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A COMPREHENSIVE CLINICAL AND GENETIC STUDY IN 127 PATIENTS WITH ID IN KINSHASA, DR CONGO

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Abstract

Pathogenic variants account for 4 to 41 % of patients with intellectual disability (ID) or developmental delay (DD). In Sub-Saharan Africa, the prevalence of ID is thought to be higher, but data in Central Africa are limited to some case reports. In addition, clinical descriptions of some syndromes are not available for this population. This study aimed at providing an estimate for the fraction of ID/DD for which an underlying etiological genetic cause may be elucidated and provide insights into their clinical presentation in special institutions in a Central African country.

A total of 127 patients (33 females and 94 males, mean age 10.03 ± 4.68 years), were recruited from 6 institutions across Kinshasa. A clinical diagnosis was achieved in 44 but molecular confirmation was achieved in 21 of the 22 patients with expected genetic defect (95% clinical

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CONFLICTS-OF-INTEREST

J.R.L. has stock ownership in 23andMe, is a paid consultant for Regeneron Pharmaceuticals, has stock options in Lasergen, Inc., and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from molecular genetic testing offered in the Baylor-Genetics Laboratories.

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sensitivity). Identified diseases included Down syndrome (15%), submicroscopic copy number variants (9%), aminoacylase deficiency (0.8%), Partington syndrome in one patient (0.8%) and his similarly affected brother, X-linked syndromic Mental Retardation type 33 (0.8%), and two conditions without clear underlying molecular genetic etiologies (Oculo-Auriculo-Vertebral and Amniotic Bands Sequence).

We have shown that genetic etiologies, similar to those reported in Caucasian subjects, are a common etiologic cause of ID in African patients from Africa. We have confirmed the diagnostic utility of clinical characterization prior to genetic testing. Finally, our clinical descriptions provide insights into the presentation of these genetic diseases in African patients.

INTRODUCTION

Genetic etiologies account for 4 to 41 % of patients with intellectual disability (ID) or developmental delay (DD), depending on the recruitment strategy, type and resolution of clinical exam and laboratory techniques applied [Battaglia and others 1999; Bernardini and others 2010; Moeschler 2008; Moeschler and Shevell 2014]. Concluding a specific genetic diagnosis often has a major impact on the management plan for a child with ID/DD and counselling of families regarding recurrence risk. Not only can this guide patient management and efficient care, but also it will make possible the implementation of preventive measures such as pre-conceptual counselling and prenatal testing. In Sub-Saharan Africa, such as in other developing countries, the prevalence of ID is thought to be higher than in developed countries, mainly attributed to environmental factors including infection and suboptimal healthcare systems [Durkin 2002; Emerson 2007; Kleinman and Eisenberg 1995; Roeleveld and others 1997]. The currently implemented public healthcare systems can result in a lack of neonatal screening programs, partial or complete absence of pediatric assessment of normal developmental milestones during well-child visits and lack of psychometric evaluation for children with delayed development. This leads to the lack of accurate figures on the prevalence of ID in Sub-Saharan Africa and the distribution by causes in this setting. In particular, data on the genetic etiology of ID in Central Africa are scarce. With the exception of some case reports, few studies have investigated a cohort of patients with ID using advanced technologies [Uwineza and others 2014; Uwineza and others 2016]. Therefore, DD/ID is still mainly attributed to 'mystical' causes in this part of the world [Abasiubong and others 2008]. A study of genetic causes will provide evidence regarding the biological bases of ID, potentially providing scientific evidence to campaign against false beliefs and help to improve information for management and therapeutic actions directed by caregivers.

Reaching a genetic etiologic diagnosis in an individual with ID is based on different but complementary approaches. Clinical evaluation generating a potential differential diagnosis is at the forefront of this process. This relies on knowledge generated from clinical presentation in previously described patients. Unfortunately, this resource is often not available for African patients. Moreover, clinical presentation shows some ethnic differences [McDonald-McGinn and others 2005; Moore and others 2007; Ofodile and others 1993; Schwartz and others 1988; Talbert and others 2014], making it difficult to clinically recognize an African patient presenting with a syndrome that is rather well described in

other world populations. Efforts are being made to supplement the scientific community with clinical descriptions of patients from diverse ethnicities [Kruszka and others 2017a; Kruszka and others 2017b; Kruszka and others 2017c] and our study is part of these efforts.

