

## A SURVEY OF GENETIC DISEASES IN RWANDA

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### ABSTRACT

The Department of Medical Genetics at the Faculty of Medicine at National University of Rwanda opened in 2006. It is available to patients from our country and surrounding regions. The offer of different genetic diagnosis and genetic counseling includes congenital malformations, chromosomal abnormalities, mental retardation, molecular defects, antenatal and prenatal genetic counseling with emphasis on primary infertility and repeated miscarriages. The remarkable and continuous increase of parents referred to our Department in recent years prompted us to present a summary of our activities, with providing a general overview of genetic diseases among Rwandan patients and confirm the need of genetic department in Rwanda and in the surrounding regions, in order to prevent and treat genetic diseases.

**Keywords:** Genetic diseases – survey - genetic analysis - Rwandan patients.

### RESUME

La Faculté de Médecine de l'Université Nationale du Rwanda a ouvert un département de Génétique Médicale en 2006. Celui-ci est accessible aux patients Rwandais et ceux provenant des régions avoisinantes. Les services offerts comprennent les analyses techniques et le conseil génétique chez les patients atteints de malformations congénitales, d'anomalies chromosomiques, de retard mental, de maladies moléculaires, d'infertilité primaire ou de fausses couches à répétitions. L'augmentation continue du nombre de consultations nous permet de donner un aperçu général des maladies génétiques chez les patients Rwandais, et souligne la nécessité d'un service de génétique au Rwanda et dans la région environnante, en offrant les actes techniques nécessaires. L'objectif est la prévention et le traitement des maladies génétiques.

**Mots-clés:** maladies génétiques - étude de surveillance - analyse génétique - patients Rwandais

### INTRODUCTION

The opening of a University Medical Genetic Department in Rwanda was related to the increasing request of the medical profession to offer genetic counseling services to the population, including technical analyses such as chromosome and molecular biology. The project of the Department also involves testing of Rwandan patients for specific genetic diseases, genetic teaching to medical doctors and their staff, and conducting research directed towards knowledge of genetic problems in Rwandan patients.

Genetic diseases include a large array of disorders, each requiring specific approaches [1, 2]. Several groups can be distinguished:

Firstly, chromosome disorders which involve numerical anomalies or chromosome rearrangements. The numerical anomalies consist of aneuploidies disorders, which refer to an abnormal number of autosomes (e.g. trisomy 21 or Down syndrome, trisomy 18, etc.), sex chromosomes (e.g. Klinefelter or Turner syndromes), or both. Rearrangements include unbalanced translocations, inversions, segmental duplications or deletions. However, chromosome studies are not limited to congenital malformations or disorders. Cytogenetic cancer studies have shown a large number of chromosome anomalies, some being specific and contributing to the diagnosis or the treatment of the disease (e.g. translocation 9/22 in chronic myeloid leukemia, etc.). Secondly, monogenic disorders which represent a group of diseases caused by mutation in one specific gene. The

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concept of mutation has been clarified with the use of molecular genetics techniques. A mutation can be limited to a single DNA base-pair, the same in all patients (sickle cell anemia, metabolic disorders), to the change of a base pair in different sites of the affected gene, patients the same diagnosis showing different mutations (cystic fibrosis disease); or to more complex situations such as the modification of the number of triplet repeats either in the gene promoter (muscular dystrophy, fragile-X syndrome), or in the coding region (Huntington chorea disease). Any of these diseases can be autosomal dominant, recessive or sex-linked.

Thirdly, polygenic disorders where a mutation of more than one gene may be responsible for a genetic disease (e.g. spina bifida, cleft palate, schizophrenia, diabetes, etc.). Very often, the number of genes involved is not yet known. But an increasing number of disorders seem to be associated with a polygenic condition, including some cancers. this an expanding field of research.

Finally, mitochondrial diseases which represent a group of disorders linked to the mutation occurring in the mitochondrial DNA. They are transmitted only by the mothers to their children of both sexes (Leber optic atrophy, etc.).

The need of a Genetic Department becomes obvious, in view of the complex origin of genetic diseases, the techniques involved in diagnosis and the large number of concerned medical specialties. The present paper reports our experience in Rwanda since the opening of our department.

## **METHODS**

### **PROBANDS**

A total number of 345 patients were referred to our Department, in University Teaching Hospitals of Kigali and Butare (CHUK and CHUB) for genetic investigations, from December 2006 to March 2010. Most of the patients presented dysmorphic traits, developmental delay and/or mental retardation.

After a complete familial history and physical examination by a geneticist, patients were classified into 2 categories: those with suspicion of chromosomal abnormalities and those with suspicion of a monogenic disease involving the mutation of a single gene, detectable or not with molecular techniques. Written informed consents were obtained from the patients or representative parents for genetic analysis and publication of cases and any accompanying images. This study was approved by Institutional Review Board from CHUK. All techniques procedures were performed in our laboratory except for molecular tests.

### **CYTOGENETIC ANALYSIS**

For patients in whom we suspected chromosomal abnormality, we performed cytogenetic analysis for

karyotype. A sample of 5 ml of peripheral blood was obtained from each patient and was cultured and harvested according to conventional protocol. Standard karyotype was performed on Q-banded metaphases spreads as described previously [3]. We used 500 resolution level for banding characterization. The karyotype was analyzed according to guidelines from the International System for Human Cytogenetic Nomenclature.

## **MOLECULAR ANALYSIS**

### **DNA extraction**

For patients in whom we suspected a molecular genetic defect (mutation), we performed DNA extraction. Five milliliters of blood sample were collected into EDTA tube and sent to the Medical Genetics Laboratory of Butare at the National University of Rwanda. A genomic DNA of each patient was extracted from peripheral lymphocytes using the phenol-chloroform standard method.

### **DNA amplification and sequence analysis**

The DNA samples were sent to the Center for Human Genetics at Liege in Belgium for most of molecular genetic investigations. The search for mutation was depending on the specific gene according to the disease we suspected.

## **RESULTS AND DISCUSSION**

The 345 patients were divided in two groups, first with patients suspected of having chromosome anomaly, and a second group of patients with a monogenic disease suspicion. For both groups, chromosome analysis were performed in our Genetic Laboratory Department at National University of Rwanda/Butare. For the second group, the symptoms were suggestive of mutation in a single gene. These cases were eligible to be investigated by molecular genetic analysis in collaboration with the Department of Human genetics at the University of Liege in Belgium .

