Article

SpainUDP: The Spanish Undiagnosed Rare Diseases Program

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Abstract: One of the IRDiRC goals for 2017-2027 is to achieve definitive diagnosis for rare undiagnosed diseases within one year, as diagnosis delay remains one of the pending issues in the rare diseases field. The Spanish Undiagnosed Rare Diseases Program (SpainUDP) was created in response to this challenging scenario to cover patients' needs and after seeing the success of the UDP in USA. SpainUDP offers a multidisciplinary approach to those patients who have long sought a diagnosis without any success. During a first phase of the protocol, undiagnosed cases are sent to SpainUDP by individual patients, patient organizations or hospitals. After a carefully analysis of phenotype, data from sequencing experiments (WES) is processed with a standard pipeline and a detailed standardized phenotypic information (mapped to HPO) is connected to genetic data. In addition, the participation of SpainUDP in international initiatives such as the European projects RD-Connect and Solve RD, the Undiagnosed Diseases Network International (UDNI), and the MatchMaker Exchange platform, allows the establishment of a global data sharing strategy across multiple projects submitting data to these international initiatives. From the official beginning of the program (at the end of 2015) until early 2018, 147 cases were accepted in SpainUDP. During this time, 37 cases (25 %) dropped out the program due to several reasons. The remaining 110 cases are distributed as follows: phenotypic and genotypic (WES) characterization was finished in 30 cases, of which 20 (67 %) were diagnosed; 21 cases are pending on variants validation by Sanger; in 25 cases, WES is ongoing and 34 cases are in a deep phenotypic characterization. As a conclusion, SpainUDP aims to achieve a diagnosis following two recommendations of the IRDiRC: the patients' diagnosis in a period of time as short as possible and the promotion of data sharing (especially genomic) at the international level.

Keywords: Diagnosis delay; rare diseases; undiagnosed programs; standardized phenotype; phenotype ontologies; whole exome analysis; international data sharing.

1. Introduction

Rare diseases (RD) constitute a very heterogeneous group of clinical entities defined by a low prevalence and characterized by a high level of clinical complexity. Their characteristics make their management in the healthcare systems not an easy task, especially in those aspects related to their diagnosis. Although substantial progress has been made toward identifying the genetic basis of rare diseases, the underlying etiologies for many of them remain undiscovered [1] and knowledge gaps continue existing regarding genotype-phenotype correlation. In addition, geographic dispersion of patients and difficulties for sharing and accessing data are still big obstacles for RD research. Efforts of individual researchers continue to multiply while remaining largely "siloed", with almost no information exchange [2], which makes extremely complicated to extract and use data for diagnostic

purposes. On the other hand, the difficulty in obtaining the correct diagnosis in the presence of pathogenic variants in multiple genes within one only patient is particularly challenging [3].

Given this complex scenario, it is not surprising that clinical assessments and conventional genetic testing lead to a diagnosis in less than half of patients [4]. The complexity of these diseases determines that a high percentage of cases has to be referred to clinical centers with a high level of specialization and, finally, to undiagnosed RD programs (where they exist), which try to translate research into medical practice through a procedure based on an individualized and exhaustive study of patients by a multidisciplinary team. One of the pioneering programs for the management and resolution of undiagnosed RD cases was the National Institutes of Health (NIH) Undiagnosed Diseases Program (UDP), which was launched in 2008 in the United States to address the diagnosis of rare and previously unrecognized diseases. Based upon its success, some years later the Undiagnosed Diseases Network (UDN) was created to extend the UDP model to other medical centers in the USA [5]. In addition, the Undiagnosed Diseases Network International (UDNI) was founded across several countries (Australia, Austria, Bulgaria, Canada, Hungary, India, Italy, Japan, South Korea, Spain, Sweden and the United States) to improve the rate of definitive diagnosis for persons living with undiagnosed conditions at the international level [6]. Inspired by the UDN, the UDNI has built a consensus framework of principles, governance and best practices [7]. After the establishment of the NIH-UDP in the United States [5], other UDP initiatives have been launched in some European countries (Austria, Bulgaria, Hungary, Sweden, Italy and Spain) and in other countries (Japan, Australia, Korea and Canada) [8]. The appearance of undiagnosed rare diseases programs has coincided with the expansion of the next-generation sequencing technology. This emerging technology has been rapidly adapted to clinical testing and is radically changing the paradigm of clinical diagnosis. Although single-gene testing and gene panels still hold great value for the diagnosis of many disorders, whole exome sequencing (WES) has shown success most often in patients who remain undiagnosed after a few traditional approaches [9]. In parallel to the enormous advances in gene identification through WES, large-scale data sharing initiatives have been developed to identify unrelated persons with pathogenic variant(s) in the same gene and an overlapping phenotype [1].