Besides the clinical evaluation, various genetic tests are often performed to further narrow the clinically generated differential diagnosis. These tests are prioritized based on the most frequent known genetic abnormalities and genes/variants for the reported phenotype. Chromosomal aberrations, including aneuploidies and submicroscopic aberrations, represent the major diagnostic category in previous studies [Boone and others 2010; Breman and others 2012; Cheung and others 2005; Devriendt and others 2003; Gambin and others 2017b; Lee and others 2013; Lu and others 2007; Morrow 2010; Rauch and others 2006; Shao and others 2008; Wiszniewska and others 2014]. Frequent submicroscopic aberrations are recurrent nonallelic homologous recombination (NAHR)-mediated copy-number variants (CNVs) [Dittwald and others 2013a; Dittwald and others 2013b]. Depending on the resolution of the applied platform, submicroscopic imbalances represent 6 to 20 % of causes [Chong and others 2014; Lupski 2015; Mannik and others 2015; Nicholl and others 2014; Rauch and others 2006; Utine and others 2014]. The most common monogenic cause of ID is the Fragile-X syndrome, which results from mutations in the *FMR1* gene (MIM 309550). For this reason, all patients with ID, especially males, are often investigated for the CGG repeat [Moeschler and Shevell 2014]. Mutations in X-linked genes are a common cause of ID and explain an excess of males with ID [Mandel and Chelly 2004; Ropers 2010]. X-linked ID is observed in 6–12 % of males with ID [Hu and others 2016; Tzschach and others 2015]. However, XLID is heterogeneous with more than 100 genes identified to date. Thus, Next Generation Sequencing (NGS) offers a more efficient and cost-effective opportunity to screen for X-linked ID [Gambin and others 2017a; Tzschach and others 2015]. X-linked inheritance is suspected when a clinically recognizable X-linked syndrome is observed or when ID segregates in the pedigree in a clear X-linked Mendelian pattern. In the absence of an established X-linked recessive pattern in pedigrees of families, skewed X-inactivation in the mother of a boy with unexplained ID is an indication of possible XL-ID. Likewise, skewed X-inactivation in a girl with unexplained ID may also indicate that an underlying X-linked mutation explained her ID [Brady and others 2015; Fieremans and others 2016]. In this study, we selected cases with a high probability of X-linked ID for NGS testing.

The overall aim of this study is to use a comprehensive approach to provide an estimate for genetic etiologic causes of ID/DD and insights into the clinical presentation of some genetic conditions in a Central African country.

MATERIALS AND METHODS

Ascertainment of patients and ethical considerations

Patients were recruited from 4 specialized schools and tertiary hospitals in Kinshasa, capital of DR Congo. Informed consent was administered in accordance with human subject studies as delineated by the Declaration of Helsinki. Procedures, risks and benefits of the study, as well as the patient's right to withdraw were explained to parents, then we received signed written consents for 127 patients with developmental delay/ID. We applied an anonymous and de-identified coding system to protect participants' privacy. Our research protocol was

approved by the ethical committee of the Public Health School of the Kinshasa, (registry number ESP/CE/008/2015). Patients were evaluated in the field by a medical expert, then clinical data and photographs were reviewed by a medical geneticist in the Centre for Human Genetics in the University hospitals of Leuven, Belgium. Body measurements were converted into standard deviation scores using the Centers for Disease Control (CDC, USA) growth charts. Clinical description was made according to the standard terminology described in the elements of morphology series [Allanson and others 2009]. Patients were classified as syndromic when they had at least 3 minor dysmorphic features, regardless of major malformations. The Informed Consent process is further described in supplemental material.

Genetic analyses

A variety of genetic laboratory analytical tools were applied in a stepwise manner as described in figure 1. Patients with recognizable syndromes underwent selective testing depending on the clinically suspected differential diagnosis. Unsolved patients were then reclassified as patients without recognizable patterns of human malformation enabling syndromic diagnoses and submitted to genetic screening starting with chromosomal microarray (86 patients with good quality DNA) and fragile-X testing. Among the remaining patients, female patients and available mothers of male patients underwent X-chromosome inactivation (XCI) assay to identify individuals with skewed X-chromosome inactivation. Female patients with skewed XCI and tested mothers with skewed XCI received whole exome sequencing (WES) when DNA sample passed the initial QC. Three samples were sequenced, including two at the Genomic Core KU Leuven and one at the Baylor College of Medicine Human Genome Sequencing Center under the Baylor Hopkins Center for Mendelian Genomics research initiative. qPCR and Sanger sequencing were used for the validation of Array-CGH and WES results, respectively.

DNA extraction—Venous blood was obtained from a peripheral vein and genomic DNA was extracted by the salt saturation method as previously described [Miller and others 1988]. The first 45 samples were extracted at the Centre for Human Genetics of the KU Leuven (Belgium) whereas the latter ones were extracted at the Institut National de Recherche Biomédicale (INRB) in Kinshasa (DR Congo).

Fragment analysis for ARX CAG repeat—We used 2 primer sets (Primer 4044 ARX-2F and JR2-FAM) to amplify the first 2 CAG stretches in the *ARX* [Allen and others 1992]. The reaction mix contained 1 µl of DNA, 5 µl of 10X amplification buffer, 5 µl of dNTP's (2 mM), 15 µl of 3X PCR enhancer, 1.5 µl of 50 mM MgSO₄, 12 µl of water and 0.5 µl of Taq DNA Pol. (5U/µl). The 35 cycles had the following conditions: 95°C for 30 secs, 58°C for 30 secs and 68°C for 60 sec. 0.1 µl of PCR product was re-suspended in 20 µl HiDi Formamide/Rox500 and loaded onto a Genetic Analyzer for Fragment separation. The separated file was analyzed with GeneMapper® v.5.

