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ARTICLE

The Incontinentia Pigmenti Genetic Biobank: study design and cohort profile to facilitate research into a rare disease worldwide

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Abstract

Incontinentia pigmenti (IP; OMIM#308300) is a rare genetic disease resulting in neuroectodermal defects, which can lead to disability. At present, there is neither definitive cure available nor are there any sufficiently reliable insights to predict the severity of the disease. We launched the Incontinentia Pigmenti Genetic Biobank (IPGB) project (http://www.igb.cnr.it/ipgb) in 2015 to establish a large-scale deposit of biological samples, to provide detailed clinical information about children diagnosed with IP and to facilitate research. We have built a cohort comprising samples of 381 clinically confirmed patients with IP and 633 healthy individuals recruited through IP patients' associations. The collection includes 269 trios, 83 duos, and 95 families with at least two affected members and represents an extensive dataset (200 cooperative medical institutes, 139 in Italy and 61 worldwide) that enables a comprehensive phenotyping. Joining the IPGB guarantees all participants access to the results including the genetic testing of IP and the long-term storage of the samples. The IPGB is the largest IP sample collection and one of the largest rare-disease-oriented collections in the world and will be open to requests for access to data by the national and international scientific community.

Introduction

Incontinentia pigmenti (IP; OMIM#308300) is a rare multisystemic genomic disorder [1] with an estimated prevalence at birth of 1.2/100,000 [2]. IP is X-linked dominant, usually lethal in males, and affects the skin and other neuroectodermal tissues in females [3]. IP is caused by variants of the *inhibitor of the K polypeptide gene enhancer in B cells, the kinase gamma* (*IKBKG*)*/nuclear factor κB (NF-κB) and the essential modulator* (*NEMO*) gene. The *IKBKG* encoded protein NEMO/IKKγ is essential for the NF-κB activation pathway, involved in a variety of physiological and cellular processes, such as immunity, inflammation, and cell proliferation/survival. Hemizygous *IKBKG/NEMO* loss of function variants are lethal in males, while *IKBKG/NEMO* hypomorphic variants, reducing but not abolishing NF-κB activation, have been identified in male patients with anhidrotic ectodermal dysplasia with immuno deficiency (EDA-ID, OMIM#300291) and in heterozygous female with IP [4]. Instead, amorphic variants, abolishing NF-κB activation by complete *IKBKG/NEMO* gene silencing, cause only IP [4]. Although the classic IP phenotype is almost entirely restricted to females, occasionally 46,XY males present an IP phenotype [5]. These rare cases show the characteristic skin lesions and, in accordance with the pathogenic model of the disease, they are postzygotic genetic mosaics for the *IKBKG/NEMO* mutation [6–8]. IP has also been diagnosed in males with a 47,XXY karyotype (Klinefelter syndrome) [9].

Despite significant advances in research, the IP/EDA-ID etiology and pathophysiology remains elusive. The rarity of such IP/EDA-ID diseases and their heterogeneous phenotypic manifestation makes it difficult to study their molecular and genetic origin as well as rendering prevention and treatment problematic. More than 30% of individuals diagnosed with IP were affected by neonatal epilepsy

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leading to a severe form of the disease [1, 10]. Until now, the absence of any genetic/phenotypic biomarkers able to predict the onset of severe forms makes such IP newborn babies vulnerable to ischemia and other risk factors.

We constituted the biobank with the aim to build a standardized collection of samples and information able to provide biological resources to support and accelerate biomedical research, clinical trials, and the evaluation of early biomarkers. Due to the nature of rare diseases, international cooperation is critical for the sharing of limited numbers of patient samples and the achievement of a critical mass. The rarity of these conditions means that the establishment of a nationwide research platform is vital to facilitate studies on pathogenesis and treatment. In addition, the establishment and maintenance of a widespread collaboration between the biobank and physicians has benefited from the contribution of rare disease patients' organizations. We have developed a strategy, in agreement with the three associations of patients affected by IP, based on genetic information provided upon informed consent and the long-term preservation of patient and family samples.

This paper provides an overview of the IP Genetic Biobank (IPGB) project, launched in 2015, and a profile of the participants who were registered in the first 3 years of the project. The IPGB collects, stores, and distributes valuable human samples and data to improve research on IP.

Methods

IPGB: definition of the database and of procedures

The IPGB involved a process (Fig. 1) with specific standard operating procedures (SOPs) that encompass.