SpainUDP is the acronym of the "Spanish Undiagnosed Rare Diseases Program", which has been implemented by the Institute of Rare Diseases Research (IIER) of the Instituto de Salud Carlos III (ISCIII), a governmental organization aimed to manage and carry out biomedical research in Spain, which also is a full member of IRDiRC. SpainUDP was launched in 2013 as a pilot program in response to the high number of consultations that IIER had been receiving for years regarding undiagnosed rare diseases cases, many of them coming from the Spanish Federation of Rare Diseases (FEDER) [10]. This program was presented during the first UDNI conference, held in Rome in 2014. In 2015, SpainUDP became fully established and an agreement was signed between the IIER-ISCIII and the Foundation for Biomedical Research of the University Hospital Puerta de Hierro (UHPH), in Madrid, for supporting detailed clinical examination in very complex undiagnosed cases. Also, SpainUDP has been included within the "Strategy for the improvement of health care for patients with rare diseases in the Community of Madrid (2016-2020)" [11]. As most of the aforementioned undiagnosed rare diseases programs, the SpainUDP's procedure is based on three main pillars: i) an exhaustive and individualized study of each case, ii) the use of next-generation sequencing techniques and iii) the establishment of data sharing systems at the international level.

Diagnosis delay remains one of the pending issues in the rare diseases field, as reflected in one of the IRDiRC goals for 2017-2027, that encourages RD community to achieve diagnosis within one year through international coordination of unsolved cases [12]. SpainUDP was created in response to this challenging scenario to offer a multidisciplinary approach to those patients who have long sought a diagnosis without any success. This paper aims to describe in detail the phases composing this program, the results obtained to date, as well as the data sharing strategy that implies the link of SpainUDP with local, national and international RD resources to maximize the possibility of finding a diagnosis.

2. Materials and Methods

The operating rules of SpainUDP (https://spainudp.isciii.es) are defined in a standardized protocol that establishes the criteria for patient selection, data collection, laboratory investigation and diagnosis. This protocol has been elaborated by the SpainUDP team, comprising several physicians from different medical specialities, geneticists, biologists, biochemist, bioinformatics and experts on dysmorphology, who support the program with their knowledge and expertise. SpainUDP also counts on the support of the Spanish Rare Diseases Biobank (BioNER, http://bioner.isciii.es), the Spanish Rare Diseases Patient Registry (SpainRDR, https://spainrdr.isciii.es) and the Spanish Mutations Database (SpainMDB, http://spainmdb.isciii.es).

2.1. Access to the program

Patients with undiagnosed diseases or their family members can apply to SpainUDP individually or through their patient organizations or hospitals. Regarding the latter, in addition to the UHPH, two more hospitals are collaborating closely with SpainUDP: the University Children Hospital Niño Jesús, Madrid, and the University Hospital Virgen del Rocío, Sevilla, since they are sharing complex pediatric undiagnosed cases in order to find a diagnosis by means of the SpainUDP approach. New agreements with other Spanish hospitals are under discussion and it is expected that they can be signed shortly, which will help to expand the action area of the program throughout the Spanish regions (although it is important to highlight that, currently, the lack of signed agreements is not a barrier for the access by patients residing in any Spanish province). Active involvement of patients' organizations is important too. Thus, after many years collaborating in different aspects (also in undiagnosed cases), a closer collaboration with the Spanish Federation of Rare Diseases Patients (FEDER) [10] has been established to provide help to undiagnosed patients and/or relatives and inform them about the possibility to apply to SpainUDP. A similar role is being played by patient associations such as "Asociación Objetivo Diagnóstico" (Diagnosis Objective Association) and "Asociación D'Genes" (D'Genes Association) [13]. Moreover, individual patients asking to be included in the patient registry without a definitive diagnosis are invited to be assessed in SpainUDP.

2.2. Inclusion criteria

The applicants have to comply a series of inclusion criteria to enter the program:

- Applicant's disease curses with clinical signs and symptoms not clearly identifiable or attributable to a well described rare disorder.
- Applicant's medical conditions have eluded diagnosis by referring specialists of the Spanish National Health System for a long time and despite extensive investigations.
- Applicants (or legal guardian) have provided consent for registering in SpainRDR.
- Applicants (or legal guardian) agree to the storage and sharing of information and biomaterials in an identified fashion amongst the SpainUDP members, and in a de-identified fashion to research sites beyond the program.
- Collaboration of medical doctors who are studying the patient at the local level is highly recommended, although it is not mandatory, since patient's autonomy in decision-making always prevails over physicians' opinions.

2.3. Deep phenotyping

Cases are sent to the program by patient associations, any clinician and/or by themselves or their families, who are required to provide all clinical information available, including photographs, imaging/radiography results and genetic studies previously performed. The SpainUDP team collects and carefully reviews the applicant's medical records and, if some documentation is lacking, it is requested. After that, they make a recommendation to accept or reject the application. Once the applicant is accepted into the program, data management is centralized by means of a secure informatics application, which has been developed to share, store and manage the collected clinical data, as well as laboratory tests, images, videos and communications between the patients or patient's relatives and the program's staff. In parallel, a close collaboration is established with local healthcare

services to avoid unnecessary journeys, thus averting discomfort for the patients and their families. On the other hand, when necessary, a plan for up to a full week of inpatient clinical testing is organized with the UHPH specialists in order to perform all of the complementary tests (with the shortest sedation, if necessary) needed to complete the deep phenotyping..