### **CHROMOSOMAL ABNORMALITIES**

A karyotype study was performed in all group of 345 patients. Among them, a series of chromosomal abnormalities were identified. The most common karyotype syndrome observed was Down syndrome (18,26 %); whereas about 4 % represented other chromosomal aberrations Table I. Most of these patients had similar clinical features mainly characterized by dysmorphic signs (hypo/hypertelorism, epicanthic fold, ear abnormalities, antimongoloid eyes, retrognathia, short neck or microcephaly); congenital malformations (cardiac defects, duodenal atresia, chest deformity, umbilical hernia, brachydactyly, etc); failure to thrive, psychomotor development delay and mental retardation.

We found it useful, for each anomaly, to propose a short

summary of main symptoms, followed by the results observed and a brief discussion.

### **TRISOMY 21 or DOWN SYNDROME**

Down syndrome or trisomy 21 is the most frequent chromosomal abnormality at birth, reported worldwide with an incidence of 1 in 800 newborns. Karyotypes of affected patients show the presence of an extra chromosome 21 which may be "free" (standard trisomy 21, with 47 chromosomes in all cells), or either "fused" with another acrocentric chromosome by the centromeres (Robertsonian translocation, with 46 chromosomes).

#### **Main symptoms**

The suggestive clinical elements are craniofacial dysmorphic features such as flat facial traits folds, mongoloids slant, epicanthic folds, ears anomalies, hypertelorism, short neck, protruding neck and uncurled hair. Musculoskeletal abnormalities include brachydactyly, single palmar crease, gap between the 1<sup>st</sup> and 2<sup>nd</sup> toes. Gastrointestinal malformations such as umbilical hernia, imperforated anus and duodenal atresia can be observed. However, mental retardation, hypotonia, hyperextensibility, developmental delay and congenital cardiac defects represent the most frequent clinical findings.

#### **Results**

Among 345 patients referred to our clinical genetic department, 63 were found to be affected by trisomy 21 and 2 had robertsonian translocation trisomy 21, Table 1. Our results suggest an important prevalence of Down syndrome in Rwandan patients. This could be explained by the absence of prenatal diagnosis as it has been demonstrated in our previous study [3].

#### **Discussion**

Several studies have shown that the advanced maternal age (> 35 years or older) remains the risk factor associated with standard trisomy 21 [4-6]. In contrast, as in our previous study [3], we found that young women represented the majority (62 %) of mothers at high risk of having a child with free trisomy 21. Similar results have been found in South African women [7, 8]. However, the meiotic non-disjunction (malsegregation), occurring during maternal oogenesis, represents the cause of free trisomy 21 in 95 % of cases [9, 10]. Down syndrome with translocation was rare in our series (2/345). Parental karyotype studies are then indicated to detect a possible balanced translocation with an increased risk to be unbalanced for subsequent pregnancies.

### **TRISOMY 13 or PATAU SYNDROME**

Trisomy 13 or Patau syndrome is not uncommon disorder, with an incidence of 1 in 10000 to 1 in 20000 at birth. The cause is the presence of an extracopy of chromosome 13. Most cases of Patau syndrome (80-85%) are caused by free trisomy 13, less frequently (10%) by translocation and rare cases of mosaicisms can occur (5%) [6].

#### **Main symptoms**

A specific triad of dysmorphic features is observed: microphthalmia, cleft lip and palate and polydactyly [11]. Other major signs of trisomy 13 include microcephaly, holoprosencephaly, hypertelorism, cardiovascular, genitourinary and ocular malformations. Patau syndrome is frequently fatal within their first week of life; most children die before completing 6 months of age [6, 11]; about 85% of the patients do not survive beyond one year. During this survey, we found 4 cases of Patau syndrome or trisomy 13, Table 1. As it is shown in Figure 1, our patients presented most of the above described clinical symptoms.



**Figure 1:** Rwandan patients with trisomy 13. Note the clinical features (A) abnormal and low set ears, (B) & (C) polydactyly, (D) Cleft lip & palate, (E), (F) & (G) dysmorphic features.

### **TRISOMY 18 or EDWARD'S SYNDROME**

Trisomy 18 or Edward's syndrome is caused by the presence of an extra chromosome 18 and it is the second most common autosomal trisomy in infant after Down syndrome, with an incidence varying from 1:3000 to 1:8000 among liveborn [11, 12].

#### **Main symptoms**

The most frequent clinical features include hypotonia at birth followed by hypertonia and psychomotor development delay. The diagnosis is made by the presence of characteristic dysmorphic features such as dolichocephaly, microcephaly, microretrognathia, hypertelorism, low set ears, limb abnormalities including polydactyly, rocker-bottom feet, overlapping fingers, visceral malformations including cardiac congenital defects, digestive and urogenital malformations. More than 90% of children affected by trisomy 18 die

**Table 1.** Karyotype results of 345 patients and frequency of chromosomal abnormalities

| Karyotype                         | Chromosomal abnormality       | Frequency  | %           |
|-----------------------------------|-------------------------------|------------|-------------|
| 47,XX,+21 or 47,XX,+21            | Free trisomy 21               | 63         | 18.26 %     |
| 46,XX,der(21;21)(q10;q10)         | Robertsonian translocation 21 | 2          | 0.58 %      |
| 47,XX,+18 or 47,XY,+18            | Free trisomy 18               | 3          | 0.87 %      |
| 47,XX,+13 or 47,XY,+13            | Free trisomy 13               | 4          | 1.16 %      |
| 46,XX,der(22)t(10;22)(p10;p10)mat | Trisomy 10p monosomy 22q      | 1          | 0.29 %      |
| 46,XY,-13q                        | Deletion 13q                  | 1          | 0.29 %      |
| 45,X0/46,XY                       | Mosaic Turner syndrome        | 1          | 0.29 %      |
| 47,XX,+mar22                      | Cat eye syndrome              | 1          | 0.29 %      |
| 47,XX,+mar22/46,XX                | Mosaic Cat eye syndrome       | 1          | 0.29 %      |
| 46,XX,t(10;22)(p10;p10)           | Balanced translocation        | 1          | 0.29 %      |
| Normal karyotype                  | -                             | 267        | 77.39 %     |
| <b>Total</b>                      |                               | <b>345</b> | <b>100%</b> |

before 12 months [13, 14]. Trisomy18 was found in three patients in our cohort Figure 2.



