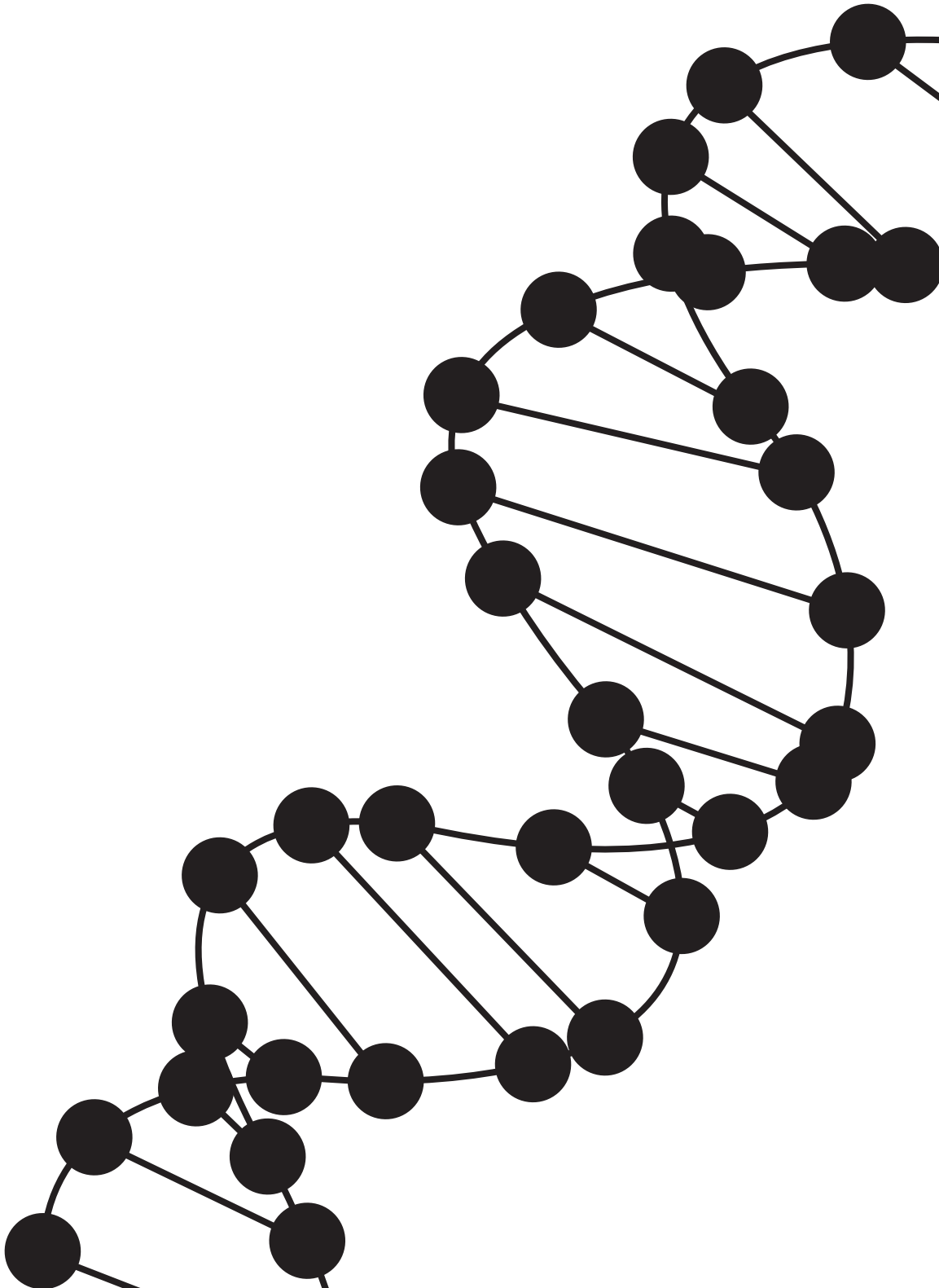
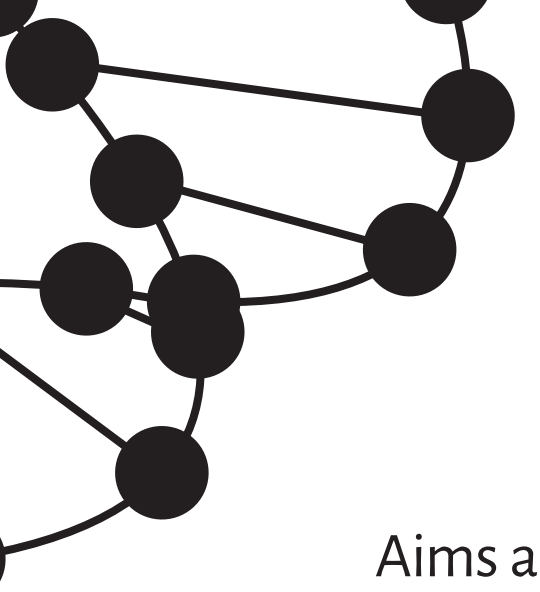




Chapter 1.2.

Aims and outline of this thesis

The objective of this thesis was to improve clinical care and management for patients with MGS and FHS syndrome by increasing insight into the phenotypic and molecular genetic characteristics of these syndromes. Furthermore, we aimed to unravel new genetic syndromes in patients with phenotypes similar to MGS or FHS syndrome.

First, we performed genotype-phenotype studies in patients with MGS and FHS syndrome, to further delineate the phenotype of these syndromes, enabling us to propose specific management advice for follow-up of patients with these syndromes.

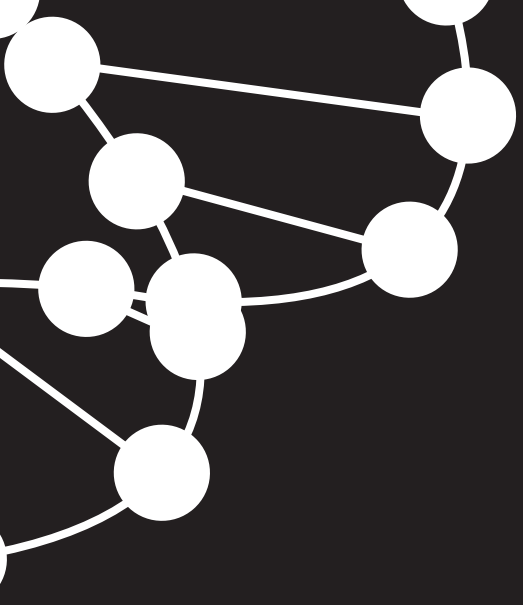
Secondly, we contemplated to identify new molecular defects by whole exome sequencing in patients with a phenotype resembling MGS or FHS, but in whom no molecular diagnosis could be made thus far. We aimed to discover new genetic syndromes, increase the diagnostic yield in our patients and subsequently improve management of these patients.

We established genotype-phenotype correlations in MGS, expanded the phenotype of MGS by providing an overview of the clinical problems of these patients (**Chapters 2.1 and 2.3**) and studying growth (**Chapter 2.2**) and proposed experience based management advice for MGS (**Chapter 2.3**).

We further delineated the phenotype of patients with FHS syndrome and provided management advice for this disorder (**Chapter 3**).

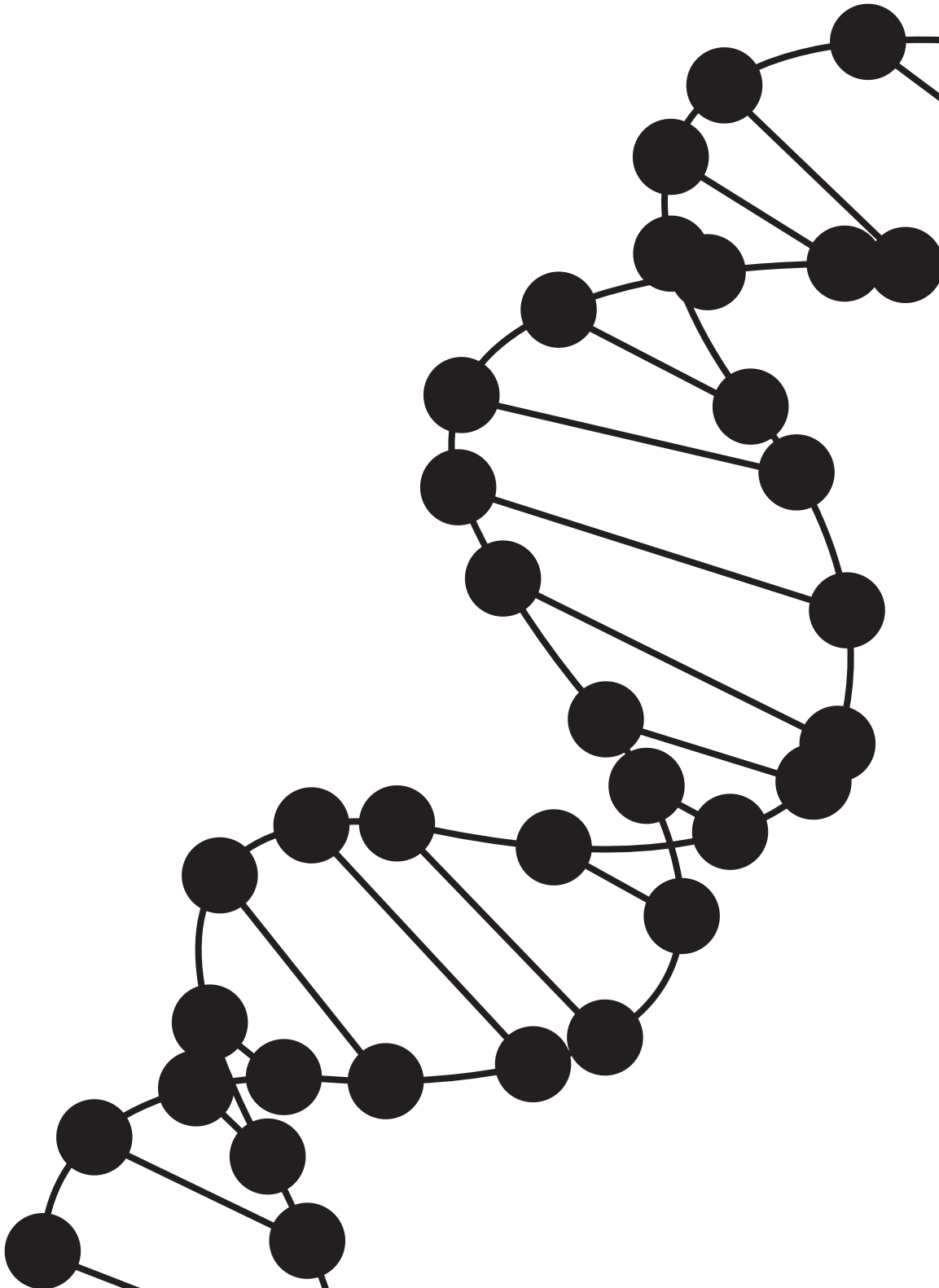
In **Chapter 4**, we described the first patient with a *de novo* nonsense mutation in *ZBTB18* and a phenotype resembling FHS without an *SRCAP* mutation. The *ZBTB18* gene is located in the chromosome 1q43q44 microdeletion syndrome region. We proposed that *ZBTB18* is a strong candidate gene for a part of the 1q43q44 microdeletion syndrome, which shows some clinical overlap with FHS.

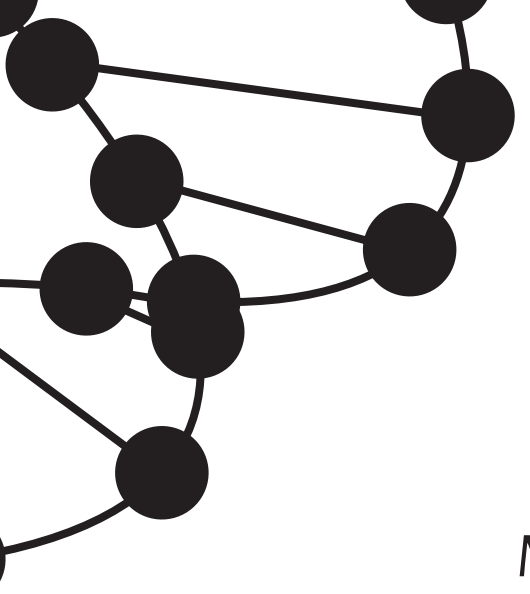
In this era of next generation sequencing, our studies highlight the importance of next generation phenotyping: establishing a correct clinical and molecular genetic diagnosis, perform genotype-phenotype studies to delineate the phenotypic spectrum of rare syndromes and unravel previously unrecognized, new genetic syndromes through whole exome sequencing.



Chapter 2

Meier-Gorlin syndrome





Chapter 2.1

Meier–Gorlin syndrome genotype–phenotype studies: 35 individuals with pre-replication complex gene mutations and 10 without molecular diagnosis

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Abstract

Meier–Gorlin syndrome (MGS) is an autosomal recessive disorder characterized by microtia, patellar aplasia/hypoplasia and short stature. Recently, mutations in five genes from the pre-replication complex (*ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*), crucial in cell-cycle progression and growth, were identified in individuals with MGS. Here, we report on genotype–phenotype studies in 45 individuals with MGS (27 females, 18 males; age 3 months–47 years). Thirty-five individuals had biallelic mutations in one of the five causative pre-replication genes. No homozygous or compound heterozygous null mutations were detected. In ten individuals, no definitive molecular diagnosis was made. The triad of microtia, absent/hypoplastic patellae and short stature was observed in 82% of individuals with MGS. Additional frequent clinical features were mammary hypoplasia (100%) and abnormal genitalia (42%; predominantly cryptorchidism and hypoplastic labia minora/majora). One individual with *ORC1* mutations only had short stature, emphasizing the highly variable clinical spectrum of MGS. Individuals with *ORC1* mutations had significantly shorter stature and smaller head circumferences than individuals from other gene categories. Furthermore, compared with homozygous missense mutations, compound heterozygous mutations appeared to have a more severe effect on phenotype, causing more severe growth retardation in *ORC4* and more frequently pulmonary emphysema in *CDT1*. A lethal phenotype was seen in four individuals with compound heterozygous *ORC1* and *CDT1* mutations. No other clear genotype–phenotype association was observed. Growth hormone and estrogen treatment may be of some benefit, respectively, to growth retardation and breast hypoplasia, though further studies in this patient group are needed.

Introduction

Meier–Gorlin syndrome (MGS; MIM #224690) is a form of primordial dwarfism, characterized by microtia, short stature and absent or hypoplastic patellae. Furthermore, pulmonary emphysema, feeding problems, various skeletal abnormalities, genitourinary anomalies and mammary hypoplasia frequently accompany this autosomal recessive disorder. Characteristic facial features, which gradually change with age, are frequently described. Infants typically have a small mouth with full lips and micrognathia, whereas in adults, a high forehead and a more prominent, narrow nose with a broad nasal bridge are distinguishable.

The first patient was reported by Meier in 1959.[1] Gorlin reported the second patient with a similar phenotype.[2] In total, only 53 cases have been described in the literature thus far.[1–21]

Recently, mutations in *ORC1*, a pre-replication complex member gene, were identified in 5 out of 204 individuals with microcephalic primordial dwarfism.[13]

As individuals with mutations in *ORC1* showed overlapping features with MGS, mutation analysis of *ORC1* was performed in 33 individuals with MGS and revealed mutations in four individuals from three families.[13] Mutation analysis of other genes of this pre-replication complex showed mutations in *ORC4*, *ORC6*, *CDT1* and *CDC6* in 14 individuals from nine families with MGS.[14] Simultaneously, beginning with a family-based mapping approach in individuals with MGS with a founder effect, Guernsey *et al*. identified mutations in *ORC1*, *ORC4* and *CDT1* in eight individuals from five families with MGS.[15] The pre-replication complex forms at origins of DNA replication and is essential to initiate genome replication.[22,23] The complex consists of the origin recognition complex (encompassing the subunits ORC1 to ORC6), two regulatory proteins (CDC6 and CDT1) and the putative helicase complex (minichromosome maintenance (MCM) proteins). The origin recognition complex is loaded onto the chromatin during M and G1 phases. Afterwards, other proteins (including CDT1 and CDC6) bind to the pre-replication complex, facilitating repeated loading of the MCM helicase. The MCM helicase unwinds DNA and recruits additional replication proteins at the beginning of the S phase, thereby initiating replication. Mutations in genes from the pre-replication complex are expected to disturb this process of DNA replication. *In vitro* studies using lymphoblastoid cell lines or skin fibroblast cell lines from two individuals with mutations in *ORC1* showed reduced levels of ORC1, ORC2 and MCM2 in chromatin-enriched cell fractions.[13] Furthermore, the S phase entry/progression in both individuals was delayed.

The five genes known to cause MGS are part of the same pre-replication complex. We posed the question whether mutations in the different genes from the pre-replication complex have a different effect on the MGS phenotype. To answer that question, we performed genotype–phenotype studies in 45 individuals with MGS, including all individuals with a molecular diagnosis of MGS thus far known.

Methods

Patients

Of the 45 individuals included in this study, 35 carried mutations in one of the five known genes for MGS. Ten individuals with a clinical diagnosis of MGS without a known molecular cause are described, including one individual carrying a monoallelic mutation in *ORC1* and two carrying a monoallelic mutation in *CDT1*. Molecular testing showed no abnormalities in five other individuals with a clinical diagnosis of MGS, but the authors had insufficient clinical information to include them in this study.

Thirty-five individuals were described previously in literature.[2–5,8,11,13–17,21] The remaining ten individuals were not yet described in literature. These ten individuals were referred to the Human Genetics departments of the Radboud University Nijmegen Medical Centre, the Netherlands and the Western General Hospital in Edinburgh, UK for molecular analysis from the Netherlands, the UK, Ireland and India. The medical history and most recent clinical data of all individuals were obtained by sending a questionnaire to the referring physicians or by physical examination by the authors. The growth data were converted to standard deviations from the mean (SDs), using two different growth charts for growth around birth and postnatal growth.[24,25] Intrauterine growth retardation was defined as weight for gestational age more than 1.3 SDs (10th centile) under the mean, postnatal growth retardation as height for age more than 2 SDs under the mean and microcephaly as head circumference for age more than 3 SDs under the mean.[26–28] Informed consent to perform molecular investigations and to publish the medical data and photographs of the patients was obtained.

Molecular data

Sequence analysis of the *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6* genes was performed on DNA isolated from peripheral blood from the affected children and the parents as previously described.[13–15] Copy number variation analysis of *ORC4* was performed in eight individuals.[15] In the individual with monoallelic mutations in *ORC1*, DNA

was screened for intragenic deletions/duplications of *ORC1*, using Multiplex ligation-dependent Probe Amplification.

Statistical analysis

Differences in birth weight, height and head circumference between the different gene categories of MGS (individuals with biallelic mutations in *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6* and individuals with a clinical diagnosis of MGS without a definitive molecular diagnosis) were analyzed using the Student's *t*-test. The significance level was set at the 5% probability level. Statistical analysis was performed using standard statistical software (SPSS version 16.0, Inc., Chicago, IL, USA).

Results

Patients and molecular data

The clinical data of all 45 individuals from 35 families with MGS are summarized in Table 1. Thirty-five individuals from 26 families had biallelic mutations in one of the five pre-replication genes underlying MGS. In ten individuals from nine families, no definitive underlying molecular cause could be identified.

Of the 35 individuals with mutations, 14 were male (40%), 21 were female (60%). The average age at most recent examination was 12 years and 9 months (ranging from 3 months to 47 years). Seventeen individuals (49%) had reached puberty or adulthood. Four individuals were deceased: two siblings with mutations in *ORC1*, of which one passed away at the age of 3 months with a severe cortical dysplasia, pachygyria and ventricular enlargement, cranial suture stenosis, congenital emphysema of the lung and absence of the pancreatic tail, in addition to the classical triad of MGS (microtia, patellar anomalies and short stature) (individual P1, Table 2).[14] His brother, deceased at 17 weeks of gestation, also exhibited microtia and severe growth retardation (individual P2, Table 2). The other two individuals were siblings with mutations in *CDT1* (I6 and I7 respectively, Table 2). One died after a sudden cardiac arrest and had congenital lobar emphysema for which he required surgery. His sister succumbed after 3 months of severe respiratory problems due to a tracheobronchomalacia with progressive pulmonary emphysema. Both showed clinical features of the classical triad of MGS.

Table 1. Clinical features of 35 individuals with Meier-Gorlin syndrome with biallelic mutations in one of the five pre-replication genes and ten individuals with a clinical diagnosis of Meier-Gorlin syndrome without a definitive molecular diagnosis, including one individual with a monoallelic mutation in *ORC1* and two with a monoallelic mutation in *CDT1*.

Clinical characteristics	*ORC1* (10 individuals)	*ORC4* (7 individuals)	*ORC6* (7 individuals)	*CDT1* (10 individuals)	*CDC6* (1 individual)	Total (35 individuals)	No definitive molecular diagnosis (10 individuals)
Sex ratio M/F	4 M/6 F	1 M/6 F	5 M/2 F	3 M/7 F	1 M/0 F	14 M/21 F	3 M/7 F
Average age at examination	13y 1m	13y 11m	8y 10m	12y 5m	15y 6m	12y 9m	11y 2m
Range age at examination	3m - 47y	5y - 23y	3y 10m – 15y 5m	4 days – 22y		3m – 47y	3y 4m – 28y 10m
Classical triad of clinical features							
Short stature (height for age <-2 SDs)	10/10	6/7	6/7	8/10	1/1	31/35 (89%)	9/10 (90%)
Microtia	9/10	7/7	7/7	10/10	1/1	34/35 (97%)	10/10 (100%)
Patellar hypoplasia/ aplasia	6/7	7/7	6/7	10/10	1/1	30/32 (94%)	9/10 (90%)
Growth (represented in mean SDs)							
Birth weight (SDs range)	-4,5 SDs (<-6.5 to -1.7)	-4.1 SDs (-6 to -2.3)	-3.2 SDs (-3.9 to -1.9)	-3 SDs (-3.9 to -0.3)	-4.1 SDs	-3.8 SDs (< -6.5 to -0.3)	-2.9 SDs (-4.4 to -2)
IUGR (birth weight <-1.3 SDs)	9/9	7/7	6/6	9/10	1/1	32/33 (97%)	10/10 (100%)
Height at examination (SDs range)	-7.1 SDs (-9.6 to -5.2)	-5.2 SDs (-6.4 to -1.8)	-2.5 SDs (-3.3 to -0.8)	-3.7 SDs (-6 to -0.4)	-3.5 SDs	-4.4 SDs (-9.6 to -0.4)	-3.3 SDs (-6.4 to -1.4)
Weight at examination (SDs range)	-6.2 SDs (-9.3 to 0.8)	-3.7 SDs (-5.3 to -1.7)	-3 SDs (-5 to -0.3)	-3.3 SDs (-5.5 to -1.4)	-3.2 SDs	-3.9 SDs (-9.3 to 0.8)	-4 SDs (-7.5 to -0.6)
Head circumference (SDs range)	-6.7 SDs (-9.8 to -4)	-2.5 SDs (-3.2 to -0.7)	-2.4 SDs (-3.3 to -1.6)	-1.3 SDs (-5 to 1.7)	-1.8 SDs	-2.9 SDs (-9.8 to 1.7)	-2.6 SDs (-5 to -1.3)
Microcephaly (OFC for age <-3 SDs)	8/8	2/5	1/6	2/9	0/1	13/29 (45%)	3/10 (30%)
Disproportionate stature	1/3	0/7	1/4	2/2	0/1	4/17 (24%)	3/9 (33%)

Table 1. Continued

Clinical characteristics	*ORC1* (10 individuals)	*ORC4* (7 individuals)	*ORC6* (7 individuals)	*CDT1* (10 individuals)	*CDC6* (1 individual)	Total (35 individuals)	No definitive molecular diagnosis (10 individuals)
Facial features							
Abnormally formed ears	6/7	2/5	3/7	7/9	1/1	19/29 (66%)	8/10 (80%)
Low-set ears	4/6	1/5	3/7	7/9	1/1	16/28 (57%)	8/10 (80%)
Posteriorly rotated ears	0/3	1/5	3/5	2/3	1/1	7/17 (41%)	6/10 (60%)
Convex nasal profile	2/3	0/2	4/4	0/4	1/1	7/14 (50%)	6/9 (67%)
Narrow nose	1/2	5/5	0/4	2/4	0/1	8/16 (50%)	4/10 (40%)
High nasal bridge	2/4	3/3	3/5	1/4	1/1	10/17 (59%)	7/10 (70%)
Microstomia	5/7	5/5	3/7	4/9	1/1	18/29 (62%)	9/10 (90%)
Full lips	7/8	2/5	5/7	6/9	1/1	21/30 (70%)	9/10 (90%)
Micro-/retrognathia	5/7	5/5	7/7	7/9	1/1	25/29 (86%)	10/10 (100%)
Downslanted palpebral fissures	1/3	0/3	5/6	2/4	1/1	9/17 (53%)	3/10 (30%)
Neurological							
Intellectual disability	1/8	0/7	0/7	0/8	0/1	1/31 (3%)	1/10 (10%)
Delayed motor development	1/8	1/7	3/7	1/9	0/1	6/32 (19%)	4/10 (40%)
Delayed speech development	2/8	1/7	2/7	0/9	0/1	5/32 (16%)	3/10 (30%)
Respiratory tract							
Respiratory problems during infancy	2/4	2/5	4/7	2/6	1/1	11/23 (48%)	5/10 (50%)
Pulmonary emphysema	3/5	1/7	0/5	7/10	1/1	12/28 (43%)	1/10 (10%)
Tracheomalacia	1/4	2/7	2/5	3/7	0/1	8/24 (33%)	3/10 (30%)
Laryngomalacia	2/4	1/7	1/5	2/7	0/1	6/24 (25%)	1/10 (10%)
Bronchomalacia	1/4	1/7	1/5	1/7	0/1	4/24 (17%)	1/10 (10%)

Table 1. Continued

Clinical characteristics	*ORC1* (10 individuals)	*ORC4* (7 individuals)	*ORC6* (7 individuals)	*CDT1* (10 individuals)	*CDC6* (1 individual)	Total (35 individuals)	No definitive molecular diagnosis (10 individuals)
Cardiac anomalies	0/9	1/7	0/5	1/8	0/1	2/30 (7%)	0/10 (0%)
Gastrointestinal							
Feeding problems during infancy	6/8	7/7	5/7	7/9	1/1	26/32 (81%)	10/10 (100%)
Nasogastric feeding/ Gastrostomy	4/8	3/6	2/7	2/9	0/1	11/31 (35%)	6/10 (60%)
Failure to thrive	2/8	1/6	3/7	4/9	1/1	11/31 (35%)	2/10 (20%)
Gastroesophageal Reflux	4/8	5/6	2/7	1/9	1/1	13/31 (42%)	2/10 (20%)
Urogenital anomalies							
Abnormal genitalia	2/10	3/7	6/7	3/10	1/1	15/35 (43%)	4/10 (40%)
Hypospadias	0/4	0/1	1/5	0/3	0/1	1/14 (7%)	0/3 (0%)
Cryptorchidism/small testes	2/4	0/1	4/5	2/3	1/1	9/14 (64%)	2/3 (67%)
Micropenis	1/4	0/1	0/5	0/3	1/1	2/14 (14%)	0/3 (0%)
Clitoromegaly	0/6	2/6	0/2	0/7	0/0	2/21 (10%)	1/7 (14%)
Hypoplastic labia minora/majora	0/6	3/6	1/2	1/7	0/0	5/21 (24%)	2/7 (29%)
Renal anomalies	1/10	0/7	0/7	2/10	0/1	3/35 (9%)	0/10 (0%)
Secondary sexual characteristics							
Mammary hypoplasia	2/2	4/4	1/1	3/3	0/0	10/10 (100%)	3/3 (100%)
Absent/sparse axillary hair	3/3	1/1	1/2	1/1	1/1	7/8 (88%)	2/4 (50%)
Absent/sparse pubic hair	0/1	0/1	1/2	0/1	0/1	1/6 (17%)	0/4 (0%)
Growth hormone treatment	2/7	5/7	1/7	1/3	1/1	10/25 (40%)	2/8 (25%)

Table 1. Continued

Clinical characteristics	*ORC1* (10 individuals)	*ORC4* (7 individuals)	*ORC6* (7 individuals)	*CDT1* (10 individuals)	*CDC6* (1 individual)	Total (35 individuals)	No definitive molecular diagnosis (10 individuals)
Musculoskeletal anomalies							
Delayed bone age	3/4	4/7	4/5	2/5	1/1	14/22 (64%)	5/8 (63%)
Genu recurvatum	4/6	2/7	0/7	3/7	0/1	9/28 (32%)	0/7 (0%)
Contractures/club feet	0/4	2/7	2/6	1/9	0/1	5/27 (19%)	1/10 (10%)
Other	1 Bifid uvula 2 Dislocated joints 1 Craniosynostosis	1 Hemivertebrae 1 Asymmetric limbs		1 Dislocated joints	1 Osteochondroma		1 Cleft palate 1 Bifid uvula 1 Craniosynostosis 1 Facial asymmetry 1 Spina bifida occulta 1 Choanal atresia 2 Polycystic ovaries 1 Dislocated joints

Table 2. Overview of mutations detected in one of the five pre-replication genes in individuals with Meier-Gorlin syndrome: biallelic mutations in 35 individuals, monoallelic mutations in three individuals.

Gene	Nucleotide alterations	Amino-acid alterations	Hetero-/ Homozygous	Putative effect	Number of individuals/ families	Individual reference number
ORC1	c.266T>A	p.Phe89Ser	Homozygous	Missense	1/1	1: P3[I]
	c.314G>A	p.Arg105Gln	Homozygous	Missense	1/1	1: P5
	[c.314G>A] + [c.1482-2A>G]	p.Arg105Gln + intron 9 splice acceptor site	Heterozygous	Missense + splice site	2/2	2: P3, P4[I]
	[c.314G>A] + [c.1999_2000delGTinsA]	p.Arg105Gln + p.Val667*fs*X24	Heterozygous	Missense + frameshift	2/1	2: P1, P2
	[c.314G>A] + [c.1996C>T]	p.Arg105Gln + p.Arg666Trp	Heterozygous	Missense	1/1	3: 1983[I]
	[c.314G>A] + [c.2159G>A]	p.Arg105Gln + p.Arg720Gln	Heterozygous	Missense	1/1	1: P4
	c.380A>G	p.Glu127Gly	Homozygous	Missense	2/1	1: P1, P2
	[c.1721C>T]***	p.Thr57Met	Monoallelic	Missense	1/1	I1 (new individual)[II]
ORC4	c.521A>G	p.Tyr174Cys	Homozygous	Missense	4/3	2: P6, P7[III] 3: 1652, 1768, 1769, 1899
	[c.521A>G] + [c.874_875insAACA]	p.Tyr174Cys + p.Ala292*fs*X19	Heterozygous	Missense + frameshift	2/2	2: P5; 3: 1939
	[c.521A>G] + CNV del	p.Tyr174Cys + del	Heterozygous	Missense + deletion	1/1	3: 1882
ORC6	[c.2T>C] + [c.449+5G>A]	p.Met1? + p.?	Heterozygous	Missense + splice site	4/3	I2, I3, I4, I5 (new individuals)
	[c.257_258delTT] + [c.695A>C]	p.Phe86X + p.Tyr232Ser	Heterozygous	Nonsense + missense	3/1	2: P8, P9, P10
CDT1	[c.196G>A] + [c.351G>C]	p.Ala66Thr + p.Gln117His (exon 2 splicing donor site)	Heterozygous	Missense + splice site	1/1	2: P12
	[c.351G>C] + [c.1385G>A]	p.Gln117His (exon 2 splicing donor site) + p.Arg462Gln	Heterozygous	Splice site + missense	1/1	2: P16
	[c832G>T] + [c.1385G>A]	pGlu278X + p.Arg462Gln	Heterozygous	Nonsense + missense	2/1	I6, I7 (new individuals)

Table 2. Continued

Gene	Nucleotide alterations	Amino-acid alterations	Hetero-/ Homozygous	Putative effect	Number of individuals/ families	Individual reference number
	[c.1081C>T] + [c.1357C>T]	p.Gln361X + p.Arg453Trp	Heterozygous	Nonsense + missense	1/1	2: P17
	[c.1385G>A] + [c1560C>A]	p.Arg462Gln + p.Tyr520X	Heterozygous	Missense + nonsense	4/2	2: P11, P13, P14, P15
	[c.1385G>A]***	p.Arg462Gln	Monoallelic	Missense	2/1	I8, I9[III]; Bongers *et al.*[3]
	c.1402G>A	p.Glu468Lys	Homozygous	Missense	1/1	3: 1627
CDC6	c.968C>G	p.Thr323Arg	Homozygous	Missense	1/1	2: P18

1[I] = Bicknell *et al.*[13]
2[I] = Bicknell *et al.*[14]
3[I] = Guernsey *et al.*[15]
[II]Individual I1 carries only one mutation in *ORC1*; Individuals I8 and I9 carry only one mutation in *CDT1*.
[III]P6 and P7 from the article of Bicknell *et al.* are the same individuals as 1768 and 1769, respectively, described in the article of Guernsey *et al.*[14,15]
***Monoallelic

Of the ten individuals without a known molecular cause, one had a monoallelic missense mutation in *ORC1* (I1, Table 2) and two siblings had a monoallelic missense mutation in *CDT1* (I8 and I9, Table 2). The healthy fathers of these three individuals carried the same mutation. Of the ten individuals, three were male (30%), seven were female (70%), with an average age at last examination of 11 years and 2 months. Three individuals had reached puberty or adulthood.

Homozygous or compound heterozygous mutations in *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6* were identified in 35 individuals. Ten had mutations in *ORC1* (29%), seven in *ORC4* (20%), seven in *ORC6* (20%), ten in *CDT1* (29%) and one in *CDC6* (3%). The mutations identified are presented in Table 2. No homozygous or compound heterozygous null mutations were detected consistent with MGS mutations acting through partial loss of pre-replication complex function. Homozygous missense mutations were detected in ten out of 35 individuals with biallelic mutations from eight families (29%), compound heterozygous missense mutations in two unrelated individuals (6%). Compound heterozygous missense and frameshift mutations were identified in four individuals from three families (11%), compound heterozygous missense and splice site mutations in eight individuals from seven families (23%), compound heterozygous missense and nonsense mutations in ten individuals from five families (29%). One individual (3%) had a heterozygous missense mutation and a partial gene deletion.