2.4. Genomic analysis

If actions carried out during this first phase are not enough to achieve a diagnosis, a second phase is performed next in the Human Genetics Department of the IIER. It consists in the execution of next generation sequencing techniques, mainly whole exome analysis (WES) performed by trio analysis (proband, father and mother). Raw genomic data from sequencing experiments of DNA samples of patients and relatives are processed through two different standardized protocols carried out in parallel in two centres: on the one hand, internally in the IIER and, on the other hand, in the "Centro Nacional de Análisis Genómicos", CNAG (National Center for Genomic Analysis). In the IIER, an in-house pipeline is applied to raw data taking as starting point best practices defined by the GATK software toolkit. In the CNAG, raw genomic data is realigned and reprocessed through the RD-Connect validated analysis pipeline and held in the centralized RD-Connect database [2, 14]. The processed data is available for online analysis through a user-friendly interface to authorized users once all the required commitments are fulfilled. As IIER is a full member of RD-Connect, it is contributing with their undiagnosed cases to the platform of this project, fulfilling all the international standards for these purposes. Moreover, phenotypic terms are extracted from clinical documents stored in the patient registry, mapped to HPO (Human Phenotype Ontology) [15] terms and uploaded into PhenoTips [16], a software tool available in the RD-Connect platform. RD-Connect genomics interface allows filtering and refining the results by mode of inheritance, population frequencies, in silico pathogenicity prediction tools and HPO codes [2]. Results of both analyses (the one carried out in the IIER and the one carried out in the CNAG) are manually reviewed by two independent researchers of SpainUDP with common criteria. After such review, a consensus is reached to select the candidate variants, which will be confirmed by Sanger sequencing in all family members. Finally, various sources of information are consulted to build a report with a detailed review of the scientific evidence that associates each detected genetic variant with a specific disease or disorder and it is discussed according to the patient's characteristics, thus concluding on the involvement and plausible causation of the identified variants with respect to the disease.

2.4. Data sharing

The third phase of SpainUDP involves data sharing from undiagnosed patients in international platforms/networks. RD-Connect platform enables a comparison of patient data from SpainUDP across multiple projects submitting data to this platform. Furthermore, it is possible to push data from PhenoTips to Phenome Central [17], a centralized repository that facilitates the matching of cases with similar clinical and genotypic profiles within larger shared international networks, such as the UDNI. Moreover, RD-Connect, Phenome Central and UDNI are participating in the Matchmaker Exchange (MME) [18], a federated platform that represents the largest effort to enable sharing specific case details to find similar cases. Since SpainUDP is contributing with its undiagnosed cases to these three international initiatives, it is indirectly connected to the remaining projects that belong to MME.

2.5. Ethical issues

Regarding ethical issues, our protocol includes the signing of several informed consents (IC) by patients admitted into SpainUDP: i) IC for registering in SpainRDR, ii) IC for storing biosamples in BioNER, iii) IC for carrying out WES analysis. Recently, we have incorporated new statements to the last cited IC in order to comply ethical requirements from Phenotips [16] and Phenome Central [17] (including specific questions suggested by both resources). It was approved by the Ethics Committee for Research of the ISCIII on the 1st September 2017 with the project identification code CEI PI

54_2017. On the other hand, the IC for storing biosamples in BioNER and patient data in SpainRDR was approved by the same Ethics Committee on the 6th February 2017 with the project identification code CEI PI 74_2016.

3. Results

From the official beginning of SpainUDP (in October 2015) to May 2018, 147 cases were accepted in SpainUDP. They were mainly pediatric, since 109 cases corresponded to patients younger than 18 years, representing 74.1 % of the total number of cases. Geographical distribution was highly variable, since the cases came from 40 of the 52 different Spanish provinces (Figure 1). Madrid (n = 50) and Seville (n = 14) were the provinces with the highest number of accepted cases.
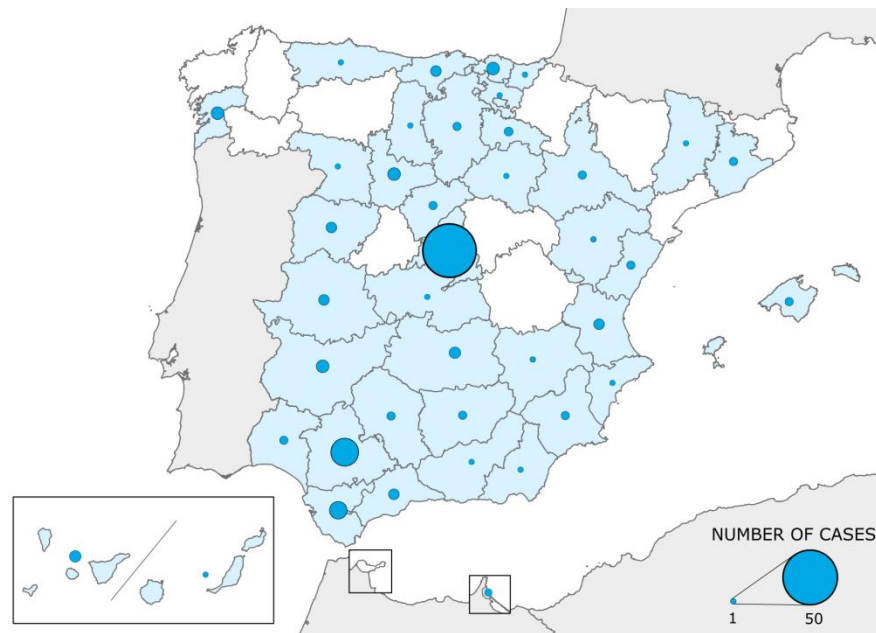

Figure 1. Geographical distribution of patients' admissions in SpainUDP.