Sample and data recruitment

Patients and their families were interviewed by staff members of the referral hospital, with the IPGB team providing permanent assistance and support (by email and phone: incontientia.pigmenti@igb.cnr.it; 00396132302). All materials used for the study, including documents and the interview guide for the focus group development, were approved by the University of Naples Federico II Ethics Committee Prof. Claudio Buccelli and by the Biobanking and Biomolecular Resources Research Infrastructure- European Research Infrastructure Consortium (BBMRI-ERIC) Ethics Committee. The patients received full information regarding the project from experts and signed specific consent forms. The consent form included an option to withdraw from the study at any time and without giving any explanation. Information and consent forms were provided in different languages for international participants who do not speak English (Step1 and Step2, Fig. 1).

Sample and data collection and storage

The Ethics Committee specified that blood to be used for scientific purposes might only be taken from under-age patients within the scope of a medical examination. Varying amounts of peripheral blood were collected, 10 ml from adults, 9 ml from children and young people (aged 3–17), 5 ml from children aged 1–3, and 2.5 ml from children below 1 year of age. By Ficoll-density centrifugation of 5 ml of blood (for adults), peripheral blood mononuclear cells and granulocytes were obtained and were stored at −80 °C. The remaining 5 ml of blood was processed to extract the DNA by using the conventional salt precipitation technique (Fig. 2) [11, 12]. Fibroblast cell lines from skin biopsies [13] were generated, when possible, and stored in liquid nitrogen.

The data included socio-demographic variables (sex, date of birth, and nationality), recorded in the IP questionnaire, which were stored in the File Maker Pro Advanced Database. The IPGB project applied a central patient and sample restricted management with unique alpha–numeric code identifiers (IDs) that were used to indicate the study participants and their study data [14] (Step3, Fig.1). The personal and genetic data in the IPGB are processed in line with general data protection regulation.

Sample and data processing: molecular IP diagnosis

The clinical data and molecular diagnosis results for the *IKBKG/NEMO* alteration were integrated, by way of a well-standardized protocol [15–17]. The IP genetic test on the DNA was performed to define the genetic profile of each IP patient. This result confirmed the clinical diagnosis (Step4, Fig. 1).

Sample and data distribution: access criteria

The collected samples and clinical data can be requested by researchers carrying out studies upon approval by the IPGB access committee. The samples and clinical data are distributed under a strict governance framework, with completion of an application form and upon signature of an institutional material transfer agreement (Step5, Fig.1).

The IPGB and its association with patients' groups

The IPGB (www.igb.cnr.it/ipgb) was founded in 2015 and obtained funding from patients' associations (IP ASSociation of Italian patients, Onlus, http://www.incontinentia pigmenti.it/, and the IP International Foundation, IPIF,