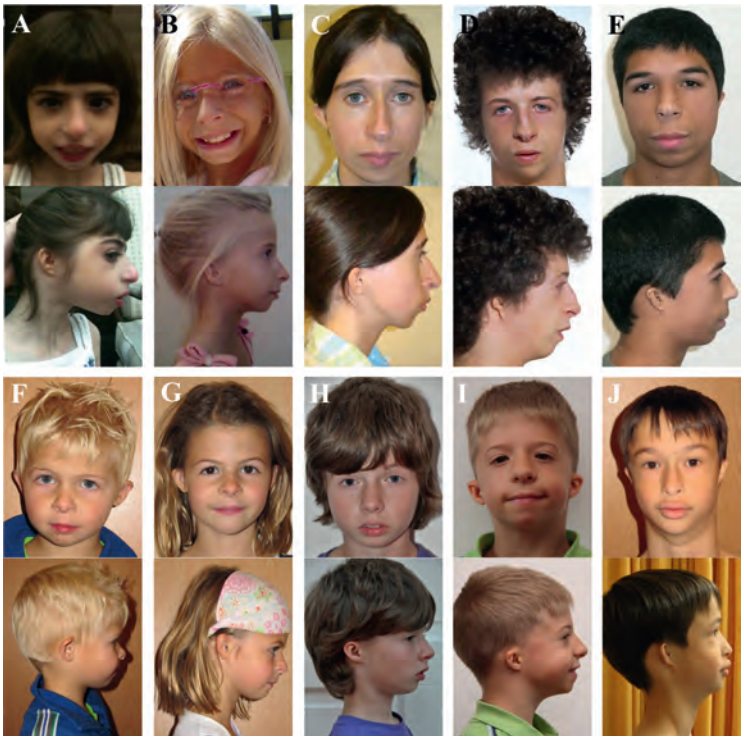
The classical triad of clinical features

The clinical diagnosis of MGS was previously based on the classical triad of microtia, absent or hypoplastic patellae and short stature. In our cohort, all three features were generally present, although not all three features had to be present to diagnose MGS. Seven individuals did not show all the features of the classical triad. They had mutations in *ORC1*, *ORC4*, *ORC6* and *CDT1*. One individual without mutations had microtia, with normal stature and normal patellae. Of the 35 individuals, four with biallelic mutations (11%; one with *ORC4*, one with *ORC6* and two with *CDT1* mutations) had a normal stature, but small ears and absent patellae. One individual (3%) with *ORC6* mutations had microtia and short stature, without patellar aplasia/hypoplasia. One individual (3%) with *ORC1* mutations had normal sized ears and normal patellae. This individual was originally diagnosed with microcephalic primordial dwarfism.[13]

Ears. Microtia was present in 34 out of 35 individuals (97%) with mutations and all individuals clinically diagnosed with MGS. One individual with mutations in *ORC1* had normal sized and shaped, though posteriorly rotated ears. Microtia ranged from

slightly small, normal shaped and positioned ears to abnormally formed (27/39; 69%), low set (24/38; 63%) and posteriorly rotated (13/27; 48%) ears (microtia grade 2) (Figure 1).[29] Conductive hearing loss was detected in two individuals with mutations in *ORC1* and *ORC6*, accompanied by a narrow ear canal in one.

Figure 1. Facial features of individuals with Meier-Gorlin syndrome. Frontal and lateral view of ten individuals with Meier-Gorlin syndrome. Note the different grades of microtia and the microstomia with full lips at young age. In the older individuals, the nose is more prominent and narrow, with a convex profile. Individuals **(A)** and **(B)** have mutations in *ORC1* (individuals P3 and P4, Table 2);[13] individual **(C)** has mutations in *ORC4* (individual P5, Table 2);[14] individual **(D)** has mutations in *CDT1* (individual P11, Table 2);[14] individual **(E)** has mutations in *CDC6* (individual P18, Table 2);[14] individuals **(F)**, **(G)** and **(H)** are new individuals with mutations in *ORC6* (individuals I2-I4, Table 2). In individuals **(I)** and **(J)**, no definitive molecular diagnosis could be made. Individual **(J)** was previously described by Bongers *et al*. in 2001.[3]



Patellae. Patellar malformations were reported in 30 out of 32 individuals (94%) with mutations, including all individuals with mutations in *ORC6*, *CDT1* and *CDC6*. The patellae were absent in 21 individuals (70%), hypoplastic in 9 (30%). The patellae of the monozygotic twins with homozygous missense mutations in *ORC4* were reported to

be palpable in early childhood, but were not palpable at several examinations during adolescence.[14,15] However, in both twins, no radiological examinations were performed. Two individuals were reported to have dimples over their knees, highly suggestive of patellar anomalies, at the age of 3 and 11 months, but no ultrasound examination was performed.

The two individuals without patellar anomalies had mutations in *ORC1* and *ORC6*.

Patellar aplasia/hypoplasia was reported in nine individuals (90%) clinically diagnosed with MGS.

Growth and growth hormone treatment

No significant difference in birth weight was detected compared for the individuals with mutations in the different genes and without a known molecular cause ($P > 0.05$). Postnatal growth ($P < 0.02$) and head circumference ($P < 0.004$) were significantly more delayed in individuals with *ORC1* mutations, compared with individuals from other gene groups (Figures 2a and b). Microcephaly was generally more apparent in older individuals.

Figure 2 a. Comparison of height (SDs) of individuals with Meier-Gorlin syndrome per gene category. 33 individuals with biallelic mutations and 9 individuals without definitive molecular diagnosis: n=9 *ORC1*; n=7 *ORC4*; n=7 *ORC6*; n=9 *CDT1*; n=1 *CDC6*; n=9 no definitive molecular diagnosis.

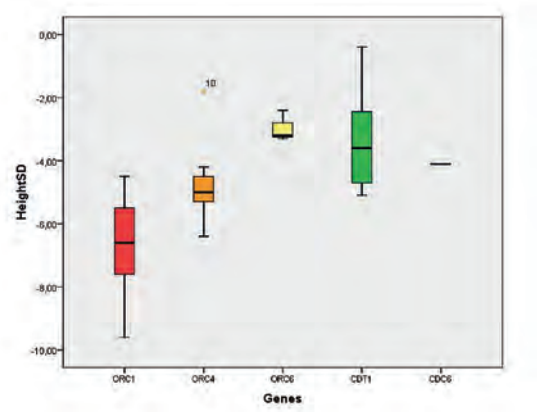
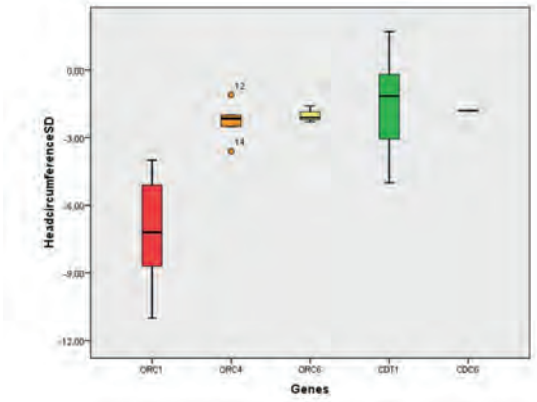


Figure 2b. Comparison of head circumference (SDs) of individuals with Meier-Gorlin syndrome per gene category. 29 individuals with biallelic mutations and ten individuals without definitive molecular diagnosis: n=8 *ORC1*; n=5 *ORC4*; n=6 *ORC6*; n=9 *CDT1*; n=1 *CDC6*; n=10 no definitive molecular diagnosis.



Intrauterine growth retardation (weight for gestational age <-1.3 SDs)[25] was established in 32 out of 33 individuals (97%) with mutations in one of the five known genes. Birth weight ranged from 1580 g after 40 weeks of gestation, to 3260 g after 41 weeks of gestation. One individual had a normal birth weight and mutations in *CDT1*. The birth weight of two individuals was unknown. IUGR was present in all ten individuals without definitive molecular diagnosis.

Postnatal growth was delayed (height for age <-2 SDs)[24] in 31 out of 35 individuals (89%) with mutations and in nine out of ten individuals (90%) clinically diagnosed with MGS. Microcephaly (OFC <-3 SDs)[24] at birth was present in three out of 14 individuals (21%) with mutations (1/1 with mutations in *ORC1*, 1/4 in *ORC4* and 1/1 in *CDC6*). Postnatally, microcephaly was present in 13 out of 30 individuals (43%) with mutations (8/8 *ORC1*; 2/5 *ORC4*; 1/6 *ORC6*; 2/9 *CDT1*). Microcephaly was reported in three out of ten individuals (30%) clinically diagnosed with MGS.

Growth hormone treatment was initiated in eight individuals with mutations in all five genes and pubertal development was delayed with a gonadotropin-releasing hormone analog in one individual with *ORC4* mutations. Positive results of growth hormone treatment were reported in one individual with mutations in *ORC4* and one with mutations in *CDC6*. The latter received treatment from age 2.5 years till age 7 years and from age 7.5 years till age 15.5 years. His height improved from -5 to -3 SDs during the first 4 years, but showed no further improvement afterwards.

The former received growth hormone treatment from the age of 3 years till the age of 9 years. Initially, her growth velocity increased, but the positive effect wore off and the treatment was stopped.

Growth hormone therapy, with positive effects, was initiated in two males without mutations. One received growth hormone from the age of 3 years and 1 month. His height increased from -6.9 to -3.1 SDs at 12 years and 7 months of age. The other male was treated with growth hormone from the age of 5 years and 5 months. His height increased from -5.8 to -4.1 SDs at the age of 6 years and 9 months.

Neurological features

Most individuals with MGS had normal intellect (39/41; 95%) and showed normal motor and speech development (31/42; 74%). A mild intellectual disability was reported in one individual with mutations in *ORC1* and one individual without mutations. Moderate learning difficulties were recorded in an individual with homozygous missense mutations in *ORC1* who suffered from intraventricular hemorrhage due to prematurity. The delay in motor development in one individual with mutations in *CDT1* might be related to congenital limb anomalies (club feet and genu recurvatum). Besides the anomalies in the individual with mutations in *ORC1* described above, structural brain anomalies were rarely observed in MGS.

Facial features

All individuals with MGS (both molecularly and clinically diagnosed) had a recognizable facial appearance with microtia (44/45; 98%), microstomia (27/39; 69%), full lips (30/40; 75%) and retro-/micrognathia (35/39; 90%) (Figure 1). The profile of the nose was less consistent, but often convex (13/23; 57%) and narrow (12/26; 46%) with a high nasal bridge (17/27; 63%) and appeared to become more prominent with age (Figure 1). Less frequent findings were strabismus (3/23; 13%), a bifid uvula (2/45; 4%) and cleft palate (1/45; 2%).

Respiratory tract

Pulmonary emphysema may be a serious complication in individuals with MGS. It was present in 12 out of 28 individuals (43%) with mutations in *ORC1* (3), *ORC4* (1), *CDT1* (7) and *CDC6* (1) and one out of ten individuals (10%) without molecular diagnosis. None of the seven individuals with *ORC6* mutations had pulmonary emphysema. Emphysema was congenital in most individuals, except for two, in whom the diagnosis was made at 4 and 7 years of age, respectively.

Structural abnormalities of the respiratory tract, comprising laryngomalacia, tracheomalacia and bronchomalacia are a relatively frequent finding in individuals with MGS. They were reported in ten out of 24 individuals (42%) with mutations and three

out of ten individuals (30%) without a known molecular cause. One individual with mutations in *ORC4* had a tracheoesophageal fistula with trachea- and bronchomalacia. She developed secondary aspiration pneumonia and required nocturnal mechanical ventilation. Ten out of 25 individuals (40%) suffered from recurrent respiratory tract infections without apparent structural malformations during infancy and young childhood, improving with aging.

Cardiovascular tract

Congenital heart defects were rarely observed in MGS. They were present in 2 out of 30 individuals (7%) with mutations. One individual with mutations in *ORC4* had a perimembranous ventricular septal defect causing congestive heart failure, which closed spontaneously.

The other individual had mutations in *CDT1* and a patent ductus arteriosus (PDA), which required interventional coiling.

Gastrointestinal tract

Feeding problems in infancy and young childhood were very common in individuals with MGS, with a prevalence of 81% (26/32) in individuals with mutations and 100% (10/10) in individuals without a known molecular cause. They may, however, partially be triggered by anxiety about the growth deficiency in these individuals. Feeding problems ranged from a small appetite (17/40; 43%) to gastroesophageal reflux (15/41; 37%) and failure to thrive (13/41; 32%). Of 41 individuals, 17 (41%) had tube feeding or gastrostomy interventions.

Urogenital tract

Minor genital anomalies were frequently described in individuals with MGS. In our cohort of 35 individuals with mutations in one of the five genes underlying MGS, 15 (43%) had genital anomalies (10/14 (71%) males, 5/21 (24%) females; mutations in all five genes). The genital anomalies in males encompassed small testes (2/14; 14%) and cryptorchidism (7/14; 50%), accompanied by a micropenis in two (14%). One individual had hypospadias (7%). In females, hypoplastic labia majora were present in four (19%). Two had clitoromegaly in addition, one also had hypoplastic labia minora. Hypoplastic labia minora alone was reported in one individual (5%). The same genital anomalies were seen in individuals without a molecular diagnosis: cryptorchidism (1/3; 33%), small testes (1/3; 33%) and hypoplastic labia majora (2/7; 29%).

Structural renal anomalies were uncommon in individuals with MGS: unilateral kidney aplasia was reported in two individuals (6%) with mutations in *CDT1*. Kidney stones were detected in one individual (3%) with mutations in *ORC1*.

Secondary sexual characteristics

Mammary hypoplasia was invariably present in all 13 post-pubertal females (100%; 10 with mutations in *ORC1*, *ORC4*, *ORC6* and *CDT1*; three without molecular diagnosis). Exogenous estrogen treatment in two siblings with *ORC4* mutations had a positive effect on breast development. Menarche and menstrual cycles were normal in all. Hypoplastic nipples were seen in one male with mutations in *CDT1*. Sparse or absent axillary hair was reported in nine post-pubertal individuals (75%; three males, six females; seven with mutations in all five genes, one with one mutation in *CDT1* and one without mutations). One individual with *ORC6* mutations also had sparse pubic hair.

Musculoskeletal features

In addition to the cardinal patellar malformation, various skeletal anomalies accompanied MGS, including a delayed bone age (14/22; 64%), slender long bones (13/20; 65%), hooked clavicles (1/15; 7%), genu recurvatum (9/28; 32%), club feet or other joint contractures (5/27; 19%) and joint subluxations (3/30; 10%). One individual with mutations in *ORC1* required craniosynostosis surgery. Another individual with *CDT1* mutations required surgery for scoliosis. One individual with mutations in *CDC6* had a mandibular osteochondroma. Three out of ten individuals (30%) without molecular diagnosis had a delayed bone age, one (10%) had contractures of the knees, one (10%) joint subluxations and five (50%) showed muscle weakness at physical examination. One individual (10%) had craniosynostosis.

Discussion

We performed genotype–phenotype studies on 35 individuals from 26 families with mutations in one of the five known pre-replication genes for Meier–Gorlin syndrome (*ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*) and ten individuals from nine families without a definitive molecular diagnosis.[13–15]

Our clinical data of the 35 individuals with MGS show that all individuals but one had at least two of the three main classical characteristics (microtia, absent/hypoplastic patellae and short stature). In the one individual with short stature only,

it is questionable whether the diagnosis MGS could be made solely on a clinical basis, indicating a broader range of phenotypes in MGS than previously expected.

In an infant with short stature or microtia, the diagnosis MGS should be considered and the patellae should be assessed with care, as hypoplasia of the patellae can be mild. In infants and young children, ultrasound investigations are preferred over radiographic investigations for demonstrating patellar aplasia, as the patella is completely cartilaginous and therefore radiolucent in the first few years of life.[30]

Follow-up of growth and development is recommended. Growth hormone treatment may be worthwhile considering, as there has been apparent benefit to some MGS individuals, however further studies need to be undertaken to determine growth trajectories and the effect of growth hormone treatment in MGS. Finally, clinicians and families should be aware that mammary hypoplasia is present in all postpubertal females with MGS. Exogenous estrogen treatment may be beneficial to breast development, but the effects need to be studied further.

All 35 individuals had mutations in one of the five known pre-replication complex genes. Ten individuals had mutations in *ORC1* (29%), seven in *ORC4* (20%), seven in *ORC6* (20%), ten in *CDT1* (29%) and one in *CDC6* (3%).

We show that individuals with *ORC1* mutations have a significantly shorter stature and smaller head circumference than individuals with mutations in the other four genes and individuals without definitive molecular diagnosis.

No other clear genotype–phenotype correlation was detected between the different gene categories. Moreover, no distinct intra- and interfamilial variation was observed. However, our data suggest that compound heterozygous missense and loss-of-function mutations have a more severe effect on the phenotype, compared with homozygous and compound heterozygous missense mutations. For instance, two individuals with compound heterozygous missense and loss-of-function *ORC1* mutations had a severe lethal phenotype with multiple congenital anomalies. Three individuals with each one missense and one loss-of-function mutation in *ORC4* had more severe growth retardation than the other four individuals with homozygous missense mutations in *ORC4* (-6.4 to -3.5 compared with -5.4 to -1.8). One of these three individuals had pulmonary emphysema, another had a congenital cardiac anomaly and two had severe feeding problems for which they required tube feeding and gastrostomy interventions (one individual with homozygous missense *ORC4* mutations also required tube feeding). Furthermore, congenital pulmonary emphysema was reported in seven out of nine individuals with compound heterozygous missense and loss-of-function *CDT1* mutations. Two of these individuals had a lethal phenotype. The only individual with

homozygous missense mutations in *CDT1* did not have pulmonary emphysema.
So far, no homozygous or compound heterozygous null mutations were identified, suggesting that these mutations cause a lethal phenotype. Two siblings with a recurrent missense mutation (p.Arg105Gln) and frameshift mutation (p.Val667*fs*X24) in *ORC1* had a lethal phenotype. A lethal phenotype was also seen in two siblings with compound heterozygous nonsense and missense mutations in *CDT1* (pGlu278X + p.Arg462Gln). We hypothesize that the presence of two null mutations or a combination of certain mutations has a disadvantage at conception or leads to early miscarriages, because of a severe effect of these mutations during embryological development. Results from studies in Drosophila of the gene *double parked (dup)*, the Drosophila ortholog of *CDT1*, support this theory.[31] Strong mutations in this gene cause embryonic lethality preceded by a failure to undergo S phase during division.
The clinical features of zebrafish with *ORC1* mutations and yeast with *ORC4* mutations show an overlap with the clinical features of individuals with MGS. Both zebrafish and yeast show a reduction in overall size.[13–15] A slight hypoplasia of jaw cartilage, reduction in number or fusion of otolith organs and smaller eye size was present in over 80% of zebrafish with a depletion of ORC1. The remaining zebrafish, with a more severe depletion of ORC1, had a more severe reduction in growth with an abnormal body curvature and reduced viability.[13]
However, individuals with MGS show additional clinical features, such as underdevelopment of the patellae and structural anomalies, such as pulmonary emphysema, brain malformations and genitourinary anomalies. Mouse models for MGS may contribute in determining the effects of the different mutations in the pre-replication complex on embryological development.
A mutation detection rate of 78% (35 out of 45 individuals) was established. The detection rate would have been lower (70%; 35 out of 50 individuals), if a clinical diagnosis of MGS could have been made in the five individuals who were excluded from the study because of insufficient clinical information.
Individuals without a molecular diagnosis had a similar phenotype to individuals with mutations. Further molecular studies, for instance parallel sequencing of pre-replication complex genes and next-generation sequencing, may be useful to diagnose MGS earlier. We suppose that the individuals without mutations in *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6* have mutations in other genes of the pre-replication complex or pathways linked to the pre-replication complex. Another possibility is the presence of intragenic deletions or duplications in one of the five known pre-replication genes underlying MGS.

In conclusion, MGS is a recognizable clinical phenotype, characterized by microtia, patellar aplasia/hypoplasia and a short stature, frequently accompanied by specific facial features, respiratory and gastrointestinal problems and skeletal and genitourinary anomalies. In three quarters of individuals with a clinical diagnosis of MGS, the diagnosis can now be confirmed by molecular analysis of *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*.

Individuals with *ORC1* mutations have the most severe growth retardation in MGS. Therefore, testing of *ORC1* first should be considered in individuals with a severe growth retardation and microcephaly. No other clear genotype–phenotype correlation was established, although compound heterozygous mutations appear to have a more severe effect on phenotype than homozygous missense mutations.

Longitudinal studies on growth/phenotype in an extended series of patients are important to investigate the effect of growth hormone and estrogen treatment on growth and mammary development, respectively.

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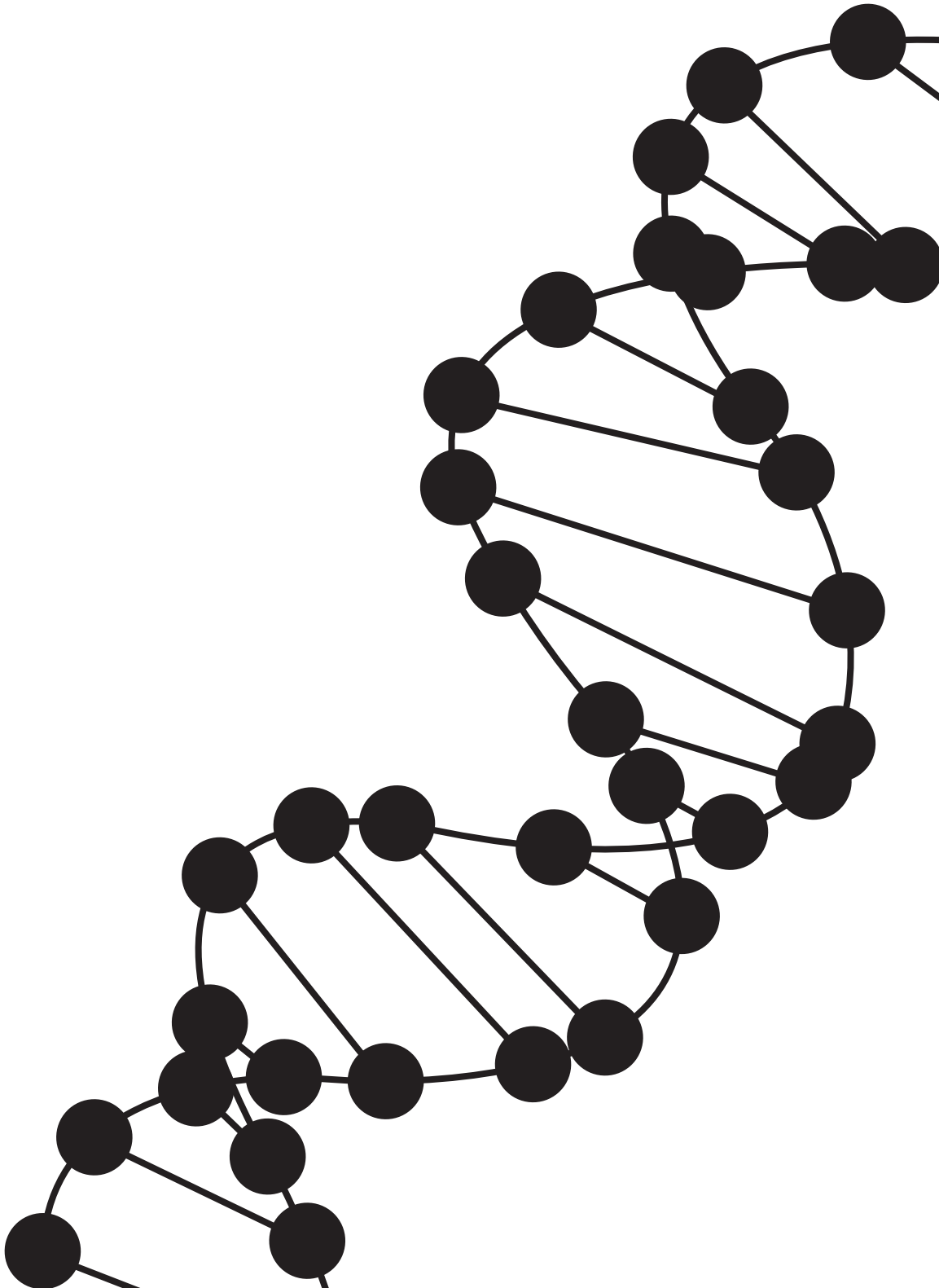

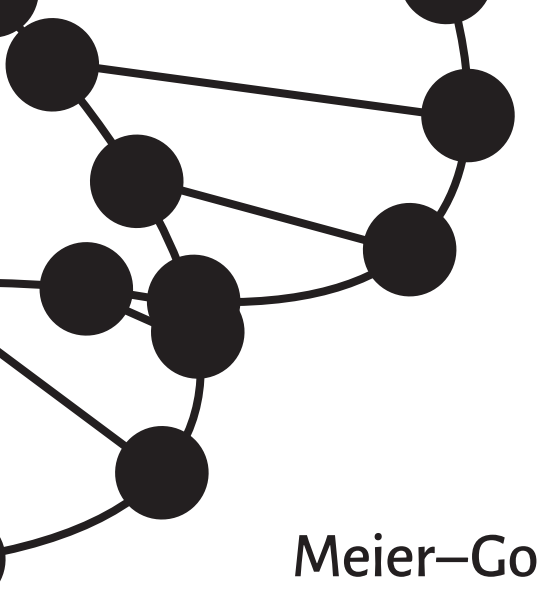

Chapter 2.2

Meier–Gorlin Syndrome: Growth and Secondary Sexual Development of a Microcephalic Primordial Dwarfism Disorder

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Abstract

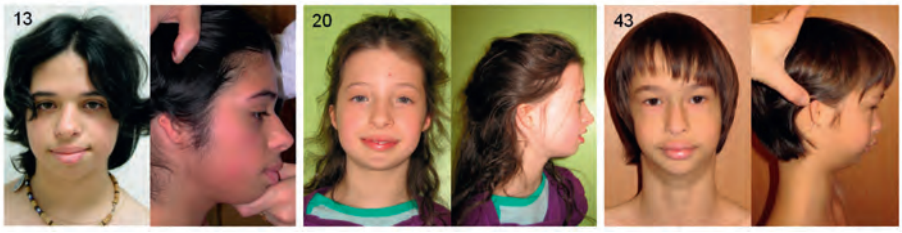
Meier–Gorlin syndrome (MGS) is a rare autosomal recessive disorder characterized by primordial dwarfism, microtia and patellar aplasia/hypoplasia. Recently, mutations in the *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6* genes, encoding components of the pre-replication complex, have been identified. This complex is essential for DNA replication and therefore mutations are expected to impair cell proliferation and consequently could globally reduce growth. However, detailed growth characteristics of MGS patients have not been reported and so this is addressed here through study of 45 MGS patients, the largest cohort worldwide. Here, we report that growth velocity (length) is impaired in MGS during pregnancy and first year of life, but, thereafter, height increases in paralleled normal reference centiles, resulting in a mean adult height of -4.5 standard deviation score (SDs). Height is dependent on ethnic background and underlying molecular cause, with *ORC1* and *ORC4* mutations causing more severe short stature and microcephaly. Growth hormone therapy (n=9) was generally ineffective, though in two patients with significantly reduced IGF1 levels, growth was substantially improved by GH treatment, with 2 SDs and 3.8 SDs improvement in height. Growth parameters for monitoring growth in future MGS patients are provided and as well we highlight that growth is disproportionately affected in certain structures, with growth related minor genital abnormalities (42%) and mammary hypoplasia (100%) frequently present, in addition to established effects on ears and patellar growth.

Introduction

Meier–Gorlin syndrome (MGS; ear patella short stature syndrome; OMIM #224690) is defined by the triad of microtia, patellar aplasia/hypoplasia and short stature. Other frequent findings include pulmonary emphysema and typical facial characteristics (Figure 1).

MGS is part of a group of autosomal recessive disorders called microcephalic primordial dwarfism, characterized by severe proportionate pre- and postnatal growth deficiency and microcephaly.[1]

Figure 1. Facial characteristics of three patients with Meier–Gorlin syndrome. Note the characteristic face with small, abnormally shaped ears, beaked nose, small mouth with full lips and retrognathia. Patient 13 was previously described by Lacombe *et al*. and Bicknell *et al*. (Case 3 and Patient 8, respectively).[7,21] Patients 20 and 43 were previously described by Bongers *et al*. (patients 4 and 2, respectively).[13]



Sixty-three cases of MGS have been described in literature, thus far.[2-23] Recently, mutations in five different pre-replication complex genes (*ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*) were identified in 67% (31/46) of patients with MGS described in literature.[20-22] The pre-replication complex consists of the origin recognition complex (subunits ORC1–ORC6), two regulatory proteins (CDT1 and CDC6) and the MCM helicase complex. The complex forms at origins of DNA replication and is essential for initiation of genome replication, a crucial step in cell cycle and cellular growth.[24,25] Growth is globally reduced in MGS, presumably as a consequence of mutations slowing cell proliferation, with reported mean adult height in females of 131.6 cm (-5.6 standard deviation score (SDs) according to Prader *et al*.; range 127–148 cm, n=5) and 147.8 cm in males (-3.3 SDs according to Prader *et al*.; range 132–157.5 cm; n=3).[9,16,17,20,21,26] Growth did not improve during growth hormone (GH) therapy performed in six patients, thus far.[7,14,18,20,22]

Reductions in growth of specific tissues are also evident, most notably affecting the patella and ear, given that microtia and patellar aplasia/hypoplasia are defining features of MGS. Microtia can vary profoundly, ranging from slightly small and normally positioned to abnormally shaped and positioned ears. Only one patient was

reported to have normal sized and shaped ears.[22] Additionally, genital growth may be specifically affected both evident at birth, resulting in minor genital anomalies and during secondary sexual development, resulting in mammary hypoplasia.[3,7,8,10,12,13,16,20,21] Detailed longitudinal growth and endocrinological studies in patients with MGS have not been described and no reference curves for height, weight and head circumference have been established. Here, we provide an overview of growth in a unique cohort of 45 MGS patients, the largest worldwide. Moreover, we describe the beneficial effect of GH treatment in two MGS patients with additional growth hormone insufficiency. Finally, we provide an overview of genital anomalies, secondary sexual characteristics and disrupted growth of the ears in MGS.

Materials and methods

Patients

Pre- and postnatal growth measurements, endocrinological findings and data regarding development of genitalia, secondary sexual characteristics and ears of 45 patients with MGS were collected retrospectively by sending clinical questionnaires to the referring physicians and prospectively by physical examination and laboratory investigations. The cohort comprised 28 females (62%) and 17 males (38%), aged between 3 months and 47 years. Twenty patients had reached postpubertal or adult age (44%; 6 males, 14 females). Gynecologic examination, including a transvaginal ultrasound, was performed in five females. The demographic data of our cohort are summarized in Tables 1a and 1b. All patients were previously described in literature.[3,5,7,11,12,13,15,16,20-23]

Table 1a. Demographic data and data on growth and sexual development of a cohort of 45 patients with Meier-Gorlin syndrome.

Demographic data	Meier-Gorlin syndrome	(% or range)
Total number of patients	45	
Number of females/males	28 /17	(62%/38%)
Age range in years	0.3 to 4.7	
Region of descent		
Europe	21	(47%)
North America	14	(31%)
North Africa	4	(9%)
Middle East	3	(7%)
Asia	1	(2%)
Middle east/North America	1	(2%)
Oceania	1	(2%)
Gene mutated		
ORC1	10	(22%)
ORC4	7	(16%)
ORC6	7	(16%)
CDT1	10	(22%)
CDC6	1	(2%)
No known molecular cause	10	(22%)
- Monoallelic mutation *ORC1*	1	
- Monoallelic mutation *CDT1*	2	
- No mutation	7	
Intrauterine growth retardation	42/43	(98%)
Mean birth weight in SDs[I]	-3.4	(-6.5 to -0.3)
Mean birth length in SDs[I]	-3.9	(-13.2 to 0.0)
Mean birth head circumference in SDs[1]	-2.1	(-5.4 to 1.5)
Number of postpubertal/adult patients (14 F/6 M)	20	(44%)
Adult height (≥ 18y)		
Mean female height in cm (7 females)	137.7	(127.0 to 150.8)
Mean male height in cm (2 males)	147.0	(136.5 to 157.5)
Mean in SDs[II] for both sexes	-4.5	(-6.4 to -2.3)
Adult BMI		
Mean female BMI (5 females)	16.8	(14.3 to 19.8)
Mean BMI in SDs[II] (5 females, 1 male)	-3.1	(-4.9 to -0.8)
Adult head circumference (≥ 15y)		
Mean female head circumference in cm (12 females)	50.3	(45.6 to 53.0)
Mean male head circumference in cm (5 males)	51.9	(44.2 to 57.4)
Mean in SDs[II] for both sexes	-2.4	(-5.8 to +1.3)

[I]SDs calculated using the growth charts of Niklasson *et al*.[27]
[II]SDs calculated using growth charts of Prader *et al*.[26]

Table 1b. Overview of mutations identified in a cohort of 45 patients with Meier-Gorlin syndrome. Biallelic mutations were found in 35 patients, monoallelic mutations in three patients. In 7 patients, no mutations were detected. Adapted from de Munnik *et al*.[23]

Gene	Nucleotide alterations	Amino acid alterations	Hetero-/ Homozygous	Putative effect	Number of patients/families
ORC1	c.266T>A	p.Phe89Ser	Homozygous	Missense	1/1
	c.314G>A	p.Arg105Gln	Homozygous	Missense	1/1
	[c.314G>A] + [c.1482-2A>G]	p.Arg105Gln + intron 9 splice acceptor site	Heterozygous	Missense + splice site	2/2
	[c.314G>A] + [c.1999_2000delGTinsA]	p.Arg105Gln + p.Val667fsX24	Heterozygous	Missense + frameshift	2/1
	[c.314G>A] + [c.1996C>T]	p.Arg105Gln + p.Arg666Trp	Heterozygous	Missense	1/1
	[c.314G>A] + [c.2159G>A]	p.Arg105Gln + p.Arg720Gln	Heterozygous	Missense	1/1
	c.380A>G	p.Glu127Gly	Homozygous	Missense	2/1
	[c.1721C>T]	p.Thr57Met	Monoallelic	Missense	1/1
ORC4	c.521A>G	p.Tyr174Cys	Homozygous	Missense	4/3
	[c.521A>G] + [c.874_875insAACA]	p.Tyr174Cys + p.Ala292fsX19	Heterozygous	Missense + frameshift	2/2
	[c.521A>G] + CNV del	p.Tyr174Cys + del	Heterozygous	Missense + deletion	1/1
ORC6	[c.2T>C] + [c.449+5G>A]	p.Met1?+ p.?	Heterozygous	Missense + splice site	4/3
	[c.257_258delTT] + [c.695A>C]	p.Phe86X + p.Tyr232Ser	Heterozygous	Nonsense + missense	3/1
CDT1	[c.196G>A] + [c.351G>C]	p.Ala66Thr + p.Gln117His (exon 2 splicing donor site)	Heterozygous	Missense + splice site	1/1
	[c.351G>C] + [c.1385G>A]	p.Gln117His (exon 2 splicing donor site) + p.Arg462Gln	Heterozygous	Splice site + missense	1/1
	[c832G>T] + [c.1385G>A]	pGlu278X + p.Arg462Gln	Heterozygous	Nonsense + missense	2/1
	[c.1081C>T] + [c.1357C>T]	p.Gln361X + p.Arg453Trp	Heterozygous	Nonsense + missense	1/1
	[c.1385G>A] + [c1560C>A]	p.Arg462Gln + p.Tyr520X	Heterozygous	Missense + nonsense	4/2
	[c.1385G>A]	p.Arg462Gln	Monoallelic	Missense	2/1
	c.1402G>A	p.Glu468Lys	Homozygous	Missense	1/1
CDC6	c.968C>G	p.Thr323Arg	Homozygous	Missense	1/1

Anthropometric measurements at birth (length, weight and head circumference for gestational age) were standardized using the growth charts from Niklasson and Albertsson-Wikland.[27] Postnatal growth measurements (height, body mass index (BMI) and head circumference for age) were standardized according to the growth charts from Prader *et al*., as used by Ranke *et al*. in their international evaluation of growth and growth hormone therapy.[26,28]

Endocrinological data (IGF1, stimulated GH, LH, FSH, estrogen and testosterone levels) of 15 patients were available and standardized according to Rikken *et al*.[29] GH treatment was initiated in nine patients. Two of these patients (P43 and P44, Table 2) were prospectively followed, seven were retrospectively analyzed.

In addition to a short stature and small head circumference, microtia is one of the most characteristic features of MGS. The ear length of 20 patients was compared to the normal values provided by Hall *et al*.[30] The ear morphology of 10 patients is shown in Figure 4. A detailed description of the ear morphology of 20 patients according to the terminology of Hunter *et al*. is provided in Supplementary Table 1.[31]

Statistical analysis

A linear mixed model with random factor patient was used to analyze the standardized height and head circumference according to Prader *et al*. (random intercept model).[26,32] Independent fixed factors were sex, molecular cause and region of descent. Age and age x age were included as fixed covariates. When the coefficient of age x age was not significant, that is, when there was no indication of a non-linear age trend, the analysis was repeated without age x age. In case of non-linearity, the relationships between age and growth during the first years and later years were estimated separately by including the variables age- and max (0, age-1; the latter variable represents the difference between the growth in the first year and second year). Growth data after the age of 20 years or after start of GH treatment were excluded from growth analysis. Five patients were completely excluded from analysis (three were treated with GH for an unknown duration; of one, no measurements before the age of 47 years were available; of one, no measurements were available after 17 weeks of gestation). Six patients were excluded from growth analysis after GH treatment was initiated. Four hundred fifty two measurements of 40 patients were obtained on different ages from birth throughout their childhood. Of six patients, only one measurement was available. Of 19 patients, five or more measurements were available.