The distribution of patients by gender without any stratification by age was very similar for both sexes (73 men and 74 women). However, the cases distribution by gender showed a different pattern depending on the age of patients (Figure 2A). Thus, taking into account only pediatric cases admitted in SpainUDP (Figure 2B), there was a higher number of boys (n=66; 60.6 %) than girls (n=43; 39.4 %), with a sex-ratio of 1.54. This gender distribution reversed in adult patients (Figure 2C) as there was a higher number of admitted women (n=31; 81.6 %) than men (n=7; 18.4 %) with a sex-ratio of 0.23.

During this time period, 37 cases (25.2 %) dropped out the program due to diverse reasons: for being diagnosed out of SpainUDP (n=24), for having common diseases instead rare diseases (n=6), for wishing to leave the program voluntarily (n=3), for a lack of collaboration from patients' reference hospitals (n=3) and for residing out of Spain (n=1). The remaining 110 cases are distributed as follows (Figure 3): phenotypic and genotypic (WES) characterization was finished in 30 cases, of which 20 (67 %) were diagnosed; 21 cases are pending on variants validation by Sanger; in 25 cases, WES is ongoing and 34 cases are in a deep phenotypic characterization.
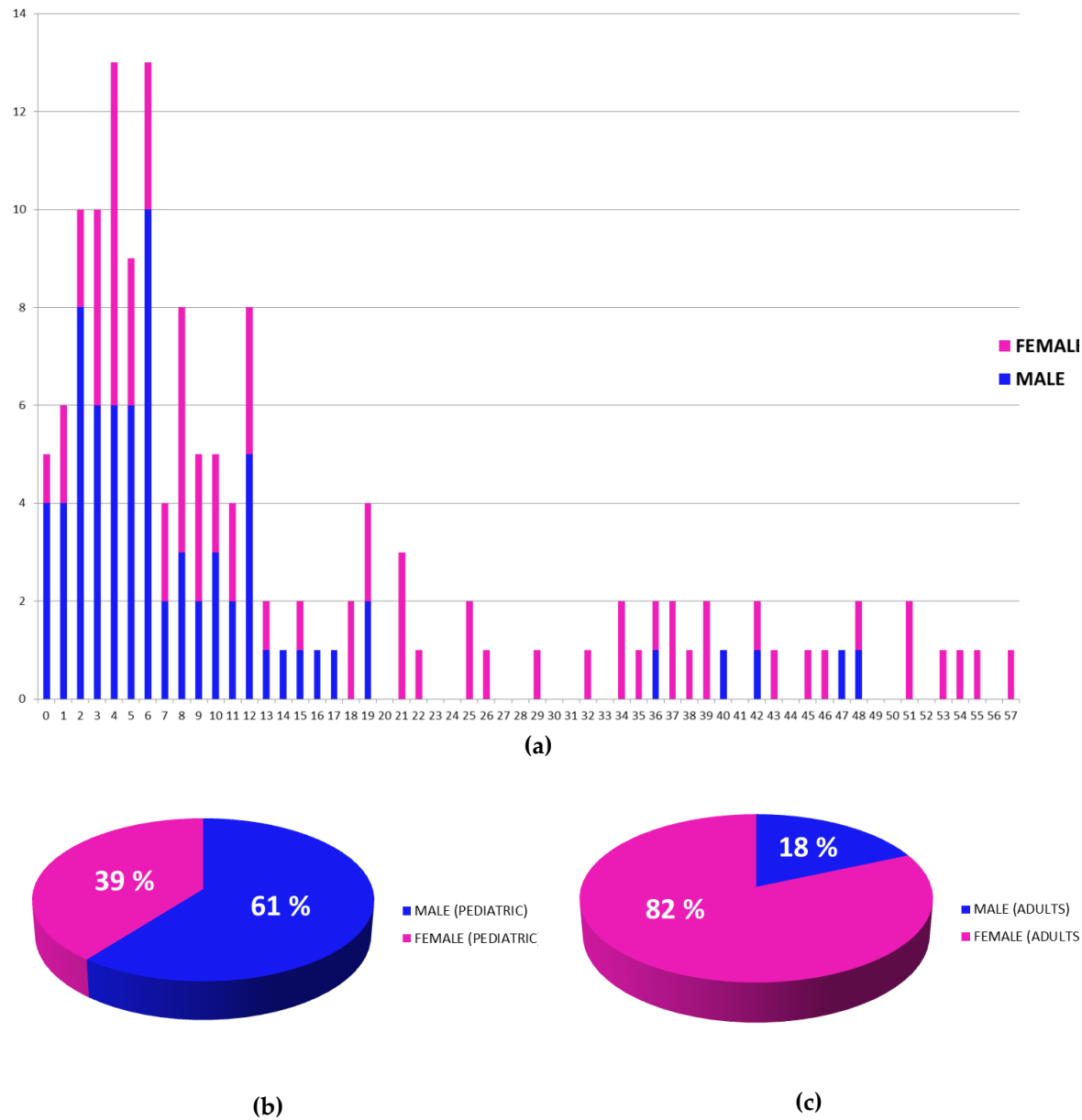
(a)

