

# Clinical information sheet

## Syndromic and nonsyndromic Heritable Thoracic Aortic and Arterial Diseases

CENTRUM MEDISCHE GENETICA UZ GENT

### Patient information

Name: \_\_\_\_\_

First name(s): \_\_\_\_\_

Sex:  Male  Female

Date of Birth: \_\_\_\_\_

Address: \_\_\_\_\_

\_\_\_\_\_

Referring physician: \_\_\_\_\_

Referring center: \_\_\_\_\_

SAMPLE:  EDTA blood  DNA  Skin biopsy  Chorionic villi  
 Heparin blood  RNA  Aortic biopsy  Amniocytes  
 Buccal swab  Fibroblasts  Paraffin embedded material  
 Other: \_\_\_\_\_

Date: \_\_\_\_\_

Sample arrived: \_\_\_\_\_

### Suspected diagnosis

- Marfan syndrome
- Ehlers-Danlos syndrome – vascular type
- Loeys-Dietz syndrome
- Shprintzen-Goldberg syndrome
- Beals-Hecht syndrome or Congenital contractural arachnodactyly
- Arterial tortuosity syndrome
- other syndromic heritable (thoracic) aortic aneurysm disease
- nonsyndromic heritable thoracic aortic aneurysm disease
- heritable arterial aneurysm/dissection disease
- bicuspid aortic valve with thoracic aortic aneurysm
- Other \_\_\_\_\_

This checklist is meant to guide genetic testing for Heritable Thoracic Aortic Disorders (H-TAD) and/or Heritable Arterial Disorders (HAD). Both syndromic and non-syndromic entities are under consideration.

Since the introduction of Next Generation Sequencing (NGS) techniques, genetic testing has evolved from serial single gene testing to parallel panel testing and is gradually evolving to whole exome (genome) sequencing. This evolution has many advantages, especially in the setting of HTAD since important clinical overlap between the different genetic entities does not allow selection of the underlying causal gene based on clinical features in many instances. This is why genetic testing has evolved from screening for one particular entity (e.g screening of the FBN1 gene in case of suspicion of Marfan syndrome) to screening of a disease entity (e.g panel sequencing for HTAD).

While this strategy will undoubtedly result in the identification of the underlying defect in more patients and families, we do want to emphasize that clinical evaluation remains essential. The NGS techniques will for example not allow mutation detection in case of (small) deletions or insertions and if there is a strong clinical suspicion for a specific disease (e.g suspicion for Marfan syndrome in case of ectopia lentis and aortic aneurysm), we will complement the NGS test with additional testing if no defect is identified.

The two clinical constellations for which specifically targeted genetic testing can/should be considered are:

1. Aortic root dilatation in combination with lens luxation: Marfan syndrome – FBN1 mutation screening
2. Recurrent arterial rupture/dissection at distinct vascular beds in young patients with no significant risk factors – vascular Ehlers Danlos syndrome – vEDS

In order to perform the appropriate set of gene tests, we are requesting clinical data. We kindly ask you to be as precise and specific as possible.

The differential diagnosis in patients referred for additional genetic testing with a clinical presentation characterized by aortic (root) aneurysm/dissection and/or arterial tortuosity is extensive. You will find an overview of possible diagnoses below. Please indicate what diagnosis you suspect in your patient and/or make sure to fill out the checklist as complete as possible so that we can set up the appropriate genetic testing.

## General recommendations for patient/family evaluation in the setting of HTAD/HAD

### Target group

Patients younger than 60 - 65 years of age with Thoracic Aortic Aneurysm (aortic diameter Z-score >2 in adults and >3 in children or aortic dissection or arterial aneurysm/dissection and without other risk factors.