Table 2. Growth hormone levels and the effect of growth hormone treatment in nine patients with Meier-Gorlin syndrome.

Patient	Sex	Gene mutated	Mutations	IGF1 (SDs)	GH stimulation	Age CA–SA (years)	Age at start GH treatment (years)	Height at start GH treatment (SDs)	Age at end GH treatment (years)	Height at end GH treatment (SDs)	Catch up ≥2SDs
5[I]	F	*ORC1*	[c.314G>A] [c.1482-2A>G]	-1.07	N	U	4.5	-7.3	6.1	-7.3	-
9[II]	F	*ORC4*	[c.521A>G] [c.874_875insAACA]	U	N	3 - 1.2	3.1	-7.1	10	-5.3	-
10[III]	F	*ORC4*	[c.521A>G] [c.521A>G]	U	U	U	U	U	15	-5.5	-
11[III]	F	*ORC4*	[c.521A>G] [c.521A>G]	U	U	U	U	U	15	-5.8	-
27[IV]	M	*ORC1*	[c.380A>G] [c.380A>G]	N	S	U	U	U	4.5	-5.2	-
38[II]	M	*CDT1*	[c.1385G>A] [c1560C>A]	U	U	U	3.5	U	7.5	-4.7	-
42[I]	M	*CDC6*	[c.968C>G] [c.968C>G]	-0.8	U	15 - 12.5	2.5 7.5	-4.0[VII] -4.3	6.7 16	-4.1 -3.5	- -
43[V]	M	U		-4.6	L	11.5 - 10	3	-6.8	14	-3.0	+
44[VI]	M	U		-3.3	N	5.3 - 3	5.4	-5.7	7.4	-3.7	+

CA = Chronological Age; SA = Skeletal Age; GH = Growth Hormone; N = Normal; S = Suboptimal; L = Low.

[I] Patients 5 and 42 were previously described by Bicknell ***et al***. (patients 4 and 18) and Bongers ***et al***. (patients 1 and 3).[13,21]

[II] Patients 9 and 38 were previously described by Bicknell ***et al***. (patients 5 and 11).[21]

[III] Patients 10 and 11 were previously described by Bicknell ***et al***. (patients 6 and 7), Bongers ***et al***. (P5 and 6) and Guernsey ***et al***. (1768 and 1769).[13,20,21]

[IV] Patient 27 was previously described by Bicknell ***et al***. (patient 1).[22]

[V] Patient 43 was previously described by Bongers ***et al***. (patient 2).[13]

[VI] Patient 44 was not previously described.

[VII] Measurement at the age of 4 years, 1.5 years after the start of GH therapy.

Results

Pre- and postnatal growth

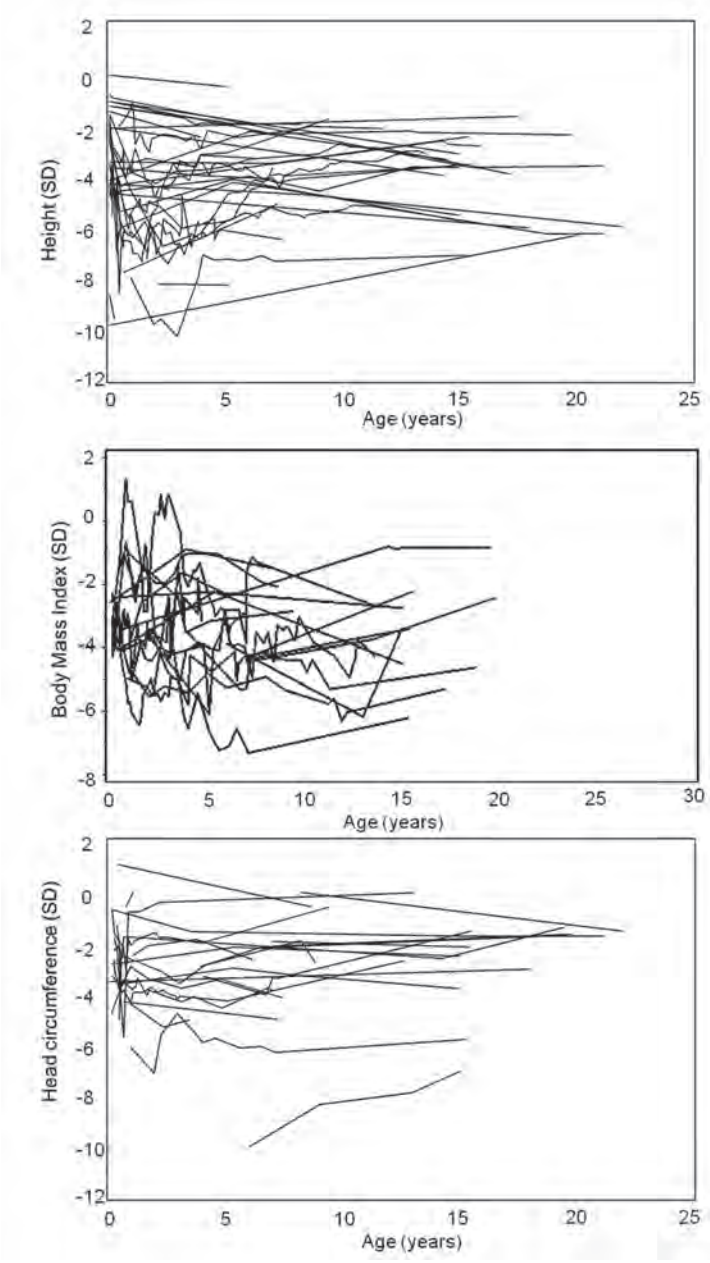
At birth, mean length was -3.9 SDs, with a mean weight of -3.4 SDs and mean head circumference of -2.4 SDs according to Niklasson and Albertsson-Wikland.[27] Mean birth length was -3.5 SDs according to Prader *et al*.[26] In the first year after birth, length dropped significant with 1.7 SDs ($P < 0.0001$) to -5.2 SDs, relative to the general population. In other words, infants with MGS are small at birth, but become even smaller in the first year of life, compared to the general population. Thereafter, height remained below, but increased in parallel with the population centiles (nonsignificant gain of 0.08 SDs/year, until age 15 years ($P > 0.05$)). Afterwards, (between age 15 and 18 years) no reliable trend could be calculated. Mean adult height (≥18 years of age) was -4.5 SDs (females 137.7 cm, males 147.0 cm), with a BMI of -3.1 SDs (females 16.8 kg/m^2, one male 15.0 kg/m^2) and head circumference of -2.4 SDs (females 50.3 cm, males 51.9 cm). Stature was proportionate, except in two previously described adult females (P21 and P22) without known molecular cause.[12] One of these females had a span of 136 cm and a height of 149 cm, the other had a span of 134 cm and a height of 143.6 cm.

Height appeared to be significantly affected by ethnic origin ($P < 0.0001$): patients from the Middle East were shortest, followed by patients from North America, Europe and North Africa. In contrast, BMI and head circumference were not significantly influenced by age or ethnic background compared to normal ($P > 0.05$).

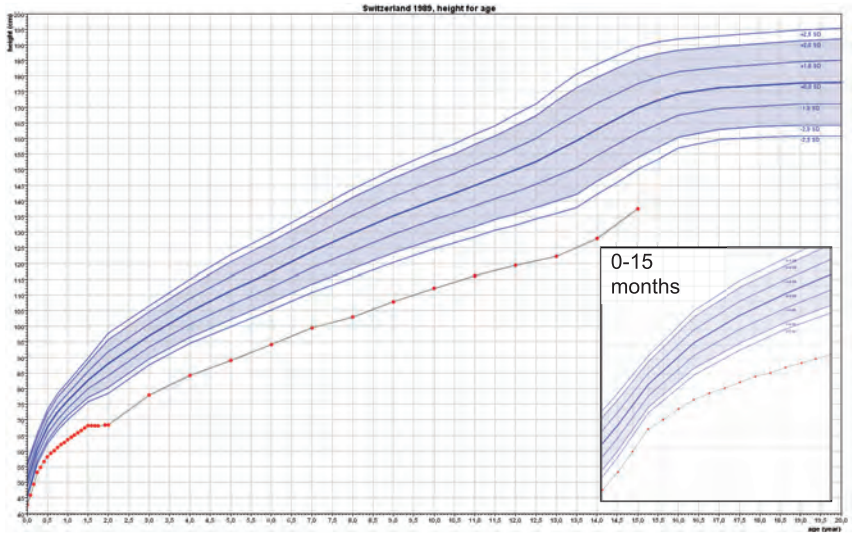
Height and head circumference were significantly influenced by the underlying molecular cause ($P < 0.0001$). Patients with mutations in *ORC1* or *ORC4* had a significantly shorter stature and smaller head circumference than patients with mutations in other genes (*ORC6*, *CDT1*, *CDC6* or unknown genes), such that they were 4.7 SDs (*ORC1*) and 3.1 SDs (*ORC4*) shorter than the others (after adjustment for ethnic origin). For head circumference, the differences were 5.0 SDs and 1.6 SDs, respectively.

Prenatal and postnatal growth data are summarized in Table 1a. The trends for height, BMI and head circumference for age, using the standardized growth charts according to Prader *et al*., are illustrated in Figure 2a.[26] In Figure 2b, the proposed reference growth chart for height of MGS patients, derived from this data analysis, is shown. The difference between the reference growth curve and the normal curve corresponds to the average difference for our patient population. For individual patients the difference may depend on ethnic background, gender and mutated gene. However, the slope of the curve (growth velocity) is independent of these factors.

Figure 2. (A) Trends for height, body mass index and head circumference of 33 patients with Meier–Gorlin syndrome. Growth measurements for both sexes were standardized according to the growth charts of Prader *et al*. Height dropped significantly with 1.7 SDs in the first year after birth ($P < 0.0001$) and increased with an average of 0.08 SDs per year afterwards, until age 15 years ($P > 0.05$).[26] Between age 15 and 18, insufficient data were available for statistical analysis. Head circumference and BMI were not significantly influenced by age ($P > 0.05$). There were no differences in growth patterns between males and females.



(B) Proposed growth charts for patients with Meier–Gorlin syndrome. This growth chart is based on the trends in a cohort of 33 patients with Meier–Gorlin syndrome. Here, the growth pattern (height for age) is shown in comparison to the growth charts for boys of Prader *et al.*[26] This growth pattern is the same for males and females. After 15 years of age, insufficient data were available to calculate a reliable statistical trend. These data can be applied to predict height in an individual MGS patient: the growth chart of an individual patient will differ from this chart in actual height (i.e., in distance relative to the normal growth chart), but follow the same pattern of growth velocity (i.e., shape) as our proposed chart.

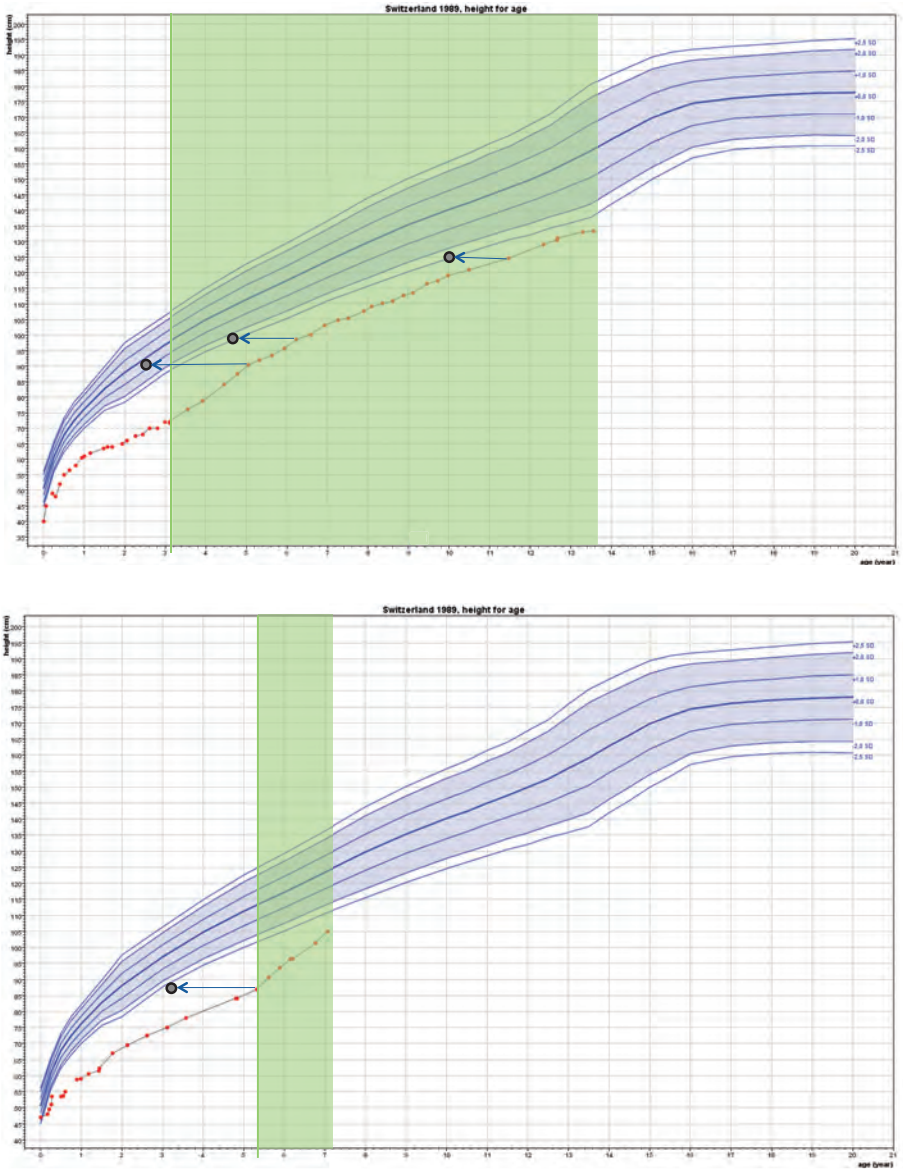


Growth hormone levels and growth hormone treatment

GH status, assessed by IGF1 and/or stimulated GH measurements, was normal in 12 out of 15 (80%) patients tested. Low IGF1 levels were detected in one female (-2.3 SDs; P21) and two males (-4.6 and -3.3 SDs; P43 and P44, respectively, described below). The female was never treated with growth hormone.

GH therapy was initiated in 9 out of 45 patients (20%). An overview of their GH status, skeletal age, the period of GH treatment and effect on height is presented in Table 2. GH status was normal in seven patients, but abnormal in two (P43 and P44). Skeletal age according to Greulich and Pyle was delayed in four patients and unknown in five.[33] A positive effect of GH treatment was seen in the two prospectively followed male patients (22%; P43 and P44, Figure 3a,b, Table 2). In both patients, height continued to decrease, even after the infancy period, up to the age of 1.5 years and to standard deviations of -7 and -6.5. In both, strikingly low IGF1 levels were detected, up to -4.6 SDs, with stimulated GH levels of 11.4 and 26 mIU/L. Growth hormone treatment resulted in an increase of height velocity in the first year of treatment from approximately 5 cm/year to more than 10 cm/year, with a total gain of 2 SDs and 3.8 SDs within 2 years after the start of treatment, respectively.

Figure 3. Growth charts of two patients with Meier–Gorlin syndrome showing a positive response to growth hormone treatment. Height for age is compared to the growth charts of Prader *et al*.[26] The green highlighted areas represent the period of GH treatment. The black/gray dots and arrows represent the bone age at that age. **(A) Growth chart of Patient 43** (Table 2; Bongers *et al*. Patient 3)[13], height improved 3.8 SDs during GH therapy. **(B) Growth chart of Patient 44** (Table 2; de Munnik *et al*. individual i)[23] height improved 2 SDs during GH therapy.



Abnormalities of genital and secondary sexual development

Minor anomalies of external genitalia were present in 19 out of 45 patients (42%). Cryptorchidism was seen in 11 out of 17 males (65%; 3 bilateral, 1 unilateral, 5 unknown), micropenis was present in two out of 17 males (12%), hypospadias in one (6%). Hypoplastic labia majora or minora were present in seven out of 28 females (25%).

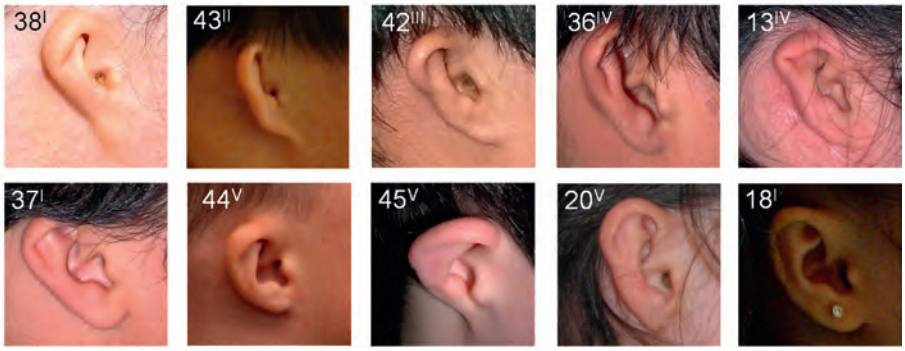
Transvaginal ultrasound investigations were performed in five out of 14 postpubertal females. A small uterus and polycystic ovaries were observed in two females, while no abnormalities were detected in two others. In the fifth female (P22), ultrasound investigations, which were performed after premature delivery, showed a shortened uterus with a probe length of 4–5 cm. This female had two miscarriages after 17 and 18 weeks, respectively. No fetal anomalies were detected by ultrasound investigation and autopsy. These were the first two pregnancies in a patient with MGS.

Secondary sexual development was affected in 17 out of 20 patients (85%, males and females). Axillary hair was sparse or absent in 9 out of 12 patients (75%; 3 males, 6 females; 8 unknown), pubic hair was sparse in 1 out of 10 patients (10%; male; 10 unknown). Mammary hypoplasia was present in all 14 postpubertal females. However, menarche had occurred at a normal age (before age 14.5 years) and menstrual cycles were regular in these females. Though endogenous hormonal levels were normal, exogenous estrogen treatment was initiated in five females. An increase in breast size was reported in two females. A very mild increase was observed in a third female, after treatment with 100 µg ethinylestradiol between 16 and 18 years of age. In contrast, no increase in breast size was seen in two other females treated with 20 µg ethinylestradiol between 13.5 and 16 years of age. Hypoplastic nipples were reported in one male, but nipples were not hypoplastic in six postpubertal females (unknown in the other eight females).

Ears

Microtia (ear length <-2 SDs) was present in 44 out of 45 MGS patients (98%). The mean right ear length was -5.8 SDs (20 patients, range -7.3 to -3.1 SDs), the mean left ear length -5.3 SDs (20 patients, range -7.9 to -3.2 SDs). Most ears were small, with a shelved antihelix, prominent crus and small or absent ear lobes. The ear anomalies of 10 MGS patients are shown in Figure 4. A detailed description according to the morphology of Hunter *et al*. of the ear malformations of 20 patients is provided in Supplementary Table 1.[31]

Figure 4. The ear morphology of 10 patients with Meier–Gorlin syndrome. The ears are arranged from most (P38) to least underdeveloped (P18, only microtia). Common features were microtia (10; 100%), a shelved antihelix (4; 40%), prominent crus helix (5; 50%) and small or absent ear lobes (6; 60%). A detailed description of the ear anomalies in 20 MGS patients is provided in Supplementary Table 1. [I]Patients 38, 37 and 18 were previously described by Bicknell *et al*. (patients 10, 11 and 17).[21] [II]Patients 43 and 20 were previously described by Bongers *et al*. (patients 4 and 2, respectively).[13] [III]Patient 42 was previously described by Bicknell *et al*. (patient 18) and Bongers *et al*. (Patient 3).[13,21] [IV]Patients 36 and 13 were previously described by Bicknell *et al*. (patients 8 and 9) and Lacombe *et al*. (patients 2 and 3).[7,21] [V]Patients 44 and 45 were previously described by de Munnik *et al*. (individual i).[23]



Discussion

In this first retrospective and partially prospective study of growth in patients with MGS, we show that the growth retardation in MGS predominantly arises prenatally and in early infancy. Mean birth length was -3.5 SDs.[26] In the first year of life, relative length further decreased to -5.2 SDs. In the following years, growth velocity stayed normal with a height curve nearly parallel to normal and a mean adult height of -4.5 SDs. This pattern is consistent with an intrinsic growth problem of reduced cellular proliferation, though, surprisingly, growth velocity post infancy is not perturbed. Alternatively, placental dysfunction could potentially cause growth impairment in MGS prenatally, while feeding problems, present in almost all MGS patients during the first year of life, might contribute to the growth retardation.

Global growth, as reflected by both height and head circumference, was influenced by the underlying molecular cause: *ORC1* mutations caused the most severe growth retardation, followed by *ORC4* mutations. This might represent a differential sensitivity to efficient DNA replication, of mutations in different pre-replication complex subunits, or simply reflect the strength of specific mutations, of which there are a limited number in each subunit so far reported. However, the limited number of patients with a mutation in one of the five pre-replication complex genes and the inclusion of patients with all features of the classical triad of clinical characteristics might have introduced ascertainment bias in these results. Centile charts for height, weight and head circumference cannot be established given the rarity of this condition,

the restricted size of our cohort and the lack of measurements at standardized ages.
Our data have clinical utility and will be useful in predicting the growth pattern during different stages of life of future patients with MGS. Our data can be used to predict the height of a patient, since height decreases with 1.7 SDs during the first year of life and the growth velocity is nearly equal to the normal growth velocity thereafter (Figure 2b). The actual height of the proposed growth chart of Figure 2b corresponds to the average height of our patient group. Corresponding curves for individual patients with different gender, ethnic background or mutated gene, would lie closer to or further away from the normal curve according to Prader *et al.*, but the growth velocity is independent of these factors, so they would all run parallel to our proposed growth chart of Figure 2b.[26] This is in contrast to growth curves in other syndromes, such as achondroplasia or Turner's syndrome.[34,35]
In two MGS patients, growth hormone treatment turned out to be successful in improving height. Growth patterns in these patients differed from the general pattern in our cohort, because growth velocity continued to be reduced after early infancy and in particular, extremely low IGF1 levels were found (Table 2). Notably though, these two patients had a classic MGS phenotype with microtia and patellar aplasia and the underlying molecular cause is currently unknown for both of them. It is tempting to speculate that the gene causing MGS in these two patients might play an important role in the growth hormone axis. Furthermore, this suggests that IGF1 measurements can be used to target treatment to a subset of MGS patients that will respond to GH therapy, in contrast to those with mutations in the pre-replication complex, who appear unlikely to benefit from GH therapy.
Mammary hypoplasia was present in all postpubertal females. Besides an early disturbance of embryonic development, (partial) insensitivity to estrogen as a cause of secondary sexual underdevelopment may be considered. This would explain the lack of response to estrogen treatment in two patients with mammary hypoplasia. However, the presence of normal menstrual cycles in these patients contradicts this theory.
In the future, further prospective growth studies would be useful to confirm our results and establish more detailed growth charts, as well as more systematically assessing the effect of GH treatment in MGS patients. Further studies of an increased number of MGS patients with a known molecular cause and elucidation of the genetic defect in MGS patients with a yet unknown molecular cause will further define the phenotypic spectrum of MGS, including growth.
Finally, animal studies (mouse models) might contribute to gain insight into the embryonic development of MGS patients and unravel pathogenic mechanisms underlying the growth retardation and underdevelopment of genitalia, secondary sexual characteristics and ears in MGS.

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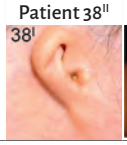
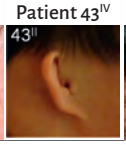
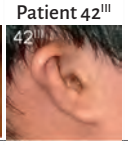
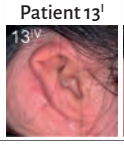
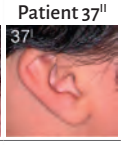
Supplementary Table 1. Overview of ear anomalies in 20 patients with Meier-Gorlin syndrome. The ear with the most severe anomalies is described first (P38), that of the patient with the least severe ear anomalies last (P18). The description follows the alphabetical order as in the article from Hunter *et al*.[31]

	Patient 38[II]	Patient 43[IV]	Patient 42[III]	Patient 36[I]	Patient 13[I]	Patient 37[II]
						
Mutated Gene	*CDT1*	U	*CDC6*	*ORC6*	*ORC6*	*ORC6*
Sex	M	M	M	M	F	M
Mutations	[c.1385G>A] [c1560C>A]	-	[c.968C>G] [c.968C>G]	[c.257_258delTT] [c.695A>C]	[c.257_258delTT] [c.695A>C]	[c.257_258delTT] [c.695A>C]
Microtia	+	+	+	+	+	+
Antihelix stem						
Prominent	+	-	+	+	-	-
Shelfed	-	-	-	+	-	+
Tragus						
Underdeveloped	-	+	+	-	-	-
Prominent	-	-	-	-	-	-
Antitragus						
Underdeveloped	+	-	-	-	-	-
Prominent	-	-	-	-	-	+
Protruding ears	-	+	-	-	-	-
Superior crus						
Underdeveloped	-	+	-	-	+	-
Prominent	+	-	+	+	-	-
Crus helix						
Underdeveloped	-	-	-	-	-	-
Prominent	+	-	+	+	+	-
Expanded terminal portion	+	-	-	+	-	-
Helix						
Darwinian notch	+	-	-	+	+	-
Darwinian tubercle	+	-	-	-	+	-
Underfolded	-	+	-	-	-	-
Overfolded	-	-	-	-	+	-
Ear lobe						
Absent/small	+	+	+	-	+	-
Forward facing	-	+	+	-	-	-
Attached	+	-	-	-	-	-

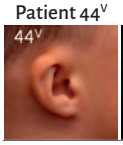
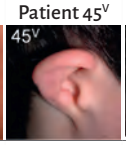
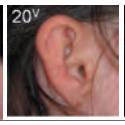
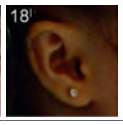
U = Unknown.

[I]Patients 13 and 36 were previously described by Bicknell *et al*. (patients 8 and 9) and Lacombe *et al*. (patients 2 and 3).[7,21]

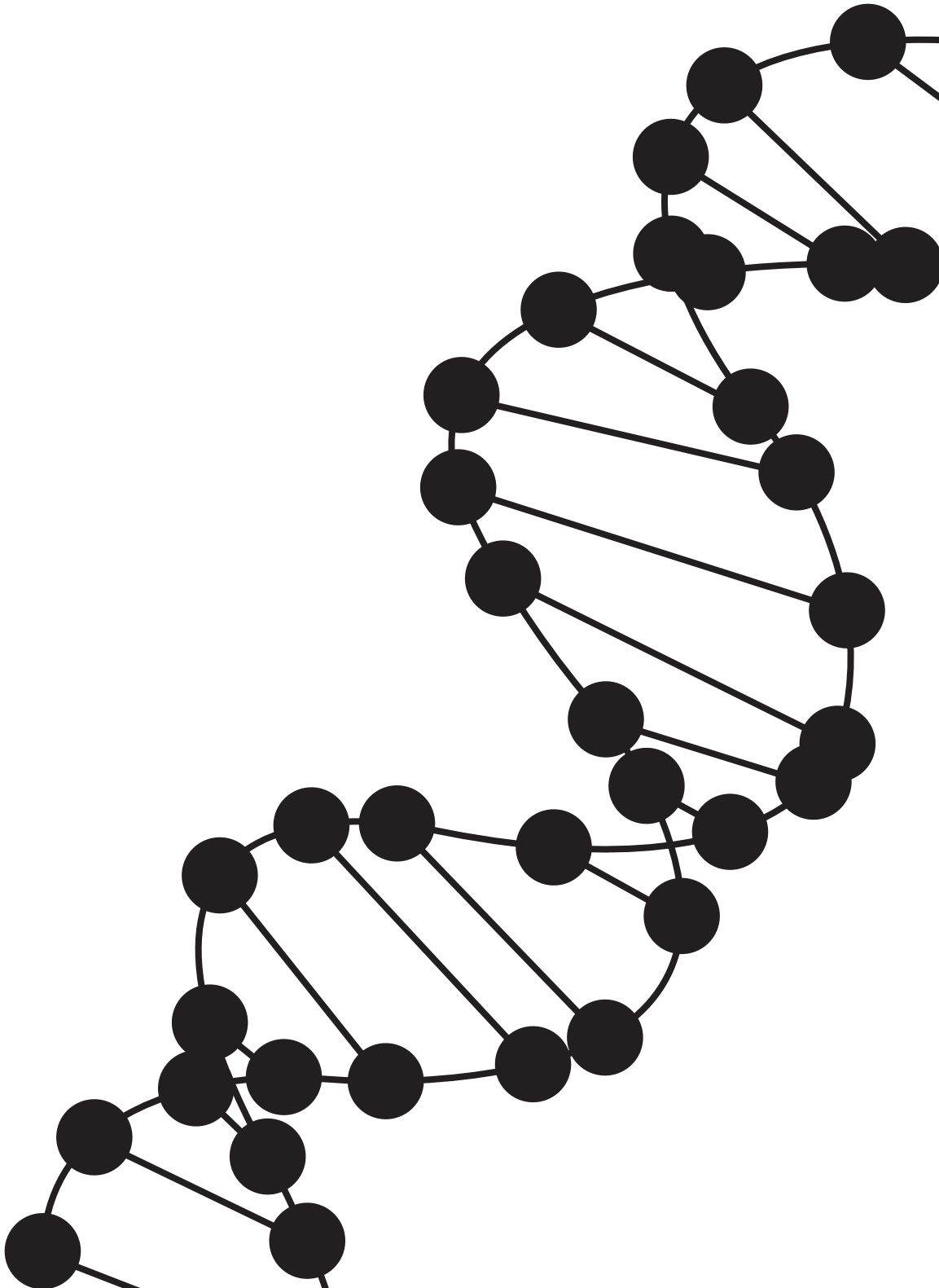
[II]Patients 9, 18, 37 and 38 were previously described by Bicknell *et al*. (patients 5, 10, 11, and 17).[21]

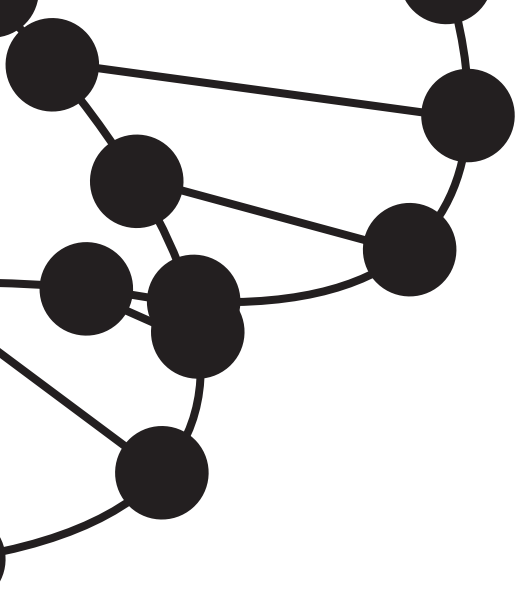
[III]Patient 42 was previously described by Bicknell *et al*. (patient 18) and Bongers *et al*. (patient 3).[13,21]

[IV]Patient 20 and 43 were previously described by Bongers *et al*. (patient 4 and 2, respectively).[13]

Patient 44[V]	Patient 45[V]	Patient 20[V]	Patient 18[II]	Patients 2, 6, 9-12 23, 24, 33, 34[2,5,6]	Total
					
U	U	U: *ORC1* Monoallelic	*CDT1*	U, *ORC1* *ORC4*, *ORC6*	
M	M	F	F	8F/2M	11F/9M
-	-	[c.1721C>T]	[c.1081C>T] [c.1357C>T]		
+	+	+	+	10/10	20 (100%)
-	-	-	-	0/10	3 (15%)
+	+	-	-	5/10	9 (45%)
-	-	+	-	1/10	4 (20%)
-	+	-	-	0/10	1 (5%)
-	+	-	-	3/10	5 (25%)
+	-	-	-	1/10	3 (15%)
-	+	-	-	1/15	3 (15%)
-	+	-	-	1/10	4 (20%)
-	-	-	-	1/10	4 (20%)
+	+	-	-	2/10	4 (20%)
-	-	+	-	5/10	10 (50%)
-	-	-	-	1/10	3 (15%)
-	-	-	-	0/10	3 (15%)
-	-	+	-	0/10	3 (15%)
-	+	-	-	0/10	2 (10%)
-	-	-	-	3/10	1 (15%)
+	+	-	-	4/10	10 (50%)
-	-	-	-	0/10	2 (10%)
-	-	-	-	1/10	2 (10%)

[V]Patients 12, 33, 34, 44 and 45 were not previously described.
[VI]Patient 2 was previously described by Bicknell *et al.*(patient 3);[22] patients 10 and 11 were previously described by Bicknell *et al.* (patients 6 and 7), Bongers *et al.* (patients 5 and 6) and Guernsey *et al.*, 2011 (patients 1768 en 1769);[13,20,21] Patient 6 was previously described by Guernsey *et al.* (patient1882);[20] patients 23 and 24 were previously described by Cohen *et al.*[5]





Chapter 2.3

Meier-Gorlin syndrome

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Abstract

Meier-Gorlin syndrome (MGS) is a rare autosomal recessive primordial dwarfism disorder, characterized by microtia, patellar aplasia/hypoplasia and a proportionate short stature. Associated clinical features encompass feeding problems, congenital pulmonary emphysema, mammary hypoplasia in females and urogenital anomalies, such as cryptorchidism and hypoplastic labia minora and majora. Typical facial characteristics during childhood comprise a small mouth with full lips and micro-retrognathia. During ageing, a narrow, convex nose becomes more prominent. The diagnosis MGS should be considered in patients with at least two of the three features of the clinical triad of microtia, patellar anomalies and pre- and postnatal growth retardation. In patients with short stature and/or microtia, the patellae should be assessed with care by ultrasonography before age 6 or radiography thereafter.

Mutations in one of five genes (*ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*) of the pre-replication complex, involved in DNA-replication, are detected in approximately 67-78% of patients with MGS. Patients with *ORC1* and *ORC4* mutations appear to have the most severe short stature and microcephaly.

Management should be directed towards in-depth investigation, treatment and prevention of associated problems, such as growth retardation, feeding problems, hearing loss, luxating patellae, knee pain, gonarthrosis and possible pulmonary complications due to congenital pulmonary emphysema with or without broncho- or laryngomalacia.

Growth hormone treatment is ineffective in most patients with MGS, but may be effective in patients in whom growth continues to decrease after the first year of life (usually growth velocity normalizes after the first year) and with low levels of IGF1. At present, few data is available about reproduction of females with MGS, but the risk of premature labor might be increased.

Here, we propose experience-based guidelines for the regular care and treatment of MGS patients.

Review

In this review, we outline the clinical symptoms of MGS and propose experience-based guidelines for care and treatment of MGS patients. The reported percentages of clinical symptoms are calculated from the frequency of these symptoms in the 38 patients with bi-allelic mutations in one of the five causative pre-replication complex genes (*ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*) described in literature.[1-5] Clinical symptoms that may affect management and that are present only in patients with clinically diagnosed MGS (in whom the molecular defect is unknown) are mentioned as well.

Disease name and synonyms

Meier-Gorlin syndrome (MGS; MIM#224690, ORPHA 2554).
Ear patella short stature syndrome (EPS).