(b)

(c)

Figure 2. Distribution of SpainUDP patients by age and gender: **(a)** Bars graphic representing the distribution of patients by age and gender. **(b)** Pie chart representing the total distribution of pediatric patients by gender. **(c)** Pie chart representing the total distribution of adult patients by gender.

Table 1 displays a brief description of each one of the solved cases. By far the most common phenotypic category of diagnosed cases was neurological disorders. In addition, these diseases could be considered as very rare conditions, since in most cases the prevalence was less than 1 per 1,000,000 inhabitants. Regarding genetic analyses, 75% of the detected causal variants corresponded to de novo mutations (mainly frameshift and stopgain variants). On the other hand, two patients (one boy and one girl) had the same genetic diagnosis (mental retardation autosomal dominant 32, associated to a mutation of the KAT6A gene).
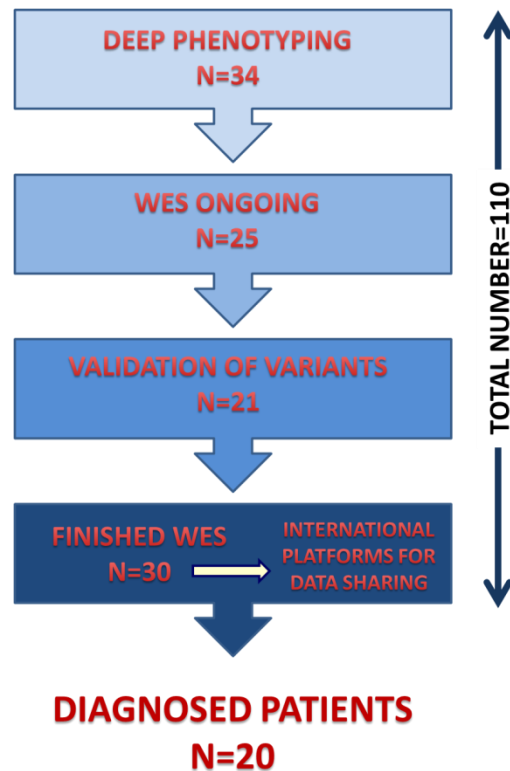
Figure 3. Main phases of SpainUDP and current number of patients within each one of them.

Table 1. Descriptive table of the cases diagnosed by SpainUDP.

Case	Candidate gene (acronym & OMIM number)	Mutation type	Mutation type	Diagnosis (disease name & OMIM number)	Inheritance[1]	Prevalence[2]
1	MEF2C (OMIM 600662)	Stopgain	De novo	Mental retardation, autosomal dominant 20 (OMIM 613443)	AD	<1/1,000,000
2	VPS13B (OMIM 607817)	Stopgain/ Splicing	Compound heterozygous	Cohen syndrome (OMIM 216550)	AR	Unknown[3]
3	SLC52A2 (OMIM 607882)	Missense	Homozygous	Brown-Vialetto-Van Laere syndrome 2 (OMIM 607882)	AR	<1/1,000,000
4	AUTS2 (OMIM 607270)	Frameshift	De novo	Mental retardation, autosomal dominant 26 (OMIM 615834)	AD	<1/1,000,000
5	CTNNB1 (OMIM 116806)	Frameshift	De novo	Mental retardation, autosomal dominant 19 (OMIM 615075)	AD	<1/1,000,000