**Figure 2 :** Rwandan patients with trisomy 18. Note the clinical features (A) & (B) facial dysmorphism, (C) limb abnormalities, (D) finger abnormalities, (E) club foot & (F) microretrognathia, short neck and low set ears

### **MOSAIC TURNER SYNDROME**

Turner syndrome has been described for the first time in 1938; a sex chromosome anomaly has been associated to the syndrome in 1960. The standard karyotype shows one X chromosome missing and the total number is 45: 45,X. However, mosaicism has been observed, usually with one 45,X cell line and one or more cells with other karyotypes: 46,XX or 46,XY; 47,XXX; etc.

#### **Main symptoms**

Standard 45,X patients show primary amenorrhea, short stature, absence of secondary sexual characteristics and craniofacial dysmorphic features. The phenotype linked to mosaic Turner syndrome such as 45,X/46,XY may be different from patient to patient sometimes with signs of full Turner syndrome, mixed gonadal dysgenesis (MGD) or female or male pseudohermaphroditism [15, 16]. Cytogenetic analysis detects the presence of Y chromosome. This is of interest for treatment. The presence of a Y chromosome is associated with virilization

in approximately 5% of patients, and gonadal neoplasia, mainly gonadoblastoma, in as many as 30% [17-20].

We observed one 45,X/46,XY mosaic in our series. Clinical and genetic details of our patient have been recently published [21]. Briefly, the patient was a 23-year-old girl presenting primary amenorrhea, absence of secondary sexual characteristics, severe delayed development and had insulin-dependent diabetes. Cytogenetic analysis, performed on her peripheral blood lymphocytes, showed that she was carrier of two patterns of karyotypes (45,X/46,XY) in one majority cell lines with 45,X [42 mitoses] and a minority cell line composed of 46,XY [23 mitoses].

### **A de novo DELETION 13q ter**

The 13q deletion syndrome is rare and characterized by a large array of clinical symptoms that depend on how much the long arm of chromosome 13 is deleted [22]. The 13q deletion is divided into 3 groups according to the genotype-phenotype correlation [23-25]. Group 1: in this group, the deletion concerns the 13q11 region. Patients usually present with growth deficiency, mild mental retardation and minor anomalies. Additionally, microcephaly, hypertelorism, a depressed nasal bridge, simian creases, and mild hypotonia may be present [23]. Patients with deletion of RB1 locus in 13q14 also have retinoblastoma [25].

Group 2 is associated with the most severe phenotype including malformations of brain, eyes, distal limbs, genitourinary and gastrointestinal tract. Patients with 13q32 deletions have invariably severe mental retardation and short stature [26].

Group 3 is characterized by mental retardation, microcephaly and genital malformations.

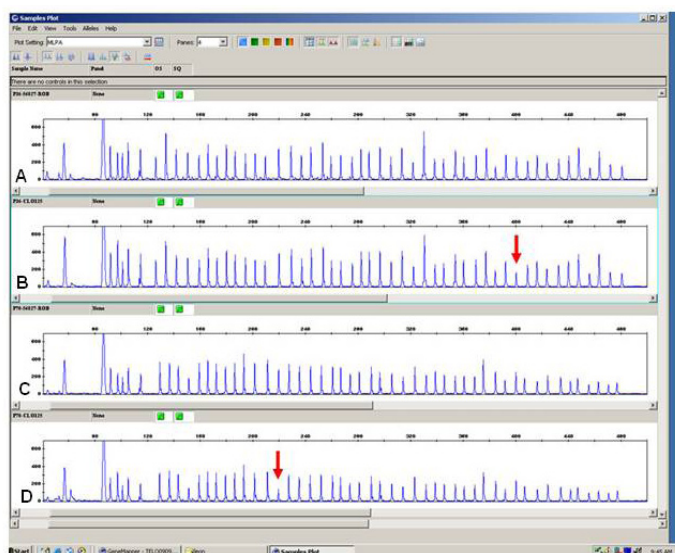
We report here a first case of Rwandan patient, carrier of a deletion 13q34 ter. He is a sixteen month-old boy born by normal delivery weighting 3.100 kg. At birth, he presented with hypotonia and genital malformation. He was referred to our clinical genetics department for



additional investigations. On physical examination, he had a psychomotor development delay; he could not seat and had a delayed speech with mild mental retardation. He presented some facial dysmorphic features characterized by hypertelorism, strabismus and webbed neck (Figure 3). He had also hypospadias. On his karyotype, the 13qter deletion was not visible. This deletion was identified using multiplex ligation probe amplification (MLPA) technique



**Figure 3 :** Rwandan patient with a de novo deletion 13q terminal. Note the clinical features (A) facial dysmorphic features characterized by hypertelorism, long philtrum, strabismus & webbed neck, (B) hypotonia, (C) large ears, and (D) hypospadias.



**Figure 4 :** Multiplex Ligand Probe Amplification (MLPA) was used for telomere's analysis. The arrows show two peaks corresponding to 13q34 deleted probes in (B) & (D) comparing with the wild-types peaks in (A) & (C). This analysis was performed using two MLPA different kits p036 & p070 from MRC-Holland Company.

(Figure 4). The same genetic analysis performed on blood samples from his parents did not reveal this 13qter deletion and thus it was a de novo chromosomal abnormality.