FMR1 testing—To evaluate the size of the CGG repeat fragment, the target region was amplified during a PRC reaction using the PRC-enhancer kit (Invitrogen) and the FRAXA-A and FRAXA-B primers. The reaction mix contained 5.6 µl of water, 2 ml of the 10X PCRx

Amplification buffer, 0.6 µl of 50 mM MgSO₄, 1 µl of dNTPs (4 mM), 8 µl of PCRx Enhancer Solution, 1.5 µl of Primer mix (10 pm/µl) and 0.25 µl of Taq DNA Polymerase from Roche. The cycling comprised an initial denaturation at 95°C for 3 minutes followed by 27 amplification cycles made of short denaturation at 95°C for 15 seconds, annealing at 64°C for 1 minute and elongation at 75°C for 1 minute. The reaction was terminated with a final elongation at 75°C for 7 minutes and cooling at 15°C ∞. PCR control was done on 2 % agarose gel with 1 kb size marker. The PCR product was resuspended with a mixture of 20 µl HiDi Formamide (Applied Biosystems) and Rox 500 (Applied Biosystems). Fragment were separated on the on ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA 94404 USA) then analyzed with GeneMapper Software.

Array-CGH—All patients without recognizable syndrome and those with unconfirmed clinical diagnostic were initially selected for aCGH. We performed whole genome copy number screening using stripped CytoSure™ ISCA v2 array 8×60k format (OGT, Oxford UK). We used the CytoSure DNA labelling kit (OGT, Oxford UK, cat. Num. 20020) for labelling, and the Oligo aCGH/ChIP-on-ChIP Hybridization kits (Agilent Technologies, cat. Num. 5188–5220) and Human Cot-1 DNA®-Fluorometric QC (Life technologies, cat. Num. 15279–101) for the hybridization, following manufacturers' instructions. Arrays were scanned with SureScan High-Resolution Technology Microarray Scanner at 2 µm wave length, followed by feature extraction using Agilent Feature Extraction Software® v. 10.10.11. Aberration detection was done by Circular Binary Segmentation (CBS) algorithm via CytoSure Interpret Software ® v.4.0. A CNV was reported as deletion when the Log2 ratio was < to -0.6 and duplication when > to +0.36. Genomic coordinates were based on the UCSC February 2009 (hg19) (NCBI build 37). We used a loop strategy to compare sample to reference [Allemeersch and others 2009]. Information of stripping procedure can be obtained upon request.

qPCR—In order to confirm results from array-CGH performed with strip slides, to evaluate parental inheritance or to confirm a clinical diagnosis of Down syndrome, we performed qPCR on LightCycler480® (Roche). The Primer3web version 4.0.0 tool (<http://bioinfo.ut.ee/primer3/>) was used to design primers (primer listed in Supplemental material, Table S1). We used the ddCt relative quantification method (Sequence Detection System bulletin 2, Applied Biosystems). For Down syndrome, *NCAM2* (NM_004540) located on 21q21.1 was used as the target and the *SCN2A* (NM_021007) located on 2q24.3 as reference gene. The standard curve experiments were derived using fourfold dilutions of genomic DNA, starting with 10 ng. The 20 µl reaction mixtures consisted of 10 µl of LightCycler® 480 SYBR Green I Master (Roche Diagnostics GmbH, Mannheim, Germany), 3 ml of PCR-grade water (Roche), 500 nM of each primer and 10 ng DNA. After an initial denaturation step for 10 min at 95°C, thermal cycling conditions were 10 s at 95°C and 30 s at 60°C for 45 cycles. When testing patients' samples, we included No-DNA templates and 3 calibrators (1 from DR Congo and 2 from Flanders) and all samples were run in Duplo following the standard 20 µl qPCR protocol.

X-chromosome inactivation pattern—Genomic DNA was subjected to the standard androgen receptor (*AR*, MIM# 313700) assay to determine X-inactivation ratios as previously described [Allen and others 1992].

Whole Exome Sequencing (WES)—Girls with highly skewed XCI and boys whose mothers showed highly skewed XCI were eligible for WES. Since one girl with skewed XCI carries a pathogenic copy deletion, WES was performed for one girl and two boys. The experiment was performed in the Genomics Core Facility (KU Leuven, Belgium) for patients DRC-0042 and DRC-0032, using the SeqCap EZ Human Exome Library v3.0 (Roche, NimbleGen) and the Illumina HiSeq2000 platform, and at the Baylor College of Medicine (Texas, USA) for patient DRC-0108, using HiSeq instrument following the procedure previously reported [Bainbridge and others 2011; Challis and others 2012; Lupski and others 2013; Reid and others 2014]. For this last sample, a sequencing yield of 6.1 Gb was generated with 92% of the targeted exome bases covered to a depth of 20X or greater. Initial bioinformatics was performed at the Baylor Hopkins Center for Mendelian Genomics following the algorithm described previously [Reid and others 2014]. Once all data were returned to the Genomics Core, they went through the inhouse bioinformatic pipeline previously described [Fieremans and others 2016] with the difference that annotated *vcf* files were converted into Excel books to allow sequential hypothesis driven data filtering. Additionally, we also reran the data analysis on Moon®, a commercial Artificial Intelligence based platform developed by Diploid (www.moon.diploid.com). Moon uses artificial intelligence to autonomously diagnose rare Mendelian diseases within a record time. This algorithm suggests a causal variant based on patient input data and extensive knowledge of rare disease genetics. Variants were classified according to the ACMG recommendations [Richards and others 2015].