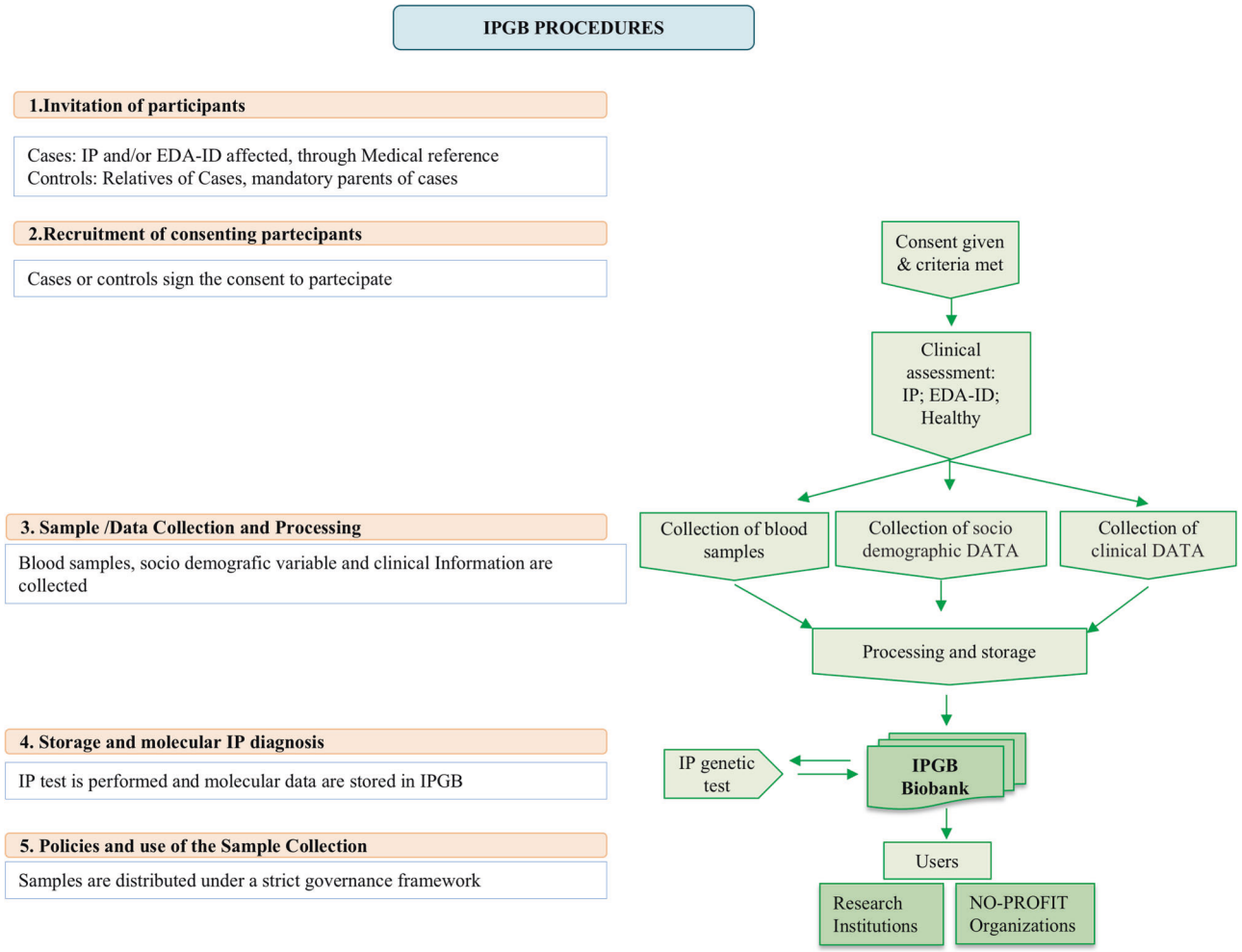

Fig. 1 IPGB Biobank procedures

http://www.ipif.org). The partnerships established with IP patients' associations (IPASSI, Onlus, and IPIF, respectively) and inputs obtained from professionals in human datasets, law, and ethics guided our proposal and sustained the project. To enhance biomedical studies in this area, we conducted a participatory study to assess the feasibility of establishing a cost-effective disease-specific biobank. This disease-specific biobank was conceived and is now administered by researchers at the Institute of Genetics and Biophysics "A. Buzzati-Traverso" CNR (IGB-ABT CNR) and at the Potenza University; the infrastructure for biobanking, responsible for processing, aliquoting, and storing the samples, is located at IGB-ABT CNR. The IPGB has formalized a partnership with the patients' organizations with a written approval signed by the IP association representative of the IPGB project submitted by the IPGB staff. In addition, the IPGB founders have maintained a collaborative scientific partnership since 2009 with the France Incontinentia Pigmenti Association (FIP, http://incontinentia-pigmenti.fr/) that is not directly involved in the IPGB project activity but supports our research programs. The agreement between the founders of IPGB and the IPIF dated back to early 2000s, while that with IPASSI Onlus started in 2011 when the association was founded. In 2016, the IPGB Biobank was recognized as an official member of the BBMRI-ERIC (https://www.bbmri.it/home).

Results

IPGB: current state of the collection

The IPGB collects biomaterials and data from patients and family members with a confirmed or suspected diagnosis of IP or EDA-ID, *NEMO/IKBKG* variants being able to cause both diseases [4]. The majority of the sample submissions (63%) came from local services and from studies carried out in Italy, for reasons of proximity: of the 247 cases from Italy, 205 belong to the IP Historical Collection (IPHC) and 42 to the recent IPGB collection (Table 1). Until now all the