### **PARTIAL TRISOMY 10p/ MONOSOMY 22p**

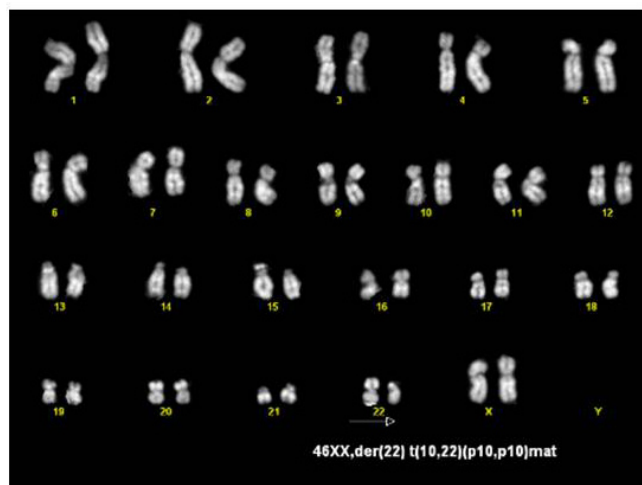
Trisomy 10p is a rare chromosomal anomaly. The short arm (p) of one chromosome 10 (10p) is represented three

times instead of two in the cells of the body. This syndrome was first described in 1974 by Schleiermacher and co-workers [27], and few other cases have been reported [28]. It is generally admitted that duplication of chromosome 10p (or partial trisomy 10p) results in a clinically recognizable chromosomal syndrome.



**Figure 5 :** A 34 month-old girl Rwandan patient with partial trisomy 10p monosomy 22p. Note the clinical signs (A) & (B) facial dysmorphic features characterized by dolichocephaly, protruding forehead, hypertelorism, long philtrum, prominent chin, (C) muscle atrophy & (D) limited movement of lower limbs.

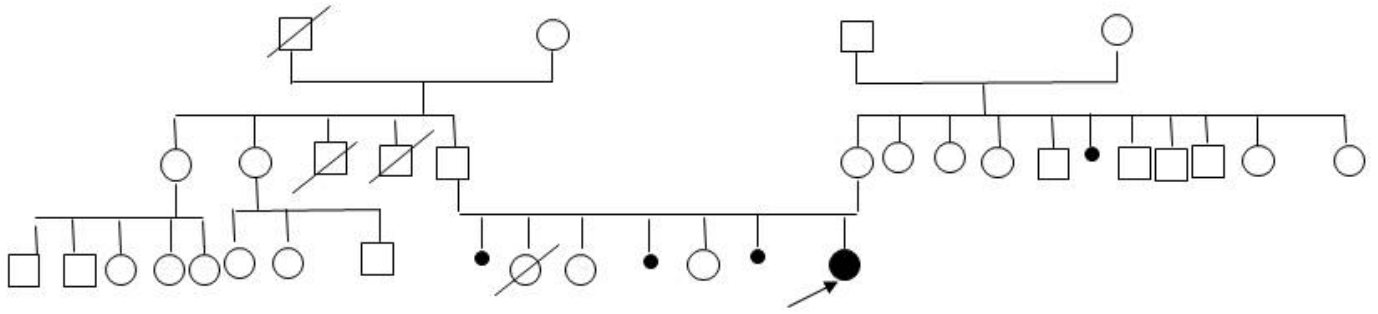
report a 34 month-old girl with severe mental retardation, psychomotor development delay associated with facial dysmorphic features (Figure 5). On physical examination, she presented with dolichocephaly and trigonocephaly, protruding forehead, upslanting eyelids, antimongoloid eyes, large philtrum, depressed nasal bridge, low set ears, prominent chin, severe muscle atrophy, abducted and flexed lower limbs. Her karyotype showed a pattern of



**Figure 6 :** Karyotype of patient with partial trisomy 10p monosomy 22p. Note the presence of 3 copies of 10p (partial trisomy) and only 1 copy of 22p : 46,XX,der(22)t(10;22)(p10;p10).

46 chromosomes with 3 copies of the short arm of the chromosome 10 (partial trisomy 10p) and only 1 copy (monosomy) of the long arm of the chromosome 22: 46,X X,der(22)t(10;22)(p10;p10)mat (Figure 6).

Several mechanisms leading to such chromosomal



**Figure 7 :** Family pedigree of Rwandan patient with partial trisomy 10p monosomy 22p. Note several miscarriages (four) in her family probably linked to this unbalanced translocation.

abnormality [28] have been proposed, but almost all cases are derived from parental reciprocal translocation between an acrocentric chromosome and chromosome 10.

Parental karyotype studies showed that the mother of our patient was carrier of a balanced translocation between chromosome 10 and 22, 46,XX,t(10;22)(p10;p10). She has had several miscarriages (Figure 7). Very likely, this could be explained by the occurrence of unbalanced translocation during fertilization probably associated with severe malformations.

### **CAT-EYE SYNDROME (CES)**

The CES is a rare genetic disorder characterized by the presence of a small supernumerary chromosome derived from the proximal part of chromosome 22. This marker usually bicentric and bisatellited, results from an inverted duplication [invdup(22)] [29, 30]. It is often present in mosaic state. The CES is characterized by a classic clinical triad of anal atresia, coloboma of the iris and preauricular tags or/and pits. Sometimes, other irregular clinical features such as craniofacial dysmorphism with hypertelorism and downslanting palpebral fissures, cardiac defects, renal malformations, male genital anomalies, skeletal defects,

and mild-to-moderate mental retardation can be observed [30].

We identified two unrelated cases of cat eye syndrome (CES), one was a newborn with a karyotype 47, XX, +mar 22 (Figure 8) present in all cells, while the second was a mosaic one. In this case, one part of cell lines expressed a normal

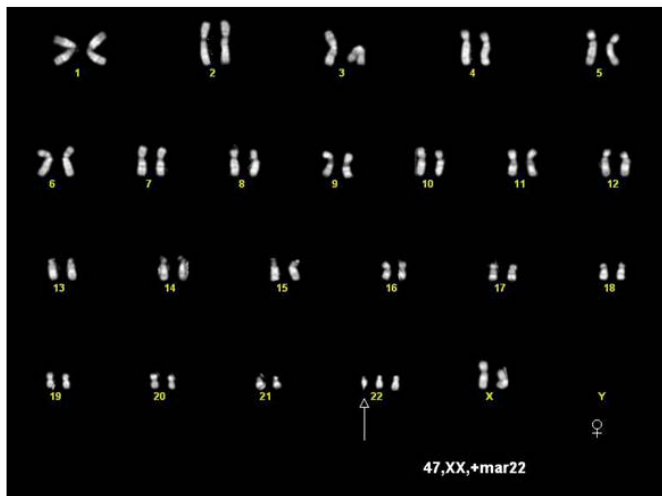


**Figure 9:** Rwandan patients with Cat-eye syndrome (CES). (A, B & C) A 10-day-old girl with CES. Note the presence of preauricular tags, facial dysmorphism and anal atresia. Her karyotype was 47,XX,+mar22. (D, E & F) A 1-month-old girl with mosaic CES : 46,XX/47,XX+mar22. Clinically, she expressed only coloboma and minor facial dysmorphism.

karyotype (46,XX), the other showed a supernumerary chromosome 22: 47, XX, +mar 22. Our probands exhibited the characteristic symptoms of this syndrome, however moderate in the mosaic case (Figure 9).