### Procedures

1. Multidisciplinary Evaluation
  - a. Clinical examination of proband:
    - i. Facial characteristics (hypertelorism, high plate, bifid uvula)
    - ii. Skeletal manifestations (armspan, pectus, arachnodactylia, flat feet, club feet, scoliosis)
    - iii. Cardiovascular manifestations (mitral valve prolapse, bicuspid aortic valve, patent ductus arteriosus)
  - b. 3 generation pedigree
  - c. If necessary, additional examination:
    - i. Ocular examination: lens luxation, iris flocculi
    - ii. X-ray, CT/MRI: osteoarthritis, arterial tortuosity, arterial aneurysms
2. Clinical evaluation in 1st degree relatives
  - a. Clinical examination as described above
  - b. If necessary, additional examination:
    - i. Echocardiography: aortic diameters, mitral valve prolapse, bicuspid aortic valve, patent ductus arteriosus
    - ii. Ocular examination: lens luxation, iris flocculi
    - iii. X-ray, CT/MRI: osteoarthritis, arterial tortuosity, arterial aneurysms

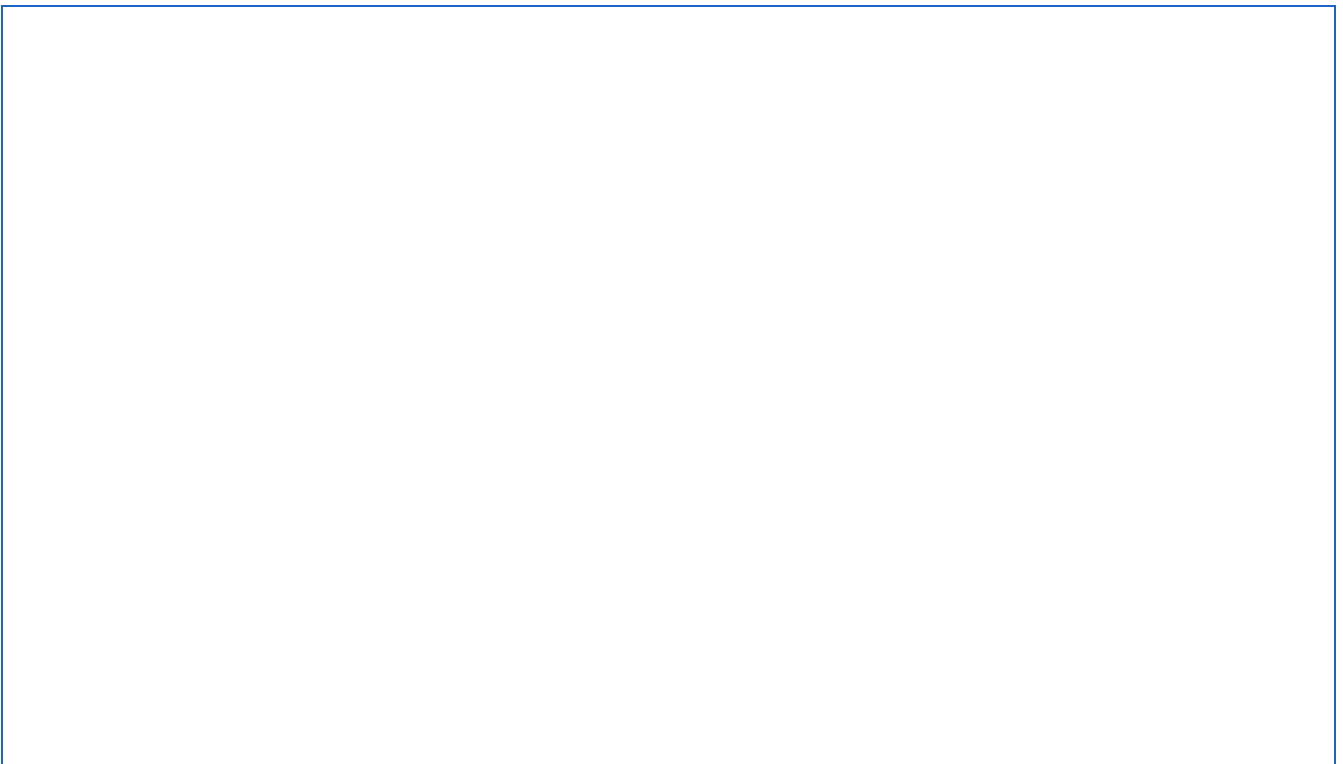
Once these two steps are completed, patients can be classified in 4 distinctive groups. The specific recommendations for each of these groups is explained below:

1. Positive family history and very specific syndromic features for MFS or vEDS as defined above: Sanger sequencing of a single gene (FBN1 or COL3A1 resp)
2. Positive family history and no or less specific syndromic features: H-TAD panel by NGS
3. Negative family history and very specific syndromic features for MFS or vEDS as defined above: Sanger sequencing of a single gene (FBN1 or COL3A1 resp)
4. Negative family history and no or less specific syndromic features (isolated TAD): Clinical follow up and in some cases consider H-TAD panel by NGS

## Clinical summary



## Pedigree



Differential diagnosis	Gene	Discriminating features
Marfan syndrome (MFS)	FBN1	Aortic root dilatation, ectopia lentis, systemic features (table 1) (diagnostic criteria Box 1)
Loeys-Dietz syndrome (LDS)	TGFR1/2 TGFB2/3 SMAD2/3/4	Bifid uvula/cleft palate, arterial tortuosity, hypertelorism, diffuse aortic and arterial aneurysms, craniosynostosis
Vascular Ehlers Danlos syndrome (vEDS)	COL3A1	Arterial dissection/rupture in multiple vascular beds, including the aorta; bowel rupture; atrophic scarring
Ehlers Danlos syndrome – valvular type	COL1A2	Severe valvular insufficiency, translucent skin
Multisystemic Smooth Muscle Cell Dysfunction Syndrome	ACTA2 p.(R179H)	Patent ductus arteriosus, congenital mydriasis, cerebrovascular lesions (Moya Moya like vascular abnormalities, white matter lesions)
Shprintzen-Goldberg syndrome (SGS)	SKI (FBN1)	Mild aortic root dilatation, mitral valve prolapse, craniosynostosis, mild-to-moderate intellectual disability
Congenital contractural arachnodactyly (CCA)	FBN2	Crumpled ears, contractures
Arterial tortuosity syndrome (ATS)	SLC2A10	Generalised arterial tortuosity, arterial stenosis, facial dysmorphism
Cutis laxa	ELN (AD)	Cutis laxa with variable involvement of internal organs (lung, aorta), association with BAV
	FBLN4 (AR)	Cutis laxa, emphysema, arterial tortuosity, aortic aneurysm, joint laxity, pectus excavatum, diaphragmatic hernia, bone fragility
Weill-Marchesani syndrome (WMS)	FBN1 and ADAMTS 10	Microspherophakia, brachydactyly, joint stiffness, short stature
Ectopia lentis syndrome (ELS)	FBN1, LTBP2, ADAMTS4	Lack of aortic root dilatation
Homocystinuria	CBS	Thrombosis, mental retardation
Nonsyndromic Heritable Thoracic Aortic aneurysm/Dissection (H-TAD) (syndromal and non-syndromal)	TGFR1/2	Lack of Marfanoid skeletal features,
	ACTA2	Livedo reticularis, iris flocculi, CVA
	SMAD3	Osteoarthritis, arterial tortuosity, arterial aneurysms and dissections, intracranial aneurysms
	TGFB2/3	Mitral Valve Prolapse, cerebrovascular disease, arterial tortuosity
	MYLK	Gastro-intestinal abnormalities
	PRKG1	Arterial aneurysms and dissections, arterial tortuosity
	MAT2A	Bicuspid aortic valve
	MFAP5	Lone atrial Fibrillation
	LTBP3	Dental anomalies and short stature
	HCN4	Left ventricular non compaction, conduction abnormalities
	MYH11	PDA
	FBN1	
H-TAD with bicuspid aortic valve (BAV)	ACTA2	BAV, lack of Marfanoid skeletal features, livedo reticularis, iris flocculi
	SMAD6	BAV, lack of Marfanoid skeletal features, coarctatio aortae