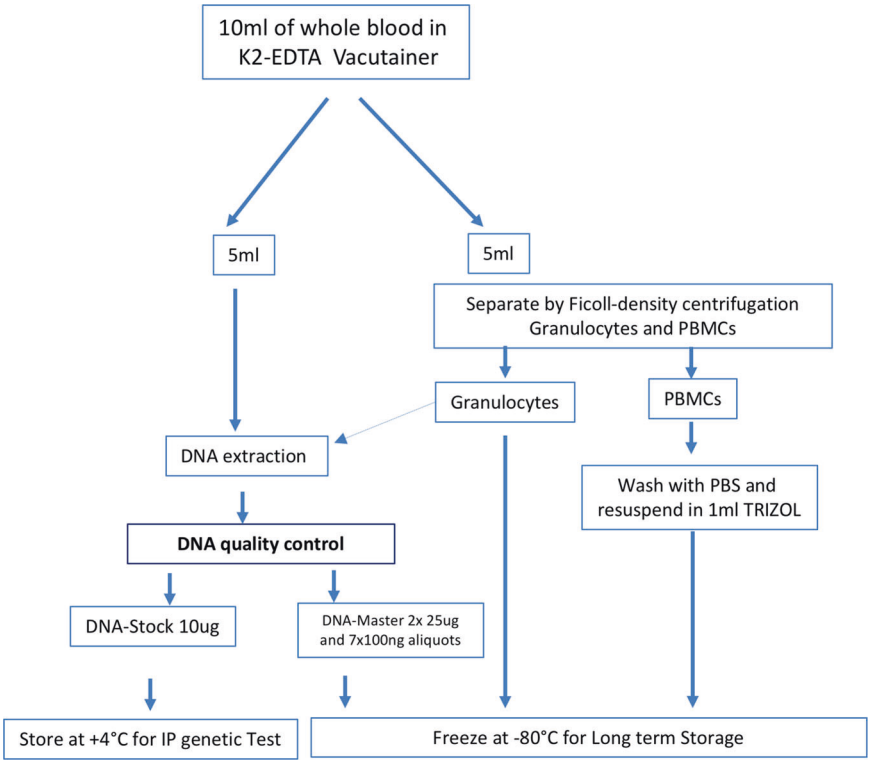

Fig. 2 Details of the sample processing procedures and blood derivative outputs

data and samples have been collected from 200 hospitals, representing 139 cooperating medical institutions located throughout Italy and 61 international structures worldwide (Table 1; Fig. 3). The future inclusion of additional hospitals/centers is scheduled. Any possible participation depends on acceptance to implement all the required workflows (Fig. 1) and the participating institutions must confirm the acceptance of the IPGB policies and of the SOPs, realized through a collaboration agreement.

Overall, the collection (the IPHC plus the new IPGB collection) is composed of 388 cases from worldwide of which mostly are IP-female index cases (342 cases, Fig. 4a), 39 samples are from IP mosaic males (Fig. 4b), and 7 from EDA-ID males (Fig. 4c; Table 2). The IPHC consists 334 cases collected since 2000 to 2015 and the new IPGB consists of 54 cases collected from 2015, the year of the foundation of the biobank, until March 2018 (Table 1). With regard to the historical collection (IPHC), it was built for research purpose by the same researchers now operating at the IPGB. Currently, the IPGB and the patients' organization (IPASSI Onlus) are collaborating to contact the source subjects, in order to obtain new consents for the IPGB project. So far, 63 IP patients from the historical collection have been contacted and, in these cases, requests for informed consent have been submitted. The whole collection (including the IPHC and the new IPGB founded in 2015, hereafter named IPGB) is a genetic biobank and preserves parental information in association with the samples. Indeed, it collects samples from index cases and their parents in a structured pedigree: trios, namely the father, mother, and the index case; and duos, namely the mother and the index case. Where possible, both biological parents and other relatives have been invited to participate to obtain complete family trios (or duos). In March 2018, the IPGB collection included 243 trios and 81 duos with female IP-index cases, 22 trios, and 2 duos with IP-male index cases and 4 trios with EDA-ID index cases (Table 3). Samples from the maternal grandparents of 32 IP females are also available (Fig. 4a). There are 55 samples from non-affected donors (47 sisters and 8 brothers of IP index cases), and 324 samples from mothers (88 IP affected) and 243 from fathers (2 IP-males) (Fig. 4a). For the IP-male index cases, samples from 22 fathers, 24 mothers, and 2 sisters have been collected (Fig. 4b) as healthy relatives. Finally, 3 mothers and 4 fathers are healthy parents of 4 EDA-ID male index cases, and one mother of EDA-ID is IP affected (Fig. 4c, Table 4).