### **MONOGENIC DISORDERS**

Genetic disorders associated with the mutation of a single gene are usually associated with a normal karyotype. Diagnosis is confirmed using molecular techniques, which allow the identification of the mutation at the DNA base level. Specific monogenic mutations associated with a genetic disease are less frequent than chromosome anomalies. However, the number of genes affected is very large, and so is equal to the number of affected patients. Therefore, molecular biology has become an essential tool in genetic diagnosis. At the demographic level, molecular studies show that the frequency of some mutations varies from population to population, so some diseases are more prevalent inside geographical regions. Thus, a study of



**Figure 8:** CES patient's karyotype with a supernumerary chromosome 22: 47,XX,+mar 22.



mutations in Rwanda, such as we report here, is important not only for the patients studied, but also to determine the nature of the most frequent genetic disorders in this country. On the long run, prevention of genetic disorders in Rwanda is concerned, and could be extended to the whole East African region.

Heredity of monogenic diseases is well known: autosomal dominant, recessive or sex-linked. As we report in the present paper, focusing on Rwandan patients, the three modes of transmission have been observed.

### **SICKLE CELL DISEASE (SCD)**

The SCD is an autosomal recessive genetic disorder resulting from a single-base mutation affecting the gene encoding beta-globin (Glu>Val). This very small change leads to the synthesis of abnormal hemoglobin (HbS) in red blood cells, which becomes hard, sticky and are shaped like sickles [31]. SCD is the most common and severe haemoglobinopathy present among the African population. The birth incidence of this disorder is 300000 children worldwide every year and up to 70% of those birth occur in Sub-Saharan Africa [32]. The clinical features of sickle cell anemia include increased risk infection, variable degree of hemolysis leading to pain and anemia, and intermittent episodes of vaso-occlusive crisis resulting in ischemia, acute and chronic organs dysfunctions [33].

Between July 2004 and July 2006, we conducted a neonatal screening study in Rwanda, Burundi and East of Democratic Republic of Congo where we collected 1825 blood dried samples with purpose of genetic testing. Using ELISA-test with a monoclonal antibody anti hemoglobin S and C and restriction PCR, we identified 60 heterozygous samples for both Hb SA and Hb CA; and two homozygous newborns for Hb SS [34]. For the whole cohort, our results indicated that the overall incidence of SS in the area was 0.11%. The prevalence of sickle cell trait (Hb SA) was 3.23 %, whereas hemoglobin C carriers were less frequent (0.22 %).

Our data provided the information about the incidence of SCD in our region and they suggested that a neonatal screening program based on ELISA test is suitable and should be extended to the national level in our country where the SCD has been previously considered to be rare.

### **HUTCHINSON-GILFORD PROGERIA SYNDROME (HGPS)**

The Hutchinson-Gilford Progeria syndrome is an extremely rare autosomal genetic disease with an incidence estimated about 1 in every 8 millions live birth. Approximately 80% of HGPS cases are caused by a de novo single-base pair substitution c.1824 C>t (GGC>GGT, p.Gly608Gly) within the exon 11 of the LMNA gene which codes for Lamin A and C proteins [35]. Patients affected by this disease are characterized by an early

onset of several clinical features including premature ageing. At birth, the patient may appear asymptomatic with a normal weight. Clinical features emerge within the first year when a profound failure to thrive occurs and patients present sclerodermatous skin changes together with almost complete absence of subcutaneous fat and baldness. There are significant changes in dentition, and the skeleton shows hypoplasia, dysplasia, severe osteolysis and pathological fractures. The most common cause of death is due to acute myocardial infarction at an average age of 13.4 years, associated with the presence of early atherosclerosis [35].

We identified a 12 year old-girl Rwandan patient with typical clinical signs of HGPS (Figure 10). The molecular



**Figure 10:** A 12-year-old girl with Hutchinson-Gilford Progeria syndrome. (A, B, C & D) premature ageing aspects, (E) skin sclerodermatous.

genetic analysis performed on her genomic DNA allowed us to identify a p.Gly608Gly heterozygous disease-causing mutation in LMNA gene [36]. Unfortunately, this patient died of cardiac complications.

### **WERDNIG-HOFFMANN DISEASE**

The Werdnig-Hoffman disease or proximal spinal muscular atrophy type 1 (PSMA1/SMA1) is an autosomal recessive disorder caused by homozygous deletion of the survival motor neuron1 (SMN1) gene which is located on the chromosome 5q12.2-q13.3 [37]. SMA type 1 is the most severe form and is characterized by its early onset of symptoms (before 6 months) [37, 38]. Specific clinical features are severe hypotonia, symmetrical muscle weakness of thorax and proximal part of the extremities, and lack of head movement. Poor sucking ability and reduced swallowing are frequent, leading to feeding difficulties. Patients present severe psychomotor development delay and respiratory failure is the common cause of death [38]. In this paper, we report on a 2 year-old-girl with severe hypotonia and muscle weakness. Molecular genetic testing performed on her genomic DNA allowed us to identify a homozygous deletion of exon 7 of SMN1 gene. Her parents were found to be carriers of this mutation thus, they had an increased risk of 25 % of having another child with Werdnig-Hoffman disease.

### **HUNTER SYNDROME**

Hunter syndrome (or Mucopolysaccharidosis type II, MPSII) is an X-linked recessive disorder due to the deficiency of iduronate 2-sulfatase (IDS) enzyme, resulting in the accumulation of heparan and dermatan sulfates in the lysosomes. The Hunter syndrome involves a wide spectrum of clinical phenotypes ranging from mild-to-severe forms. The severe clinical manifestations of Hunter syndrome include an early onset at 2 to 4 years of age, coarse facial features, short stature, joint stiffness, organomegaly, cardiovascular and respiratory disorders, skeletal abnormalities, development delay, progressive mental retardation and death before 15 years. Normal intelligence, short stature and survival until adulthood characterize the mild form.