Sanger sequencing—Sequencing primers used for Sanger validation are listed in Supplemental material (Table S2). The 50 µl amplification reaction contained 5 µl of DNA (50 ng), 5 µl of PCR reaction buffer + Mg²⁺ (Roche), 5 µl of each Primer solution (2.5 pmol/µl), 5 µl of dNTP's (2 mM), 0.5 µl (2.5 U) of Taq DNA Polymerase (Roche) and 24.5 µl of Ultrapure water (Baxter). The PCR was performed on a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA 94404 USA) using the following program: initial denaturation stage at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and elongation at 72 °C for 45 s; and a final extension stage at 72 °C for 5 min. PCR products were sequenced using Big Dye termination method and detected with ABI 3730xl DNA Analyzer instrument (Applied Biosystems, Foster City, CA 94404 USA).

RESULTS

General clinical characterization of the cohort and laboratory workflow

We collected clinical information and obtained DNA from 127 patients (33 females and 94 males), with a mean age of 10.03 ± 4.68 years (ranges 1.24 – 24.65). Only 7 patients were aged equal to or above 18 years, 120 were below 18 years. A general outline for the workflow of the study is shown as Figure 1. The IQ scores were available for 73 (57.48 %) patients (details in supplemental figure 1), and ranged from borderline to profound. The

remaining patients were either not yet evaluated or data were not available. Positive family history (up to third degree) was reported in 8 patients (details in supplemental figure 2).

Personal history and clinical examination revealed neurological manifestations in 24 patients, including seizures in 15, deafness in 4, cerebral palsy in 3 and dystonia in 2. Nine patients had a major malformation at birth including congenital heart defect in 2, congenital inguinal hernia in 2 and one for each of coloboma of eyelid, congenital cataract, cryptorchidism, limb reduction defect, micro-penis and a congenital testicular cyst. Microcephaly (OFC -2 SD) was present in 38 patients and macrocephaly (OFC $+2$ SD) in 10. The OFC was not available in 1 patient.

From the 127 patients included in this study, we reached a clinical diagnostic in 24 patients (18.89 %), considered to have a recognizable syndrome (Figure 1). Among those without recognizable syndromes (103 patients), 47 patients (45.63 %) had three or more minor anomalies and were thus considered to be dysmorphic.

Clinically recognized syndromes

In 19 patients, the clinical diagnosis of Down syndrome was made (figure 2 and table 2). In addition to these subjects, we clinically diagnosed one patient each, respectively, with Williams-Beuren syndrome (patient DRC-0000, extensively reported in [Lumaka and others 2016]), Partington syndrome (patient 416848, has a similarly affected brother), Oculo-Auriculo-Vertebral Spectrum (patient DRC-0020, clinical details in S3) and Amniotic Bands Sequence (patient DRC-0156, clinical details in S3). In one patient, we suspected Noonan syndrome (patient DRC-0098) and in 8 other individuals the most likely clinical diagnosis was acquired ID given the onset subsequent to a neurological injury (birth trauma or infection). With exception for the Noonan syndrome, all genetic diagnoses were confirmed with qPCR for the 19 Down syndrome patients, with FISH on nuclei for the William-Beuren syndrome and Sanger sequencing for pathogenic allele associated with Partington syndrome (detection of the 24 bp duplication in exon 2 (c.428–451dup) previously reported [Laperuta and others 2007; Poirier and others 2006; Stepp and others 2005]).

Fragile-X syndrome

The size of the *FMRI* CGG repeat was determined in the 103 patients without a clinically recognizable syndrome, the suspected Noonan syndrome patient and the patient with OAVS. Among the 130 alleles, including 25 girls and 80 boys, not a single premutation or full mutation was detected. Allele sizes ranged from 18 to 48 and the mean was 28.55 ± 2.84 . The most common allele was 29 (24.61 %). Only two male patients presented with intermediate size alleles (47 and 48 repeats).

Microarray-CGH

Chromosomal microarray-CGH was initiated for the same group that received *FMRI* testing. In 19 cases, the DNA samples did not pass the quality criteria for array-CGH and were thus excluded. Hence the results presented below are from 86 patients. CNVs were classified following the ACMG classification guidelines [Kearney and others 2011]. Ten CNV's were considered to be likely pathogenic or pathogenic for ID in 10 out of 86 tested

patients (11.6 %). All identified CNVs were confirmed using an independent technique, qPCR (in 9/10) or SNP-array (in 1/10 patient with a mosaic deletion) (table 1). Facial photographs of patients with relevant CNVs are presented in figure 3 whereas detailed clinical description can be found in table 2.