Due to the competence of the founders of the IPGB and the likely high contribution of genetic factors to the phenotypes of interest, the IPGB was mainly conceived as a genetic and data resource. We collected 1326 DNA samples (1132 from a unique withdrawal of blood and 194 from repeated withdrawal of blood of the same donor) extracted from peripheral blood of participants at the cooperating hospitals (Figs. 3 and 4). From 832 donors we have received DNA extracted from blood. From 494 donors we have received peripheral blood samples: for 241/494 we also obtained granulocytes preparation and for 269/494

Table 1 Geographical distribution of index cases collection

Countries	Case collection	IPHC collection	IPGB collection	Cooperative medical institutions
Australia	4	3	1	4
Brasile	1	1	0	1
Bulgaria	1	0	1	1
Croazia	2	2	0	2
Czech Republic	3	3	0	1
Denmark	2	2	0	1
Finland	1	1	0	1
France	20	20	0	2
Greece	1	1	0	1
Japan	1	1	0	1
India	3	2	1	3
Ireland	8	8	0	2
Italy	247	205	42	139
Malaysia	1	1	0	1
Mexico	2	1	1	2
Perù	2	1	1	2
Poland	4	4	0	3
Serbia	4	4	0	1
Singapore	1	1	0	1
Spain	42	40	2	7
Srilanka	1	1	0	1
Sweden	1	1	0	1
Turkey	10	8	2	8
UK	22	22	0	12
USA	1	1	0	1
Venezuela	3	0	3	1
Total	388	334	54	200

IPHC incontinentia pigmenti historical collection

we obtained lymphocytes (Table S1). We have collected six skin biopsies: three from IP-female index cases, two from IP-male index cases, and one from an IP female mother. Fibroblast cell lines from skin biopsies of affected tissue of five index case participants (four IP females and one IP male) are stored in liquid nitrogen. The DNA genomic extraction, performed by using the standard method [11, 12], produced on average yields of 114.09 µg ± 81.29 DNA with, in terms of quality, absorbance ratios of 260/280 nm and 260/230 nm (means 1.89 ± 0.35 and 1.81 ± 0.39, respectively) on 179 samples.

Clinical and genetic profiles of the participants

We collected baseline clinical information through interviews and reviews of medical records. All the clinical data were obtained for each patient through their completion of a clinical IP questionnaire developed by the IPIF, further extended by the FIP, and by the IPASSI, and validated by the three IP association scientific advisors. The common items addressed during each interview included all phenotypic aspects of IP, and the patient's and her/his family history of the diseases. All the surveys were performed by medical coordinators at the cooperating hospital. Clinical data for each affected family members were obtained via medical reports (doctor's letters or a clinical questionnaire) that were provided by the physicians (IPGB reference) or by the patients themselves. Research staff attempted to follow up any missing data through phone calls or e-mails to the patient's families. For families with multiple affected individuals, clinical information was collected for all affected members. The standardization and comprehensiveness of the clinical data collected allows the classification and stratification of the participants with IP/EDA-ID according to clinical phenotype.

The IPGB is nationally recognized as an expertise center for IP molecular diagnosis. We diagnose all the participants for any genetic alteration in the IP locus upon the acquisition of DNA from affected, under-age donors and non-affected siblings in addition to medical examinations. As reported in [1], the genetic and clinical profiles of our IP collection revealed heterogeneity of the IP-associated genotypes and phenotypes. About 30% of the IP index cases (117/388 cases) showed a severe form of IP, involving central nervous system defects (neonatal seizures, ischemic stroke, white matter alterations, etc.), 19.6% of which (23/388, 5.9% cases) had inherited the *IKBKG/NEMO* mutation from an IP mother with a mild phenotype (intrafamilial heterogeneity). These data are collected by the IPGB to perform genotype–phenotype correlation studies that are at present still not applicable in IP. In addition, we registered socio-demographic variables and clinical and genetic detections.

Because IP is an X-linked dominant disease, lethal in males [3] who can survive only when genetic mosaicism for the *NEMO/IKBKG* mutation [6] or hypomorphic *NEMO/IKBKG* variants are present [4], the male-to-female ratio of the affected cases was very low. We calculated 13.4% are males and 86.5% are females. The IP index cases were collected at a mean age of 8.72 years and mode age of 0.083 years (29.88 days from birth), suggesting that the clinical diagnosis is often made very early during infancy (Table S2). We calculated the relative frequency of the age distribution of the appearance of the first dermatological sign corresponding to the clinical diagnosis of IP. The age of onset of the first sign of disease is registered in the clinical questionnaire or, in adults we asked when they had the Stage I/II skin lesions. We observed that in 61% of cases this occurred between birth and 5 years of age ($0 < x < 5$ years) (Table S3), and in 45.8% of cases within the first year