## Checklist for Syndromic and nonsyndromic Heritable Thoracic Aortic and Arterial Diseases

Suspected clinical diagnosis (see list above):	
Maximal aortic diameter	mm
Age at measurement	yrs
Localisation of the maximum dilatation	
Sinus Valsalva	<input type="checkbox"/>
Sinotubular Junction	<input type="checkbox"/>
Ascending Aorta	<input type="checkbox"/>
Aortic arch	<input type="checkbox"/>
Descending Aorta	<input type="checkbox"/>
Abdominal Aorta	<input type="checkbox"/>
Aortic dissection:	
thoracic type A – type B	A – B
abdominal	<input type="checkbox"/>
Arterial tortuosity	<input type="checkbox"/>
Peripheral arterial dissection (please specify):	
Bicuspid Aortic Valve	<input type="checkbox"/>
Other cardiovascular lesions (please specify):	
Other systemic features (please specify):	
<ul style="list-style-type: none"> <li>• Ocular:</li> <li>• Osteo-articular:</li> <li>• Central Nervous:</li> <li>• Skin:</li> <li>• Gastro-intestinal:</li> </ul>	

## Revised Ghent Criteria for Diagnosis of Marfan syndrome and related conditions

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### In the absence of family history:

- (1) Ao ( $Z \geq 2$ ) + EL = MFS
- (2) Ao ( $Z \geq 2$ ) + FBN1 = MFS
- (3) Ao ( $Z \geq 2$ ) + Syst ( $\geq 7$ pts) = MFS
- (4) EL + FBN1 with known Ao = MFS

### In the presence of family history:

- (5) EL + FH of MFS (as defined above) = MFS
- (6) Syst ( $\geq 7$  pts) + FH of MFS (as defined above) = MFS
- (7) Ao ( $Z \geq 2$  in adults,  $Z \geq 3$  in children) + FH of MFS (as defined above) = MFS

Z: Z-score (aortic root diameter corrected for age and BSA); EL: ectopia lentis; FBN1: Fibrillin 1 mutation; Syst: systemic score (see below); FBN1 with known Ao: FBN1 mutation linked to aortic aneurysm in other patients/families (Loeys et al, Journal of Medical Genetics 2010)

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### Required clinical data

Aortic diameter at the level of the sinus of Valsalva	mm
Age at measurement	yrs
Height	cm
Weight	kg
Ectopia Lentis	Y/N
Systemic score	/20

Family History (please specify):

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Systemic features	Yes	No	NE	Score
Wrist AND thumb sign	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	3
Wrist OR thumb sign	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
Pectus carinatum deformity	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2
Pectus excavatum or chest asymmetry	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
Hindfoot deformity	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2
Pes Planus	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
Pneumothorax	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2
Dural ectasia	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2
Protrusio acetabuli	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2
Reduced US/LS AND increased arm/height AND no severe scoliosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
Scoliosis or thoracolumbar kyphosis	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
Reduced elbow extension	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
Facial features (3/5) (dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
Skin striae	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
Myopia > 3 diopters	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
Mitral valve prolapse (all types)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1
<b>TOTAL SCORE*</b>				<b>/20</b>

NE= not examined

\* Maximum total: 20 points; score > 7 indicates systemic involvement

# Informed consent

## for patients willing to participate in diagnostic and scientific studies concerning heritable connective tissue and blood vessel disorders

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### CENTRUM MEDISCHE GENETICA UZ GENT