6	EHMT1 (OMIM 607001)	Stopgain	De novo	Kleefstra syndrome 1 (OMIM 610253)	AD	<1/1,000,000
7	SCN2A (OMIM 182390)	Frameshift	De novo	Epileptic encephalopathy, early infantile, 11 (OMIM 613721)	AD	Unknown
8	KMT2D (OMIM 602113)	Frameshift	De novo	Kabuki syndrome 1 (OMIM 147920)	AD	1-9/100,000
9	PIK3R1 (OMIM 171833)	Missense	De novo	Short syndrome (OMIM 269880)	AD	<1/1,000,000
10	DDX3X (OMIM 300160)	Frameshift	De novo	Mental retardation, X-linked 102 (OMIM 300958)	XLD, XLR	<1/1,000,000
11	PNPT1 (OMIM 610316)	Stopgain/ Missense	Compound heterozygous	Combined oxidative phosphorylation deficiency 13 (OMIM 614932)	AR	<1/1,000,000
12	KAT6A (OMIM 601408)	Frameshift	De novo	Mental retardation, autosomal dominant 32 (OMIM 616268)	AD	<1/1,000,000
13	SLC6A1 (OMIM 137165)	Frameshift	De novo	Myoclonic-atonic epilepsy (OMIM 616421)	AD	Unknown
14	SYNGAP1 (OMIM 603384)	Frameshift	De novo	Mental retardation, autosomal dominant 5 (OMIM 612621)	AD	Unknown
15	SLC9A6 (OMIM 300231)	Frameshift	De novo	Mental retardation, X-linked, syndromic, Christianson type (OMIM 300243)	XLD	<1/1,000,000
16	KCNQ2 (OMIM 602235)	Missense	De novo	Epileptic encephalopathy, early infantile, 7 (OMIM 613720) & Seizures, benign familial neonatal, 1 (OMIM 121200)	AD	Unknown
17	NKX2-1 (OMIM 600635)	Frameshift	De novo	Choreoathetosis and congenital hypothyroidism with or without pulmonary dysfunction (OMIM 610978)	AD	<1/1,000,000

18	VPS13D (OMIM 608877)	Missense/ Missense	Compound heterozygous	Movement disorder with ataxia and spasticity (no OMIM code yet)	AR	Unknown
19	FRMPD4 (OMIM 300838)	Missense	Hemyzygous	Mental retardation, X-linked 104 (OMIM 300983)	XLR	Unknown
20	KAT6A (OMIM 601408)	Missense	De novo	Mental retardation, autosomal dominant 32 (OMIM 616268)	AD	<1/1,000,000

[1] AD=autosomal dominant, AR=autosomal recessive, XLD=X-linked dominant & XLR=X-linked recessive.

[2] Prevalence data extracted from Orphanet database (accessed on 17th May 2018).

[3] Prevalence is unknown and only 200 have been described to date worldwide.

Table 2 shows age and gender data of the 20 diagnosed patients. Among these patients, 12 (60.0 %) were male. The age at diagnosis ranged from 3.0 to 27.7 years (only one patient was over 18 years of age at the time of diagnosis) and the average age was 8.9 years. The mean time for diagnosis in SpainUDP was 12.5 months and ranged from 1.5 and 23.9 months.

Table 2. Gender and age statistics for the cases diagnosed by SpainUDP.

Case	Gender	Age at SpainUDP entry (years)	Age at diagnosis (years)	Diagnosis delay in SpainUDP (months)
1	female	5.3	6.8	18.3
2	female	3.1	3.2	1.8
3	female	26.8	27.7	10.6
4	male	2.2	3.0	9.4
5	male	4.6	5.3	8.3
6	female	11.1	12.5	16.6
7	male	3.9	4.1	1.5
8	male	7.0	7.2	2.4
9	male	8.5	9.9	16.0
10	female	9.9	11.4	18.7
11	male	1.5	3.0	18.1
12	female	6.4	8.1	19.5
13	female	6.3	7.3	11.4
14	male	14.0	14.9	11.2
15	male	7.2	8.1	10.5
16	male	6.1	6.9	10.1
17	male	12.7	13.6	10.8

18	female	8.5	9.7	13.5
19	male	5.6	7.6	23.9
20	male	6.6	8.1	17.8
	Mean ± SD	8.0 ± 5.5	8.9 ± 5.6	12.5 ± 6.2
	Median	6.5	7.8	11.3
	Minimum value	1.5	3.0	1.5
	Maximum value	26.8	27.7	23.9
	10th percentile	2.3	3.1	1.9
	90th percentile	13.9	14.8	19.4

After WES and despite great efforts and different strategies, ten cases remain unsolved to date:

- Case 1: a boy with a very complex phenotype including global developmental delay, osteoporosis and irregular hyperpigmentation.

- Case 2: a young woman with primary hyperparathyroidism, brown tumors and pathological fractures.

- Cases 3 and 4: two sibblings with similar phenotypes characterized by global developmental delay, autistic features and hypotonia.

- Case 5: a boy with myoclonic epilepsy, autism spectrum disorder and frequent diarrheas.

- Case 6: a boy with severe lower limb amyotrophy, sensorimotor neuropathy and dysmorphic features.

- Case 7: a boy with a speech disorder and autistic behavior.

- Case 8: a girl with a systemic autoinflammatory disorder with a relevant digestive component (refractory to treatment).

- Case 9: a boy with remarkable dysmorphisms, such as prognathism, dental malocclusion and macrocephaly, and also absence crises, unexplained fevers, intolerance to exercise and decreased muscle mass.

- Case 10: a boy who probably has two genetic diagnoses, as he has been diagnosed with epileptic encephalopathy early infantile type 4 (mutation of the STXBP1 gene) which only explains part of his phenotype, but he also has bilateral sensorineural hearing impairment of unexplained origin.

Although analysis of WES is currently finished, further analyses of these cases are being accomplished and it is expected that ongoing investigations lead to more diagnoses in the near future.