In our study, we identified 2 male siblings with clinical symptoms consistent with the diagnosis of severe form of Hunter syndrome. Molecular analysis performed on their genomic DNA showed a deletion of a T-nucleotide in the exon 2, resulting in a stop codon (p.Y54X) in IDS2 gene [39]. In addition, their mother was tested and found to be carrier as well as their two sisters.

### **CYSTIC FIBROSIS (CF)**

Cystic fibrosis is an autosomal-recessive genetic disorder due to mutation in CF transmembrane conductance regulator (CFTR) gene located on chromosome 7q31.3. Alterations in the protein lead to changes in the characteristics of exocrine excretions. A lack of functional CFTR in the epithelial cell membrane results in production of sweat with a high salt content (associated with a risk of hyponatremic dehydration) and mucus secretions with an abnormal viscosity (leading to stasis, obstruction and bronchial infection). The diagnosis of CF is often difficult to assess in developing countries, because the phenotype of this disease is very similar to that of frequent pathologies in Africa such as protein energy malnutrition (PEM), chronic pulmonary infections, HIV or TB. In addition, many of these countries do not have any genetic laboratory to carry out sweat chloride test and molecular genetic analysis [40].

According to the 1998 Cystic Fibrosis Foundation Consensus conference, the diagnosis is suspected in children following these elements: suggestive clinical manifestations such as chronic sinopulmonary disease, gastro-intestinal manifestations (e.g. pancreatic insufficiency, meconial ileus, diarrhea, vomiting or constipation); failure to thrive, salt loss syndrome, and other manifestations such as diabetes mellitus or nasal polyps [41]. The diagnosis is suspected on the basis of sweat test results (chloride concentration above 60 mmol/L) and is confirmed by identification of a CFTR mutation. Recently, mutations in ENaC genes have been characterized to be involved in CF [42,43, 44].

In our study, we selected 60 Rwandan patients on the

basis of CF-like clinical symptoms. A sweat test was positive in 37 patients. However a negative sweat test result cannot fully rule out a CF disease. We therefore searched for CFTR and ENaC mutations in all 60 patients using dHPLC (Denaturing High-Performance Liquid Chromatography) screening method followed by sequencing of positive CF-exons and all coding regions of ENaC genes. Three CFTR mutants, including one previously undescribed missense mutation (p.A204T), and a 5T/7T variant were identified in five patients. ENaC gene sequencing in these 5 patients detected 8 ENaC variants: c.72T>C and p.V573I in SCNN1A; p.V348M, p.G442V, c.1473+28C>T, and p.T577T in SCNN1B; and p.S212S, c.1176+30G>C in SCNN1G. In the 55 CF-like patients without any CFTR mutation, we identified five of these eight ENaC variants, including the frequent p.G442V polymorphism, but we did not detect the presence of the p.V348M, p.T577T, and c.1176+30G>C ENaC variants. Moreover, these last three ENaC variants, p.V348M, p.T577T, and c.1176+30G>C, were not found in the control group. Our data suggest that CF-like syndrome in Africa could be associated with CFTR and ENaC mutations [45].

### **SPINOCEREBELLAR ATAXIA TYPE 2**

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant neurodegenerative disease that results from the expansion of an unstable trinucleotide CAG repeat encoding for a polyglutamine tract. The clinical phenotype of SCA2 includes a progressive cerebellar ataxia with additional features such as ophthalmoplegia, extra-pyramidal or pyramidal signs and peripheral neuropathy [46].

In our survey, we identified a large family with SCA2 disease. The index case was a 14 year-old female with several family members affected by the disease. She had a clinical history of progressive cerebellar ataxia since the age of 12. She presented cerebellar limb ataxia associated with dysarthria, dystonia and mild akinesia. Physical examination revealed slow eye movements, lower limb decreased reflexes, supranuclear ophthalmoplegia with horizontal nystagmus, facial myokimia, postural tremor with fasciculation, amyotrophy in the lower limbs and proximal weakness in the upper limbs. She had mild pyramidal signs, but there was no mental deterioration. She could not walk unassisted. The family pedigree showed that other family members including her father presented similar symptoms with a decreasing age at the onset of symptoms in four successive generations but unfortunately most of them had died. Molecular analysis of SCA2-CAG expansion in the proband revealed one major CAG pathological allele (CAG19/CAG43) for a normal range between 14 and 31 repeats [47].

### **HEREDITARY MULTIPLE EXOSTOSIS**

Hereditary multiple exostosis (HME) is an autosomal dominant skeletal disorder mainly characterized by multiple