**Diensthofd**  
prof. dr. B. Poppe

prof. dr. F. Malfait  
prof. dr. O. Vanakker  
prof. dr. B. Callewaert

**Psychologische begeleiding**  
S. Hellemans  
A. Van Tongerloo

**Genetische counseling**  
V. Szymczak  
A. Van Mullem  
L. Wildero-Van Wouwe

**DNA Laboratorium**  
prof. dr. K. Claes  
prof. dr. E. De Baere

**Laboratorium Cytogenetica**  
prof. dr. B. Menten  
prof. dr. F. Speleman  
prof. dr. N. Van Roy  
dr. A. Dheedene

**Klinische Genetica**  
prof. dr. J. De Backer  
prof. dr. A. De Paepe  
prof. dr. B. Dermaut  
prof. dr. B. Leroy

dr. S. Janssens  
dr. R. De Putter

**Kwaliteitsbeheer**  
Lic. G. Van der Cruyssen

**Bindweefsellaboratorium**  
prof. dr. P. Coucke  
dr. S. Symoens

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## Subject of the study

Genetic diagnostics of heritable connective tissue- and blood vessel disorders

## General information and objectives of this study

Connective tissue disorders comprise a heterogeneous group of disorders, characterized by abnormalities involving the skin, eyes, cardiovascular system, the lungs, the skeleton and joints. These disorders are often caused by specific genetic defects and are in these cases heritable. Connective tissue plays various important roles in the body such as providing strength to the different organs and tissues and enabling communication between tissues, required for their growth and normal functioning.

Connective tissue diseases can present in very different ways (according to the system(s) that is (are) mainly affected). E.g. problems of connective tissue of the bone and skeleton can result in an increased risk of fractures and bone deformities. These are important characteristics of osteogenesis imperfecta or brittle bone disease. Other heritable connective tissue disorders, such as the Ehlers-Danlos syndromes, are mainly characterized by weakness of the soft connective tissue and are associated with symptoms such as hyperelasticity and fragility of the skin, abnormal and delayed wound healing and joint hypermobility. The latter finding often leads to recurrent joint dislocations and chronic joint pain. Other types of connective tissue disorders such as Marfan syndrome are associated with dilatation and/or fragility of blood vessels.

Because your (child's) condition can be heritable, it is useful to perform genetic analysis first to confirm the diagnosis and second to perform further screening of family members.

Genetic analysis is mostly performed on blood and in some cases it can be useful to investigate other tissues (see below).

1. The aim of this study is to identify the genetic defect(s) (mutation(s)) that is (are) causing or influencing your (child's) health problems or those in (one of) your family members. This knowledge can be of importance for the treatment and management of your (child's) disease. The identification of a mutation also enables exclusion or confirmation of the diagnosis in family members who are at risk of having this condition or who have already developed symptoms. Prenatal genetic diagnostics (the genetic diagnosis of congenital or heritable disorders in the future/unborn child) can be performed when there is a significant risk for future or unborn children. It is possible that the underlying genetic defect can not be found at short term. When in the future new scientific insights permit to find the genetic defect responsible for your (child's) condition, this (these) analysis (analyses) can be performed on your (child's) sample and/or data received by analysis of your (child's) sample. However, this analysis will not automatically be performed. At any time you can inquire about further diagnostic and/or research modalities.

It is possible that with this genetic analysis (a) genetic variant(s) is (are) found from which is it not clear whether this (these) variant(s) is (are) responsible for your (child's) condition. In that case the options to further explore this (these) variant(s) will be discussed with you.

2. Besides analysis of the genetic defect(s) related to your disease, further studies aimed at exploring the underlying pathogenic mechanisms of this disorder can be performed on the prelevated anonymized material (see below). These studies lead to a better understanding of the mechanisms, inheritance and evolution of connective tissue disorders. This knowledge may be important for you (your child) and your family and also enables better management, treatment and care for all patients affected by this disorder.
3. These investigations can be performed on the following material that has been taken from you (your child) (check the appropriate boxes):

**Blood sample**

These will only be used for the study of connective tissue disorders. From this blood sample, we can extract and preserve DNA and/or generate a lymphoblast cell line (white blood cell line). The lymphoblast cell line can be preserved to extract DNA at a later time.