Definition

Meier-Gorlin syndrome (MGS) is characterized by the triad of microtia, absent or small patellae and short stature. At least two of these three clinical features are present in 97% (32/33) of patients with MGS, the combination of patellar a-/hypoplasia and microtia being the most prevalent. Microtia, however, can be mild. One patient was described with short stature, but without microtia or patellar anomalies, indicating that the clinical phenotype might be more variable than previously suspected.[3,4]

Epidemiology

Until now, 67 patients with MGS have been described since the first reported patients by Meier in 1959 and Gorlin in 1975 (30 males; 36 females; 1 of unknown sex; age range 0.3-55 years).[1-22] The underlying molecular defect is known in 38 of these patients.[1-5]
The exact prevalence of MGS has not been determined, but is estimated to be less than 1-9/1,000,000 based on the number of cases described in literature. However, this might be an underestimation, due to underreporting and missed diagnoses.

Clinical description

Patients with MGS present with a recognizable phenotype. The classical triad of clinical features comprises microtia, patellar aplasia or hypoplasia and pre- and postnatal growth retardation.

Microtia is present in almost all patients with MGS (34/36; 94%). Severity ranges from mild to severe microtia, where the ears appear underdeveloped and low-set. Narrow ear canals and conductive hearing loss may accompany microtia.

Patellar anomalies are among the most frequent findings in MGS (31/33; 94%). In most patients, patellae are absent, but they may be hypoplastic.

Prenatal growth is delayed in the majority of patients with MGS. Intra uterine growth retardation (IUGR) was present in 97% (35/36), with a mean birth weight of –3.8 SDs (range < –6.5 SDs to –0.3 SDs), five infants were born prematurely (5/30; 17%; terms ranging from 28 to 36 weeks of gestation).[2-4] In three cases, the pregnancy was terminated because of severe IUGR, in combination with congenital anomalies.[5]

We studied growth extensively in our cohort of 45 MGS patients (35 patients with a known and 10 with an unknown molecular defect).[23] Postnatal growth was delayed during the first year of life. Growth velocity was almost normal thereafter, with patients growing parallel to the normal growth charts without significant catch up growth. In this cohort, height varied considerably between MGS patients and ranged from –9.6 SDs to –0.4 SDs. The average adult height in the MGS patients with a known underlying molecular defect was –5.5 SDs (five patients).

Microcephaly (head circumference < –3 SDs) was present in 43% (13/30) of patients, with a head-circumference ranging from –9.8 SDs to +1.7 SDs. Height differed significantly between the gene mutated, ethnic background and gender. Patients with mutations in *ORC1* and *ORC4* had a significantly shorter stature (a difference of 4.7 SDs and 3.1 SDs, respectively) and smaller head circumference (a difference of 5.0 SDs and 1.6 SDs, respectively), than patients with mutations in *ORC6*, *CDT1* or *CDC6*.[4,23]

Growth hormone therapy was unsuccessful in MGS patients with a known molecular defect. However, growth hormone therapy was successful in two patients with a clinical diagnosis of MGS, whose growth velocity continued to be low after the first year and who had low levels of IGF1 (–3.3 SDs and –4.6 SDs).[23]

All postpubertal females (10/10; 100%) had mammary hypoplasia. Transvaginal ultrasound investigations were performed in five adult females (clinically diagnosed with MGS). A small uterus was reported in three of them and polycystic ovaries were reported in two of these five females.[23]

Axillary hair is often sparse or absent, while pubic hair generally is normal in both males and females.

Furthermore, patients with MGS have a recognizable facial phenotype with microstomia, full lips and retro-/micrognathia at young age (Figure 1). The nose can be narrow and convex with a high nasal bridge. These characteristics of the nose become more prominent with age.

Figure 1. Facial features of Meier-Gorlin syndrome. (A) A girl aged 7 years and 9 months with two mutations in *ORC4*. **(B)** A 14 year old girl, who carries one mutation in *ORC1*. Both show the characteristic facial features of MGS, including microtia, a prominent nose with a convex nasal profile, a small mouth with full lips and retro-/micrognathia.

Intellect is normal in the majority of patients with MGS (30/31; 97%), although delayed motor development and/or speech development without intellectual disability (ID) were present in 19% (6/32) and 16% (5/32) of patients, respectively. A mild ID was present in one patient (3%).
Respiratory tract anomalies are relatively frequent. Congenital pulmonary emphysema was reported in 43% (12/28) of patients with MGS. Congenital pulmonary emphysema is usually detected in the neonatal period or early in childhood (10/12; 83%), but can be diagnosed late in childhood. Bronchomalacia, laryngomalacia and/or tracheomalacia are reported in 42% (10/24) of subjects. Recurrent respiratory tract infections occur often during childhood, but disappear thereafter.
Congenital cardiac anomalies appear to be rare: they have been described in two patients (2/30; 7%: one ventricular septal defect, one patent ductus arteriosus).
Feeding problems are seen in approximately 80% (26/32) of patients with MGS during infancy and young childhood. Problems range from a small appetite (which may correlate with the short stature of infants), to gastroesophageal reflux with administration of tube feeding or gastrostomy interventions in 35% (11/31).
Anomalies of the urogenital tract are frequently reported, especially cryptorchidism in males (9/14; 64%) and hypoplastic labiae in females (5/21; 24%).
A delayed bone age and genu recurvatum are often seen in patients with MGS. Joint contractures, including club feet, were present in 23% (7/30) of patients.
Not much is known about the reproduction of patients with MGS. Recently, a previously described woman clinically diagnosed with MGS gave birth prematurely twice after 17 and 18 weeks of gestation, respectively.[23]

Aetiology

MGS is an autosomal recessive disorder. Mutations in five genes of the pre-replication complex (*ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*), were detected in patients with MGS. Mutation detection rate was approximately 78% in a cohort of 45 patients with MGS.[4]
The pre-replication complex (PRC) assembles on genomic DNA at origins of replication. The exact underlying mechanism for the clinical features of MGS remains to be elucidated. The impaired function of the PRC is presumed to reduce the G1 phase of DNA replication and to limit the available time for origin licensing. This limitation is hypothesized to become rate-limiting, thereby impeding cellular proliferation. This would result in a reduction of the total cell number and thereby diminish overall growth.[24,25] Another possible mechanism affecting cell cycle progression was identified in ORC1 depleted cells, where a defect in the rate of formation of primary cilia was

demonstrated to influence cell cycle progression.[26] Alternatively, the ORC1 subunit of the PRC contains distinctive domains that regulate centriole and centrosome copy number. It is suggested that mutations in these domains alter centrosome duplication and thereby contribute to the growth retardation and microcephaly in MGS.[27]

Mutations in *ORC1* and *ORC4* appear to cause a more severe short stature and microcephaly than mutations in other genes. Furthermore, compound heterozygous missense- and loss of function mutations appear to have a more severe effect on the phenotype than homozygous or compound heterozygous missense mutations.[4] No patients are known with homozygous or compound heterozygous loss of function mutations, suggesting that these mutations cause a lethal phenotype. No additional genotype-phenotype correlations were recognized.

Diagnostic methods

The clinical diagnosis of MGS can be established in the presence of microtia, patellar aplasia or hypoplasia and proportionate short stature. However, about 18% (6/33) of patients show only two out of three cardinal features and one patient was described with short stature only.

The diagnosis MGS should be considered in patients with short stature or microtia and the presence of these features necessitates comprehensive examination of the patellae. In infants, ultrasound investigations are advised, since patellae are radiolucent in the first 5–6 years of life and will not be visible by conventional radiography.

Furthermore, the characteristic facial features and mammary hypoplasia are specific findings for MGS and will assist in diagnosing MGS.

In a patient clinically suspected to have MGS, the diagnosis can be confirmed by detecting compound heterozygous or homozygous mutations in one of the five pre-replication complex genes (*ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*). Mutations were detected in approximately 78% of patients clinically suspect for MGS.[4] In patients with a severe short stature and/or microcephaly, starting with the analysis of *ORC1* and *ORC4* should be considered, since mutations in these two genes are associated with a significantly shorter stature and smaller head circumference than mutations in the other genes.[4,23]

Differential diagnosis

The association of microtia, patellar anomalies and short stature as such has not been described in other syndromes.

MGS is a part of the primordial dwarfism spectrum. These disorders are characterized by microcephaly and prenatal short stature. Other primordial dwarfism disorders include Seckel syndrome and Microcephalic Osteodysplastic Primordial Dwarfism (MOPD) type I, II and III. However, even though these syndromes show considerable overlap with MGS, they are clinically distinct disorders. In Seckel syndrome, short stature is proportionate, as in MGS. However, height and head circumference are generally much smaller in Seckel syndrome than in MGS (average –7.1 SDs and –8.7 SDs, respectively)[28], the facial appearance differs from MGS and patients generally have ID. These patients can have patellar anomalies and microtia, but these findings are not frequently present, contrary to MGS, where they are mandatory features.

Patients with MOPD type 1 and 2 have a disproportionate short stature and a skeletal dysplasia, clearly distinguishing these disorder from MGS. MOPD type 3 is rare and it is suggested this is the same disorder as MOPD type 1. Stature in patients with MOPD type 3 was proportionate, though.

Patellar anomalies and short stature are cardinal features of RAPADILINO and genitopatellar syndrome. However, these syndromes differ from MGS in the lack of microtia (both), the presence of radial ray defects (RAPADILINO) and developmental delay and agenesis of the corpus callosum (genitopatellar syndrome).

If patellar anomalies are present and microtia and short stature are absent, nail patella syndrome should be considered. In nail patella syndrome, familial patellar a-/hypoplasia is almost always accompanied by nail dysplasia, including the pathognomic tri-angular lunulae.

An overview of other syndromes with patellar anomalies, such as small patella syndrome, patella aplasia-hypoplasia and trisomy 8 mosaicism is provided by Bongers *et al*.[29]

In patients with microtia, other diagnoses to contemplate are branchio oto renal syndrome, Townes-Brocks syndrome, Treacher-Collins syndrome, Nager syndrome, Miller syndrome and CHARGE association.

Genetic counselling

MGS is an autosomal recessive disorder. The recurrence risk for a couple with an affected child is 25%. Parents are obligate heterozygotes. Heterozygotes are asymptomatic.

In literature, three patients with a classic MGS phenotype and mono-allelic mutations (one in *ORC1*, two in *CDT1*) were described. However, their healthy fathers carried the

same mutation. Therefore, it is expected that these patients either carry a second mutation in *ORC1* or *CDT1* that cannot be detected with the current molecular techniques, or that their symptoms were caused by a different molecular defect.
To our knowledge, so far, no patients with MGS have had any children. However, future reports are expected. Offspring of a patient with MGS are obligate heterozygotes.
The risk of having an affected child with MGS is low (<1%), since carrier frequencies and thus the risk of a partner being a carrier are low.

Antenatal diagnosis

When the underlying molecular defect is known, prenatal diagnosis by chorionic villus sampling or amniocentesis is possible. If no causative gene defects are identified, prenatal ultrasound investigations at 18–20 weeks of gestation may contribute to the recognition of MGS. However, it is unknown whether overall growth is already delayed at this term. Microtia and congenital pulmonary emphysema can be seen and certain facial features, such as micrognathia may be detected. Nonetheless, because of the variable expression of the disorder and the lack of structural congenital anomalies, ultrasound abnormalities may be absent or mild and difficult to interpret. The ethical issues of prenatal diagnosis and possible termination of a pregnancy for a disorder with a variable expression and a relatively low chance of life-threatening complications or intellectual disability, should be discussed.

Management

Especially during childhood, multidisciplinary care is needed.
Here, we propose guidelines for regular care and treatment, based on clinical experience. An overview of the proposed guidelines for diagnostic evaluation and management of patients with MGS is presented in Supplementary Table 1.

Ears

Microtia can be associated with narrow ear canals and conductive hearing loss. Therefore, at the time of diagnosis, a patient suspected to have MGS should be examined by an ear-nose-throat specialist. Afterwards, examinations are indicated when hearing loss appears to be present.

Patellae

Patellar aplasia or hypoplasia may lead to instability of the knee joint, pain and early gonarthrosis. A multidisciplinary approach, including examination by an orthopedic

surgeon and rehabilitation physician is advised. Special shoes and advise about strength enhancing exercises and sports, may be provided, especially since patients with MGS often have hypermobile joints and are more prone to develop pes planus.

Growth and growth hormone treatment

Growth hormone treatment was effective in only four patients, one with *ORC4* mutations, one with *CDC6* mutations and two without mutations.[4,23] In one patient with *CDC6* mutations, height improved from –5 SDs to –3 SDs during the first 4 years. In two patients without mutations, height improved from –6.8 SDs to –3.0 SDs and from –5.7 SDs tot –3.7 SDs.

In a child with MGS, endocrinological evaluation (IGF1 and stimulated growth hormone measurements) is advised. Growth hormone treatment should be considered in a patient with low IGF1 levels and further delay of growth after the first year.

Feeding problems

Feeding problems can be severe, necessitating tube feeding and a gastrostomy to optimize nutrition. Gastroesophageal reflux as a cause should be considered.

Congenital pulmonary emphysema

Surgical resection can be performed when respiratory distress is life-threatening or debilitating. In older patients with MGS and congenital pulmonary emphysema treatment with selective Beta2-antagonists can be considered.

Congenital cardiac anomalies

Since 7% of the reported MGS patients had a congenital cardiac anomaly, we advise cardiac screening (physical examination, ECG and ultrasound investigations) at the time of diagnosis.

Mammary hypoplasia

Treatment of mammary hypoplasia with exogenous estrogen can be debated in females with MGS. Estrogen therapy was reported to be successful in two females with MGS. In a third female, minor effects were reported. In two other females, no effect was seen.[4,23] Breast augmentation surgery can be considered.

Reproduction

Males born with cryptorchidism may have fertility problems. One female with MGS had two consecutive miscarriages. Her uterus was described to be small. No information about other pregnancies is available, but preconception gynaecologic examinations should be considered and possible preterm labor should be a point of attention in obstetric care of females with MGS, especially since gynecologic evaluation in four other females with MGS revealed a small uterus and polycystic ovaries in two of them.[23]

Prognosis

Life span is expected to be normal for most individuals with MGS. Osteoarthritis of the knees, secondary to patellar anomalies, may occur at a younger age than usual.

Life threatening complications that may accompany MGS are severe congenital pulmonary emphysema and cortical malformations. When severe congenital anomalies and/or severe respiratory problems are absent, however, there is no reason to expect an abnormal life span.

Four reported patients with MGS were deceased (11%) and three pregnancies were terminated because of ultrasound abnormalities, including severe IUGR (8%).[3-5,16]

One patient (with *ORC1* mutations) passed away after 3.5 months. He had a severe cortical dysplasia, pachygyria and ventricular enlargement, cranial suture stenosis, congenital emphysema of the lung and his pancreatic tail was absent. His brother died in utero after 17 weeks of gestation. Both patients exhibited microtia and a severe growth retardation. A third patient (with *CDT1* mutations) deceased after a sudden cardiac arrest. He had congenital pulmonary emphysema for which he required surgery. His sister passed away after 3 months. She had severe respiratory problems due to a tracheobronchomalacia with progressive pulmonary emphysema.

Unresolved questions

The long-term risks of congenital pulmonary emphysema in patients with MGS need to be evaluated through long-term follow-up. Furthermore, future reports about reproduction in patients with MGS have to be awaited to assess the exact reproductive risks.

Approximately 22% of patients with a classical clinical phenotype of MGS lacked mutations in one of the five known genes of the pre-replication complex.[4] These patients might have mutations in one of the five known genes we are unable to detect with current sequencing techniques, or their symptoms may be caused by mutations in other genes of the pre-replication complex or in pathways connected to this complex.

It is currently unknown why patients with *ORC1* and *ORC4* mutations appear to have shorter stature and smaller head circumference compared to patients with mutations in other genes. Further understanding of the role of the different genes of the pre-replication complex during growth and development might contribute to gain insight in these differences.

Conclusions

Here, we provide an overview of the clinical features of Meier-Gorlin syndrome and guidelines for management and treatment of associated problems in this rare disorder. MGS is an autosomal recessive primordial dwarfism disorder characterized by microtia, patellar a-/hypoplasia and short stature and often accompanied by feeding problems, respiratory problems, mammary hypoplasia and urogenital anomalies.
Multidisciplinary care, directed towards diagnosis and treatment of ear anomalies and/or hearing loss, patellar anomalies, feeding problems, respiratory problems and possible reproductive issues, is necessary to assure optimal medical care. Endocrinologic testing should be routinely performed in all children with MGS, although growth hormone treatment is not beneficial in most patients with MGS.
Molecular analysis of the five known genes for MGS (*ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*) is important in genetic counseling. A molecular diagnosis assists in determining the exact recurrence risk and enables future reproductive options, such as preimplantation genetic diagnosis and invasive diagnostic testing during pregnancy.
Since a molecular diagnosis cannot be established in 20-35% of patients with MGS, further studies are necessary to identify the underlying genetic defects in these patients.

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Supplementary table 1. Management advice for diagnostic evaluation and management of patients with Meier-Gorlin syndrome based on clinical experience.

Age	Organic system	Signs and symptoms	Investigation	Period of evaluation	Treatment options
From birth till late childhood	*Ears*	Small external auditory meatus Conductive hearing loss	Otoscopy Hearing tests	At diagnosis Annually when indicated	Hearing aids
	Skeleton	Patellar a-/hypoplasia Knee complaints, hypermobile joints, pes planus	Knee imaging: ultrasonography ≤ 5-6y, X-rays > 5-6 y. Orthopedic assessment	At diagnosis In case of signs and symptoms	Rehabilitation advice Arch support Surgical intervention
	Growth	Impaired growth velocity after 1 year of age Low IGF1	1. Evaluation of growth 2. Complete endocrine work-up	1. At diagnosis and annually 2. At diagnosis and in case of signs and symptoms	Growth hormone treatment
	Central nervous system	Delayed motor development, muscle weakness Speech delay	Developmental assessment	Annually	Speech therapy Extra assistance at school
	Gastrointestinal tract	Poor weight gain, poor intake Gastro-esophageal reflux	1. Monitor feeding and growth 2. Esophageal pH monitoring	1. Annually 2. In case of signs and symptoms	Nasal tube feeding GERD treatment[1]
	Respiratory tract	Respiratory tract infection Laryngo-/tracheomalacia Congenital pulmonary emphysema	1. Physical examination 2. Evaluation by pulmonary specialist; CAT scan	1. At diagnosis 2. In case of signs and symptoms	Antibiotics when indicated Surgery when life-threatening/ disabilitating Selective Beta2-agonists when indicated
	Genitourinary tract	Cryptorchidism in males Hypoplastic labiae in females	1. Physical examination 2. Ultrasound investigations of the inguinal region in males	1. At diagnosis 2. In case of symptoms and signs	Orchidopexy
	Heart	Congenital cardiac defect	1. Physical investigation 2. Cardiac ECG and ultrasound	1. At diagnosis 2. At diagnosis	Cardiac surgery
From childhood onwards	*Skeleton*	Knee complaints, arthrosis	Orthopedic assessment	In case of signs and symptoms	Surgical intervention
	Secondary sexual development	Mammary hypoplasia in females Sparse/absent axillary hair in both males and females	Physical examination	Annually during puberty In case of signs and symptoms	Estrogen treatment, breast augmentation surgery

Supplementary table 1. Continued

Age	Organic system	Signs and symptoms	Investigation	Period of evaluation	Treatment options
	Genitourinary tract	Irregular menses, polycystic ovaries, small uterus	Gynecologic assessment[II]	In case of signs and symptoms	Hormonal treatment when diagnosed with polycystic ovaries[III]
	Pregnancy	Possible risk of premature delivery	Antenatal care in a secondary care centre	During pregnancy	

[I]Gastroesophageal reflux disease (GERD) treatment according to the guidelines of the Dutch society of Pediatrics[1]:
- Symptoms of GERD < 18 months of age: conservative treatment by thickening feeds can be considered, but treatment with ranitidine or proton pump inhibitors for 2-4 weeks can be started directly.
- Symptoms of GERD > 18 months of age: consider treatment with proton pump inhibitors.
[II]In case of polycystic ovaries, assessment according to the Rotterdam guidelines[2]:
- Physical examination of the external sexual characteristics and signs of hyperandrogenism
- Ultrasound investigations of the internal sexual organs
- Laboratory assessment to establish androgen excess and/or ovalutory dysfunction.
- [III]Hormonal treatment[3]:
- Consider treatment with oral contraceptives if menses occurs less than four times a year.
- In case of a child wish, consider ovulation induction by clomifene citrate.

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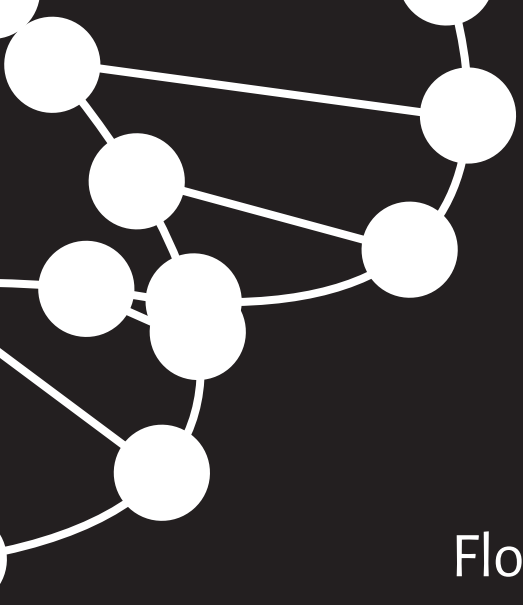
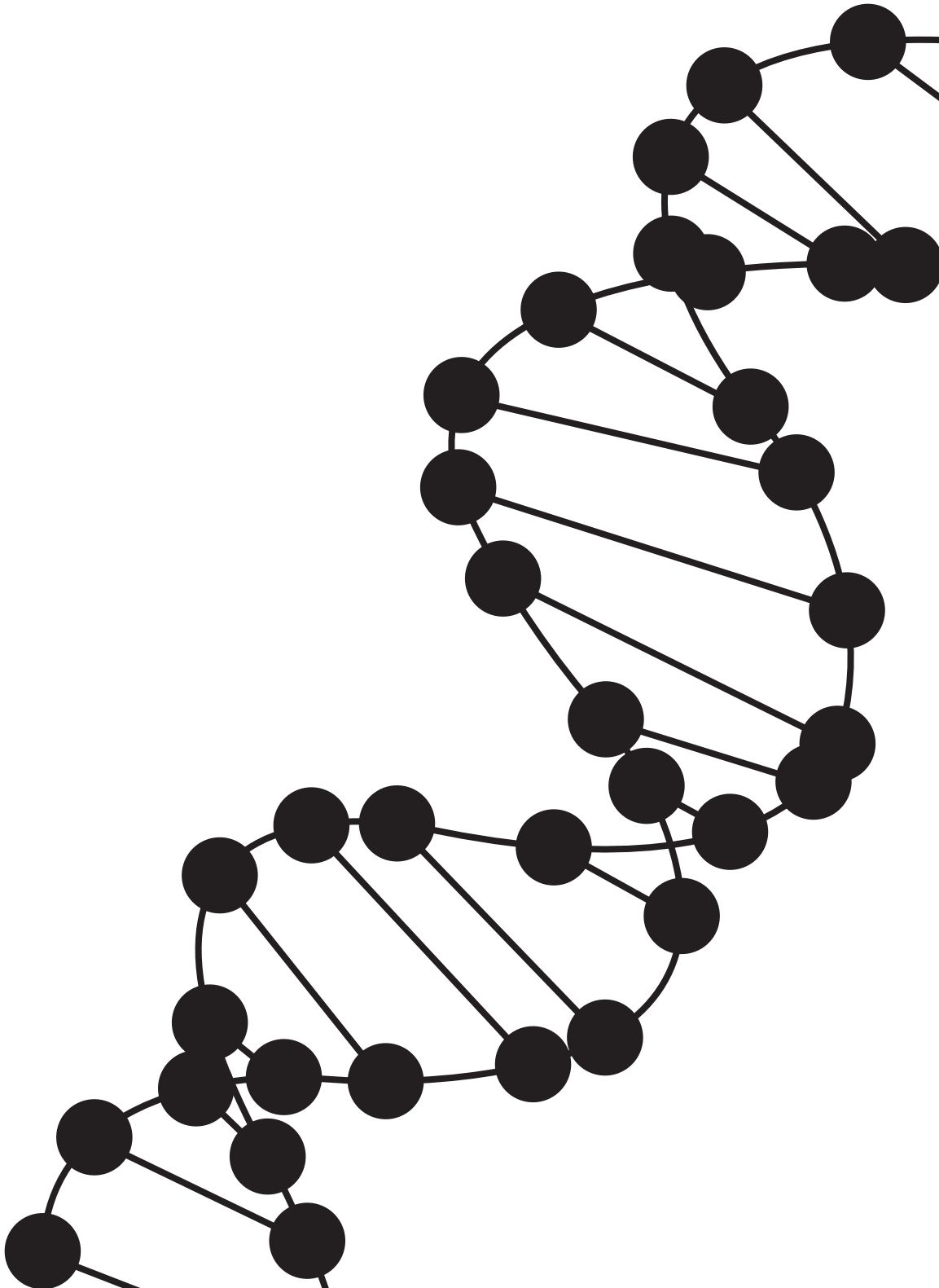
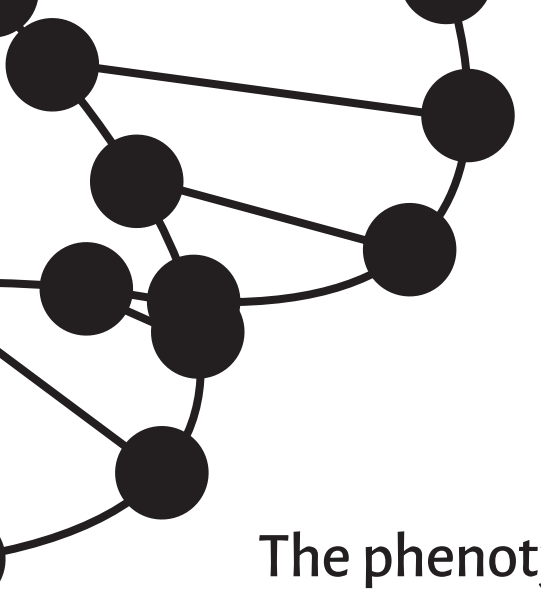


Chapter 3

Floating-Harbor syndrome





Chapter 3

The phenotype of Floating-Harbor syndrome: clinical characterization of 52 individuals with mutations in exon 34 of *SRCAP*

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Orphanet J Rare Dis 2013;8:63

Abstract

Background Floating-Harbor syndrome (FHS) is a rare condition characterized by short stature, delays in expressive language and a distinctive facial appearance. Recently, heterozygous truncating mutations in *SRCAP* were determined to be disease-causing. With the availability of a DNA based confirmatory test, we set forth to define the clinical features of this syndrome.

Methods and results Clinical information on fifty-two individuals with *SRCAP* mutations was collected using standardized questionnaires. Twenty-four males and twenty-eight females were studied with ages ranging from 2 to 52 years. The facial phenotype and expressive language impairments were defining features within the group. Height measurements were typically between minus two and minus four standard deviations, with occipitofrontal circumferences usually within the average range. Thirty-three of the subjects (63%) had at least one major anomaly requiring medical intervention. We did not observe any specific phenotype-genotype correlations.

Conclusions This large cohort of individuals with molecularly confirmed FHS has allowed us to better delineate the clinical features of this rare but classic genetic syndrome, thereby facilitating the development of management protocols.

Background

Floating-Harbor syndrome (FHS; MIM 136140) is a rare disorder characterized by short stature with delayed bone age, deficits in expressive language and a distinctive facial appearance. The name of the syndrome is derived from the two hospitals where the first patients were reported over 35 years ago.[1,2] Recently, we used exome sequencing to investigate a cohort of 13 unrelated individuals with classic features of FHS and identified heterozygous mutations in *SRCAP* (MIM 611421) as causative of this disorder.[3] All reported mutations were truncating and occurred between codons 2,407 and 2,517 in exon 34 resulting in loss of three C-terminal AT-hook motifs. *SRCAP* encodes a SNF2-related chromatin-remodeling ATPase that serves as a coactivator for CREB-binding protein, better known as CBP, the major cause of Rubinstein-Taybi syndrome (RTS). The disrupted interaction between these two proteins likely explains some of the clinical overlap between FHS and RTS.[4] The mechanism of disease in FHS is suspected to be dominant-negative due to the non-random clustering of truncating mutations in the final exon that results in the loss of the major transactivation function of SRCAP located in a 655 residue C-terminal fragment, evidence that expression of a construct solely consisting of the CBP interaction domain of SRCAP strongly inhibits CREB-mediated transactivation in a dominant-negative fashion and the existence of patients with haploinsufficiency of *SRCAP* who do not have features of FHS.[3,5]

Many of the features of FHS are non-specific (short stature, delayed bone age and language delays) and if the distinctive facial features are not recognized, this diagnosis can be difficult. Several years ago, Feingold provided a thirty-two year follow-up on the first reported patient accompanied by a review of the literature.[6] He suggested that some of the patients reported to have FHS did not fit the classical description and likely had a different condition. With the availability of a molecular test, we are now able to further delineate the distinctive and recognizable features of this syndrome.

Methods

Subjects and clinical data

Individuals with a presumptive clinical diagnosis of FHS were invited to be part of this study. Clinical data was collated from three sources: FORGE Canada Consortium (Finding of Rare Disease Genes in Canada), based at the Children's Hospital of Eastern Ontario, the Manton Center for Orphan Disease Research at Boston Children's

Hospital and the Radboud University Nijmegen Medical Centre. All samples that were referred for analysis were accepted for the study. Approval of the study design was in compliance with the Helsinki Declaration and was obtained from each of the participating institutions' research boards. Free and informed consent was obtained from each study subject (or guardian, if appropriate) prior to enrollment. Recruitment e-mails were sent to all members of the Floating-Harbor syndrome support group. Interested families or physicians contacted the genetic counselor at the Manton Center. A medical history questionnaire was administered to the family or physician via telephone, which reviewed all pertinent medical and developmental history, as well as FHS-specific questions (Supplementary File 1). Referring providers who submitted cases directly to the above institutions completed the same questionnaire. In most cases, clinical photographs were available prior to molecular testing and the likelihood of finding a mutation was noted. Due to the diversity of the sample sources, there was wide pre-test probability of referred individuals actually having FHS, as this is a rare condition and most clinicians do not have familiarity with it. The clinical information from the first 13 subjects described by Hood *et al*. was also included.[3]

Molecular analysis

Sanger sequencing of exons 31–34 of *SRCAP* was performed using DNA samples from individuals with suspected FHS (Supplementary Table 1). When available, parental studies were performed to determine *de novo* or inherited status. The clinical information, from twenty-seven individuals who did not carry a mutation in exons 31–34 of *SRCAP*, was used to help clarify key diagnostic features. For three individuals, who most closely resembled the FHS phenotype and for which no mutations were identified in exon 34, complete sequencing of the *SRCAP* gene was performed (primer sequences available on request).

Results and discussion

Molecular

In total, 24 males and 28 females were identified with mutations in *SRCAP*; 39 new individuals and 13 previously reported.[3] Ages at time of data collection ranged from two years to 52 years of age. The average age of diagnosis was 8 years. Two mother/daughter pairs and a number of the other subjects have been previously reported in the literature.[1,3,6,7,9–11] All the mutations identified in our cohort were truncating

(nonsense or frameshift) alleles (Table 1). Two mutations are recurrent; the Arg2444* mutation was observed in about half (24/52) (including the original patient described by Pelletier and Feingold)[1], while the Arg2435* mutation was present in approximately one quarter (13/52) of the individuals with FHS. In our original cohort of 13 patients with FHS we delineated the boundaries of the critical region to between codons 2407 and 2517. The extended cohort of molecularly-defined patients we present here extends the critical region to between codons 2389 and 2748, a further 249 amino acids in exon 34. Interestingly, the boundaries of this critical region are delineated by mutations observed in our two mother-daughter pairs (Table 1), however, the significance of this finding is unclear.

Table 1. Mutations detected in exon 34 of *SRCAP* in individuals with FHS.

	c.DNA	Frequency (52)	Comments
Glu2389*	c.7165G>T	2	Mother/Daughter[8]
Gln2407*	c.7219C>T	1	
Gln2407*fs**35	c.7218_7219delTC	1	
Asn2410*fs**32	c.7230insA	1	
Thr2425*fs**17	c.7274insC	1	
Arg2435*	c.7303C>T	13	2nd Recurrent mutation
Ala2440*fs**3	c.7316dupC	1	
Arg2444*	c.7330C>T	24	Most frequent recurrent mutation
Pro2459*fs**125	c.7374dupT	2	
Pro2459*fs**16	c.7376delC	1	
Thr2512*fs**5	c.7533_7534insAA	1	
Gln2517*fs**5	c.7549delC	1	
Asn2618*fs**11	c.7852insC	1	
Arg2748*	c.8242C>T	2	Mother/Daughter[7]

Facial gestalt

The face of FHS is the most distinctive aspect of this syndrome (Figures 1, 2 and 3) and although there are changes with age, the cardinal features, as originally described, remain constant.[1,2,4] The overall facial shape is triangular. The nose is narrow at the root and broadens to the tip. The columella is low hanging, nares are large and the philtrum is often short. The upper vermillion is typically thin and the lower lip is often everted. The lips tend to be in a horizontal plane at rest or when smiling. The eyes are frequently deep set and the eyelashes tend to be long. The ears can be low set and large in appearance. As seen in the photos, the FHS phenotype is more difficult to recognize in infancy.

Figure 1. Facial photographs of 6 females with FHS with the common Arg2444* mutation.

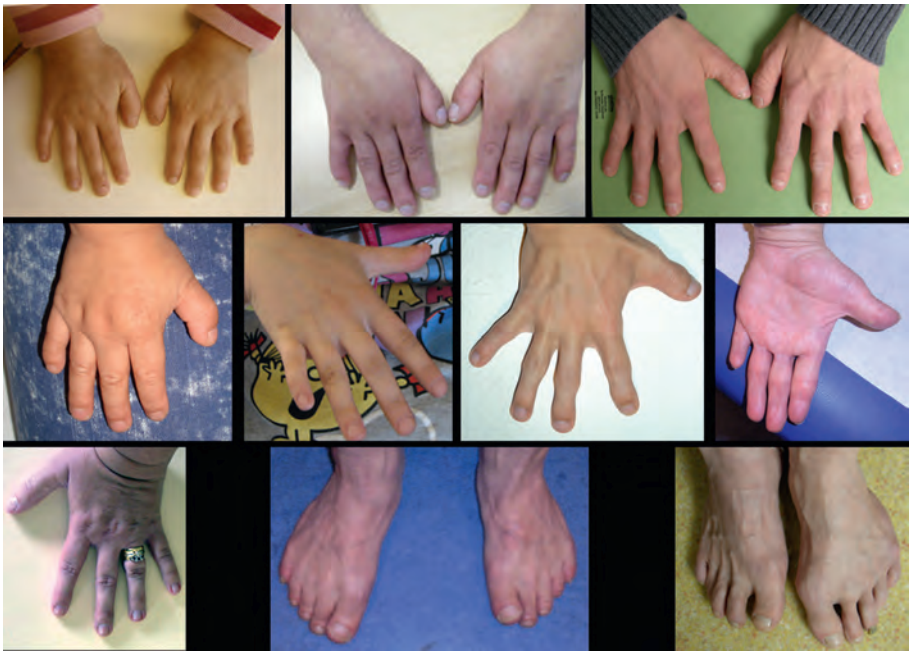
Figure 2. Facial photographs of 4 individuals with FHS of varying ages with the Arg 2435* mutation.

Figure 3. Facial photographs of 7 individuals with FHS as examples of the other mutations. (A) A female with the Gln2407* mutation. **(B)** A male with the Ala2440*fs**3 mutation. **(C)** A female with the Asn2618*fs**11 mutation. **(D)** A female and male with the Pro2459*fs**125 mutation. **(E)** A mother and daughter with the Arg2748* mutation.