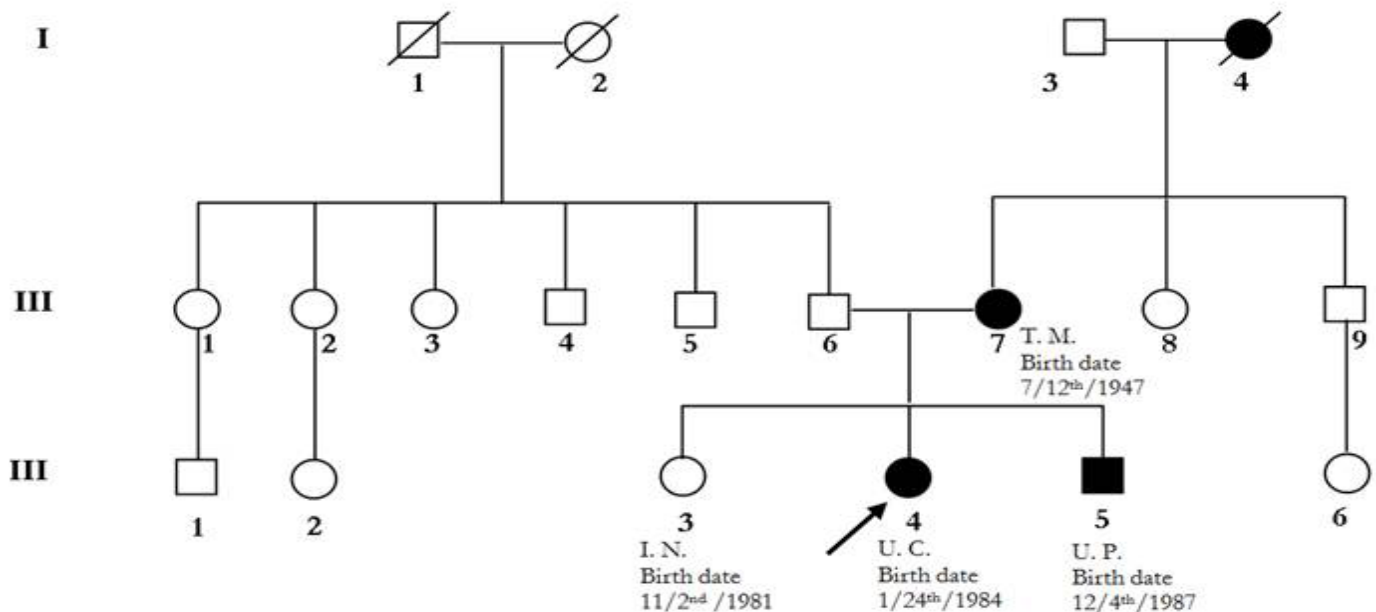


osteochondromas located at the growth plates of long bones. HME is a genetically heterogeneous disorder and results from mutations in EXT1 and EXT2 genes located on chromosome 8q23-q24 and 11p11-p12, respectively [48, 49].

We report a case of a 15-year-old girl who presented characteristic clinical features of HME. The same clinical symptoms were observed in her relatives (Figure 11). The Arg340Cys mutation of EXT1 gene was found in proband confirming the molecular diagnosis. A surgical management was carried out in this patient to remove the osteochondroma and this specimen was sent to anatomopathology unit analysis. Removed osteochondroma should be examined for malignant transformation towards secondary peripheral chondrosarcoma. Patients should be well instructed and regular follow-up for early detection of malignancy seems justified.

### OTHER GENETIC DISORDERS

Besides a clear-cut class of monogenic diseases, there are other genetic diseases for which diagnosis is more difficult. This is linked to at least three factors.



**Figure 11:** Family pedigree of Rwandan patients with hereditary multiple exostosis (HME) syndrome. Affected patients are highlighted in black symbols. The arrow indicates the index case.

Firstly, there is still no known candidate gene. Secondly, the disease is polygenic and mutations may increase susceptibility, not being directly the cause of the disease; the number of genes involved is not known or a threshold effect is not determined. Thirdly, the involved gene is very large and a possible mutation site is difficult to identify. Nevertheless, in these cases the genetic nature of the disease is suspected on several grounds: presence at birth, some other cases in the family, similarity with a proven genetic disease, etc. However, in some patients

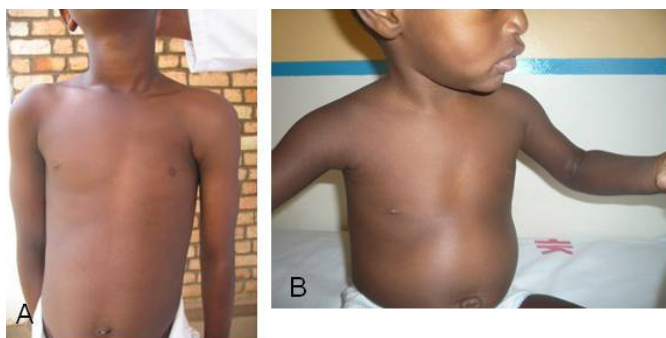
the sporadic event of the disease makes the genetic origin hypothetical.

### POLAND SYNDROME

We report here on 2 patients affected by Poland syndrome (Figure 12). The first patient has been published recently [50]. Poland syndrome is a rare congenital condition. Its incidence is estimated at about 1:7,000 and 1:100,000 births. It is characterized by absence of unilateral chest wall muscles and sometimes ipsilateral symbrachydactyly. The condition typically presents with unilateral absence of the sternal or breast bone portion of the pectoralis major muscle which may or may not be associated with the absence of nearby musculoskeletal structures. The exact etiology of the Poland syndrome is unknown. It is assumed that the aplasia of the pectoralis muscles and associated chest defects, as the athelia, aplasia of costal cartilages, are consequences of an interruption of early embryonic blood supply of subclavicular artery branches. Geneticists currently hold that Poland syndrome is rarely inherited and generally is a sporadic event.

### FREEMAN-SHELDON SYNDROME

Freeman-Sheldon syndrome (FSS) referred to distal arthrogryposis type 2A, known also as craniocarpotarsal dysplasia or Whistling face-Widmill vane hand syndrome, is a rare autosomal genetic disorder mainly characterized by facial dysmorphism and joint abnormalities. Three specific clinical abnormalities include microstomia with pouting lips, camptodactyly with ulnar deviation of the finger and talipes equinovarus [51]. There are other clinical signs less



**Figure 12:** Rwandan patients with Poland syndrome. Note the hypotrophic left anterior chest wall (A) and hypoplastic pectoralis major muscle (A & B).

common such as microglossia, short nose, long philtrum, H-shaped, chin dimple and sunken eyes, short stature and scoliosis. The intelligence is usually normal. The FSS is caused by a mutation in the embryogenic heavy chain (MYH3) gene located on chromosome 17p13.1 [52]. Most cases are sporadic but families with autosomal recessive inheritance have been reported so far [53, 54].

In the present study, we identified a newborn with typical features of FSS (Figure 13). His past medical history suggested that one of his siblings had died of the same syndrome during the neonatal period at 10 months of age. Their parents were apparently healthy and the family pedigree was suggestive of an autosomal inheritance condition.



**Figure 13:** Rwandan patient with Freeman-Sheldon syndrome. Note characteristic dysmorphic features (A & B), camptodactyly (C), club foot and joint stiffness (D & E).

### **RECESSIVE DYSTROPHIC EPIDERMOLYSIS BULLOSA (RDEB)**

Epidermolysis bullosa is a group of various inherited disorders characterized by the blistering of the skin and mucous membrane which occurs after exposure to mechanical trauma, but also internal organs can be affected. The simplex forms are characterized by blister

formation at the level of basal keratinocytes within the epidermis. In both patients, clinical picture was compatible with DEB syndrome [55].