**Skin biopsy**

This biopsy is used for culturing fibroblasts (connective tissue cells). The fibroblast culture can be stored and used at a later time. Fibroblast cells can be used to study the extracellular matrix proteins they produce and can be used to extract DNA. The extracted DNA will only be used to perform genetic tests relevant to the study of connective tissue disorders.

**Aortic biopsy**

In case of aortic or arterial surgery, part of the aortic or arterial wall, which would normally be removed and destroyed, can be used to establish a cell culture or to perform additional analyses. As such, no extra material needs to be removed for establishing this cell culture.

**Urine sample**

A urine sample can be used for the diagnosis and further study of connective tissue disorders.

**Skeletal muscle biopsy**

This tissue is used for assessment of the respiratory chain of the mitochondria (energy production entities) and for immunohistological staining, and electron microscopy.

Your (child's) samples and data will be stored in the laboratory of the Center for Medical Genetics Ghent (Medical Research Building 1). Once the Belgian law concerning biobanking and storage of human samples of 19/12/2008 (article 22) is implemented, your (child's) samples will be stored in the biobank of the department of Medical Genetics (Medical research building 1).

It may be necessary to take a new blood sample and/or skin biopsy for study purposes when no/insufficient DNA is left in the laboratory or when no skin biopsy was performed in diagnostic setting, but that a skin biopsy is of importance for studying the underlying mechanisms of your (child's) condition.

## What are the risks involved when taking part in this study?

### Blood sampling

Puncturing the skin causes short lasting pain. Occasionally, some prolonged bleeding of the puncture site can occur or a bruise can appear. This will disappear within two weeks. There is a small risk of fainting or of infection at the puncture site.

### **Prelevation of a skin biopsy**

A biopsy with a maximal diameter of 5 mm will be taken from a non-visible place, mostly at the inner side of the upper arm. The biopsy will be taken after injection of a local anesthetic. After the procedure, the skin will be sutured or Steri-strips will be applied. The injection of a local anesthetic can cause short lasting pain. There is a small risk of prolonged bleeding or wound infection after the procedure. The wound should be healed within two weeks after the procedure, and can leave a small round scar.

### **Prelevation of an aortic biopsy**

If aortic surgery is being performed on you or your child, part of the aortic wall, that would normally be removed and destroyed, will be preserved for research purposes. As such, no extra material needs to be removed and there are no additional risks associated with this procedure.

There are no risks involved in the prelevation of a **urine sample**.

### **Prelevation of a muscle biopsy**

A skeletal muscle biopsy is taken on the anterior muscle of the upper leg (quadriceps muscle). This is done under full anesthesia. The operation site may hurt a little in the days following the surgery. The associated risks are those of regular low-risk surgery including adverse reactions on anesthesia, local pain, bleeding, infection.

## **Injury resulting from participation in the study?**

The researcher provides compensation and/or medical treatment in case of damage and/or injury resulting from participation in the study. For this purpose, we have a no-fault insurance in accordance with the Act of May 7th 2004 concerning experiments on humans. At that moment your (child's) data may be passed to the insurer.

## **What will happen with your (child's) medical information obtained during the course of this study?**

In accordance with the Belgian law of December 8th 1992 and August 22th 2002, your (child's) privacy will be guaranteed and you will have access to the collected data. At your request, incorrect information can be adjusted. Representatives of the principal investigator, auditors, the ethical committee and the certified governmental instances will have direct access to your (child's) medical records to control the experimental procedures and/or data, without violating the confidentiality of these records. This access is confined within the boundaries set by the aforementioned legislation. By signing this informed consent form, you agree with this.