X-chromosome inactivation analysis

From our cohort of individuals with ID we investigated the X chromosome inactivation (XCI) profile in all 27 unrelated females index patients, as well as 41 mothers of male patients from whom DNA was available. In total, 6 highly skewed profiles were identified. Of 41 mothers of boys with ID tested, 3 (7.32 %, CI: 1.89 – 18.63) had a highly skewed XCI (i.e. 90%). In addition, 3 other mothers (7.32 %) had moderate skewed XCI (i.e. 80% XCI<90%), 8 (19.51 %) had mild skewed XCI (70% XCI<80%), whereas 27 (65.85 %) showed random X-inactivation (i.e. <70). Among the 27 female indexes with ID, we detected 3 (11.11 %) with a highly skewed X-inactivation. Five (18.52 %) had moderate and mild skewing, respectively; and 14 (51.85 %) had random X-inactivation.

Whole Exome Sequencing

The WES was performed in the boy with suspicion of Noonan syndrome (DRC-0098) and 3 patients from XCI study, including 2 boys (DRC-0018 and DRC-0042) and 1 girl (DRC-0032). One of the girls with highly skewed XCI profile (DRC-0018) had a pathogenic CNV, thus was not considered for WES. DNA from the 2 other patients with history of highly skewed XCI did not pass the quality criteria for WES.

In patient DRC-0018, novel missense variant, potential candidate, was identified in the X-linked gene *TAFI*. This patient is considered for publication in a large cohort of patients with *TAFI* mutations and is thus not further discussed here.

In Patient DRC-0042, we did not identify any plausible X-linked variant. However, we identified two variants in the AMINOACYLASE 1 gene (*ACY1*, MIM 104620) of this patient. One of these variants was inherited from the mother and the father was not available for testing consistent with the subject having biallelic variants for an AR disease trait. The maternally inherited variant (*ACY1*: NM_000666:exon15:c.C1156T;p.R386C; rs2229152) was previously reported in the homozygous state in 1 patient and classified as pathogenic after functional analysis revealed reduced enzymatic activity [Sommer and others 2011]. However, this variant is present in the homozygous state in 3 individuals of African origin listed in gnomAD where the phenotype is not specified nor whether this sample was sequenced in a cohort with ID or neurocognitive phenotypes such as ASD or schizophrenia. The previous patient presented with several episodes of febrile seizures, severe mental retardation, autistic features and lack of speech development and brain cortical dysplasia with grey matter heterotopia. This clinical presentation is far more severe than the manifestations observed in our patient (non-syndromic ID and autistic-like behavior). Given the rather limited clinical overlap and the presence of this variant in the homozygous state in unaffected individuals, and despite the functional study, this variant remains a variant of unknown clinical significance (VUS) according to the ACMG guidelines [Richards and others 2015].

The second *ACY1* variant is a rare missense variant (*ACY1*: NM_000666:exon2: c. C49T;p. R17C; rs749167670). This variant has not been reported as pathogenic, but is only observed in the heterozygous state in 3 individuals in gnomAD. In addition, this variant is predicted to be damaging by the vast majority of *in silico* prediction tools (SIFT, Polyphen, Mutation Taster, Mutation Assessor and CADD score included). However, previously reported pathogenic variants are mainly truncating mutations with the premature truncating variant (PTV) positioned where the message is predicted to undergo nonsense mediated decay resulting in a loss-of-function, LoF, allele. Thus, by clinical molecular diagnostic criteria the variant remains a VUS. The previously detected patients were identified by the assessment of urinary organic acids, which is unavailable clinically in DR Congo.

Patient DRC-0032 (Case 4), has highly skewed XCI and had a mother with ID. We filtered the exome data for X-linked and autosomal dominant as well as autosomal recessive inheritances. We did not find a plausible candidate variant for the patient's phenotype.

Also, no clinically relevant variant was identified through WES in the patient DRC-0098 with suspicion of Noonan syndrome.

DISCUSSION

We investigated 127 individuals with ID, mostly children, recruited in Kinshasa, capital of DR Congo in Central Africa. Our data were obtained from children attending special schools or institutions in Kinshasa. In the local cultural and economic context, children with more severe ID, those with major malformations and those from disadvantaged families rarely attend school, as reflected in Supplemental figure 3. This cohort can be considered as selected and not necessarily reflecting the general population in the country. Nevertheless, cognitive phenotypes are notoriously challenging to clinically assess and quantify.

Clinically, we established a diagnosis in 24 out of the 127 patients (almost 19 %), including two clinical diagnosis without clear genetic bases (amniotic band syndrome and oculo-auriculo-vertebral syndrome). Like in our patients, ID has previously been observed in these two conditions [Beleza-Meireles and others 2014; Ruggieri and others 2007]. Interestingly, our clinical diagnostic was molecularly confirmed in 21 of the 22 patients with expected genetic defect (95% clinical sensitivity).

With 19 cases out of 127 (14.8 %; CI: 9.531 % – 21.97 %), Down syndrome is the most prevalent genetic cause in our cohort. This prevalence of Down syndrome is also observed in most series investigating ID [Devriendt and others 2003; Rauch and others 2006]. However, our frequency is seemingly higher than 9.2 % reported by Rauch et al. in Switzerland, and 7.1 % reported by Devriendt et al in Flanders. This could be due to the absence of prenatal screening programs in DR Congo and to the larger size of families potentially resulting in more mother's with advanced maternal age, thus exposing the last pregnancy to higher risk for Down syndrome. More than 75% of mothers of children with Down syndrome were aged 35 years, which is consistent with the contention of advanced maternal age contributing to our observations as this is a well-known risk factor for nondisjunction trisomy 21 [Csermely and others 2015; Snijders and others 1995].