Here we present two patients affected by DEB. Patient 1, a 5-day-old girl, showed blistering predominantly on hands, legs and feet since his birth (Figure 14 A). In the course of the disease, bullae became dystrophic, occurred mechanically, induced also on the head and trunk and healed with scarring.

Patient 2, aged 10 days, showed at birth extensive blistering of the hands and feet (Figure 14 B). Later, he developed blisters on arms, legs and trunk after mechanical trauma.

In both cases, family pedigrees were suggestive of an autosomal recessive inheritance for DEB. In addition, one of the patient 1 sibling had died at 1 month of age suffering from the disease symptoms.



**Figure 14:** Rwandan patients with Recessive Dystrophic Epidermolysis Bullosa. (A & B) Patient 1, a 5-day-old girl with blisters legs and feet. (C, D & E) Patient 2, a 10-day-old boy with skin fragility showing blisters scarring on arms, leg and trunk.

### **USHER SYNDROME**

Usher syndrome is a genetic disorder characterized by hearing loss or deafness and progressive vision loss. The loss of vision is caused by a disease called retinitis pigmentosa (RP), which affects the retina. To date, three major types have been identified [56]. Usher syndrome is inherited in an autosomal recessive pattern [57]. It is thought to be responsible for 3 % to 6% of all childhood deafness and about 50% of deaf-blindness in adults. Vision loss occurs as the light-sensing cells of the retina gradually deteriorate.

We report here three cases of children from the same family aged 15, 11 and 8 years, two males and one female, respectively. They were first referred to the eye clinic department due to deafness associated with progressive visual loss and after were evaluated by a geneticist. They



were all deaf and dumb since birth. They belong to a family of 4 children; their parents were healthy and had one brother of 4 years of age who was not affected.

### **PRUNE BELLY SYNDROME**

Prune-belly syndrome is a rare congenital disease. It is characterized by congenital absence or deficiency of the abdominal musculature associated abnormalities of the genitourinary tract, including dilated urinary bladder and ureters, hydronephrosis and bilateral undescended

microphthalmos on the left orbit, with well developed extraocular muscles and optic nerves. Abdominal CT SCAN revealed left renal agenesis, hypoplastic uterus and urinary bladder. The ophthalmologist decided to perform right eyelid and orbital reconstruction; but found a normal eyeball, with vascularised and opaque cornea, firmly attached to the underlying, partially developed eyelid tissue; then decided to close the wound.

Fraser syndrome phenotype is complex and pleiotropic disease which shows significant overlap with other malformations syndromes such as Walker-Warburg or



**Figure 15:** Rwandan patient with Prune-Belly syndrome (A & B) characterized by abdominal musculature deficiency.

testes [58]. The case we present here had an abdomen protruding, thin-walled, with visible intestinal loops (Figure 15). All abdominal wall muscles were absent. Due to the aplasia of rectus abdominis muscles, the thorax was erected thus impairing the respiration movement. The newborn had a bilateral cryptorchidism. We have not yet excluded urinary tract abnormalities as the newborn died before ultrasound exam.

### **FRASER SYNDROME**

Fraser syndrome is a rare autosomal recessive disorder mainly characterized by cryptophthalmos, ear, nose and skeletal malformations, craniofacial dysmorphism, syndactyly of fingers and toes, laryngeal stenosis, urogenital malformations and mental retardation in survivors [59]. It has a recurrence risk of 25 % among siblings. We report a case of a newborn aged 20 days who presented multiple abnormalities and was born to non consanguineous Rwandan parents. Clinically, the newborn had cryptophthalmos and his nose was large with depressed nasal bridge. She had bilateral syndactyly of 1st, 2nd, 3rd and 4th fingers and her external genitalia were ambiguous (Figure 16). However, the cytogenetic study performed on blood lymphocytes showed that she was a female with normal karyotype. Head CT-scan performed showed normal brain, normal right eyeball,

Peters' Plus syndromes [60]. However, Thomas et al. in 1986 have established diagnostic criteria that distinguish Fraser syndrome from other similar syndromes (Table 2)



**Figure 16:** Rwandan patient with Fraser syndrome. (A) cryptophthalmos, (A & B) syndactyly, (C) external genitalia ambiguous.

[61]. In addition, prenatal diagnosis of Fraser syndrome should be done through prenatal ultrasound. The diagnosis should be confirmed according to above criteria due to great variability of possible malformations but also using molecular testing of mutation causing disease.



**Table 2.** Diagnostic criteria of Fraser syndrome [61]

| Major criteria               | Minor criteria                    |
|------------------------------|-----------------------------------|
| Cryptophtalmos               | Congenital malformation of nose   |
| Syndactyly                   | Congenital malformation of ears   |
| Abnormal genitalia           | Congenital malformation of larynx |
| Sibling with Fraser syndrome | Cleft lip $\pm$ palate            |
|                              | Skeletal defects                  |
|                              | Umbilical hernia                  |
|                              | Renal agenesis                    |
|                              | Mental retardation                |

## GENETIC COUNSELING AND CONCLUSION

Genetic counseling is the process of providing individuals and families with information on the nature, inheritance and implications of genetic disorders to help them make informed medical and personal decisions. It deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members.

In this survey, genetic counseling has been provided to all concerned patients and their relatives. Couples whose infant has a genetic disease have been advised on the presence or not of a recurrence risk. For chromosomal abnormality, the recurrence risk is lower except in case of balanced translocation or advanced maternal age. Patients affected by an autosomal dominant disease have a 50 % risk of having an affected child. In case of a recessive disorder, the risk of recurrence, if parents are carriers, is 25 %. This risk is increased in case of consanguinity. Finally, in case of X-linked recessive disease half of the children of a carrier mother will be affected but often a severely male affected will be identified. The recurrence risk is 50 % in case of X-linked dominant disease. However, a spontaneous mutation can be observed in all these modes of inheritance. The parents and relatives have been also informed of the possibility of prenatal diagnosis which we hope to implement in the near future in our country.

In conclusion, our survey revealed a large number of genetic diseases in Rwandan patients and suggests that genetic investigations should be mandatory in case of suspicion of any genetic disorder suspicion. However, as most of these investigations are very expensive and not accessible to all Rwandan population, it would be advisable to benefit a support from health institutions such as health insurances.

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