Skeletal

Of the 17 individuals where thumb morphology was formally assessed, broad thumbs were only seen in 10 individuals indicating that they are a frequent but not mandatory finding in FHS. The differential diagnosis of broad thumbs includes Rubinstein-Taybi syndrome, where they are a cardinal feature. FHS is also in the differential, which is logical as SRCAP interacts with CBP. Other skeletal findings include broad first toes and brachydactyly. Broad fingertips are seen frequently and the fingers are often described as being clubbed, although would be more accurately classified as having broad fingertips (Figure 4). Leisti *et al*. reported a right-sided pseudoarthrosis-type anomaly of the clavicle noted at age two in one of their patients.[2] Four individuals in our series have uni- or bilateral clavicular anomalies including pseudoarthroses or hypoplasia. Two individuals have 11 pairs of ribs and four have hip dysplasia.

Figure 4. Hands and feet of individuals with FHS. Clinical photos demonstrating the variability of features ranging from unremarkable to brachydactyly, short broad thumbs and big toes, broad fingertips.



Growth

Where available, growth parameters were plotted on aggregate graphs. Thirteen of 49 individuals had birth weights less than the third percentile (Figure 5). For females, the maximum height was at the 20th percentile, with most data points between minus two and minus four standard deviations (SDs) (Figure 6). For the males, the height measurements varied more widely, with maximum height at the 25th percentile and two adult heights below 4 SDs (Figure 7). Occipito-frontal circumferences (OFC) were more variable, with most being well within the average range (Figures 8 and 9). Seven individuals had OFCs less than 2 standard deviations and only one measurement was less than minus 3 SDs. This suggests relative sparing of head size in relation to stature. Body weights were not consistent to suggest a particular body habitus for this syndrome and probably reflect the variability seen in the general population.

Figure 5. Birth weights of individuals with FHS. Male birth weights - blue dots; Female birth weights – pink dots. The mean, 5th and 95th confidence intervals are indicated.

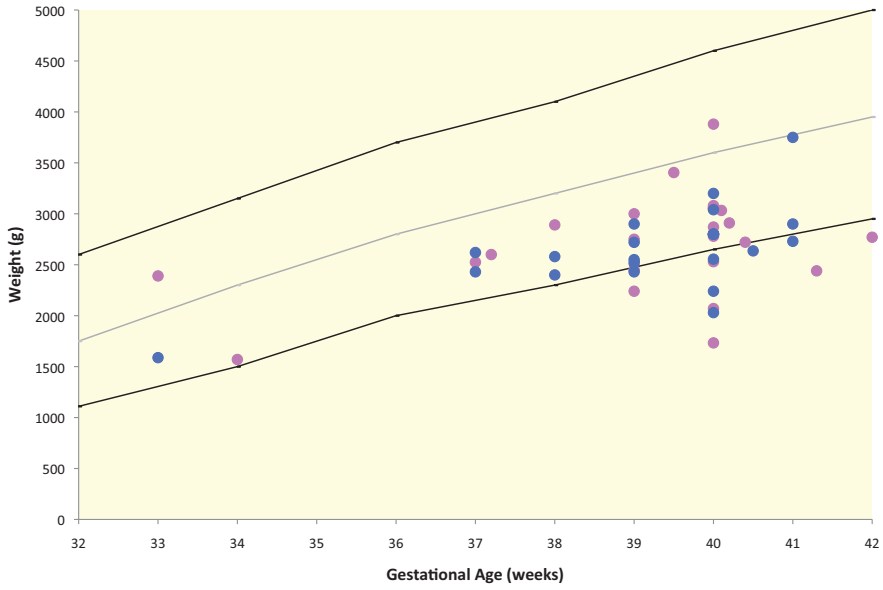


Figure 6. Height and weight of female individuals with FHS. Each point represents a single individual's measurements at the time of data collection.

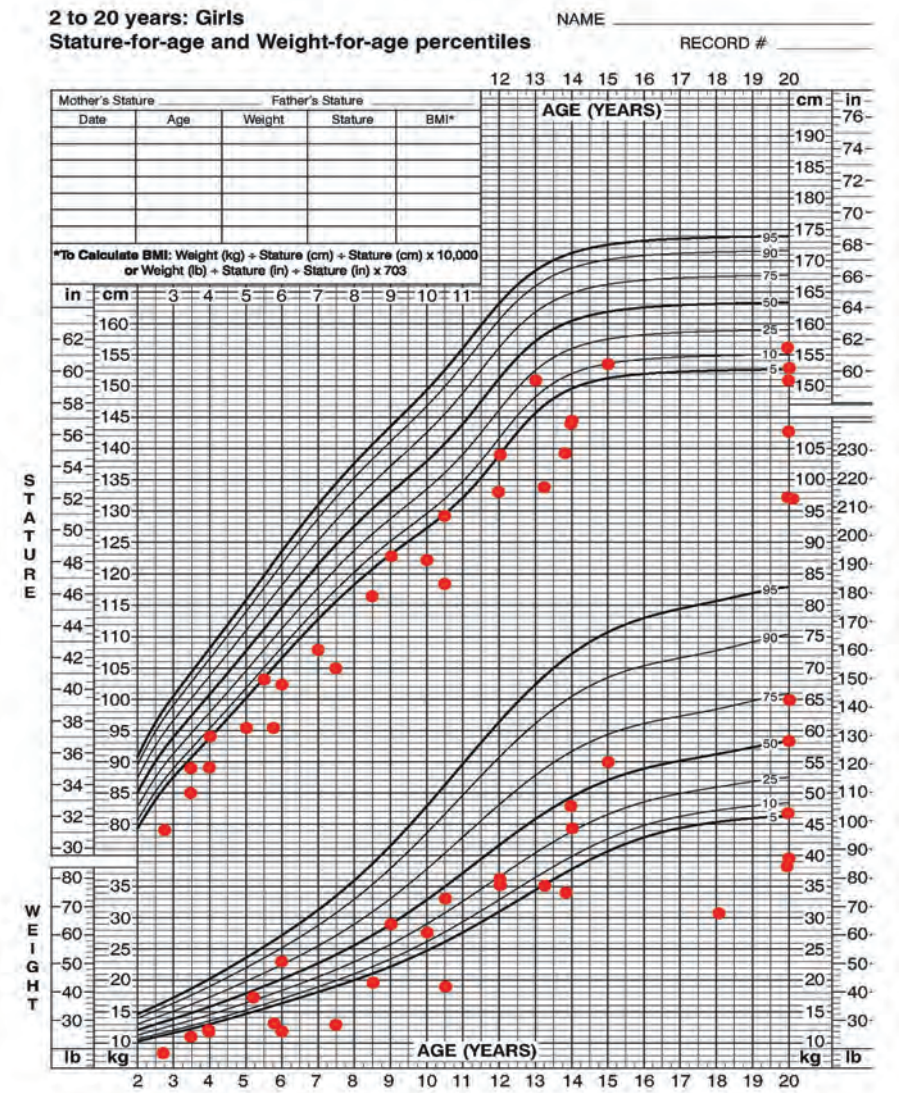


Figure 7. Height and weight of male individuals with FHS. Each point represents a single individual's measurements at the time of data collection.

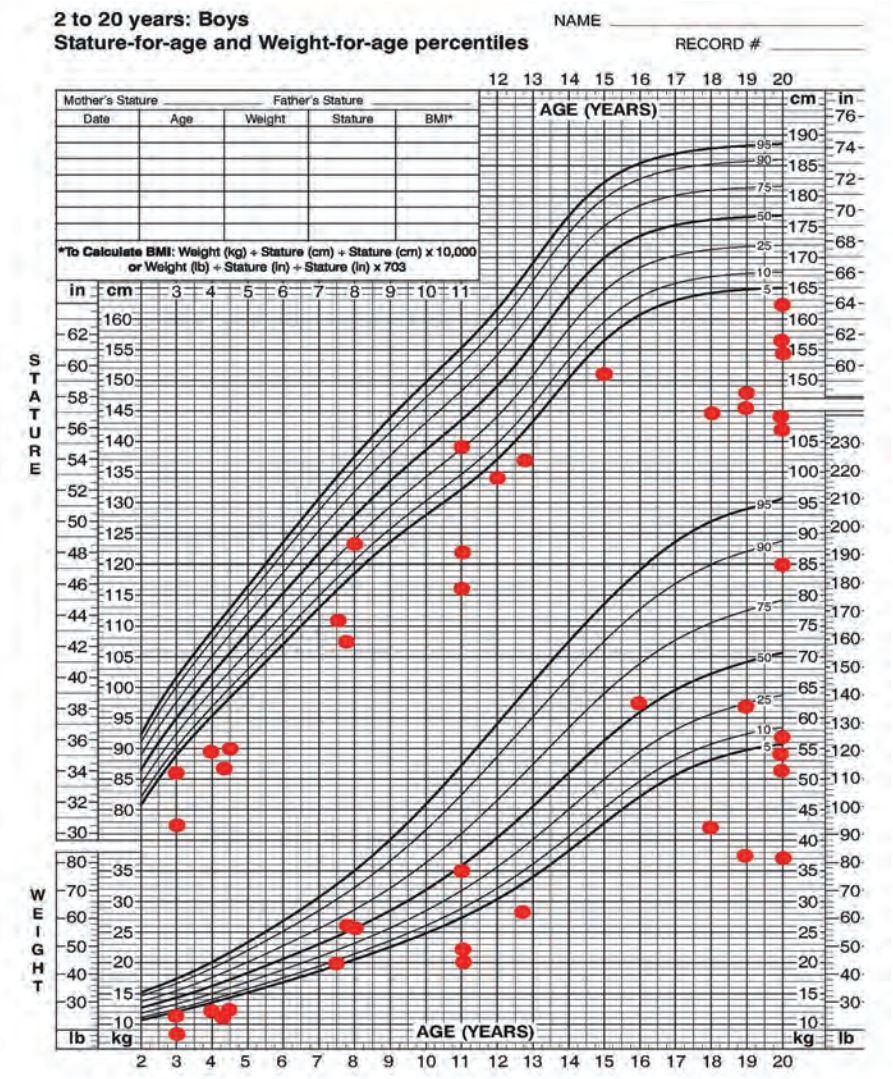


Figure 8. OFCs of females with FHS. Each point represents a single individual's measurements at the time of data collection. The mean, 5th and 95th confidence intervals are indicated.

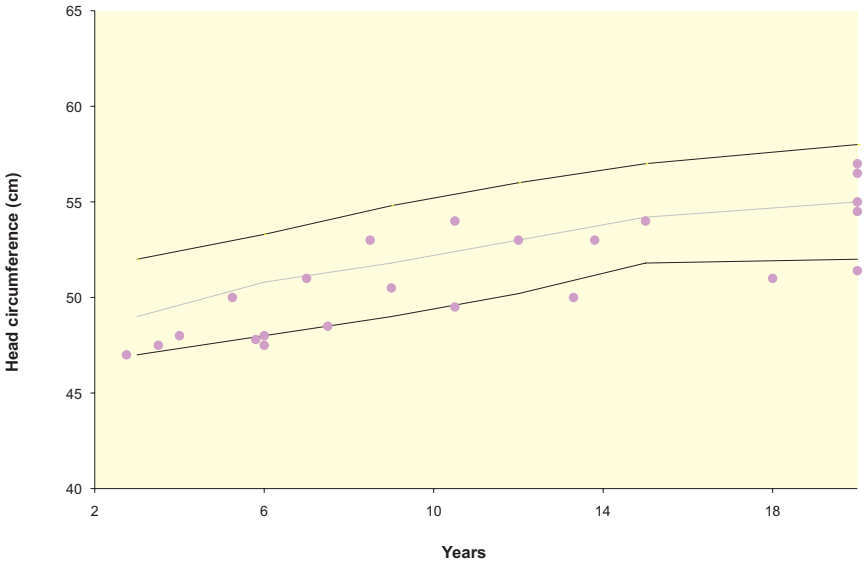
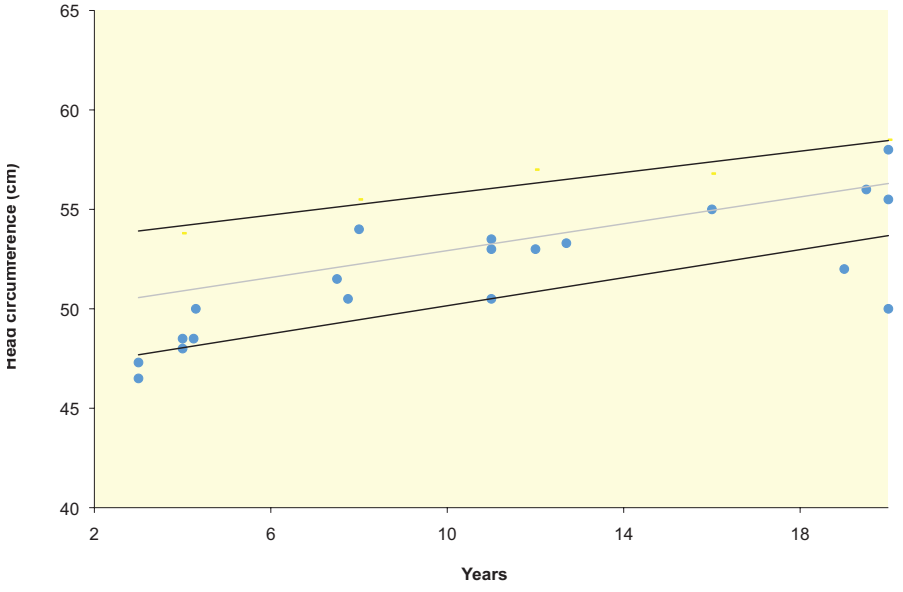


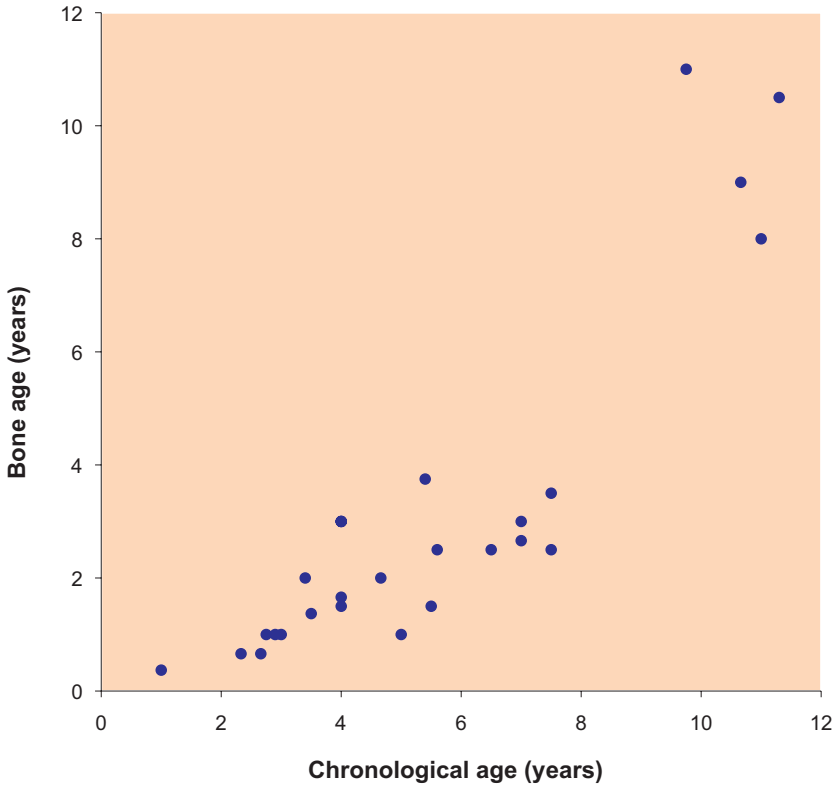
Figure 9. OFCs of males with FHS. Each point represents a single individual's measurements at the time of data collection. The mean, 5th and 95th confidence intervals are indicated.



Bone age and endocrine

Bone age values were plotted against chronological age (Figure 10) and all values in subjects less than 8 years old showed significant delays. There were no data values between ages 8–10 years, however, the bone ages approached the chronological age or became advanced after age 10 years. A number of participants in this study have been on growth hormone (GH) therapy, which may alter the natural history of growth in this population. Two of our subjects have been assessed in more detail regarding this issue.[9,10] Some GH treated individuals with FHS had documented GH deficiency, while others had modest responses to treatment despite normal levels of GH.[9,10,12] Early puberty has previously been reported in FHS and was documented in four individuals in our study.[13] Some of our subjects are currently pre-pubertal, while others could not accurately report pubertal timing, rendering the data incomplete. However, early puberty could explain the advanced bone age seen in teenage individuals with FHS as well as contributing to shorter adult heights.

Figure 10. Bone age values plotted against chronological age for 25 individuals with FHS.



Structural anomalies

A number of structural anomalies were detected in our cohort (Table 2), but no particular finding was seen with enough frequency to consider it a distinguishing feature of this syndrome. However, as some anomalies may affect clinical management, comprehensive screening is necessary in this population.

Table 2. Frequency of different clinical features in individuals with FHS.

Clinical features	Frequency reported
Eyes	
Strabismus	7/43
Hyperopia	5/43
Nystagmus	1/43
Ears	
Recurrent otitis media/T-tube placement	6/52
Hearing loss	9/52
Cochlear anomaly	1/U[I]
Other ENT	
Cleft lip and pseudocleft lip	2/52
Velopharyngeal insufficiency	2/U
Choanal atresia	1/U
Dental issues	
Small teeth/increased spacing	13/38
Cavities	6/38
Malocclusion/underbite	3/38
Cardiac malformation[II]	3/52
Gastrointestinal	
Motility issues (reflux/constipation)	13/52
Colonic stricture	1/U
Celiac disease	2/52
Genitourinary	
Cryptorchidism	5/24
Renal/collecting system anomalies	7/U
Seizures	6/52
Hypothyroidism	2/52

[I]U denominator unknown
[II]Mild aortic coarctation, atrial septal defect, Tetralogy of Fallot.

Voice quality and language

A high-pitched voice is often commented upon in individuals with FHS and was reported in 8/11 individuals. Others noted a nasal quality to the voice. An additional individual had documented velopharyngeal insufficiency (VPI), which may indicate

that VPI is under-recognized. Expressive language delay is a cardinal feature of this syndrome and was reported in all subjects. There was significant variability in severity with one individual who was bilingual, while another could only speak a few words as an adult. However, language development could potentially be hampered by the high frequency of recurrent otitis media and conductive hearing loss found in our cohort.

Cognition

The cognitive abilities in individuals with FHS range from average (IQ of 104) to significant intellectual impairment in a few instances. Most individuals had some modifications of their schooling (37/41). Obtaining full psychoeducational assessments on this cohort was beyond the scope of this study. However, when assessing global cognition in an individual with FHS, one must consider the language impairments, and in some instances sensory impairments and adjust accordingly.

Behaviour

The caregivers, in comparison to physicians, who filled out the questionnaires, often commented upon behavioral issues for their children (5/25). It is likely that these issues are under-recognized in this population. Rigid mannerisms were observed (7/25), as were some obsessive tendencies (e.g. skin picking). Parents often described their children as anxious individuals and attention deficit hyperactivity disorder (ADD or ADHD) was common (9/32).

We acknowledge that the data collection in our study was incomplete as data was obtained from a number of sources without a centralized clinical assessment. We also recognize that the ethnic backgrounds of the study subjects were mostly Caucasian and that FHS may be more difficult to diagnose in other populations. However, three individuals of Chinese origin were clinically diagnosed and identified to have mutations in *SRCAP*. In addition to growth and developmental issues, all of these subjects had classical FHS facial features, which were distinct from those of their family members.
Lastly, we evaluated for the presence of a genotype-phenotype correlation in FHS. Upon review of the clinical data, no clinical features were identified which discriminated between the different mutations. Given that all mutations cause truncation in a very defined area of the gene, this observation was not entirely unexpected.

Development of diagnostic criteria

The indication for analysis of the *SRCAP* gene was a presumptive diagnosis of FHS. The majority of those who underwent testing had short stature, delayed bone age, language delays and a distinctive facial appearance, usually with a prominent nose. Clinicians very familiar with FHS were able to distinguish those who ultimately carried a mutation in *SRCAP*, by his/her clinical information and facial photographs, from those who did not have a mutation. Those individuals who were referred who did not have a mutation detected often had dysmorphic facial features, but these were distinct from the classical FHS gestalt, making facial features the defining characteristic of FHS. The nose is quite distinctive in FHS with its overall triangular appearance, the orientation and size of the nares and the low hanging columella. The linear orientation of the mouth, at rest or when smiling, is also an important defining feature.

Additional consistent features of those who tested negative were a formal diagnosis of autism or head circumferences at a comparatively smaller OFC percentile than that for height. Russell-Silver syndrome and 3-M syndrome are included in the differential diagnosis for FHS, but we do not believe any of the patients in our negative group had either of these diagnoses.

Three individuals, whose phenotype most closely resembled FHS, had sequencing of the entire *SRCAP* gene to explore the possibility of mutations outside of exons 31–34. However, no mutations were detected. It is plausible that their phenotypes could be due to a mutation in another gene that codes for a protein, which interacts with SRCAP and CBP. Further research is needed to elucidate this possibility. Given that we have no evidence of genetic heterogeneity within our cohort, we conclude that the detection of a truncating mutation in exon 34 of *SRCAP* is a mandatory feature for a diagnosis of FHS. This is contrary to the report put forth by Le Goff *et al*.[14] Six of their nine subjects were found to have mutations in exon 34 within the boundaries we describe and they proposed that their three mutation-negative individuals indicate genetic heterogeneity for FHS. However, we reviewed the two photographs of their *SRCAP*-negative patients and did not believe their facial features were consistent with a diagnosis of FHS.

A high frequency of associated anomalies was seen in this study (33/52 had at least one major anomaly requiring medical intervention); however, none are pathognomonic for FHS. This large cohort of FHS individuals clarifies which clinical features are observed frequently and informs patient management guideline development. For example, celiac disease was initially thought to be more common in FHS, however, only 2 of 52 subjects had this finding. Although this is more than expected in comparison to the general population, the numbers are not such to suggest generalized screening.

In comparison, genitourinary, ocular and dental issues were seen often enough to warrant investigations.

Suggestions for management

Based on our clinical data, we suggest the following guidelines for the care of individuals with FHS:

1. Sequencing of *SRCAP* exons 31–34 in all suspected cases to confirm the diagnosis
2. Complete assessments of auditory and visual systems
3. Renal and urinary tract ultrasound
4. Neurologic assessment if there is a suspicion of seizures
5. Dental hygiene to prevent cavities and to monitor for malocclusion
6. Evaluation for growth hormone deficiency at baseline, to be repeated if loss of growth velocity occurs
7. Monitoring of bone age and pubertal timing. In cases of precocious puberty, referral to a pediatric endocrinologist
8. Psychoeducational assessments corrected for deficiencies in expressive language and sensory issues
9. Monitoring of behavioral disturbances and provision of early intervention
10. Counseling for families regarding recurrence risk (extremely low) and to offspring of individuals with FHS (50% chance).

Conclusions

We have assembled the largest cohort of individuals with Floating-Harbor syndrome; documenting pathogenic mutations in *SRCAP* in 52 affected individuals. Characteristic clinical findings include short stature, delayed bone age, distinctive facial features, expressive language delay and broad thumbs. If the characteristic facial gestalt is not present, the likelihood of finding a mutation in *SRCAP* is very low. It is not uncommon for an individual with FHS to have additional anomalies and health complications that require medical intervention and thus comprehensive baseline screening and surveillance is warranted. In general, individuals with FHS are healthy and despite some impairments, enjoy a good quality of life.

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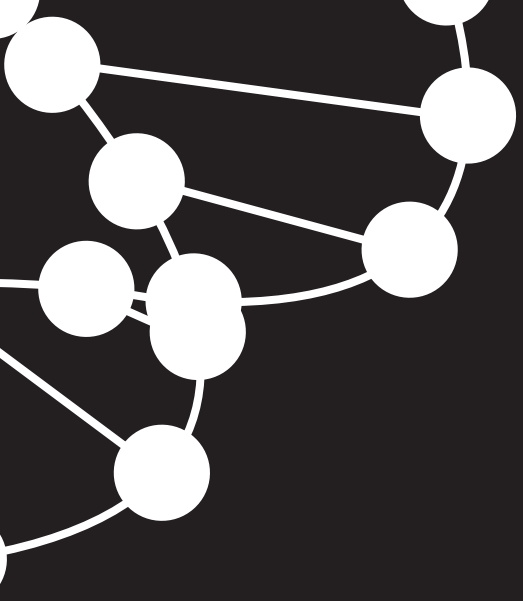
Supplementary File 1. Floating-Harbor questionnaire

Patient	Wide mouth
Sex	Low set ears
Ethnicity	Broad thumbs
Paternal age (years)	Broad fingertips
Gestation (weeks)	Brachydactyly
Birth weight (g)	Clinodactyly
Age at diagnosis	Other skeletal
Age at last assessment (ALA[1])	Dental issues
Head circumference (cm) ALA	Gastrointestinal problems
Weight (kg) ALA	Seizures
Height (cm) ALA	Other health issues/serious illnesses/hospitalizations
Age at puberty	Behaviour issues
Pre-pubertal height	Attention Deficit/Hyperactivity
Bone age years versus chronological age	Psychiatric issues/Anxiety/Depression
Triangular face	High pitched voice
Distinctive nose	Speech delay
Low-hanging columella	Intellectual development/Developmental Delay/ Mental Retardation
Short philtrum	
Thin upper vermilion	

[1]ALA – at last assessment

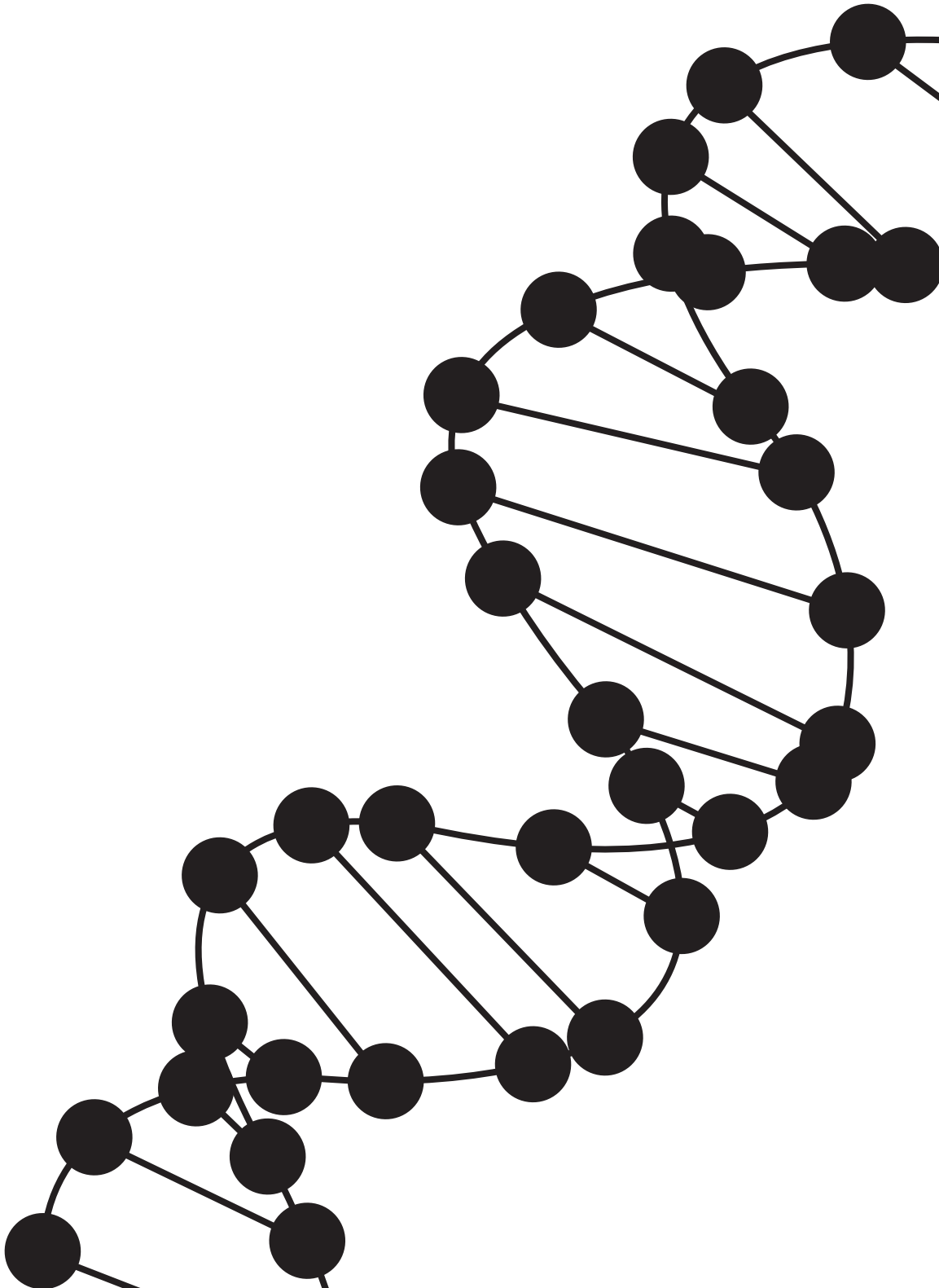
Supplementary Table 1.

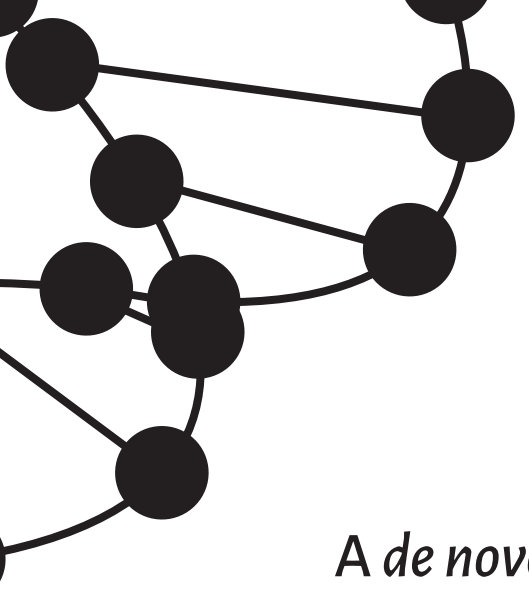
FHS Primers			
1	SRCAPexon34_A.F	TGACCTGCTGGCCTTAACTT	381
2	SRCAPexon34_A.R	GGCGGATATGACAGGAGTGT	
3	SRCAPexon34_B.F	CACCGGCGCAGTAAAAAG	400
4	SRCAPexon34_B.R	TATGAGCTGGTGGAGGGGTA	
5	SRCAPexon34_C.F	TCAGCCCCAAATCCAATAAC	374
6	SRCAPexon34_C.R	AGGATCTCCACAGCAACTGG	
7	SRCAPexon34_D.F	GCATTGGCATCTCCAGAGTC	368
8	SRCAPexon34_D.R	TCTGTCAGCTCTTCCGAGGT	
9	SRCAPexon34_E.F	GTGAGGAGTTGCCCCTGT	499
10	SRCAPexon34_E.R	ATGGGAGGGGAGGATTCTG	
11	SRCAPexon34_F.F	GGCTGGTAACTGTGGTAGAGGA	488
12	SRCAPexon34_F.R	CCCAGGAATAAGCTGTGGTTC	
13	SRCAPexon34_G.F	ACCTTGAAGGGAAAAACCAATG	457
14	SRCAPexon34_G.R	AGCTCTCGAGAACAGAAGTGCT	
15	SRCAPexon34_H.F	CCTCCCAAGAATCCTCCATC	500
16	SRCAPexon34_H.R	GCTTCAGCCTCAGACTCCTCTA	
17	SRCAPexon34_I.F	TGCGTCCAGGGTCTCTAGTC	494
18	SRCAPexon34_I.R	TGACAACCAGTTGCCTACCC	



Chapter 4

ZBTB18





Chapter 4

A *de novo* nonsense mutation in *ZBTB18* in a patient with features of the 1q43q44 microdeletion syndrome

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Abstract

The phenotype of patients with a chromosome 1q43q44 microdeletion (OMIM 612337) is characterized by intellectual disability with no or very limited speech, microcephaly, growth retardation, a recognizable facial phenotype, seizures and agenesis of the corpus callosum. Comparison of patients with different microdeletions has previously identified *ZBTB18* (*ZNF238*) as a candidate gene for the 1q43q44 microdeletion syndrome. Mutations in this gene have not yet been described. We performed exome sequencing in a patient with features of the 1q43q44 microdeletion syndrome that included short stature, microcephaly, global developmental delay, pronounced speech delay and dysmorphic facial features. A single *de novo* nonsense mutation was detected, which was located in *ZBTB18*. This finding is consistent with an important role for haploinsufficiency of *ZBTB18* in the phenotype of chromosome 1q43q44 microdeletions. The corpus callosum is abnormal in mice with a brain-specific knock-out of *ZBTB18*. Similarly, most (but not all) patients with the 1q43q44 microdeletion syndrome have agenesis or hypoplasia of the corpus callosum. In contrast, the patient with a *ZBTB18* point mutation reported here had a structurally normal corpus callosum on brain MRI. Incomplete penetrance or haploinsufficiency of other genes from the critical region may explain the absence of callosum agenesis in this patient with a *ZBTB18* point mutation. The findings in this patient with a mutation in *ZBTB18* will contribute to our understanding of the 1q43q44 microdeletion syndrome.

Introduction

The phenotype of patients with a submicroscopic deletion of chromosome 1q43q44 (OMIM 612337) is characterized by intellectual disability, microcephaly, growth restriction, dysmorphic features, agenesis of the corpus callosum (ACC) and cardiac, gastroesophageal and urogenital anomalies.[1–8] Even for patients with an overlapping deletion, the phenotype varies considerably. So far, no direct association has been identified between the phenotype and the deleted genes. On the basis of overlapping microdeletions, a number of candidate genes have previously been suggested for microcephaly and corpus callosum agenesis. These include *AKT3*, *ADSS*, *CEP170*, *Clorf100*, *Clorf101*, *Clorf121*, *Clorf199*, *EFCAB2*, *FAM36A*, *HNRNPU*, *HNRNPU-AS1*, *PLD5*, *PNAS-4* and *SDCCAG8*.[1,3,8–14] Specifically, *ZBTB18* has repeatedly been identified as a strong candidate gene for microcephaly and/or ACC.[7,9,11,14,15] *ZBTB18* is particularly compelling since a brain-specific knock-out of this gene in mice causes microcephaly and callosal anomalies.[16] However, other studies suggest critical regions that do not include *ZBTB18*.[8,10,12]

To our knowledge, no point mutations in *ZBTB18* have been described so far. Here, we describe a patient with a *de novo* nonsense mutation in *ZBTB18* and phenotypic features of the 1q43q44 microdeletion syndrome.

Materials and methods

Clinical features

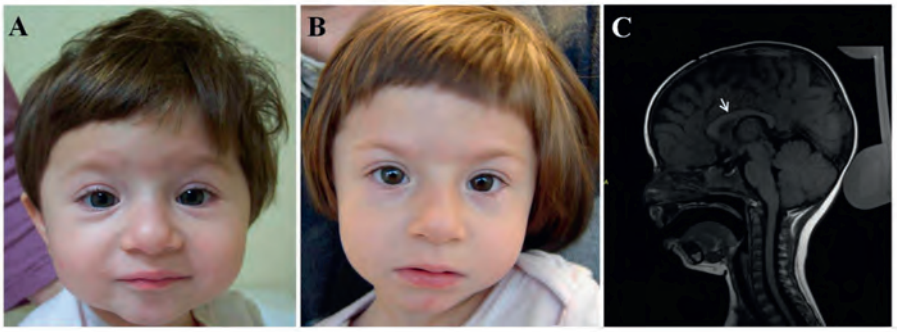
This female was the first child of healthy, non-consanguineous parents. The pregnancy was established through *in vitro* fertilization, because of subfertility due to oligospermia. Amniocentesis, performed because of raised maternal serum markers, revealed a normal female karyotype. On ultrasound at 20 weeks of gestation bilateral pyelectasia and choroid plexus cysts were noted. Labor was induced at 36 weeks of gestation because of intra-uterine growth restriction.