The index with Partington syndrome (MIM 309510) as well as his similarly affected brother had the typical clinical presentation as described in Caucasian patients in the literature [Cossee and others 2011; Gedeon and others 1994; Partington and others 1988]. We could not find records of similar cases from Central Africa. In one child with clinically suspected Noonan syndrome, no mutation was detected in the panel of genes currently associated with this condition. To date, in approximately 15% of Noonan syndrome cases, no genetic cause is identified (<http://www.ncbi.nlm.nih.gov/books/NBK1124/>). Moreover, no novel candidate gene in which pathogenic variation might cause the Noonan phenotype was elucidated through exome sequencing. The high yield of clinical diagnoses demonstrates that a clinical genetic approach is still the basis of genetic diagnosis in patients with ID from D Congo as has been advocated in Europe [Rauch and others 2006].

A broad genetic screening was performed for the remaining patients. We prioritized *FMR1* testing and microarray-CGH in the current study to be consistent with the guidelines for genetic screening tests in unexplained ID [Miller and others 2010; Moeschler and Shevell 2014]. The throughput and added value of aCHG has been widely shown. We did not identify a single case with Fragile X syndrome. However, the presence of intermediate size alleles suggest that fragile X is likely to occur in Congolese. By means of microarray-CGH, relevant CNVs were identified in 11.6 % with good quality DNA. In one individual, a del15q1.2 was identified. This microdeletion is a known risk factor for developmental disorders, including ID (with an estimated penetrance of 10 %), autism spectrum disorders, schizophrenia and epilepsy [De Wolf and others 2013]. The deletion was also present in his father. This is in line with the observation that the vast majority of del15q11.2 are inherited, mostly from a normal parent. Except for the Williams syndrome and Smith-Magenis syndrome, we could not find records of publication of the other 9 CNVs in Africa. Thus, observations from our study provide a relevant insight into the clinical presentation of African patients with those 9 CNVs.

The proportion of mothers with highly skewed XCI is not significantly different from the normal population of adult females where highly skewed X-inactivation was observed in 3.6 % (22 out of 415 females) [Amos-Landgraf and others 2006]. However, when considered amongst the 68 female patients and mothers of affected boys, our prevalence (13.6 % is statistically higher compared to 9.8 % general proportion reported by Amos-Landgraf [Amos-Landgraf and others 2006]. Both the small size of the population and the selective character of the sample may explain the apparent enrichment of skewed XCI in this cohort.

One boy was compound heterozygous for two variants in the *ACY1*. Mutations in *ACY1* are responsible for Aminoacylase 1 deficiency, an autosomal recessive metabolic disease characterized by ID, seizures, hypotonia, motor delay and, in one family, sensorineural hearing loss [Ferri and others 2014; Van Coster and others 2005]. Interestingly, one of the 2 mutations (p.R386C) was previously reported as pathogenic and proven to down-regulate the activity of the Aminoacylase-1 [Sommer and others 2011]. Confirmation of this disorder can be done by metabolic testing on urine. However, this is not available locally for the moment. In the other boy, a likely causal mutation was identified in *TAF1*, a gene recently identified as candidate gene for XL-ID [Hu and others 2016; O'Rawe and others 2015]. However,

more clinical evidence and functional studies of the found variant are needed in order to confirm that this is a causal mutation.

Taken together, the cause of ID was suspected in 44 (34.7 %) patients (Figure 4). Altogether, genetic causes were identified in 34/127 patients, the majority of them chromosomal aberrations, including submicroscopic aberration in 11 patients. We also confirmed the usefulness of a good clinical characterization of patient. This can guide toward specific types of testing and thus prevent unnecessary and expensive genome screening. Our study confirms that array-CGH is the first tiers diagnostic test for developmental delay/ID even in African patients. With our results, we have provided insights into the clinical presentation of genetic diseases in African patients.

This is one of the first studies on genetic aspects of ID in central Africa. The results clearly show that the same range of genetic causes are identified in Africa compared to other societies. However, the interpretation of rare variants is limited by control data from subject originating from this part of the world. Nevertheless, this finding of genetic contributions to ID in DR Congo has important ramifications for patients, families, and for public health initiatives. Moreover, it is an important message for families of children with ID and caregivers, to refute commonly held ideas and traditional mystical beliefs on the causes of ID. This study confirms the usefulness of the implementation of both clinical genetics and laboratory genetics services in Africa.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Study algorithm and overview of screening results.

Legend: After a consent process, we performed systematic clinical evaluation of recruited patients. Genetic assays were prioritized based on the presence or absence of a clinical hypothesis. For those without clinical diagnostic or with non-confirmed clinical diagnostic, genome wide screening was performed, leading to a selection of handful patients for WES.

Figure 2.

Clinical photographs of patients with Down syndrome.