4. Discussion

Lack of screening tests and limited knowledge among health professionals about how to recognize the signs and symptoms of rare diseases lead to diagnostic delays [19]. A high rate of cases remains undiagnosed for many years and, as a consequence, during a great part of their lives these patients and relatives do not know health implications of the disease or disease progression and they do not have access to appropriate treatments. Programs aimed to these cases are currently growing and developing in many countries. In this regard, the UDNI is trying to validate the cooperation in this important field. The "Spanish Undiagnosed Rare Diseases Program" (SpainUDP), developed by the IIER-ISCIII, aims to solve some of these cases in Spain in close collaboration with the UDNI and other European projects such as RD-Connect and Solve-RD.

The majority of patients studied in SpainUDP were younger than 18 years. This is not surprising as the onset of rare diseases usually occurs during the first years of life [19]. It was also observed that

the pediatric admissions into the program was higher in males than females, since 60.6% were boys, what means about 1 girl for every 2 boys. Similarly, 63.2% of the diagnosed pediatric patients were male. A similar finding has been described in many birth defects and autism spectrum disorders, which are currently more commonly diagnosed in males than females [20]. Several theories could explain the male-female sex based discrepancy, such as a higher risk in males of being affected by X-linked recessive disorders and phenotypic differences in the presentation or severity between sexes. However, with our current data, we are not able to explain the reasons for these gender-based differences found in the number of pediatric admissions and it would be necessary to check them in the next future with a bigger set of data. In the case of adult patients, a much higher admission of women (81.6%) was registered. The reason for these sex differences is unclear, but it could be partially explained by two reasons: women tend to be affected by chronic diseases at younger ages than men [21] and, on the other hand, many menopausal women are more susceptible to chronic fatigue due to changing hormone levels, which might be easily confounded with a rare disease. Regarding the later, based on our experience in SpainUDP, we consider that the diagnostic process in adults is frequently hard to manage as many interacting factors are contributing to the clinical status of patients. Psychiatric disorders play an important role at this point. Thus, sometimes psychiatric symptoms might be confounded by the patients, their families or even health care providers with symptoms produced by rare diseases. This is a challenging scenario not so easy to manage within SpainUDP, as it is difficult to demonstrate from the clinical point of view that patients are suffering a psychiatric common disease instead of a rare disease and it usually requires additional strategies. Currently, three adult patients belonging to SpainUDP with suspicion of suffering psychiatric disorders are being deeply studied to discard the possibility that they might have a rare disease (results not included in Table 1, since some tests are still pending). But in any case, this is probably transversal for all or many undiagnosed diseases programs. In this regard, similar findings were described in the article published by Gahl et al. [22], where 6 of 39 diagnosed patients were adults with fibromyalgia or common psychiatric disorders such as somatization, psychogenic movement disorder and psychogenic tic cough.

Regarding the geographical differences on patients' admissions into SpainUDP, it was observed a high variability depending on patients' residence provinces, being Madrid the province with the highest number of admitted patients. It is not an unexpected result as two of the three hospitals collaborating actively in SpainUDP (University Hospital Puerta de Hierro and University Children Hospital Niño Jesús) are located in Madrid and also this province has several millions of inhabitants. Patients who live in this province can freely choose the hospital to go to be attended by medical specialists, so they can be examined by the medical teams of the aforementioned hospitals without any administrative impediments. However, patients living outside Madrid need to request a transfer from their local hospital to UHPH and, unfortunately, they sometimes find some administrative obstacles. As a consequence, the access to our program by these patients requires more effort. This is why SpainUDP has had to adapt to these administrative constrains allowing that patients living in other provinces different to Madrid can be also investigated remotely through intense collaboration with local physicians who facilitate us clinical reports of patients or even carry out different medical tests required for a deep analysis of their phenotype. Nonetheless, the administrative obstacles found for some patients may be playing a role in the withdrawal of SpainUDP, since 29 (78.4%) of a total of 37 patients that dropped out the program lived out of Madrid. All this means that we have identified this problem as an issue to which a special attention should be paid and for which some administrative solution should be found to avoid frustration among patients who could benefit from this strategy and promote both equality and equity.

A deep phenotyping combined with exome sequencing allowed the diagnosis of 20 patients who were undiagnosed for a long time. Our results (see Table 2) indicate that the average age at SpainUDP entry for these 20 patients was around 8 years and, since the onset for the majority of rare diseases occurs during the first years of life, we might assume that the average of the diagnosis delay in this group of patients before entering SpainUDP was approximately 8 years. Although the time period needed for diagnosis in SpainUDP was much shorter, with a mean value of 12.5 months, we would

like to indicate that only in 55.0% of the cases a diagnosis was achieved in less than one year, that is the "gold standard" established by the IRDiRC [12]. On the other hand, after WES, 10 cases remain unsolved and diagnosis delay in these cases is much higher than one year. Therefore, this is another problem to pay attention and currently it is one of the most important challenges in SpainUDP, so we have to focus our efforts on reducing the time necessary for diagnosis.