Birth length was 45.5cm (-2.3 SDs), with a weight of 2.15 kg (-2.3 SDs) and a head circumference of 30 cm (-2 SDs). Neonatal examination was unremarkable. Ultrasound investigations of the brain and the abdomen showed no abnormalities.

The girl developed feeding problems after the introduction of formula milk and weight gain was poor. After elimination of cow's milk from the diet, her weight gain remained delayed. Gastroenterological examinations (including biochemistry,

endoscopy, small bowel biopsy and abdominal ultrasound and upper GI tract X-rays) revealed gastroesophageal reflux, but no other explanation for her growth restriction. Because of developmental delay and suspected absence seizures associated with vomiting episodes, neurological assessment was performed which was unremarkable. Additional investigations included a cerebral MRI, EEG, metabolic screen and hearing tests. All results were normal (Figure 1c).

Figure 1. Female with a *de novo* nonsense mutation in *ZBTB18*: c.397G>T (p.(Glu133*))
(A) Patient at age 16 months. Dysmorphic features include arched eyebrows with telecanthus, short palpebral fissures, a long nose with a prominent tip, short philtrum and micrognathia; **(B)** Patient at age 34 months. Dysmorphic features are similar to those at age 16 months, however, the nasal tip is less prominent, the philtrum is longer and the upper lip is thinner; (**C)** Lateral view of the brain at age 15 months. Note the presence of a normal corpus callosum (white arrow).



At the age of 16 months, she was referred to the genetics clinic for further assessment. At this time, all growth parameters were below -2.5 SDs. Her height was 70.5 cm, weight 7 kg and head circumference 41.5 cm. On clinical examination, she had proportionate short stature, mild facial dysmorphic features (Figure 1a and b) and slightly broad first fingers and toes. At later reviews, her delay became more evident and more marked in the speech domain. She walked unsupported at 26 months. At 34 months, her cognitive development was at the level of 2 years old with prominent speech delay. Her speech was hyper nasal. Her height was 79 cm (-4.5 SDs), with a weight of 7.9 kg (-4.6 SDs) and a head circumference of 43 cm (-4.1 SDs).
At this time, a diagnosis of possible Floating-Harbor syndrome was considered.
Subsequent mutation analysis of the *SRCAP* gene revealed no abnormalities. MLPA assays for telomeric and recurrent genomic rearrangements (MRC-Holland kits) were also normal, as was a genomic microarray.

Molecular analysis

Exome sequencing was performed in a female patient from a cohort of patients with short stature, developmental delay, predominantly speech delay, with or without facial dysmorphic features. Exome sequencing was done on DNA from the female and both parents as described previously.[17,18] In brief, genomic DNA was isolated from blood with the use of a QIAgen DNA mini kit (QIAgen, Venlo, The Netherlands). Exomes were enriched using a SOLiD-optimized SureSelect human exome kit (Agilent v2, 50Mb, Agilent Technologies Inc., Santa Clara, CA, USA), followed by sequencing performed using the 5500xl platform (Life Technologies, Carlsbad, CA, USA). After sequencing the trio, candidate *de novo* events were selected by excluding variants seen in either parent, whereas recessive models were evaluated using bi-allelic variants inherited from both parents. Candidate mutations were validated by conventional Sanger sequencing methods in DNAs of the patient and her parents (Supplementary Tables 1–3) and annotated according to HGVS recommendations. After publication, the variant will be submitted to the Human Gene Mutation Database (HGMD; URL: http://www.hgmd.cf.ac.uk/ac/index.php).

Results

Exome sequencing detected three *de novo* variants. Two *de novo* missense variants were not confirmed with Sanger sequencing (Supplementary Table 3). Sanger sequencing confirmed the presence of a single *de novo* nonsense mutation located in *ZBTB18*: c.397 G>T (p.(Glu133*)) (NM_205768; UCSC, hg19). This mutation is predicted to cause a premature stop codon and may lead to nonsense mediated decay. No additional mutations in *ZBTB18* were detected through Sanger sequencing in nine patients with an overlapping phenotype. Furthermore, neither stop mutation nor any other truncating event (stop-loss, frameshift mutation) in *ZBTB18* was identified in 6500 individuals from the exome variant server (Exome Variant Server, NLHBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) (accessed July 2013)).

Discussion

We report a *de novo* nonsense mutation in *ZBTB18* in a patient with global developmental delay, prominent speech delay, microcephaly, short stature and discrete facial dysmorphisms. These clinical features are consistent with the phenotype of patients with microdeletions of chromosome 1q43q44 (Table 1). Overlapping clinical features include microcephaly (97%), developmental delay (100%), intra-uterine growth retardation (62%) and postnatal growth retardation (45%). We suggest that haploinsufficiency of *ZBTB18* contributes to these features of the chromosome 1q43q44 microdeletion syndrome.

Table 1. Comparison of the clinical features of a patient with a *de novo* nonsense mutation in *ZBTB18*, c.397G>T (p.(Glu133*)) and the phenotype of patients with a chromosome 1q43q44 microdeletion including *ZBTB18* described in literature.

Features	Patient with *ZBTB18* mutation	Patients with 1q43q44 microdeletion described in literature (N= 29) [1,8-12]	
Deletion size (range in Mb)	NA[I]	1.26 - 4.9	
Mutation	397G>T (p.(Glu133*))	NA	
Sex	F	16F/12M	(57%/43%)
Growth parameters			
Intra-uterine growth retardation	+	6/15	(40%)
Postnatal growth delay	+	11/26	(42%)
Microcephaly (OFC < -2 SDs)	+	28/29	(97%)
Neurology			
Developmental delay	+	18/18	(100%)
Speech delay	+	3/3	(100%)
Corpus callosum anomalies	-	23/28	(82%)
Hypotonia	-	14/18	(78%)
Seizures	-	19/29	(66%)
Facial dysmorphic features			
Hypertelorism	-	5/19	(26%)
Strabismus	-	2/18	(11%)
Prominent nasal tip/Bulbous nose	+	10/19	(53%)
Abnormal philtrum	+	6/19	(32%)
Abnormal lips	+	8/19	(42%)
Micro-/retrognathia	+	1/19	(5%)
Abnormal ears	-	4/19	(21%)
Abnormal fingers/toes	+	8/19	(42%)
Cardiac anomalies	-	7/19	(37%)
Urogenital anomalies	-	6/19	(32%)
Gastrointestinal problems	+	8/17	(47%)

[I]NA = Not Applicable

There are a number of reports on patients with deletions of 1q43q44 not including *ZBTB18*.[1,8–12] Out of 27 patients with deletions distal or proximal (including the *AKT3* gene) to *ZBTB18*, only 5 (19%) showed corpus callosum abnormalities and 10 (37%) had microcephaly, compared with 82 and 97%, respectively, of patients with deletions including *ZBTB18*.[1,8–12] Furthermore, IUGR was seen in 3 out of 14 of these patients (21%) and postnatal growth retardation was seen in 7 out of 23 patients (30%). Collectively, these results support a crucial role for *ZBTB18*, but with contributions to the phenotype of other genes in patients with larger deletions.

ZBTB18, also known as ZNF238 or RP58, acts as a transcriptional repressor of key proneurogenic genes such as Neurogenin2 and NeuroD1.[16] ZBTB18 is activated during neuronal differentiation in pin-like cells of the ventricular zone and in migrating multipolar cells.[19] ZBTB18 participates in neuron and astrocyte differentiation by mediating cell-cycle control of neural stem cells.[20] Mice with loss of ZBTB18/RP58 die at birth with neocortical defects. CNS-specific loss of ZBTB18 is associated with microcephaly, ACC and cerebellar hypoplasia.[16] ZBTB18-mutant brains maintain precursor pools, but have reduced neuronal and increased glial differentiation. This is consistent with a role for ZBTB18 in favoring neuronal differentiation and brain growth by repressing multiple proneurogenic genes in a timely manner. On the basis of the phenotypes observed in mutant mice, Xiang *et al*[16] proposed *ZBTB18* as a strong candidate gene for the 1qter deletion syndrome. Our finding of intellectual disability and microcephaly in a female with a *de novo* nonsense mutation in *ZBTB18* strongly supports this hypothesis.

Dysmorphic features in our female were mild, whereas variable facial features have been previously reported with 1q43q44 microdeletions of different sizes and gene content. Clearly, other genes from the 1q43q44 region may still contribute to the phenotypic outcome in patient cases with the 1q43q44 microdeletion syndrome. Most (24 out of 29) previously reported patients with a 1q43q44 microdeletion including *ZBTB18* had corpus callosum abnormalities on brain imaging. The majority (66%) developed seizures.[1,8–12,15] Incomplete penetrance is a possible explanation for the absence of ACC in this girl, given that the corpus callosum was apparently normal in at least five patients with 1q43q44 microdeletions that included *ZBTB18* as described previously.[1,9,12] We note that brain-specific loss of ZBTB18 in mice leads to a small brain phenotype with ACC and cerebellar hypoplasia.[16]

In conclusion, we describe the first patient with a *de novo* nonsense mutation in *ZBTB18*. The phenotype of the patient reported here suggests that *ZBTB18* haploinsufficiency contributes to the microcephaly and global developmental delay that are core features of the 1q43q44 microdeletion syndrome.

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Supplementary Table 1. Exome mapping statistics for a female patient with global developmental delay, prominent speech delay, microcephaly, short stature and mild dysmorphic features, and her parents. Enrichment was performed with Agilent's v2 (50Mb) exome; sequencing was performed using Life Technologie's 5500xl platform; mapping and variant calling was done using Life Technologie's LifeScope v2.1.

	Child	Mother	Father
Total mapped bases [hg19]	5.245.459.026	4.984.680.534	5.788.453.725
Bases on target	80.6%	81.7%	81.4%
Bases near target (+/- 50nt)	7.3%	7.1%	7.2%
Bases off target	12.1%	11.2%	11.4%
Median target coverage	64.5	60.7	71.8
Average target coverage	81.4	78.0	91.2

Supplementary Table 2. Variant filtering of exome data for a female patient with global developmental delay, prominent speech delay, microcephaly, short stature and mild dysmorphic features. Trio - *de novo* analysis, similar to Vissers *et al*. 2010; de Ligt *et al*. 2013.[1,2]

All variants		45803
	coding and splice site variants	19863
of which	private[I]	314
	non synonymous	217
	high quality *de novo* candidates[II]	3

[I] Variant not present in dbSNP132 nor in 1051 in-house exomes.
[II] Variant not present in maternal or paternal exome data, ≥5 unique variant reads, ≥20% variant reads.

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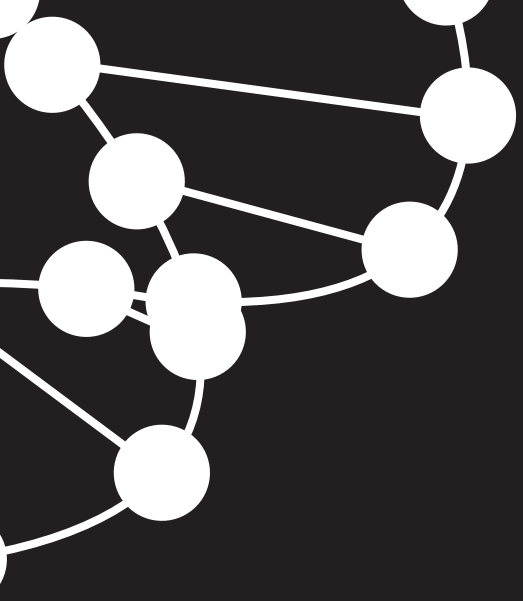
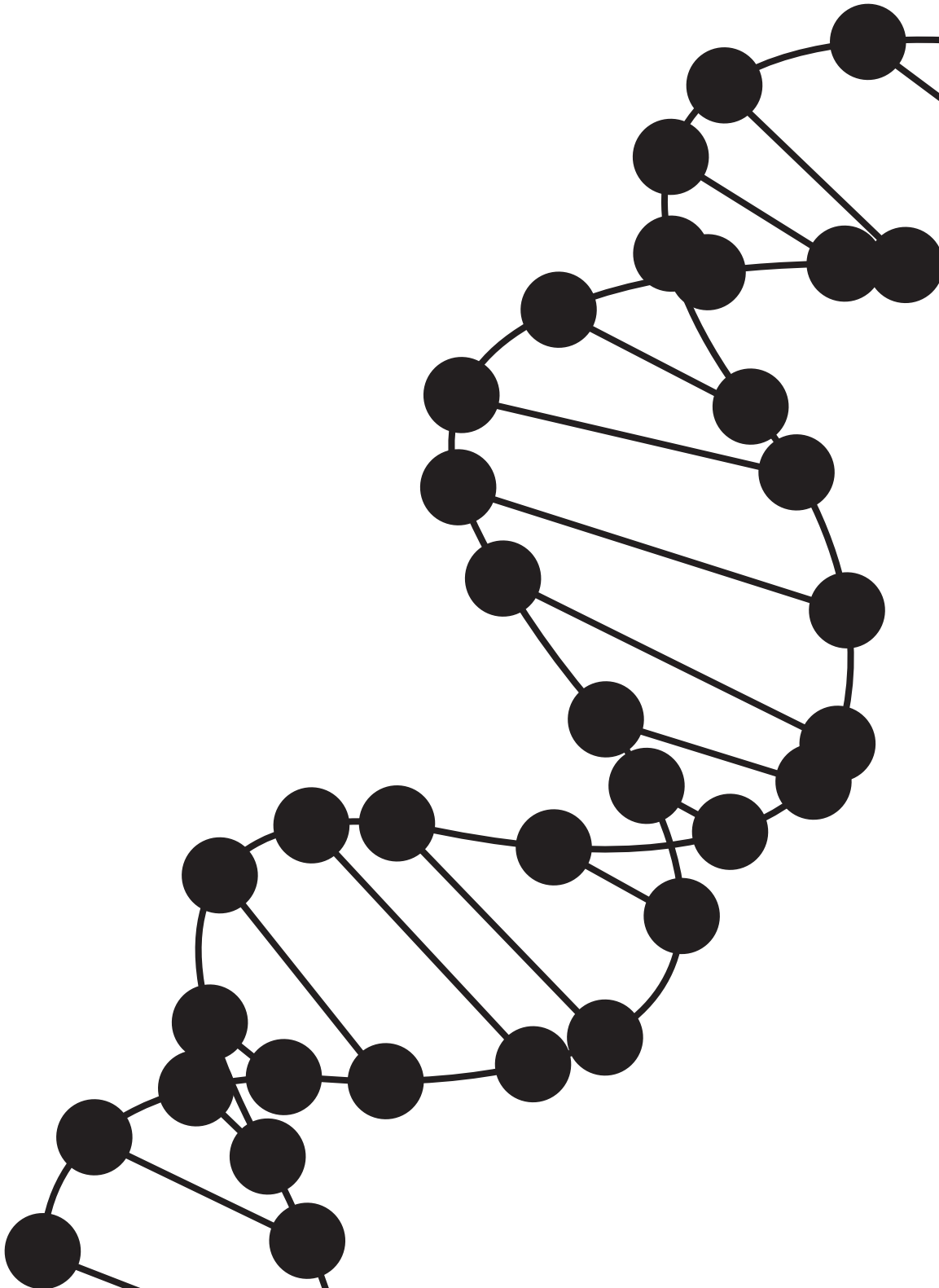
Supplementary Table 3. Candidate *de novo* variants in a patient with global developmental delay, prominent speech delay, microcephaly, short stature and mild dysmorphic features.

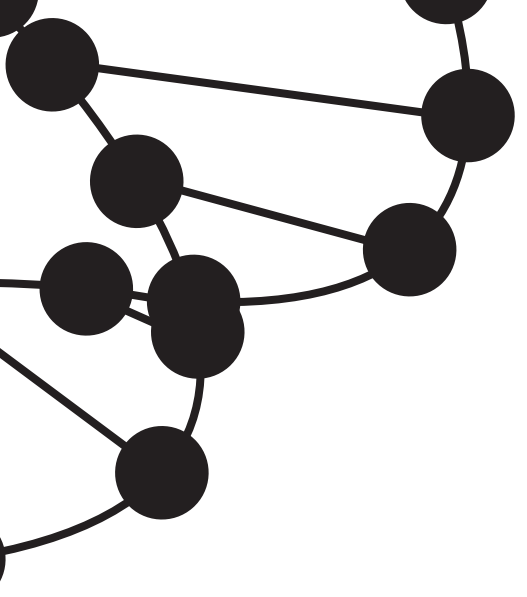
Chromosome	Genomic position [hg19]	Reference	Mutation	Reads	Variation reads	% variation	Gene name	Gene id	Reference Amino Acid	Mutation Amino Acid	Amino Acid changes	mRNA changes	phyloP	Grantham score	Validated	*De novo*
Chr1	244217473	G	T	45	23	51.1	ZNF238/ ZBTB18	NM_205768	E	*	E133*	397G>T	5.555	NA	Mutation detected	Yes
Chr7	158449251	T	G	26	6	23.1	NCAPG2	NM_017760	Q	P	Q736P	2207T>G	0.396	76	No mutation detected	NA
Chr9	125865392	T	C	15	6	40.0	RABGAP1	NM_012197	L	P	L1037P	3110T>C	3.336	98	No mutation detected	NA



Chapter 5

Summary, general discussion and future prospects





Chapter 5.1

Summary

In this exciting new era of genomics, the introduction of next generation sequencing (NGS) has precipitated the development of 'next generation phenotyping'. In the field of clinical genetics, new syndromes can be recognized by integrating the clinical phenotype of patients with molecular variants detected by whole exome sequencing. Furthermore, through whole exome sequencing, the molecular cause of many known syndromes has been identified, enabling genotype-phenotype studies for these syndromes (**Chapter 1**).

In this thesis, we have put next generation phenotyping into practice: we performed genotype-phenotype studies in two syndromes accompanied by short stature after their molecular causes had been identified through NGS: Meier-Gorlin syndrome (MGS) and Floating-Harbor syndrome (FHS). We provided clinically based management advice for these conditions (**Chapters 2 and 3**) and we used the results of whole exome sequencing to establish a new syndrome (**Chapter 4**). Consequently, we increased the knowledge and awareness of these conditions.

Chapter 2. Meier-Gorlin syndrome

The discovery of *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6* mutations as the underlying cause of MGS in 2011 enabled us to perform the genotype-phenotype studies described in **Chapter 2.1**.[1-3]

We performed these studies in a cohort of 35 MGS patients with biallelic mutations in one of the five above mentioned genes of the pre-replication complex and in ten additional MGS patients in whom the diagnosis of MGS was based exclusively on the clinical findings, since no underlying genetic defect was detected.

The classical triad of microtia, patellar a-/hypoplasia and short stature was present in 83% of patients with a clinical and molecular diagnosis of MGS. Four patients (one with *ORC1* mutations, one with *ORC6* mutations and two with *CDT1* mutations) had microtia and patellar aplasia with a normal stature. One patient with *ORC1* mutations had microtia and short stature, without patellar a-/hypoplasia. Finally, one individual with *ORC1* mutations had short stature, but the ears and patellae were of normal size.

These findings show the variability of the phenotypic spectrum of MGS and should prompt clinicians to consider the diagnosis of MGS more often, since MGS can be present in patients with just one of the three cardinal features.

Other frequent clinical findings in our cohort were typical facial features (including

a small mouth with full lips and retro-/micrognathia and a narrow nose with broad nasal bridge and convex nasal profile), respiratory problems (including congenital pulmonary emphysema and trachea-, laryngo- and/or bronchomalacia) and feeding problems during infancy, mammary hypoplasia and genital anomalies.

Our studies revealed that patients with *ORC1* mutations show the most severe growth retardation, reflected by the fact that these patients are shortest in height and have the smallest head circumference, with mean standard deviation scores of -7.1 SDs (range -9.6 to -5.2 SDs) and -6.7 SDs (range -9.8 to -4 SDs) respectively.

Furthermore, our data suggest that compound heterozygous nonsense and missense mutations have a more severe effect on the phenotype than homozygous missense mutations. Growth was more delayed in three patients with one missense and one null mutation in *ORC4* and these patients had more congenital anomalies (congenital pulmonary emphysema, congenital heart defect and severe feeding problems) in comparison with four patients with two missense mutations in *ORC4*. Furthermore, the only individual with homozygous missense mutations in *CDT1* had no congenital pulmonary emphysema, while all seven patients with one missense and one null mutation in this gene were diagnosed with congenital pulmonary emphysema.
Moreover, four patients with compound heterozygous null and missense mutations had a lethal phenotype: one boy with one frameshift and one missense mutation in *ORC1* had a severe cortical dysplasia with pachygyria and ventriculomegaly, craniosynostosis, congenital pulmonary emphysema and pancreatic tail agenesis. He passed away at the age of 3.5 months. His brother deceased after 17 weeks of gestation. A boy with one nonsense and one missense mutation in *CDT1* passed away due to a sudden cardiac arrest, his sister died of severe respiratory problems due to a tracheobronchomalacia with progressive congenital pulmonary emphysema.
Thus far, no patients with MGS and homozygous or compound heterozygous null mutations have been described, suggesting that the effect of these mutations is not compatible with life. This hypothesis is supported by results from Drosophila studies described in literature, in which null mutations in *ORC6* and *CDT1* cause embryonic lethality.[4,5]

In **Chapter 2.2**, in depth studies were performed in our cohort of 45 MGS patients in order to establish a specific growth pattern, enabling us to predict growth in patients with MGS.

Trend analyses of growth showed that the growth retardation in MGS predominantly arises prenatally. Prenatal growth retardation was present in 98% of patients with MGS, with a mean birth weight of -3.9 SDs and a mean length of -3.4 SDs. In the first year of life, height decreased further to -5.2 SDs. In the subsequent years, growth velocity remained normal to slightly increased, resulting in a mean adult height of -4.5 SDs.
These growth studies showed the growth pattern of MGS patients: height decreases with an average of 1.7 SDs in the first year of life and growth velocity is near to normal thereafter.

Height was significantly influenced by ethnic origin and the underlying molecular cause. These findings confirmed and expanded our previous findings described in **Chapter 2.1**. We showed that patients with mutations in *ORC1* and *ORC4* were significantly shorter (4.7 SDs and 3.1 SDs shorter, respectively) than patients with mutations in *ORC6*, *CDT1* and *CDC6* or without molecular diagnosis. Their head circumferences were 5.0 SDs (*ORC1*) and 1.6 SDs (*ORC4*) smaller than the head circumference of patients with mutations in *ORC6*, *CDT1* and *CDC6*.
Growth hormone treatment was successful in two patients with a clinical diagnosis of MGS. In these patients, growth hormone treatment was initiated when growth velocity continued to be decreased after the first year of life and extremely low levels of IGF1 were detected. These findings suggest that IGF1 measurements can be used to predict the success of growth hormone treatment in a subset of patients with MGS.

Recent clinical findings have shown the presence of congenital pulmonary emphysema in two MGS patients diagnosed with asthma/asthmatic symptoms (unpublished data). Although future studies are necessary to assess its exact incidence and long-term effects, the presence of congenital pulmonary emphysema should be considered in all patients with MGS who present with airway problems resembling asthma.

The results of our molecular and longitudinal clinical studies (with follow-up data of over 15 years) in patients with MGS have led us to propose experience based advice for diagnostic evaluation and management of patients with MGS in **Chapter 2.3**. Medical care should be directed towards growth and secondary sexual development, motor and speech development, respiratory and skeletal problems.

Chapter 3. Floating-Harbor syndrome

After the identification of heterozygous truncating mutations of the *SRCAP* gene as the underlying molecular cause of FHS in 2012, we performed genotype-phenotype studies in 52 patients with FHS. The results of these studies are described in **Chapter 3**.[6]

In these 52 patients, we reported 14 different mutations in the *SRCAP* gene. All 14 detected mutations were truncating mutations between codons 2389 and 2748 in exon 34. Two mutations were recurrent: Arg2444* and Arg2435*, present in 50% and 25% of patients, respectively. Only one instance of parent-to-child submission was observed.

We further delineated the clinical spectrum of FHS and identified expressive language delay and the facial phenotype as the core, mandatory features of FHS. In patients with these cardinal features, mutation detection rate was 95%. The likelihood of detecting a mutation in *SRCAP* in patients without distinctive facial features was very low.
The characteristic facial features include a triangular face with deep-set eyes and long eyelashes, a narrow nasal root that broadens towards the tip, a low columella, large nares, short philtrum and thin upper vermillion with everted lower lip. A key feature is the horizontal lineage of the lips at rest or when smiling.
The expressive language delay was present in all patients, but varied considerably, since one patient was bilingual, while other adults with FHS could only speak a few words. Cognitive development varied from normal to profound intellectual disability.
Other frequent, non-obligatory findings included broad thumbs and first toes and brachydactyly with broad fingertips.
Growth analysis showed that prenatal growth retardation (birth weight below -2 SDs) was present in approximately 25% of FHS patients and that growth velocity decreased after birth, with a postnatal growth retardation (height below -2.5 SDs) present in 92% of patients. Bone age was significantly delayed before the age of eight.
Microcephaly (head circumference below -2 SDs) was present in only a minority of patients and only one patient had a head circumference below -3 SDs, indicating that growth of the brain is relatively spared in FHS.

At least one major anomaly was present in 63% of patients. However, none of them was present frequently enough to be distinguishing. Gastro-intestinal (motility issues, colonic stricture, celiac disease), genitourinary (cryptorchidism, abnormalities of the renal/collecting system) and eye anomalies (strabismus, hyperopia, nystagmus) were

described relatively frequently, as well as seizures, hearing loss and dental issues (small teeth, cavities, malocclusion). Cardiac malformations (mild aortic coarctation, atrial septal defect, Tetralogy of Fallot) and hypothyroidism were seen less frequently.

Based on our genotype-phenotype studies, we suggested that management of FHS patients should be directed towards auditory and visual problems, genitourinary malformations, dental issues, growth, bone age and possible precocious puberty, psychomotor and speech development, possible seizures and behavioral problems.

FHS is a highly recognizable, monogenic disorder, indicating that patients without mutations in *SRCAP* either were misdiagnosed with FHS in the past, or have mutations in a second FHS gene harboring a broader clinical spectrum.

Chapter 4. *ZBTB18*

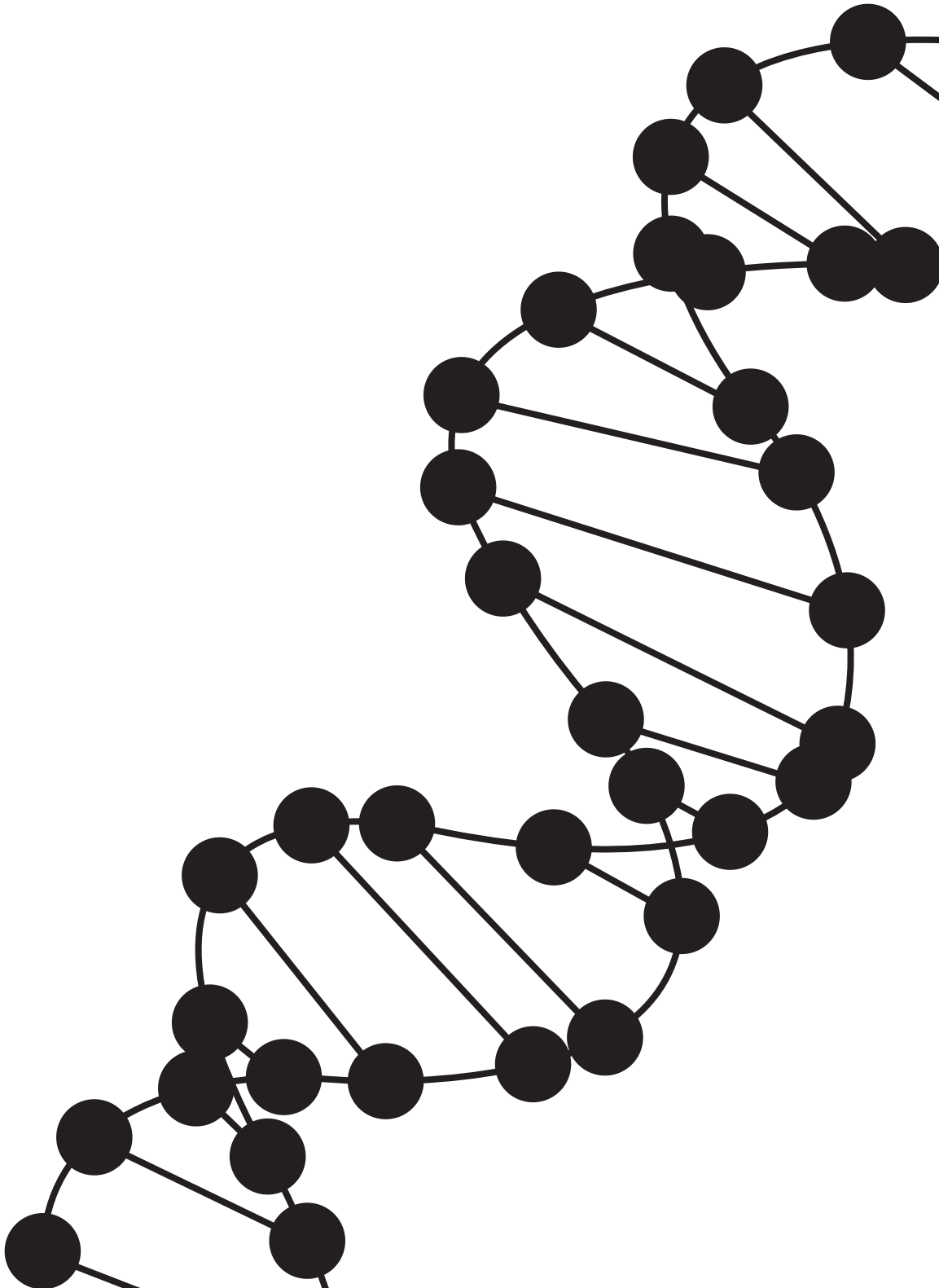
As described in **Chapter 3**, Floating-Harbor syndrome is characterized by short stature, a typical facial phenotype and expressive language delay.
In 2011, we saw five patients with a clinical diagnosis/suspicion of FHS. A molecularly confirmed diagnosis of FHS could be made in only two of the five patients. Whole exome sequencing led to the identification of a novel disease gene in one of the three remaining patients.
We reported this case in **Chapter 4**: the first patient with a *de novo* nonsense mutation in *ZBTBT18* and a phenotype including short stature, microcephaly, a developmental delay (especially of the speech) and facial dysmorphic features (arched eyebrows, telecanthus, short palpebral fissures, a long nose with a prominent tip, short philtrum, thin upper lip and micrognathia), showing some resemblance to FHS.

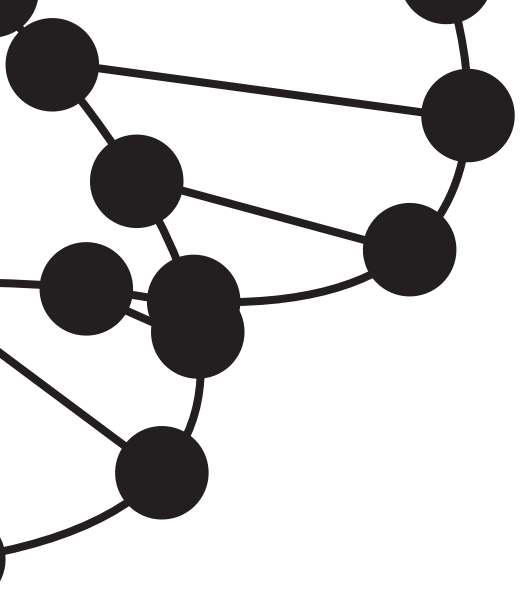
ZBTB18 (also known as *ZNF238*) is located on chromosome 1q44, within the chromosome 1q43q44 microdeletion region.[7] It encodes a C2H2-type zinc finger protein that acts as a transcriptional repressor of genes involved in neuronal development.[8]
The phenotype of patients with 1q43q44 microdeletions and a smallest region of overlap including *ZBTB18* suggested this gene as a strong candidate gene for microcephaly and/or agenesis of the corpus callosum.[9-13]
Clinical findings in patients with microdeletions of chromosome 1q43q44 may resemble the FHS phenotype. Overlapping features include short stature and language

deficits. Additionally, structural defects such as cardiac, gastro-intestinal and urogenital anomalies have been described in both syndromes. Dysmorphic features of patients with 1q43q44 microdeletions include a round face with deep set eyes, epicanthic folds, low set ears, a prominent metopic ridge, short nose with prominent/broad nasal tip, a thin upper lip, wide spaced teeth and retro-/micrognathia.
The absence of facial features distinctive for FHS and the presence of microcephaly and corpus callosum abnormalities in patients with 1q43q44 microdeletions distinguish these patients from patients with FHS.

The clinical features of our patient have all been described in patients with 1q43q44 microdeletions that include the *ZBTB18* gene. However, our patient did not have corpus callosum abnormalities, even though these are seen in 82% of patients with deletions. Furthermore, the facial features of our patient overlapped only partially with those of the 1q43q44 microdeletion syndrome (prominent nasal tip, smooth philtrum, thin upper lip and micrognathia).
The absence of corpus callosum abnormalities in our patient could be attributed to incomplete penetrance or to a contribution of other genes in the 1q43q44 region to corpus callosum development.

Our study suggests that *ZBTB18* haploinsufficiency contributes to the phenotype of the 1q43q44 microdeletion syndrome: the non-mandatory pre- and postnatal growth retardation (present in 40% and 42% of patients) and the core features, particularly microcephaly and developmental delay, present in 97% and 100% of patients with this syndrome, respectively. A lethal phenotype with neocortical defects is seen in *Zbtb18* knockout mice and *ZBTB18*-mutant brains show reduced neuronal and increased glial differentiation.
Furthermore, central nervous system-specific loss of ZBTB18 causes microcephaly, agenesis of the corpus callosum and cerebellar hypoplasia, providing supportive evidence for this hypothesis.[8]





Chapter 5.2

General discussion and future prospects

In this thesis, we have put next generation phenotyping into practice. We performed genotype-phenotype studies in three syndromes with short stature as a cardinal feature, after the elucidation of their underlying genetic causes through whole exome sequencing: Meier-Gorlin syndrome (MGS) and Floating-Harbor syndrome (FHS) (**Chapters 2 and 3**).[1-3,6]
Furthermore, through whole exome sequencing, we identified a *de novo ZBTB18* mutation in a patient previously suspected to have FHS and proposed *ZBTB18* as a candidate gene for (a part of) the phenotype of the 1q43q44 microdeletion syndrome (**Chapter 4**).

Implications for medical care

Our findings illustrate the relevance of next generation phenotyping, including genotype-phenotype studies in rare disorders and the importance of a correct and molecularly confirmed diagnosis in individual patients.
In an individual with a syndrome accompanied by a short stature, it can be very challenging to identify a definitive molecular diagnosis. When next generation techniques are used as a first tier diagnostic tool, knowledge of the phenotypic spectrum of genetic disorders is essential to assess the relevance of detected variants. For this purpose, next-generation phenotyping, including genotype-phenotype studies, is of crucial importance in daily clinical practice.

MGS and FHS syndrome

We have further delineated the phenotypic spectrum of MGS and FHS syndrome, thereby facilitating the assessment of health risks for patients with these syndromes. In addition, we have developed experience-based guidelines for the management of these patients, enabling appropriate medical follow-up for individual patients who are diagnosed with MGS and FHS syndrome.

Our genotype-phenotype studies and overview of the frequencies of clinical features of patients with MGS and FHS syndrome will assist physicians to clinically recognize these syndromes and should prompt them to examine these specific features in patients with MGS and FHS syndrome. Moreover, our data can help physicians to determine whether these syndromes should be part of the differential diagnosis of an individual patient.
Our genotype-phenotype studies increase the likelihood of making a correct clinical and molecular diagnosis in patients with clinical features in the spectrum of MGS or FHS syndrome, thereby reducing the number of misdiagnoses.