Legend: Detailed clinical description of patients with Down syndrome are presented in table 1.

Figure 3.
Facial picture of patients with clinically relevant CNVs.
Legend: detailed description is provided in table 2.

Figure 4.

Overview of etiological categories

Legend: about a third of patients received a diagnostic. Chromosomal aberrations (aneuploidy and submicroscopic aberrations) was the major etiological group.

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Clinical description of patients with Down syndrome

Table 1.

ID	Gender	Age (Y)	Maternal age	OFC (z-score)	Weight (Z-score)	Height (Z-score)	Uplant of eyes (14/15)	Hypertelorism (14/15)	Epicanthus (13/15)	Snidal gap (13/15)	Flat face (11/15)	Brachycephaly (10/15)	Transverse Palmar crease (10/15)	Small/l flat nose (7/15)	Brachydactyly of toes (7/15)	Tongue protrusion (6/15)	Low posterior webbing (4/19)	Low set ears (3/15)	Syndactyly toes 2-3 (2/15)	Karyotype (6/15)	aCGH (6/15)	qPCR (15/19 out) (19/19)
DRC-0100	M	4.21	37.97	-2.06	0.70	-0.72	+	+		+	+		+			+		+		NA	T21	1,587401
DRC-0015	M	4.52	36.66	-3.6	NA	NA	+	+			+			+						NA	T21	1,52274
437634	M	5.08	NA	-2.74	-1.80	-0.91	+		+	+			+							T21	NA	1,543993
437587	M	6.88	40.25	-1.43	NA	NA		+		+			+					+		T21	NA	1,470867
447754	M	7.00	39.62	-0.42	NA	-2.77	+	+		+	+		+	+						T21	NA	1,491399
437573	M	7.70	39.41	-2.21	-1.64	-1.82	+	+	+		+			+		+				T21	NA	1,450617
DRC-0051	M	12.52	35.62	-2.2	0.11	-1.6	+	+		+	+		+	+			+			NA	NA	1,501773
437632	M	13.38	31.76	-3.07	NA	NA		+		+				+						T21	NA	1,570982
DRC-0161	M	13.98	36.79	-3.21	-2.39	-3.14		+		+	+			+		+	+		+	NA	NA	1,538652
437590	M	14.65	36.49	-2.7	NA	NA	+	+		+							+			T21	NA	1,522737
DRC-0157	M	17.03	26.15	-2.57	-1.89	-4.24	+	+							+			+		NA	T21	1,203025
DRC-0041	F	17.08	42.02	-2.58	<-3	-3.69		+	+		+				+					NA	NA	1,470867
DRC-0013	F	1.86	24.32	NA	NA	NA	+	+		+	+		+	+	+					NA	NA	1,506987
DRC-0177	F	8.32	37.71	-3.69	-1.73	-3.33		+			+		+	+						NA	NA	1,881218
DRC-0058	F	9.51	26.43	-3.95	-2.84	-3.58	+		+	+			+	+					+	NA	NA	1,538652
DRC-0152	F	9.93	40.47	-2.56	-2.88	-2.41	+	+		+	+			+		+	+			NA	T21	1,707241
DRC-0174	F	11.03	40.79	-2.8	-2.34	-2.4	+		+	+				+						NA	T21	1,649086
DRC-0062	F	12.73	42.92	-2.28	-1.89	-3.09	+	+		+			+							NA	NA	1,517469
DRC-0171	F	13.73	43.27	-2.6	0.93	-1.47	+	+	+	+	+				+					NA	NA	1,396356

Table 2.