If information is derived from this study that is relevant for your (child's) health and/or your (child's) treatment and follow-up, the Center for Medical Genetics will invite you (and your child) to discuss these results. If information relevant to family members results from this study, we will ask you to contact your family or – if you do not wish to do this yourself – to give us permission to contact your family. If you do not wish that family members are informed, this decision will be respected.

If the study yields information that is relevant to healthcare in general, the results may be published in a scientific journal. This means that results obtained from specific clinical investigations such as echocardiography, ophthalmologic examination, medical imaging, etc. can be included after anonymization. Exceptionally, it may be relevant to publish clinical photographs. In this case, you will be asked to sign a separate informed consent form.

## **Who to contact in case you (your child) have additional questions about this study or you (your child) want to terminate your (child's) participation in this study?**

It is recommended to directly contact the physician who enrolled you (your child) in this study. You can find his or her contact information on the last page of the informed consent form. If you are unable to reach this physician, you can contact the Center for Medical Genetics Ghent at (0032)9/332.36.03. You will be put in contact with one of the responsible investigators, as soon as possible.

## Informed consent

**You will receive a copy of this informed consent.**

**The original will be kept in your (child's) medical record at the Center for Medical Genetics Ghent.**

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I, \_\_\_\_\_

voluntarily give my consent for participation in this study for which analyses on my (child's) genetic material will be performed to identify genetic disorders in the context of my (child's) condition

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I have received and read the patient information leaflet and I had sufficient time to ask questions. I have been sufficiently informed about the objectives of this study. I have had the opportunity to ask questions about this study and all my questions have been answered in sufficient manner.

I am aware of the fact that this study has been approved by the independent ethics committee of the Ghent University Hospital and that the study will be performed according to the rules of good clinical practice (ICH/GCP) and the Declaration of Helsinki (i.e. ethical principles regarding human experimentation). The fact that this study has been approved by the ethics committee did not influence my decision to take part in this study.

I therefore agree with the use of information derived from questioning (personal history taking), physical and technical examinations for scientific purposes only. My identity will be kept confidential, will not be disclosed and the professional confidentiality will be respected. I have been informed and consent that my personal details and medical records will be processed and stored for at least 20 years. I am aware that I have access to and can correct this information. Since these data are being processed in the context of medical-scientific purposes, I understand that access to these records can be postponed until completion of this study. If I want to access my personal data, I will contact the supervising physician, responsible for data processing.

I understand that auditors, representatives of the principal investigator, the ethics committee or the certified governmental instances may want to inspect my records to control the collected information. By signing this document, I consent to this. Furthermore, I am aware and I consent that my (child's) anonymized samples/data may be passed on to the principal investigator and may be shared with (non-)European collaborators. My privacy will be guaranteed at all time. My (child's) data/samples will not be used for commercial purposes.

I have understood the content of this research and I am prepared to participate or have my child(ren), mentioned below and represented by me, participate in this study. At any time, I will have the possibility to withdraw from this study.

**(1) Consent for adults**

place and date

---

name and signature

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**(2) For patients incapable of giving consent,  
represented by the legal representative**

name patient

---

**CONSENT**  
place and date

---

name and signature of the legal representative

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**(3) For child(ren) in the presence  
of their parents**

name child

---

name child

---

**CONSENT**  
place and date

---

name and signature of the father

---

name and signature of the mother

---

**(4) For child(ren) not under the responsibility of  
their parents**

name child

---

**CONSENT**  
place and date

---

name and signature of tutor, relative or  
legal representative

---

relation to the child(ren)

---

justification for replacing the parents

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**(5) For child(ren) over 12 years of age**

**CONSENT**

**place and date**

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**name and signature of the child over 12 years of age:**

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**CONSENT**

**place and date**

---

**name and signature of the child over 12 years of age:**

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**(6) Consent physician**

I hereby declare to have sufficiently informed the  
aforementioned participant about the aims of this study.  
The participant agreed to participate voluntarily by signing  
and dating the informed consent form.

**place and date**

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**name and signature**

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