Currently, next generation sequencing techniques are still (largely) unavailable in many countries. In these countries, clinicians still depend on targeted DNA analysis following a clinical suspicion/diagnosis of a genetic disorder. However, many syndromes are accompanied by short stature: a search for proportionate short stature in the Winter-Baraitser London Dysmorphology Database (version 1.0.36) reveals 960 hits at present.[14] It can be challenging and difficult to clinically distinguish between these syndromes, especially when clinical features are similar, when the disorders lack pathognomonic features or when certain features can only be recognized after a certain age.

The molecular cause of many syndromes remains unknown, enabling only clinical diagnoses at present. Erroneous clinical diagnoses are regularly made, even by experienced clinical geneticists.[15-17]
The parents of the three patients previously diagnosed with FHS, in whom the diagnosis of FHS was discarded after *SRCAP* analysis, had focused on the wrong disorder and management of these patients was incorrectly directed towards FHS. One of the patients who displayed behavioral problems turned out to have KBG syndrome (KBG after the initials of surnames of the first three patients described).[18] Even though behavioral problems have been described in patients with FHS, they are a cardinal feature in KBG syndrome.[19-22] After the patient was diagnosed with KBG syndrome, his problems could be attributed to this syndrome and parents were brought in contact with the parents of other patients with KBG syndrome.

After a patient is diagnosed with a syndrome, questions of parents may arise: will our child survive? Will he or she develop significant other health problems? Will my child have a normal intellect?
The findings of this thesis can be shared with the families of patients with MGS or FHS syndrome and thereby help to answer their questions and obtain the best medical care.

In syndromes with a short stature, parents also wonder how tall their child will become. Often, little is known about the expected growth and adult height of patients with rare syndromes. We successfully used a new method to perform in depth growth studies in a cohort of 45 MGS patients (**Chapter 2.2**). The usual methods based on statistical analyses of large groups of children could not be applied to our small cohort of MGS patients, since there was insufficient power to perform the statistical analyses necessary to create classic growth charts.[23]

We therefore developed a linear mixed model with random factor patient. This model can be extrapolated to predict the growth pattern and adult height in other rare syndromes with short stature as well.

ZBTB18 mutations

We reported the first *de novo* nonsense mutation in *ZBTB18*, detected in the second of our three patient, who were previously diagnosed with FHS (**Chapter 4**). Her phenotype consisted of a developmental delay, especially of the speech, short stature, microcephaly and facial dysmorphic features including a round face with deep set eyes, epicanthic folds, low set ears, a prominent metopic ridge, short nose with prominent/ broad nasal tip, a thin upper lip, wide spaced teeth and retro-/micrognathia. Since we described only a single patient, studies to establish the phenotypic spectrum of *ZBTB18* mutations and genotype-phenotype correlations could not be performed at the time.

However, recently, a second patient with a *de novo* mutation in *ZBTB18* was reported.[24] This 4-year old infant had a developmental delay, hypotonia, spastic diplegia with hyperreflexia and dystonia, stereotyped behaviors and a thin corpus callosum and cerebellar vermis hypoplasia. Furthermore, whole exome sequencing revealed a *de novo ZBTB18* mutation in a third patient, with a severe developmental delay with marked speech delay, seizures in infancy and facial dysmorphic features including deep set eyes, full arched eyebrows, a full nasal tip, broad mouth with full lips and a pointed chin (unpublished data).

Even though a cohort of three patients is still insufficient to perform genotype-phenotype studies, these patients confirm our hypothesis that *ZBTB18* mutations cause a syndromic form of intellectual disability/ global developmental delay and that ZBTB18 haploinsufficiency contributes to the phenotype of the chromosome 1q43q44 microdeletion syndrome.

Genetic counseling

One of my main reasons to engage in research is to help patients and their families by increasing the knowledge of rare genetic disorders, not only to improve medical care for these disorders, but to optimize genetic counseling as well.

Diagnosing a child with a specific syndrome can help parents to come to term with the problems of their child. In many cases, the search for a classifying diagnosis is a process that extends over numerous years. Parents often feel guilty and wonder if there was anything they could have done to prevent the disorder in their child, especially during pregnancy. Once a diagnosis is made, genetic counseling is important to support

patients and their parents to "understand and adapt to the medical, psychological and familial implications of genetic contributions to disease".[25]

Establishing the molecular diagnosis of MGS or FHS syndrome proves to parents that they could not have averted the disorder of their child and that they are not to blame. Furthermore, insight into the definitive diagnosis helps patients and their parents in the process of accepting their problems and can be of benefit in obtaining support for special education and adaptations at home.

However, feelings of sorrow that the condition is incurable can be present and, unfortunately, any feelings of guilt do not always abate with this understanding.[26,27] Discussing these emotional aspects is an important facet of genetic counseling.

Following the molecular confirmation of the diagnosis and mutation specific testing in both parents, the exact recurrence risk can be determined, providing parents and patients (and sometimes other family members) with more options for family planning, either through reassurance in the case of a *de novo* mutation, or through DNA analysis in chorionic villi or preimplantation diagnosis (PGD) in the case of inherited variants.[28,29] In the future, non-invasive prenatal testing, in which DNA analysis of the fetus can be performed in maternal blood without risk of fetal loss, may be available for many syndromes in which the molecular diagnosis is established.[30-32]

Implications for molecular diagnostics

Next to the implications for medical care, the results of our genotype-phenotype studies influence the molecular diagnostic process.

We established that FHS is a highly recognizable, monogenic (homogeneous) disorder, indicating that patients without mutations in *SRCAP* likely were misdiagnosed with FHS in the past or have a disorder similar to FHS that is caused by mutations in a separate gene.

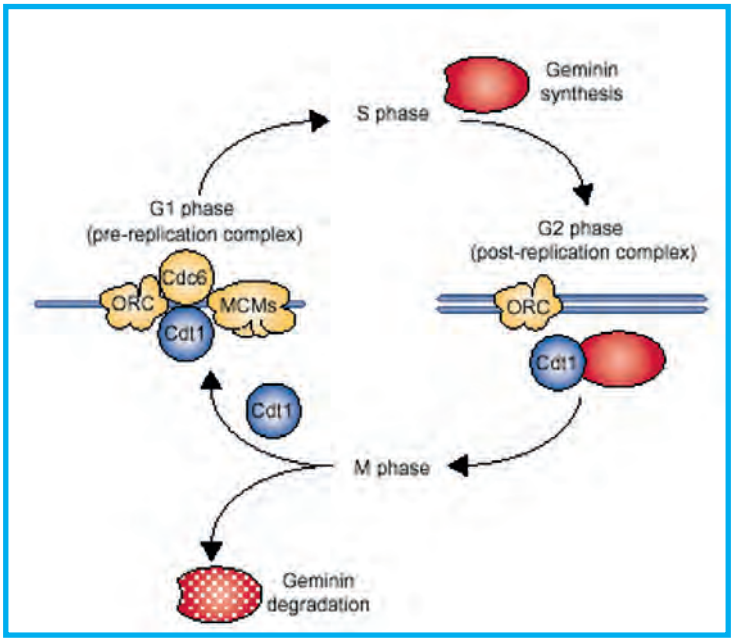
MGS, on the other hand, is a heterogeneous disorder. We detected mutations in one of the five known genes (*ORC1*, *ORC4*, *ORC6*, *CDT1*, *CDC6*) in approximately 70% of MGS patients. In 22% of patients with a classical phenotype of MGS, no mutation could be identified.

Recently, however, we performed WES in four MGS patients and recognized a *de novo* heterozygous mutation in *GMNN* in one patient[A]. Simultaneously, another group detected a *de novo GMNN* mutation in another MGS patient. Afterwards, we performed Sanger sequencing of the *GMNN* gene in seven MGS patients and detected a *de novo* mutation in a third patient.[33]

A This patient is described in this thesis as individual j (**Chapter 2.1**) and patient 43 (**Chapter 2.2**). In these chapters, he is included as a patient in whom no definitive diagnosis could be made.

The *GMNN* gene encodes the DNA replication inhibitor Geminin. Geminin interacts with CDT1, a DNA replication factor involved in the regulation of cell cycle progression and replication (Figure 1).[34-36]

Figure 1. Model illustrating how Cdt1 and geminin limit DNA replication to exactly one round per cell cycle.[90] The origin-recognition complex (ORC) remains bound throughout the cell cycle. During mitosis Cdt1 is sequestered by geminin; upon exit from metaphase, geminin is degraded, releasing Cdt1. Cdt1 and Cdc6 bind to DNA, allowing the mini-chromosome maintenance (MCM) complex to bind to DNA during G1 phase, thereby 'licencing' DNA for a single round of replication. The MCM complex, Cdt1 and possibly Cdc6 are displaced from DNA during S phase. Newly synthesized geminin binds to displaced Cdt1 during S, G2 and M phases, preventing re-licencing of DNA within the same cell cycle. (published with permission of Nature Cell Biology)



These are the first three patients with an autosomal dominant form of MGS. All three mutations were *de novo*, reducing the recurrence risk from 25% to less than 1% for parents, while increasing the recurrence risk for the offspring of patients from less than 1% to 50%.

The classical triad of microtia, patellar anomalies and short stature was present in all three patients. Compared to our cohort of MGS patients with mutations in *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*, however, the patients with mutations in *GMNN* are at the severe end of the spectrum, with heights ranging from -3.9 to -6.8 SDs.

In addition, the patients with *GMNN* mutations have certain clinical features that are rare in MGS: one of the patients was previously described as one of two patients with extremely low IGF1 levels and a positive response to growth hormone treatment.

Furthermore, two of the three patients have marked lumbar lordosis and developmental delay and/or cognitive impairment. Moreover, two patients share fullness of the peri-orbital region, a feature not previously recognized in MGS. Even though these three patients fulfill the clinical criteria of MGS, the clinical data of more patients needs to be analyzed to determine whether these patients really have MGS or a clinical phenotype resembling MGS.

GMNN mutations (and *ORC1* mutations) may perturb DNA replication to a greater extent than mutations in the other MGS associated genes. Furthermore, *GMNN* mutations may have a more profound effect on the replicative burst and cell growth required for brain development and head size expansion and height. Lack of geminin in mice resulted in preimplantation mortality.[37]

In three other MGS patients[B], we were able to identify two recurrent mutations in *ORC6* through whole exome sequencing. Previously, these mutations were not detected after whole exome sequencing on a different platform that did not cover the entire protein coding region of known genes at sufficient read depth, a well recognized restriction of whole exome sequencing.[38]

In the five remaining patients of our cohort, including three patients with mono-allelic mutations in *ORC1* and *CDT1*, the molecular defect remains to be elucidated. These remaining patients could have syndromes resembling MGS and have been diagnosed with MGS unjustly. Mutations could also be located in one of the regulatory elements present in the remaining 98-99% of our DNA which we are unable to sequence with current molecular techniques such as whole exome sequencing.
The introduction of whole genome sequencing may lead to the identification of the molecular cause in these patients: in whole genome sequencing, the entire genome is sequenced, enabling the detection of mutations in non-coding regions. Furthermore, whole genome sequencing is more effective in detecting variants in the coding regions of our DNA, due to an increased coverage of these regions.[39] The introduction of whole genome sequencing is expected to further increase the diagnostic yield in MGS, as was shown for severe early-onset epilepsy and intellectual disability.[40,41]
Moreover, the discovery of *de novo* dominant mutations in *GMNN* has altered our belief that MGS is solely an autosomal recessive disorder.

B All three patients are included in **Chapter 2.1** and **Chapter 2.2** as patients without definitive molecular diagnosis. One of these patients is described as individual i (**Chapter 2.1**) and patient 44 (**Chapter 2.2**). The other two patients were not specified.

To unravel the genetics of MGS (and other syndromes in which the underlying molecular defect is unknown) we may benefit from animal studies as well. The phenotype of animals with mutations in an orthologous gene may help to select candidate genes (both before and after exome sequencing) and to establish or support the pathogenicity of mutations in novel genes detected with WES.[1,42] Moreover, clinical features seen in animal studies may be relevant to our human patients and thereby influence diagnostic management and follow-up of these patients.

Insights into pathophysiology and developmental pathways

Establishing the molecular defect in rare genetic syndromes followed by thorough genotype-phenotype studies can provide valuable insights in the pathophysiology of these disorders. Knowledge of the mechanisms through which mutations in the concerning genes cause these syndromes is crucial. It can help to order genes in developmental pathways and provide clues for possible therapeutic strategies.

The other way around, identification of genes involved in specific developmental pathways may lead to the selection of candidate genes for specific syndromes with a phenotype likely to be caused by disturbance of that pathway or in which mutations in other genes from that pathway have already been described.

For instance, mutations in genes from intracellular signaling pathways influencing growth, including the RAS-MAPK/ERK and PI3K-mTOR pathways, can cause several syndromes in which growth is affected, such as Noonan-syndrome, FGFR3-related skeletal dysplasias (the RAS-MAPK/ERK pathway) and PTEN-hamartoma tumor syndrome (the PI3K-mTOR pathway). Especially in the RAS-MAPK/ERK pathway, knowledge of the genes involved in this pathway has enabled researchers to identify new 'Rasopathies'.[43-66]

The pre-replication genes *ORC1*, *ORC4*, *ORC6*, *CDT1* and *CDC6*, that are known to cause MGS, are all part of the pre-replication complex (preRC), a complex that is essential for DNA replication.[67,68] *GMNN*, our recently identified MGS gene, interacts with this complex through interaction with CDT1 (Figure 1). The precise pathogenic mechanism of MGS is yet to be unraveled, but several mechanisms have been proposed. Mutations in these genes are presumed to delay cell cycle progression and may become rate limiting during very rapid cell cycles, slowing cell cycle progression and subsequently reduce total cell number and thereby organ size.[69,70]

A second possible mechanism affecting cell cycle progression is a defect in the rate of formation of primary cilia, which was demonstrated in ORC1 depleted cells.[71] Finally, mutations in distinctive domains of the ORC1 subunit of the preRC, that regulate

centriole and centrosome copy number, are suggested to alter centrosome duplication and thereby contribute to the growth retardation and microcephaly in MGS.[72]

The mutations in the *SRCAP* gene that cause FHS are clustered in the C-terminal region of the gene.[6] *SRCAP* encodes a SNF2-related chromatin remodeling ATP-ase, a co-activator of CREB-binding protein. Mutations in *SRCAP* may lead to an altered protein that interferes with normal activation of the *CREBBP* gene. CREBBP plays an important role in regulating cell growth and division. Studies have linked CREBBP to the RAS-MAPK pathway by demonstrating that mitogen-activated protein kinase (MAPK) phosphorylation of Ets-1 and Ets-2 resulted in enhanced transactivation by preferential recruitment of the co-activators CREBBP and p300.[73]

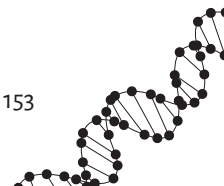
So far, the *ZBTB18* gene could not be linked directly to any of the known pathways for growth.

Further functional studies of the genes that cause MGS and FHS syndrome and the *ZBTB18* gene are necessary to determine their exact role in embryological development and the pathophysiologic mechanism of mutations in these genes. Again, animal studies may help in providing these insights and may assist in developing future therapies, as was the case in Leber congenital amaurosis, where studying animal models eventually led to RPE65 gene therapy.[74-77]

Future studies: a molecular diagnosis and data sharing are fundamental

We intend to carry our next generation phenotyping studies forward and further delineate the phenotypic spectrum of MGS, FHS syndrome, *ZBTB18* mutations and disorders resembling these syndromes by additional longitudinal genotype-phenotype studies in extended cohorts of patients with these syndromes. We aim to incorporate our mixed linear growth model to study growth in these disorders. These studies will further assist physicians in interpreting results from next generation sequencing and accommodate them in establishing a clinical diagnosis in countries where the use of whole exome sequencing in a diagnostic setting is not standard.

Genotype-phenotype studies can only be performed when the underlying molecular defect is known. However, in OMIM (Online Mendeliance in Man; http://omim.org/), there are over 1600 phenotypic descriptions (entities) of patients or groups of patients in whom the molecular diagnosis remains to be elucidated.[78]



Current molecular tests (e.g. linkage studies, genome-wide SNP-array studies including homozygosity mapping and whole exome sequencing) are used to detect the underlying molecular cause in syndromes of which the cause is yet to be established.
As clinical geneticists, we play a key role in this process by recognizing clinical syndromes, selecting cohorts of patients and interpreting results of molecular investigations. Furthermore, we assist in the unraveling of new syndromes caused by mutations in novel genes by proper phenotyping, recognizing patients with overlapping phenotypes and performing segregation analysis in families.
A more widespread use of whole exome sequencing can increase the number of established correct diagnoses and further reduce the number of phenotypic descriptions with an unknown molecular defect. New techniques, such as whole genome sequencing, are expected to contribute to this process even further.[40,41,79]

A prerequisite for next generation phenotyping is data sharing. When the underlying molecular defect of a rare syndrome is identified, the formation of a cohort of patients with the same genetic defect is essential to perform genotype-phenotype studies. Due to the low prevalence of many genetic syndromes, however, it can be very difficult to obtain sufficient data and perform these studies. (Inter)national cooperation to establish large scale data sharing is crucial to form cohorts of patients with rare disorders.
The same accounts for the unraveling of molecular defects in patients with rare genetic syndromes: in daily practice, it is often difficult to find a second patient with a mutation in the same gene or to collect more patients with the same phenotype in whom you can perform WES simultaneously in order to detect the underlying genetic defect in these patients. In our studies, we encountered these difficulties when we detected a *de novo ZBTB18* mutation in a single patient (**Chapter 4**). Identification of more patients with mutations in this gene is essential to enhance the knowledge of this disorder and improve patient care.

This problem is recognized by professionals, patients and politicians.
At present, the European Union 'helps to pool scarce resources that are currently fragmented across individual EU countries and helps patients and professionals share expertise and information across borders' by enabling the formation of national centers of reference for rare diseases that join together to form European networks of reference (http://ec.europa.eu/health/rare_diseases/european_reference_networks/).
Professional driven initiatives have led to the development of programmes for secured data sharing between clinicians and scientists. They aim to facilitate in

establishing a correct diagnosis by indicating overlapping clinical features between a patient and known syndromes or between two patients without a known diagnosis, thereby enabling us to learn more about known rare disorders and to unravel new genetic syndromes. An example of such a platform is Matchmaker Exchange (http://matchmakerexchange.org), which aims to 'facilitate the matching of cases with similar phenotypic and genotypic profiles (matchmaking) through standardized application programming interfaces (APIs) and procedural conventions'. These APIs enable submitters to search for overlapping genetic variants and phenotypes in multiple databases with just one query. Phenome Central (https://phenomecentral.org), Leiden Open Variation Database (http://www.lovd.nl), GeneMatcher (http://www.genematcher.org), Cafe Variome (www.cafevariome.org) and Decipher (https://decipher.sanger.ac.uk) are examples of databases that are part of the Matchmaker Exchange platform.[80-83]

A patient participation portal is Genomeconnect, part of the clinical genome resource (http://www.clinicalgenome.org). It aims to 'connect people who are interested in sharing de-identified genetic and health information to improve the understanding of genetics and health' and is associated with the Matchmaker Exchange platform.

An example of the successful use of these databases is the identification of *de novo HNRNPK* mutations in two patients with ID, specific facial dysmorphic features and skeletal and connective tissue abnormalities.[84]

The matchmaking process can be challenging, though. To increase the chances of successful matchmaking, the use of standardized measures for capturing phenotypic abnormalities is of major importance.[85] The Human Phenotype Ontology project (http://www.human-phenotype-ontology.org) aims to 'provide a standardized vocabulary of phenotypic abnormalities encountered in human disease'.[86] So far, it 'provides a structured, comprehensive and well-defined set of 10,088 classes (terms) describing human phenotypic abnormalities and 13,326 subclass relations between the HPO classes'.[87]

HPO has assisted in unraveling genetic defects in 707 cases with unexplained bleeding and platelet disorders, demonstrating the power of this standardized vocabulary.[88]

Another initiative to increase the chance of successful matchmaking is a computational tool called 'Phenolyzer'.[89] This tool prioritizes Mendelian and complex disease genes, based on disease or phenotype terms entered as free text, thereby utilizing existing disease nomenclature systems to interpret the user input, sometimes including multiple diseases or phenotype terms.

Thus, the widespread use of HPO-terms in daily practice and data sharing programmes could accommodate physicians in making a correct diagnosis, enable genotype-phenotype studies by forming cohorts of patients with rare disorders and assist in elucidating new genetic syndromes by connecting the phenotypes of patients without a diagnosis.

Next to professional-based initiatives, patient organizations play an upcoming, important role in data sharing and educating patients and their surroundings, by providing them with information about their disorder. Moreover, they keep patients and their parents informed about new developments (i.e. potential therapies, DNA-investigations) and stimulate research participation.
Nowadays, patients often organize themselves through social media, such as facebook or patient organization websites. Patients or parents of patients with the same disorder form their own groups, enabling contact between people who live on other sides of the world. Social media are a good platform for patients to increase the awareness of their condition and to help collect data from other patients with the same disorder for research purposes. A good example of a patient organization that is active on social media and aims to 'educate, increase awareness and promote research for the support and enrichment of individuals living with Koolen-de Vries Syndrome and their families', is 'Supporting Families with Koolen-de Vries Syndrome Mission': www.supportingkdvs.com and https://ww.facebook.com/supportingkdvs.
To our knowledge, no active patient organizations exist for MGS and FHS syndrome (the information on the Floating Harbor syndrome support group dates back to 2013 (www.floatingharborsyndromesupport.com)) and our research groups. These patients could also benefit from patient organizations that remain up to date.

For future studies, sharing DNA or other tissues is equally important as sharing clinical data. Biobanks store biological samples of patients for research purposes. Scientists can obtain DNA or other tissues from several patients with the same disorder, to search for the underlying molecular cause of a syndrome or to perform functional studies to acquire more insight in the pathophysiology of disorders.
Two examples of such biobanks are AGORA (Aetiologic research into Genetic and Occupational/environmental Risk factors for Anomalies in children) of the Radboud university medical center, a data- and biobank of patients with congenital malformations (such as malformations of the kidneys and urinary tract, intestines and anus, lip and palate and the heart, but also childhood cancers; www.agoraproject.nl)

and the 'Dutch Parelsnoer initiative', a collaboration of the eight Dutch Universities to collect clinical data and biological samples from patients with chronic diseases (www.parelsnoer.nl).

All initiatives are important steps towards proper worldwide data sharing. In an ideal world, all clinical and molecular information would be shared in one database accessible to physicians, scientists and patients worldwide and DNA of all patients would be stored in biobanks and be available for researchers worldwide through a single coordination centre.

In conclusion

In this thesis, we performed genotype-phenotype studies in Meier-Gorlin syndrome (MGS) and Floating-Harbor syndrome (FHS) syndrome and proposed experience based management advice after the molecular cause for these disorders was identified through whole exome sequencing. We have increased the knowledge of the phenotypic spectrum of these syndromes and improved clinical care for these patients. Additionally, we established a *de novo* nonsense mutation in *ZBTB18* in a girl with features resembling FHS syndrome through whole exome sequencing.
To take next generation phenotyping to a higher level, sharing of clinical and molecular data is essential. For example, data sharing will enable us to form a cohort of patients with *ZBTB18* mutations and even larger cohorts of patients with MGS and FHS syndrome in whom we can perform genotype-phenotype and follow-up studies to further improve diagnosis and management of these patients. Furthermore, functional studies aimed to increase our knowledge about the underlying molecular mechanism of these disorders may assist us to select new candidate genes for MGS and disorders resembling MGS and FHS syndrome and may contribute to explain the phenotypic variability of these syndromes and potentially provide valuable clues for therapeutic strategies.

Overall, the era of genomics provided valuable new techniques with a tremendously increased diagnostic yield, leading to the identification of the molecular genetic defect in patients with clinically well-known disorders and many new genetic syndromes. In this era, clinical genetic practice is slowly shifting from a phenotype-first approach to a predominantly genotype-first approach.

Major challenges still to overcome comprise genotype and phenotype data sharing of patients and interpretation of the clinical significance of genetic variants by integrating clinical and molecular data, combined with knowledge from model systems and biological network data.

Phenotyping of each individual remains of the utmost importance to interpret detected genetic variants, make the correct diagnosis and ensure optimal medical care.

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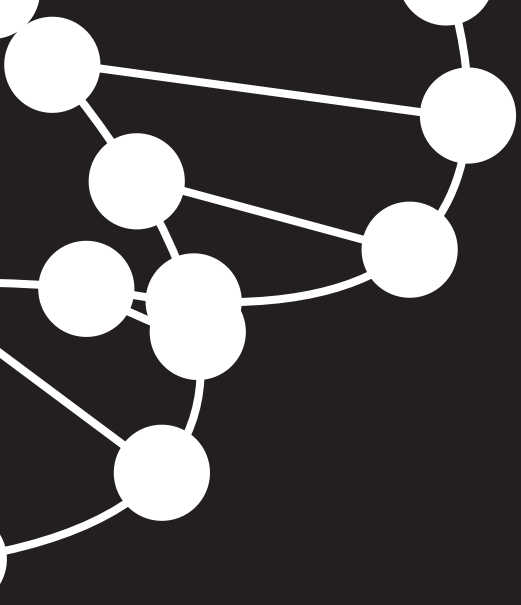
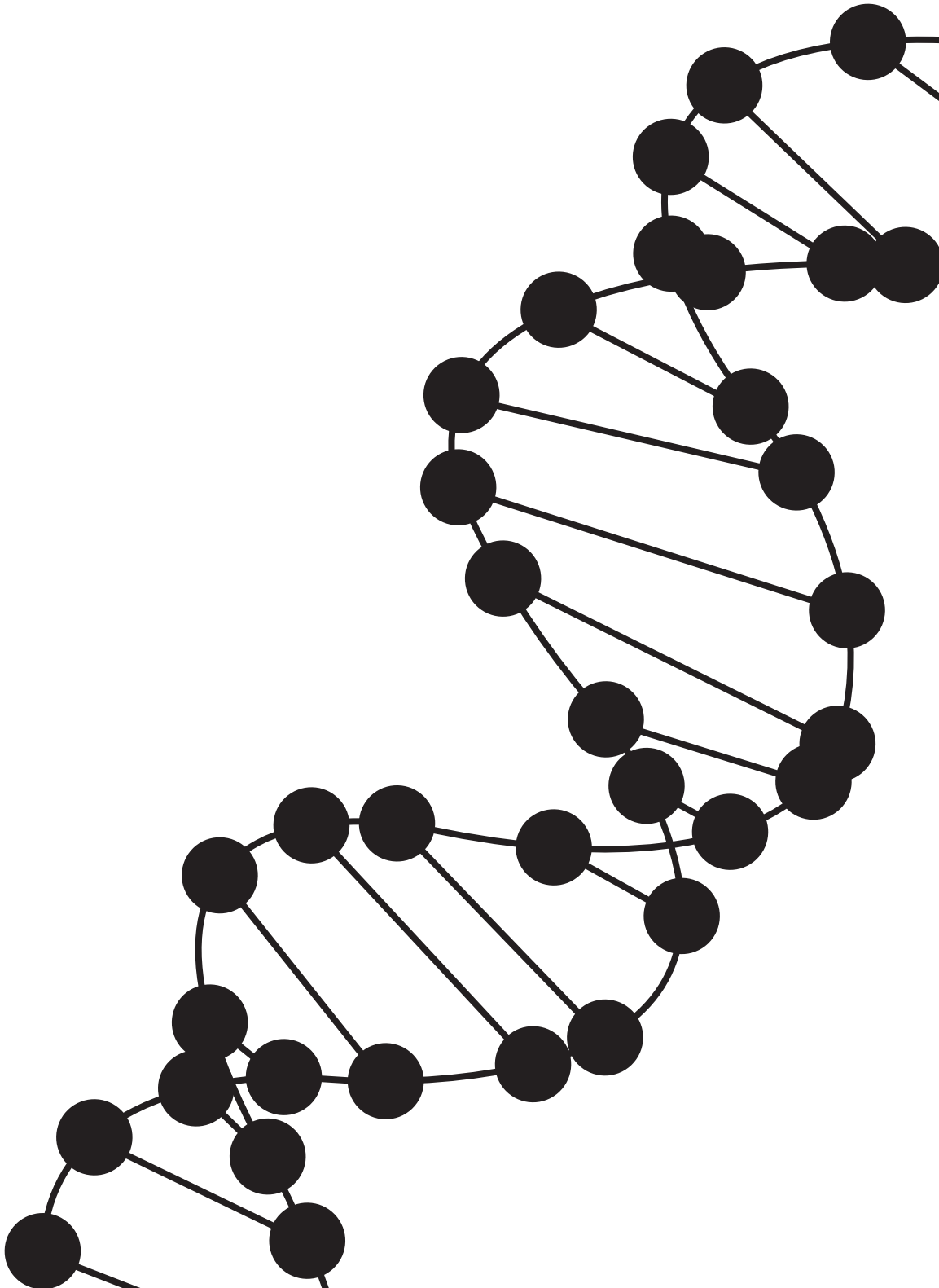
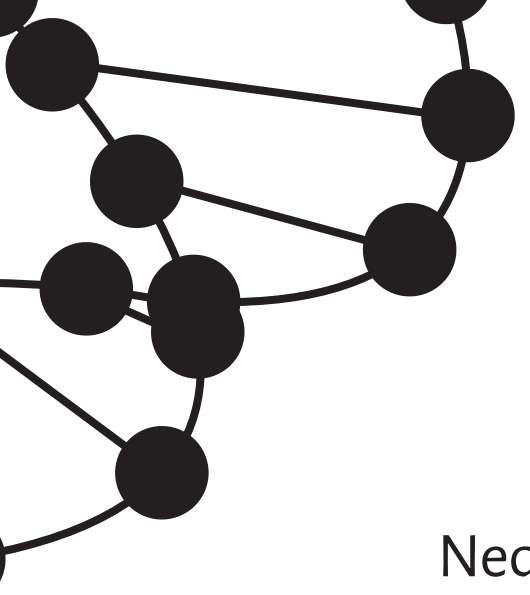


Appendix





Nederlandse samenvatting

Op dit moment leven we in het 'Genomics' tijdperk. Dit tijdperk wordt gekenmerkt door een nieuwe generatie genetische onderzoeken ('next generation sequencing' (NGS)), waarbij veel genen tegelijkertijd onderzocht kunnen worden, terwijl het voorheen slechts mogelijk was om één gen per test te onderzoeken. Met behulp van deze nieuwe technieken zijn we veel vaker in staat om een diagnose te stellen, maar komen we ook voor nieuwe uitdagingen te staan. De introductie van NGS heeft tot een nieuwe werkwijze binnen de klinische genetica geleid: 'next generation fenotypering'. Met deze werkwijze worden diagnoses gesteld en nieuwe syndromen ontdekt door het associëren van de overeenkomstige klinische kenmerken ('fenotype') van patiënten met DNA-afwijkingen ('mutaties') in genen die gevonden zijn met NGS. Bij kinderen met een verstandelijke beperking bijvoorbeeld, worden tussen de 0 en 7 DNA-varianten per individu gevonden, die bij het kind nieuw zijn ontstaan en dus niet bij beide ouders worden teruggevonden. Om te kunnen bepalen welke van deze varianten daadwerkelijk de oorzaak is van de verstandelijke beperking, is het belangrijk om de klinische kenmerken van een patiënt gedetailleerd in kaart te brengen. Deze vergelijken we vervolgens met het fenotype van andere patiënten (of dieren) met DNA-afwijkingen (mutaties) in dezelfde genen.
Daarnaast zijn we er in geslaagd om met behulp van NGS de onderliggende genetische oorzaak voor veel bekende syndromen te identificeren, waardoor genotype-fenotype studies voor deze syndromen mogelijk zijn geworden. Om nieuwe syndromen te ontdekken en meer kennis over deze syndromen te vergaren, is het belangrijk om het fenotype van een patiënt zo goed mogelijk in kaart te brengen. Door middel van (inter)nationale samenwerking en het delen van data in internationale databases is het mogelijk om de kenmerken van meerdere patiënten met mutaties in hetzelfde gen te bestuderen en zo meer inzichten te krijgen in nieuwe syndromen. Het inzicht in het klinische spectrum van deze aandoeningen wordt hierdoor vergroot, waardoor patiënten met deze aandoeningen en hun ouders en behandelend artsen weten welke gezondheidsproblemen zouden kunnen optreden. Bovendien kunnen we op deze manier patiënten met hetzelfde zeldzame fenotype vinden en de resultaten van hun genetisch onderzoek vergelijken om de onderliggende oorzaak voor hun syndroom op te sporen.

In dit proefschrift hebben we, nadat de onderliggende genetische oorzaak met behulp van NGS was vastgesteld, genotype-fenotype studies verricht voor twee syndromen, die beiden onder andere gekenmerkt worden door een kleine lengte: Meier-Gorlin syndroom (MGS) en Floating-Harbor syndroom (FHS). Op basis van deze studies

presenteerden we als eerste adviezen voor de medische zorg van patiënten met deze syndromen (**hoofdstukken 2 en 3**). Doordat artsen nu de klinische kenmerken van MGS en FHS beter met die van hun patiënt kunnen vergelijken, wordt het makkelijker om een klinische (waarschijnlijkheids)diagnose te stellen en te bepalen welk aanvullend genetisch onderzoek aangewezen is. Tenslotte hebben we met NGS een nieuw ontstane mutatie in *ZBTB18* gevonden bij een patiënt bij wie in eerste instantie gedacht werd aan FHS (**hoofdstuk 4**). Vervolgens hebben we de medische gegevens van andere patiënten met *ZBTB18* in kaart gebracht (ongepubliceerde data) en hiermee een nieuw syndroom geïdentificeerd.

Hoofdstuk 2. Meier-Gorlin syndroom

Het Meier-Gorlin syndroom (MGS) is voor het eerst beschreven in 1959. In 2011 werd ontdekt dat MGS wordt veroorzaakt door mutaties in de *ORC1*, *ORC4*, *ORC6*, *CDT1* en *CDC6* genen in ongeveer 70% van de patiënten. Deze vijf genen maken onderdeel uit van het pre-replicatie complex, een complex dat betrokken is bij het kopiëren van het DNA ('DNA-replicatie'), dat een cruciale stap in de celdeling is.
Deze ontdekking stelde ons in staat om de genotype-fenotype studies te verrichten, die zijn beschreven in **hoofdstuk 2.1**.

We hebben genotype-fenotype studies verricht in een cohort van 35 MGS patiënten met twee mutaties in één van de vijf bekende genen van het pre-replicatie complex en 10 MGS patiënten bij wie de onderliggende genetische afwijking onbekend was. De diagnose MGS werd bij deze 10 patiënten gesteld op basis van klinische kenmerken.

De klassieke trias van klinische kenmerken van MGS omvat kleine oren ('microtie'), te kleine/afwezige knieschijven ('patella a-/hypoplasie') en een kleine lengte. Deze klassieke trias werd gezien in 29 van de 35 MGS patiënten met mutaties in een van de vijf bekende genen. Vier patiënten (één met *ORC1* mutaties, één met *ORC6* mutaties en twee met *CDT1* mutaties) hadden microtie en patella aplasie, maar een normale lengte. Één patiënt met *ORC1* mutatie had microtie en een kleine lengte, maar normale patellae. Een andere patiënt met *ORC1* mutaties had een kleine lengte, zonder microtie en patella afwijkingen.
Deze bevindingen tonen aan dat het fenotypische spectrum van MGS breder is dan werd gedacht voordat genetische diagnostiek mogelijk was. Aangezien na moleculaire

diagnostiek van patiënten is gebleken dat bij een klein deel van de patiënten met MGS slechts één van de drie klassieke kenmerken van MGS aanwezig is, wordt aan artsen geadviseerd de diagnose MGS vaker in hun differentiaal diagnostische overwegingen mee te nemen.

Andere frequente klinische kenmerken in ons cohort van patiënten met MGS waren een typisch gelaat (o.a. een klein mond met volle lippen en een smalle neus met een brede neusrug), ademhalingsproblemen tijdens de vroege kinderleeftijd (inclusief aangeboren long emfyseem, en trachea-, laryngo- en/of bronchomalacie), voedingsproblemen tijdens de kinderleeftijd, onderontwikkelde borsten ('mamma hypoplasie') en afwijkingen van het genitaal.