Relevant CNVs and observed phenotype

Num	Patient	ISCN nomenclature	Type	Size (bp)	Validation (tested gene)	Inheritance	Clinical features
1	DRC-0033	arr 2p16.1(59,886,015–61,213,851)x1	Del	1,327,837	qPCR [<i>BCCL1A</i>]	Mother normal, father NA	Girl, 14.89 years. Developmental: hypotonia, feeding difficulties, motor delay, learning difficulties. Seizures until the age of 3. IQ not available. Normal growth. Dysmorphisms: narrow forehead, upslant of the eyes, ptosis, protruding lips and small mouth and chin, a remnant of post axial polydactyly (right hand), a left single palmar crease, pes planus and short toes (figure 3A).
2	437575	arr [hg19] 2q24.3(165,828,304–166,616,001)x1	Del	787,698	qPCR [<i>SCN3A</i>]	Parents NA	Boy, 10.40 years. Development: delayed speech and learning difficulty. Hand biting behavior. IQ not available. Normal growth. Dysmorphisms: truncal obesity, mildly hypoplastic thumbs, clinodactyly of the 5th toes and short toenails (figure 3B).
3	DRC-0018	arr [hg19] 8p23.3-p23.1(61,749–11,985,357)x1	Del	11,923,609	qPCR [<i>GATA4</i>]	Mother normal, father NA	Girl, 8.31 years. Mother has hearing problems. Patient presented neonatal distress, treated in a NICU. Development: gross motor delay (crawling at 12 months, walking at 2 years after physical therapy), fine motor delay, speech was delayed, and a hearing difficulty notice by the family but not further assessed. Behavior problems. IQ not available. Growth within normal range. Dysmorphisms: Congenital heart defect, not otherwise described, diagnosed at 2 months. Mild strabismus, prominent nasolabial folds, flat philtrum, thin upper lip vermillion with absent cupid's bow, mild retrognathia and long fingers and toes (figure 3C). Note highly skewed XCI profile (8.97 %).
4	DRC-0136	arr [hg19] 13q14.2-q32.1(49,189,356–97,091,014)x1	Mosaic Del	47,901,659	SNP Array with Illumina v2.1 which revealed the deletion in about 53 % of cells	Parents NA	Girl, 14.66 years. Development: delayed fine motor, speech and learning development. IQ estimated at 63 (Bonhomme test). Growth: short stature (height –3.63 SD), relative macrocephaly (OFC 1.92 SD), weight at –1.58 SD. Dysmorphisms: mild lower limb asymmetry, coarse facial features with a prominent forehead, depressed glabella, hypertelorism, strabismus, broad nose, flat philtrum, bilateral preauricular pits, short thumbs, deep palmar creases, dry palmar skin, bilateral clino-and brachydactyly of 5 th fingers, swan neck deformity of fingers, swollen feet, hemangioma on the anterior right upper leg and on the posterior left lower leg (figure 3D).
5	DRC-0114	arr [hg19] 15q24.1-q24.2(72,963,962–75,535,357)x1	Del	2,571,396	qPCR [<i>PCSK2</i>]	De novo	Boy, 10.6 years. Development: speech regression from age 2, reported to have moderate ID and autistic behavior. Growth within normal range.

Num	Patient	ISCN nomenclature	Type	Size (bp)	Validation (tested gene)	Inheritance	Clinical features
6	437633	arr [hg19] 17p11.2(16,782,547–20,294,010)x1	Del	3,511,464	qPCR [<i>RAI1</i>]	Parents NA	Dysmorphism: prominent nasolabial folds, pointed small chin, small and protruding ears, multiple skin creases on the neck, long and slender fingers, brachydactyly of toes 4–5, high sandal gap and phalangeal joint hyperlaxity (figure 3E). Boy, 5.14 years. Development: mood problems, anxiety and self-injury behavior, limited speech, reported to have severe ID. Normal growth parameters. Dysmorphism: broad forehead, grossly hypotonic face, short bulbous nose, tented upper lip, upslant of the palpebral fissures, short philtrum, dysplastic ears, scars from self-injuries on the forehead and on both hands (figure 3F).
7	437582	arr [hg19] 20q11.22-q11.22(33,186,305–34,775,792)x1	Del	1,589,488	qPCR [<i>GDF5</i>]	Parents NA	Boy, 15.62 years. Development: pervasive behavior and learning problems, IQ of 51 (Bonhomme). Short stature (–2.48). Dysmorphism: hypertelorism, square chin, hypertelorism with downslant of the eyes, camptodactyly of the 5th fingers, brachydactyly of toes 4–5 and metatarsus adductus (figure 3G).
8	DRC-0040	arr [hg19] 20p13-q11.22(60,734–33,254,059)x3	Dup	33,193,326	qPCR [<i>PCSK2</i>]	Father normal, mother NA	Boy, 17.7 years. Neonatal distress at birth. Development: delayed development, speech regression at the age of 2 years, reported to have severe ID with autistic behavior. Normal growth parameters (OFC:0.53 SD, weight –1.44 SD, height 1.13 SD). Dysmorphism: marked facial dysmorphism with a low set anterior and posterior hairline, thick and arched eyebrows, ptosis, broad nose with broad nasal ridge, flat nasal tip, broad and everted nostril, short philtrum, thick and everted vermillion of the lips, camptodactyly and calluses on the 5th fingers and fetal fingertip pads (figure 3H).
9	416840	arr [hg19] 22q13.31q13.33(44,985,665–51,220,923)x1	Del	6,235,259	qPCR [<i>SHANK3</i>]	Parents NA	Boy, 5.14 years. Development: speech and motor delay, drooling, global hypotonia (unable to stand without support), autistic behavior and reported to have severe ID. Growth: relatively tall (height 1.79 SD) for head circumference (OFC –1.63 SD) and weight (0.51). Dysmorphism: arched eyebrows, long palpebral fissures, short and bulbous nose, tented upper lip, large ears, slender fingers and toes (figure 3I).
10	447761	arr [hg19] 15q11.2(22,698,520–23,217,513)x1	Del	518,994	qPCR [<i>CYP17</i>]	Paternal	Boy, 17.65 years. Development: reportedly normal until the age 13, then parent noticed school problems, pervasive behavior and increased mood instability. Ongoing treatment at 17 years: multiple drugs including Haloperidol, Valproic acid, Clomipramine, Trifluoperazine and Promazine. Growth: macrocephalic (OFC 3.8 SD) with normal weight (0.72 SD) and height (0.92 SD).

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Num	Patient	ISCA nomenclature	Type	Size (bp)	Validation (tested gene)	inheritance	Clinical features
Dysmorphism: thick eyebrows and high sandal gap. (figure 3D).							