The majority of the solved cases corresponds to children with pediatric neurological disorders, who form a particularly difficult-to diagnose patient population. These disorders are often very complex and, in addition to intellectual disability, patients usually have wide and diverse phenotypes, such as micro or macrocephaly, developmental delay, feeding problems, epilepsy, hypotonia, brain structural anomalies, dysmorphic facial features, etc. The complexity of these disorders causes that a high percentage of this kind of unsolved patient cases is referred to undiagnosed diseases programs, such as SpainUDP. As an example, in a study carried out by Yang et al. [23] for diagnostic evaluation of 2,000 patients with suspected genetic disorders, patients were primarily pediatric (88%) with diverse clinical manifestations, most often including nervous system dysfunction. The extremely low prevalence of the diagnosed diseases in SpainUDP (see Table 1) is one of the reasons that could explain why these patients had not been diagnosed in the National Health System and had to be referred to specialized programs such as SpainUDP.

The use of the RD-Connect platform was very fruitful for the purposes of SpainUDP, since it enhanced the analyses, integration and sharing of very complex phenotypic and genotypic data managed in this program. However, unfortunately, to date the actions carried out in SpainUDP to match our unsolved cases with similar phenotypic and genotypic profiles in platforms such as MME have failed. A truly optimizing of this type of case-based matching on a global scale would require a major communication among researchers [1]. Although one of the strongest trends identified by IRDiRC is that "data sharing is the leitmotif, especially for genomics data" [24], and technical conditions are created to establish networks of patients data resources visible to clinician and researchers regardless of the geographical location, there are still many open questions and future challenges that need to be solved. In order to researchers "feel free and sure to share data", issues related to intellectual property rights would need to be assessed and handled in accordance with fundamental ethical rules and principles [25].

Current challenges for SpainUDP include the validation of variants of unknown significance through functional assays that demonstrate their pathogenicity. Thus, some pathogenic variants are being evaluated using cultured cells and animal models for functional assays that allow characterizing their mechanisms of pathogenicity. In this regard, currently, collaborations with some researchers are being established to accomplish this kind of experimental studies. In addition to other European projects and strategies, Solve-RD might be a relevant tool to achieve these goals, since this project pursues an integrated "beyond the exome" approach consisting of the use of sophisticated combined omics approaches and a "genetic knowledge web".

5. Conclusions

In summary, the recognition of difficulty for diagnosis by rare diseases community has triggered the emergence of special programs to investigate patients with undiagnosed disorders worldwide, such as SpainUDP. It aims to make appropriate diagnoses in rare diseases patients who still have not had a confirmed diagnosis, usually for a long time. At the same time, this multidisciplinary program, linked to a rare diseases research institute, aims to foster the discovery of new diseases through a translational approach. We also would like to emphasize the involvement of patients and their families with the diagnostic process carried out in SpainUDP, which has resulted in an extraordinary collaboration on their part, perhaps due to finding in this strategy a different approach to their "diagnostic odyssey".

This article describes SpainUDP and synthesizes the first results obtained in its initial 2 years and how the collaboration with the UDNI and other international initiatives will open some hope for those patients who are still without a diagnosis. It also highlights the challenges and limitations in this long way for sharing experiences among rare diseases researchers.

Author Contributions: E.L. and M.P. designed the article. B.M. and J.A. participated in the genomic analyses. E.B. and M.P. contributed to the deep phenotyping of patients. E.L. and M.P. uploaded cases data into international platforms for data sharing. E.L., E.B. and M.P. analyzed patient data through the RD-Connect platform. E.L. carried out statistical analyses of data. E.L. prepared the draft and B.M., E.B., J.A. and M.P. critically reviewed it; and E.L. finished the manuscript.

Appendix A: SpainUDP Network

Name	Institution	Department	City
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Sara Monzón	Instituto de Salud Carlos III	Bioinformatics	Majadahonda (Madrid)
Julián Lara	University Hospital Puerta de Hierro	Pediatrics	Majadahonda (Madrid)
Rosario Cazorla	University Hospital Puerta de Hierro	Pediatrics	Majadahonda (Madrid)
Gemma Iglesias	University Hospital Puerta de Hierro	Pediatrics	Majadahonda (Madrid)
Enriqueta Román	University Hospital Puerta de Hierro	Pediatrics	Majadahonda (Madrid)
Purificación Ros	University Hospital Puerta de Hierro	Pediatrics	Majadahonda (Madrid)
Pablo Tutor	University Hospital Puerta de Hierro	Internal Medicine	Majadahonda (Madrid)
Susana Mellor	University Hospital Puerta de Hierro	Internal Medicine	Majadahonda (Madrid)
Carlos Jiménez	University Hospital Puerta de Hierro	Neurology	Majadahonda (Madrid)
Mª José Cabrejas	University Hospital Puerta de Hierro	Clinical Biochemistry	Majadahonda (Madrid)
Emiliano González	University Hospital Puerta de Hierro	Clinical Biochemistry	Majadahonda (Madrid)
Luis González	University Children Hospital Niño Jesús	Pediatrics	Madrid
Marcos Madruga	University Hospital Virgen del Rocío	Pediatrics	Sevilla

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