Door het fenotype van MGS patiënten met mutaties in de vijf verschillende genen onderling te vergelijken, kwam aan het licht dat patiënten met *ORC1* mutaties de meest ernstige groeiachterstand hadden: zij waren het kleinst en hadden de kleinste schedelomtrek, met een gemiddelde standaard deviatie score van respectievelijk -7.1 SDs (range -9.6 tot -5.2 SDs) en -6.7 SDs (range -9.8 tot -4 SDs).

Daarnaast hebben we het fenotype van patiënten met verschillende types mutaties (met een verschillend effect op de vorming van het eiwit) in hetzelfde gen met elkaar vergeleken. Deze data suggereerde dat compound heterozygote nonsense en missense mutaties een ernstiger effect op het fenotype hebben dan homozygote missense mutaties. Drie patiënten met een missense mutatie en een nonsense mutatie in *ORC4* hadden een meer uitgesproken groeiachterstand en meer aangeboren afwijkingen (aangeboren long emfyseem, een aangeboren hartafwijking en ernstige voedingsproblemen) dan vier patiënten met twee missense mutaties in *ORC4*. Daarnaast had de enige patiënt met een homozygote missense mutatie in *CDT1* geen aangeboren long emfyseem, terwijl dit bij alle zeven patiënten met een missense en een nonsense mutatie in *CDT1* wel vastgesteld was.
Vier patiënten met compound heterozygote nonsense en missense mutaties zijn overleden: een jongen met een nonsense en een missense mutatie in *CDT1* is overleden ten gevolge van een plotse hartstilstand, terwijl zijn zus op de leeftijd van drie maanden overleed aan ernstige ademhalingsproblemen veroorzaakt door een tracheobronchomalacie met progressief aangeboren long emfyseem. Een jongen met een frameshift mutatie en een missense mutatie in *ORC1* overleed na 3.5 maanden. Hij had een ernstige corticale dysplasia met pachygyrie en ventriculomegalie,

craniosynostose, aangeboren long emfyseem en een agenesie van de pancreasstaart. Zijn broer overleed na 17 weken zwangerschap. Hij had een ernstige groeiachterstand en microtie.

Vooralsnog zijn er geen MGS patiënten beschreven met homozygote of compound heterozygote nonsense mutaties, hetgeen suggereert dat het effect van deze mutaties dusdanig ernstig is, dat dit niet verenigbaar is met het leven. Deze hypothese wordt ondersteund door de uitkomsten van diermodel studies met Drosophila-vliegen: vliegen met nonsense mutaties in *ORC6* en *CDT1* overlijden tijdens de embryologische fase.

In **hoofdstuk 2.2** beschreven we de resultaten van een groei studie die we hebben verricht in ons cohort van 45 MGS patiënten om een specifiek groei patroon te kunnen herkennen. Dit met als doel de groei en het groeipatroon van MGS patiënten te kunnen voorspellen.
Trend analyses van de groei toonden aan dat de groei achterstand van MGS patiënten voornamelijk tijdens de zwangerschap optreedt. Een intra-uteriene groei achterstand werd gezien bij 98% van de MGS patiënten. Het gemiddelde geboortegewicht lag op de -3.9 SDs, met een gemiddelde lengte van -3.4 SDs. In het eerste levensjaar boog de lengte verder af tot -5.2 SDs, maar in de jaren daarna was de groeisnelheid normaal tot licht verhoogd in vergelijking met kinderen zonder MGS. Dit laatste resulteerde in een gemiddelde volwassen lengte van -4.5 SDs. Deze studie resultaten maken het mogelijk om het groeipatroon van MGS patiënten te voorspellen: de lengte neemt in het eerste levensjaar af met gemiddeld 1.7 SDs, waarna de groeisnelheid nagenoeg normaal wordt.

De groei werd significant beïnvloed door etniciteit en de onderliggende moleculaire oorzaak.
Dit bevestigde onze bevindingen die beschreven zijn in **hoofdstuk 2.1** en gaf ons verder inzicht in de groei. We toonden aan dat patiënten met *ORC1* en *ORC4* mutaties significant kleiner waren (respectievelijk 4.7 SDs en 3.1 SDs kleiner) dan patiënten met mutaties in *ORC6*, *CDT1* en *CDC6* of MGS patiënten zonder moleculaire diagnose.
De schedelomtrek van patiënten met *ORC1* en *ORC4* mutaties was 5.0 SDs (*ORC1*) en 1.6 SDs (*ORC4*) kleiner dan de schedelomtrek van patiënten met mutaties in *ORC6*, *CDT1*, *CDC6* en patiënten zonder moleculaire diagnose.

In ons cohort van MGS patiënten werden twee Nederlandse patiënten (bij wie de diagnose MGS werd gesteld op basis van klinische kenmerken) vanaf de leeftijd van 3 en 5 jaar succesvol behandeld met groeihormoon (een inhaalgroei van 2 SDs of meer). Bij deze twee patiënten bleef de groeisnelheid na het eerste levensjaar vertraagd, waardoor de groei verder afboog van het gemiddelde. Ook hadden zij extreem lage IGF1 waarden. Deze bevindingen suggereren dat de IGF1 waarde bij een aantal MGS patiënten kan worden gebruikt om te voorspellen of behandeling met groeihormoon zinvol is.

Recent werd aangeboren long emfyseem vastgesteld bij twee MGS patiënten met astma/verschijnselen die op astma lijken (niet gepubliceerde data).
Ofschoon meer onderzoek nodig is om vast te stellen hoe frequent aangeboren long emfyseem daadwerkelijk voor komt bij MGS patiënten, stellen we dat nader onderzoek naar aangeboren long emfyseem altijd geïndiceerd is bij MGS patiënten met luchtwegklachten die lijken op astma.

Op basis van de resultaten van onze moleculaire en longitudinale klinische studies van MGS patiënten (met follow-up data van meer dan 15 jaar) hebben we in **hoofdstuk 2.3** een voorstel gedaan voor de diagnostiek, controles en behandeling van patiënten met MGS. De belangrijkste aandachtspunten voor de medische zorg zijn de groei, de ontwikkeling van secundaire geslachtskenmerken, de motorische en spraak-taal ontwikkeling, luchtwegklachten en orthopedische problemen.

MGS is een heterogene aandoening, waarbij er behoudens de lengte en schedelomtrek, geen duidelijke verschillen in fenotype zijn tussen de vijf bekende genen.
Bij ongeveer 30% van de MGS patiënten werd met DNA-onderzoek tot recent geen onderliggende oorzaak gevonden. In 2015 werd bij drie MGS patiënten (waaronder twee Nederlandse patiënten) één nieuw ontstane mutatie in *GMNN* gevonden. Bij twee van de drie patiënten werd deze mutatie opgespoord met behulp van exoom sequencing. Hierna werd de mutatie bij de derde patiënt met behulp van Sanger sequencing opgespoord. Het *GMNN* gen codeert voor het eiwit geminin, dat door een interactie met het eiwit CDT1 een essentiële rol heeft in DNA replicatie. Opvallend is dat deze bevinding wijst op een autosomaal *dominante* overerving, terwijl tot nu toe alleen aanwijzingen werden gevonden voor een autosomaal *recessieve* overerving. De patiënten met *GMNN* mutaties presenteerden zich met de bekende klassieke trias van MGS, maar hun groeiachterstand leek ernstiger (lengte tussen de -3.9 en -6.8 SDs)

en zij hadden andere bijkomende kenmerken (bij twee van de drie patiënten): een evidente holle rug (versterkte 'lumbale lordose'), een ontwikkelingsachterstand en/of verstandelijke beperking en prominent weefsel rond de ogen. Één van de patiënten was een van de twee Nederlandse patiënten die goed reageerde op de behandeling met groeihormoon.

Daarnaast waren we in staat om bij drie patiënten met exoom sequencing twee mutaties in *ORC6* te vinden. Tot nu toe hadden we deze mutaties niet opgespoord na exoom sequencing met een ander platform.

Van ons cohort zijn er nog vijf patiënten bij wie het genetische defect nog niet opgehelderd is (waaronder twee patiënten met één mutatie in *ORC1* en *CDT1*). Deze patiënten kunnen een syndroom hebben dat klinisch lijkt op MGS. Zij kunnen ook mutaties hebben die we met de huidige technieken nog niet kunnen detecteren.

Hoofdstuk 3. Floating-Harbor syndroom

Nadat in 2012 werd vastgesteld dat het Floating-Harbor syndroom (FHS) veroorzaakt wordt door truncerende mutaties in het *SRCAP* gen, hebben we genotype-fenotype studies verricht bij 52 FHS patiënten met 14 verschillende mutaties in dit gen. De resultaten van dit onderzoek zijn beschreven in **hoofdstuk 3**.

We stelden vast dat alle 14 beschreven mutaties in het *SRCAP* gen truncerende mutaties zijn, gelegen tussen codons 2389 en 2748 in exon 34. Er waren twee terugkerende mutaties: Arg2444* en Arg2435*, die bij respectievelijk 50% en 25% van de patiënten gevonden werden.

We hebben de klinische kenmerken van FHS verder in kaart gebracht. Karakteristieke kenmerken zijn een kleine lengte (92%; range -1 tot -4.5 SDs; met name postnataal), achterblijvende skeletleeftijd, karakteristieke gelaatskenmerken, brede duimen en een expressieve spraakachterstand. Het faciale fenotype was in ons cohort de beste voorspeller voor de aanwezigheid van een *SRCAP* mutatie. Wanneer de typische faciale kenmerken niet aanwezig waren, was de kans dat er een *SRCAP* mutatie werd gevonden erg klein.

De karakteristieke faciale kenmerken zijn een driehoekig gelaat met diepliggende ogen, lange wimpers, een smalle neusbasis die breder wordt richting de neuspunt, een lage columella, grote neusgaten, een kort filtrum en een dunne bovenlip met een naar buiten gekeerde onderlip. Een typerend kenmerk is de horizontale lijn van de boven- en onderlip.
De expressieve spraak-taal achterstand werd bij alle patiënten beschreven, maar varieerde in ernst: één patiënt sprak twee talen, terwijl andere volwassen FHS patiënten slechts enkele woorden spraken. De verstandelijke ontwikkeling varieerde van normaal (IQ 104) tot ernstig verstandelijke beperkt (enkele patiënten).

Microcefalie werd slechts bij een minderheid van de patiënten vastgesteld en slechts één patiënt had een schedelomtrek die onder de -3 SDs lag, hetgeen suggereerde dat de groei van de hersenen van FHS patiënten relatief gespaard wordt.

Ongeveer 63% van de FHS patiënten had ten minste één aangeboren afwijking, onder andere in het maag-darmstelsel (motiliteitsproblemen, een vernauwing van het colon, coeliakie), het urinewegstelsel (cryptorchidisme, nier-/nierbekken afwijkingen), het hart (milde coarctatie van de aorta, atrium septum defect, tetralogie van Fallot), de ogen (strabismus, hypermetropie, nystagmus) en het gebit (kleine tanden, cariës, malocclusie). Gehoorsverlies en epilepsie werden relatief frequent gezien.

Op basis van deze resultaten, pleiten we ervoor dat de zorg voor FHS patiënten gericht wordt op de herkenning en behandeling van gehoor- en visusproblemen, urogenitale malformaties, tandafwijkingen, vertraagde skeletleeftijd en groeiretardatie, mogelijke vervroegde puberteit, de psychomotore en spraak-taalontwikkelingsachterstand, epilepsie en gedragsproblemen.

FHS is een monogene aandoening met een klinisch goed herkenbaar fenotype. Patiënten met klinische kenmerken lijkend op FHS, maar zonder mutatie in *SRCAP*, hebben hoogstwaarschijnlijk geen FHS, maar een mutatie in een tweede 'FHS gen', waarbij het klinische spectrum breder is.

Hoofdstuk 4. *ZBTB18*

Zoals beschreven in **hoofdstuk 3**, wordt Floating-Harbor syndroom (FHS) gekenmerkt door een kleine lengte, typische gelaatskenmerken en een expressieve spraak-taal achterstand.
Bij slechts twee van de vijf patiënten die we in 2011 in ons universitair medisch centrum zagen, kon de diagnose FHS moleculair bevestigd worden. In één van de overige drie patiënten heeft whole exome sequencing geleid tot de identificatie van een mutatie in een nieuw gen, *ZBTB18*.
In **hoofdstuk 4** beschrijven we de laatstgenoemde patiënt: de eerste patiënt met een *de novo* nonsense mutatie in *ZBTB18* had als klinisch beeld een kleine lengte, microcefalie, een ontwikkelingsachterstand (voornamelijk van de spraak) en faciale dysmorfieën (gebogen wenkbrauwen, telecantus, korte palpebrale fissuren, een lange neus met een prominente punt, een dunne bovenlip en een kleine kin). Op een MRI scan van de hersenen werden geen afwijkingen gezien. De klinische kenmerken overlapten deels met het fenotype van FHS (o.a. kleine lengte, spraakontwikkelingsachterstand).

Het *ZBTB18* gen (ook bekend als *ZNF238*) ligt op chromosoom 1q44, in de regio van het chromosoom 1q43q44 microdeletie syndroom. Het gen codeert voor een C2H2-type zinc vinger eiwit dat de transcriptie remt van genen die betrokken zijn bij de ontwikkeling van het centrale zenuwstelsel.

De klinische kenmerken van patiënten met microdeleties van chromosoom 1q43q44 die in de literatuur beschreven zijn komen deels overeen met die van FHS patiënten. Overeenkomstige kenmerken zijn een kleine lengte en spraak-taalproblemen. Ook aangeboren afwijkingen, zoals hartafwijkingen, gastro-intestinale afwijkingen en urogenitale afwijkingen zijn beschreven bij beide syndromen. Typische gelaatskenmerken van patiënten met 1q43q44 microdeleties zijn onder andere een rond gelaat met diepliggende ogen, epicantusplooien, laagstaande oren, een prominente metopische naad, een korte neus met prominente/brede neuspunt, een dunne bovenlip, wijd uiteen staande tanden en retro-/micrognatie.

Het fenotype van FHS onderscheidt zich van het 1q43q44 microdeletie syndroom door de aanwezigheid van karakteristieke gelaatskenmerken en de afwezigheid van microcefalie en corpus callosum afwijkingen. Microcefalie en corpus callosum agenesie zijn wel aanwezig bij het grootste deel van de patiënten met 1q43q44 microdeleties.

Deze patiënten hebben vaak geen herkenbare, karakteristieke gelaatskenmerken.
In de literatuur zijn meerdere genotype-fenotype studies gepubliceerd van patiënten met 1q43q44 microdeleties inclusief het *ZBTB18*-gen, waarin wordt gesuggereerd dat *ZBTB18* een belangrijk kandidaatgen is voor microcefalie en/of corpus callosum agenesie.

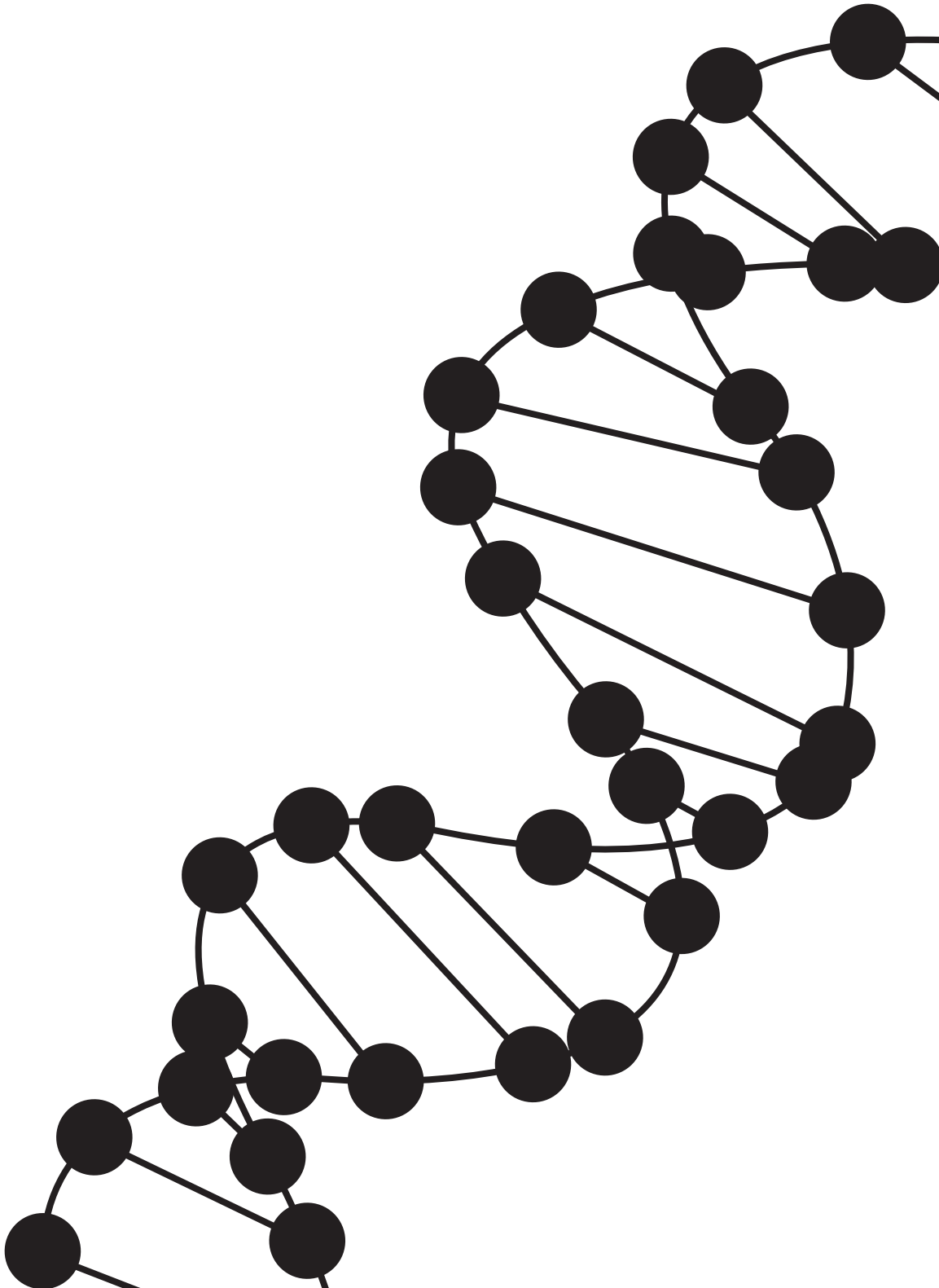
De aanwezigheid van een *de novo* nonsense mutatie in *ZBTB18* in onze patiënt met een kleine lengte, microcefalie, een ontwikkelingsachterstand en opvallende gelaatskenmerken suggereerde dat haploinsufficiëntie van het *ZBTB18*-gen bijdraagt aan zowel de pre- en postnatale groeiachterstand (aanwezig bij 40% en 42% van de patiënten), de microcefalie (aanwezig bij 97% van de patiënten) als de ontwikkelingsachterstand (aanwezig bij 100% van de patiënten) van het 1q43q44 microdeletie syndroom.
Deze hypothese wordt ondersteund door het feit dat *zbtb18* knockout muizen een lethaal fenotype met neocorticale afwijkingen hebben en dat verlies van zbtb18 in het centrale zenuwstelsel leidt tot microcefalie, corpus callosum agenesie en hypoplasie van het cerebellum. Bovendien laten *zbtb18*-gemuteerde hersenen een verminderde neuronale en toegenomen gliale differentiatie zien.

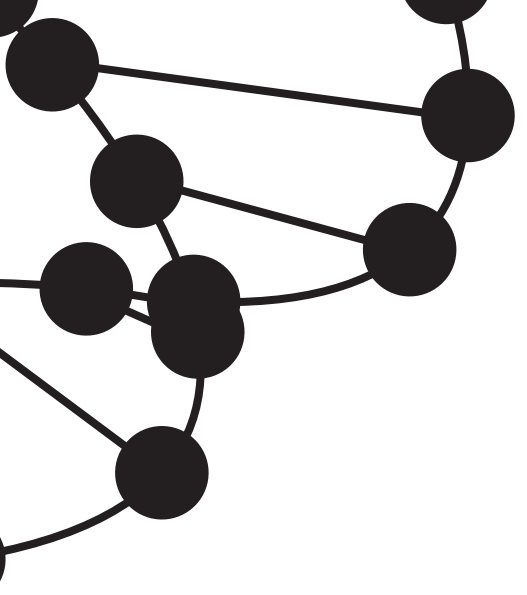
Alle klinische kenmerken van onze patiënt zijn beschreven bij patiënten met 1q43q44 microdeleties, waarbij het *ZBTB18*-gen in het 1q43q44 deletie-gebied ligt. Onze patiënt had echter geen afwijkingen van het corpus callosum, terwijl deze bij 82% van de patiënten met deleties worden gezien. Bovendien kwamen de faciale kenmerken van onze patiënt slechts gedeeltelijk overeen met die van patiënten met het 1q43q44 microdeletie syndroom (een prominente neuspunt, een vlak filtrum, een dunne bovenlip en een kleine kin).
De aanwezigheid van corpus callosum afwijkingen bij het 1q43q44 microdeletie syndroom zou verklaard kunnen worden door een variabele expressie, of door een bijdrage van andere genen in het microdeletie gebied.

We beschreven de eerste nieuw ontstane (nonsense) mutatie in *ZBTB18*. Aangezien dit slechts één patiënt betrof, waren we niet in staat om genotype-fenotype studies te verrichten.
Recent werd bij twee andere patiënten een *ZBTB18* mutatie geïdentificeerd. Een 4 jaar oud meisje had een ontwikkelingsachterstand, een lage spierspanning, spasticiteit met dystonie, stereotype gedrag en een dun corpus callosum met onderontwikkeld

middendeel van de kleine hersenen (cerebellaire vermis hypoplasie). Een 15 jaar oude jongen had een ernstige ontwikkelingsachterstand met een uitgesproken spraak-taalachterstand, epilepsie en opvallende gelaatskenmerken, waaronder diepliggende ogen, volle gebogen wenkbrauwen, een volle neuspunt, een brede mond met volle lippen en een puntige kin (niet gepubliceerde data).
Deze gegevens bevestigen onze hypothese dat *ZBTB18* mutaties een syndromale vorm van een ontwikkelingsachterstand/verstandelijke beperking veroorzaken en dat verlies van ZBTB18 bijdraagt aan het fenotype van het chromosoom 1q43q44 microdeletie syndroom.

Het genomics tijdperk heeft ons waardevolle nieuwe technieken van genetisch onderzoek gebracht, waardoor de onderliggende genetische oorzaak voor veel patiënten met bekende en nieuwe syndromen geïdentificeerd is.
In dit tijdperk verschuift de klinisch genetische praktijk langzaam van een 'fenotype-eerst' naar een voornamelijk 'genotype-eerst' benadering, wat vraagt om een nieuwe werkwijze, die aangeduid wordt als 'next generation fenotypering'.
In dit proefschrift beschrijven we genotype-fenotype studies voor MGS en FHS en hebben we adviezen geformuleerd voor de behandeling en medische controles van patiënten met deze aandoeningen, nadat de genetische oorzaak voor deze syndromen was geïdentificeerd met behulp van exoom sequencing. We hebben de kennis van het fenotypisch spectrum van deze syndromen vergroot en hebben de medische zorg voor deze patiënten verbeterd. Daarnaast hebben we met behulp van exoom sequencing een nieuw ontstane mutatie in *ZBTB18* aangetoond bij een meisje met een klinisch beeld lijkend op FHS.
Om next generation fenotypering verder te verbeteren, is het essentieel om de klinische en moleculaire data geanonimiseerd te delen in internationale databases die vrij toegankelijk zijn. Dit zal ons helpen om een cohort van patiënten met *ZBTB18* mutaties te vormen en om de cohorten van patiënten met MGS en FHS te vergroten. Hierdoor wordt het mogelijk om (uitgebreidere) genotype-fenotype studies te verrichten, met als uiteindelijke doel de diagnostiek te verbeteren en gepersonaliseerde medische zorg voor deze patiënten te realiseren.





Abbreviations

µg	Micrograms
ACC	Agenesis of the corpus callosum
ADD	Attention deficit disorder
ADHD	Attention deficit hyperactivity disorder
ADSS	*Homo sapiens* Adenylosuccinate Synthase gene
AGORA	Aetiologic research into genetic and occupational/environmental risk factors for anomalies in children
AKT3	*Homo sapiens* V-Akt Murine Thymoma Viral Oncogene Homolog 3 gene
ALA	At last assessment
ANKRD11	*Homo sapiens* Ankyrin repeat domain-containing protein 11 gene
APIs	Application programming interfaces
ASD	Autism spectrum disorder
ASHG	American Society of Human Genetics
ATP	Adenosinetriphosphate
ATRX	Alpha-thalassemia X-linked intellectual disability syndrome
BMI	Body mass index
BRAF	*Homo sapiens* V-Raf Murine Sarcoma Viral Oncogene Homolog B1 gene
BRWD3	*Homo sapiens* Bromodomain- and WD repeat-containing protein 3 gene
CA	Chronological age
C_2H_2	Acetylene
CBL	*Homo sapiens* Cas-br-m murine ecotropic retroviral transforming sequence homolog gene
CDC6	*Homo sapiens* Cell Division Cycle 6 gene
CDT1	*Homo sapiens* Chromatin licensing and DNA replication factor 1 gene
CEP170	*Homo sapiens* Centrosomal Protein 170kDa gene
C1orf100	*Homo sapiens* Chromosome 1 open reading frame 100 gene
C1orf101	*Homo sapiens* Chromosome 1 open reading frame 101 gene
C1orf121	*Homo sapiens* Chromosome 1 open reading frame 121 gene
C1orf199	*Homo sapiens* Chromosome 1 open reading frame 199 gene
cm	centimeters
CREB	*Homo sapiens* cAMP Response Element-Binding Protein 1 gene

CBP/CREBBP	*Homo sapiens* CREB-Binding Protein gene
DMD	*Homo sapiens* Dystrophin gene
DNA	Desoxyribonucleïnezuur
ECG	Electrocardiogram
EEG	Electroencephalogram
EFCAB2	*Homo sapiens* EF-Hand Calcium Binding Domain 2 gene
e.g.	Exempli gratia; for example
ENT	Ear nose throat
EPS	Ear patella short stature
ERK	Extracellular signal-regulated kinases
ESHG	European Society of Human Genetics
Ets-1	Protein C-ets-1
Ets-2	Protein C-ets-2
F	Female
FAM36A	*Homo sapiens* Family with sequence similarity 36, member A gene
FGFR3	*Homo sapiens* Fibroblast Growth Factor Receptor 3 gene
FGS	FG syndrome, FG are the initials of the surnames of the first patients described
FHS	Floating-Harbor syndrome
FISH	Fluorescence in situ hibridization
FLNA	*Homo sapiens* Filamin A gene
FSH	Follicle-stimulating hormone
g	grams
GERD	Gastroesophageal reflux
GH	Growth hormone
GI	Gastrointestinal
GMNN	*Homo sapiens* Geminin gene
HGMD	Human gene mutation database
HGVS	Human Genome Variation Society
HNRNPK	*Homo sapiens* Heterogeneous Nuclear Ribonucleoprotein K gene
HNRNPU	*Homo sapiens* Heterogeneous Nuclear Ribonucleoprotein U gene
HNRNPU-AS1	*Homo sapiens* Heterogeneous Nuclear Ribonucleoprotein U antisense RNA1 gene
HPO	Human Phenotype Ontology
ID	Intellectual disability
IGF1	Insulin-like Growth Factor 1

IUGR	Intra-uterine growth retardation
KANSL1	*Homo sapiens* Kat8 Regulatory NSL Complex, Subunit 1 gene
KBG	The initials of the first three patients with KBG-syndrome described in literature
KRAS	*Homo sapiens* V-Ki-Ras2 Kirsten Rat Sarcoma Viral Oncogene Homolog gene
L	Low
LH	Luteinizing hormone
M	Male
MAP2K1	*Homo sapiens* Mitogen Activated Protein Kinase Kinase 1 gene
MAPK	Mitogen-activated protein kinase
Mb	Mega base pairs (= 1.000.000 base pairs)
MCM	Minichromosome maintenance
MED12	*Homo sapiens* Mediator Complex Subunit 12 gene
MGS	Meier-Gorlin syndrome
MIM	Mendelian Inheritance in Man
mIU	Milli-international units
MLPA	Multiplex ligation-dependent probe amplification
MOPD	Microcephalic osteodysplastic primordial dwarfism
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
n	number
N	Normal
NA	Not applicable
NGS	Next generation sequencing
NRAS	*Homo sapiens* Neuroblastoma Ras Viral Oncogene Homolog gene
OMIM	Online Mendelian Inheritance in Man
OFC	Occipitofrontal circumference
ORC1	*Homo sapiens* Origin Recognition Complex subunit 1 gene
ORC4	*Homo sapiens* Origin Recognition Complex subunit 4 gene
ORC6	*Homo sapiens* Origin Recognition Complex subunit 6 gene
PCR	Polymerase chain reaction
PGD	Preimplantation genetic diagnosis
PI3K	Phosphoinositide 3-kinase
PLD5	*Homo sapiens* Phospholipase D family, member 5 gene

PNAS-4	Now known as *DESI2*: Homo *sapiens* Desumoylating Isopeptidase 2 gene
PND	Prenatal diagnosis
PreRC/PRC	Pre-replication complex
PTPN11	*Homo sapiens* Protein-Tyrosine Phosphatase, Nonreceptor-Type, 11 gene
PTEN	*Homo sapiens* Phosphatase and Tensin Homolog gene
RAF1	*Homo sapiens* V-Raf-1 Murine Leukemia Viral Oncogene Homolog gene
RAS	Rat Sarcoma
RIT1	*Homo sapiens* Ric-like Protein without Caax Motif 1 gene
RP58	Retinitis Pigmentosa Protein 58
RPE65	Retinal Pigment Epithelium-Specific 65 kDa Protein
RTS	Rubinstein-Taybi syndrome
S	Suboptimal
SA	Skeletal age
SADDAN	Severe achondroplasia with developmental delay and acanthosis nigricans
SDCCAG8	*Homo sapiens* Serologically Defined Colon Cancer Antigen 8 gene
SDs	Standard deviation score
SHOC2	*Homo sapiens* Soc-2 (Suppressor of Clear, C Elegans) Homolog gene
SNF2	Sucrose Non-Fermentable Protein 2
SNP	Single Nucleotide Polymorphism
SOS1	*Homo sapiens* Son of Sevenless, Drosophila, Homolog 1 gene
SRCAP	*Homo sapiens* Snf2-Related CREBBP Activator Protein gene
UK	United Kingdom
USA	United States of America
UPF3B	*Homo sapiens* Up Phrameshift Suppressor 3 Homolog B gene
VKGN	Vereniging Klinische Genetica Nederland
VPI	Velopharyngeal insufficiency
WES	Whole exome sequencing
ZBTB18	*Homo sapiens* Zinc Finger and BTB Domain Containing 18 gene
ZNF238	*Homo sapiens* Zinc Finger Protein 238 gene

Dankwoord

En dan is daar opeens het laatste deel van mijn proefschrift: het dankwoord. Ik ben dankbaar dat ik de mogelijkheid heb gekregen om wetenschappelijk onderzoek te verrichten en dit proefschrift te schrijven. Ik heb de afgelopen jaren heel erg veel geleerd, dat neem ik mee in mijn werk als arts en bij het onderzoek dat ik in de toekomst nog wil verrichten.

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De leden van de manuscriptcommissie: prof. dr. Willemsen, prof. dr. Noordam en dr. Bijlsma, bedankt voor het beoordelen van mijn manuscript.

Lies, Dorien en Jeroen. Dit onderzoek had zonder jullie hulp niet gedaan kunnen worden. Lies, jij had veel goede ideeën over kandidaatgenen en strategieën over het vinden van nieuwe (kandidaat)genen. Jij zag altijd mogelijkheden en ruimte om onderzoek in te passen binnen jullie drukke klinische werkzaamheden. En na het vertrek van Lies naar Rotterdam heb jij, Dorien, met jouw kennis van ciliopathieen, dit op een zelfde wijze voortgezet.
Jeroen, we hebben samen heel wat tijd besteed aan het opzetten van nieuwe genen en kandidaatgenen en het (leren) analyseren van exoom data. En jij hebt heel wat kandidaatgenen getest in ons cohort. Bedankt voor alles!

Lieve Charlotte, bedankt dat je mijn paranimf wilt zijn. We zijn bijna vanaf het begin collega's en ik denk met veel plezier aan onze borrels, etentjes en de ski weekenden in Damuels. Alhoewel afspreken de laatste tijd met al die zwangerschappen en kleine kinderen wat minder lukt, kijk ik uit naar onze etentjes en weekendjes in de toekomst!

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oefententamen ging nooit goed. Het advies luidde dan steevast: 'maak je niet druk, het komt allemaal goed. Je kunt nu toch niets meer in je opnemen, ga lekker een wijntje drinken in de stad.' Een goed advies, want goed kwam het daarna uiteindelijk altijd. Ook met mijn onderzoek hebben jullie mij gesteund, bedankt voor de goede zorgen tijdens de week en weekenden die ik bij jullie heb mogen werken.

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Curriculum Vitae

Sonja de Munnik werd geboren op 13 februari 1984 in Emmen. Na het behalen van haar eindexamen Gymnasium aan het Dr. Nassau College in Assen, begon zij in 2001 aan haar studie geneeskunde aan de Rijksuniversiteit Groningen. Zij behaalde haar doctoraal examen in 2005 en haar artsendiploma in 2007.
Na haar studie werkte zij vijf maanden als ANIOS op de sectie klinische Genetica van de afdeling Genetica van het Radboud universitair medisch centrum in Nijmegen. Aansluitend werd zij vanaf augustus 2008 tot juni 2013 opgeleid tot klinisch geneticus door Prof. Dr. V.V.A. van Slobbe-Knoers en Dr. I. van der Burgt. Naast haar opleiding heeft zij vanaf 2010 onder leiding van Prof. Dr. H.G. Brunner, Prof. Dr. V.V.A. van Slobbe-Knoers en Dr. M.H.F. Bongers wetenschappelijk onderzoek verricht, resulterend in dit proefschrift 'From genotype to phenotype: clinical syndrome delineation in the era of genomics'. Momenteel is zij werkzaam als klinisch geneticus bij de sectie Klinische Genetica van de afdeling Genetica in het Radboud universitair medisch centrum, met als aandachtsgebieden prenatale diagnostiek, erfelijke neurodegeneratieve aandoeningen en erfelijke stollingsstoornissen.
Sonja is getrouwd met Arno Otterman en samen hebben zij twee kinderen: Hannah en Lars.

Curriculum Vitae

Sonja de Munnik was born on February 13th, 1984 in Emmen, The Netherlands. She graduated from secondary school (gymnasium at the Dr. Nassau College) in Assen and started Medical School at the Rijks Universiteit Groningen in 2001. She obtained her doctoral degree in 2005 and her MD degree in 2007.

After finishing college, she worked as a junior doctor at the Clinical Genetics division of the department of Human Genetics of the Radboud University Medical Center in Nijmegen for five months. Afterwards, she was trained as a Specialty Registrar in Clinical Genetics from August 2008 till June 2013, by Prof. Dr. V.V.A. van Slobbe-Knoers and Dr. I. van der Burgt. Simultaneously, from 2010, she worked on her PhD under supervision of Prof. Dr. H.G. Brunner, Prof. Dr. V.V.A. van Slobbe-Knoers and Dr. H.M.F. Bongers, resulting in this thesis: 'From genotype to phenotype: clinical syndrome delineation in the era of genomics'.

Currently, she works as a Consultant in Clinical Genetics at the department of Human Genetics of the Radboud University Medical Center Nijmegen, specializing in prenatal diagnosis, hereditary neurodegenerative disorders and hereditary coagulation disorders.

Sonja is married to Arno Otterman, and together they have two children: Hannah and Lars.

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