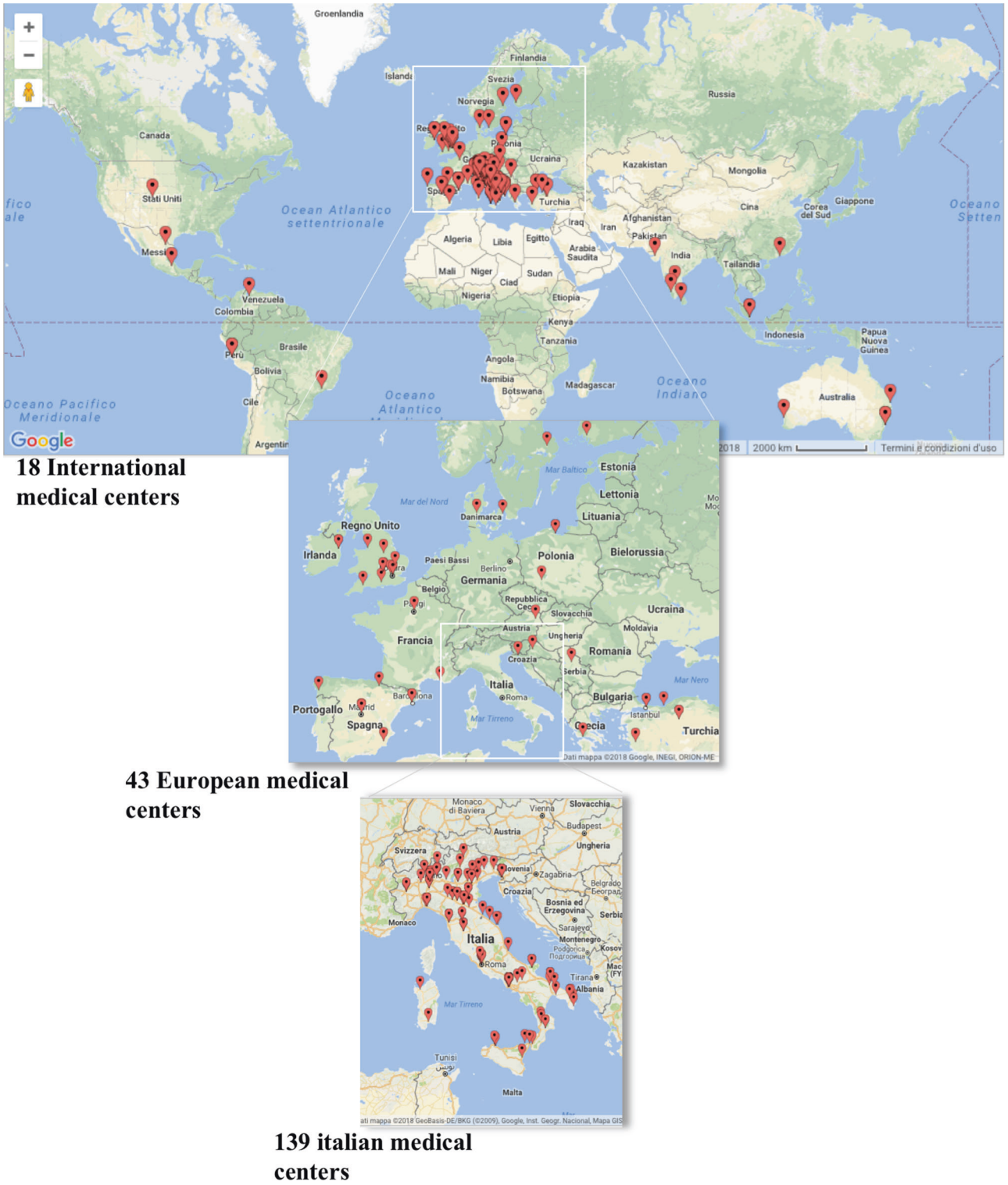

Fig. 3 Geographical distribution of the cooperating hospitals

(Table S4), often between the first and second month of life (20.7%) (Table S5). Currently, the patients' age range from 1 to 58 years, and about the 59% of them are between 5 and 20 years. The analysis of the IP-male index cases showed that the corresponding values were 5.83 years old (mean age) and 1.58 years (mode age) (31 cases available); for the EDA-ID males the age of clinical diagnosis was 2.19 years (Table S2). To analyze the burden of the IP disease on the

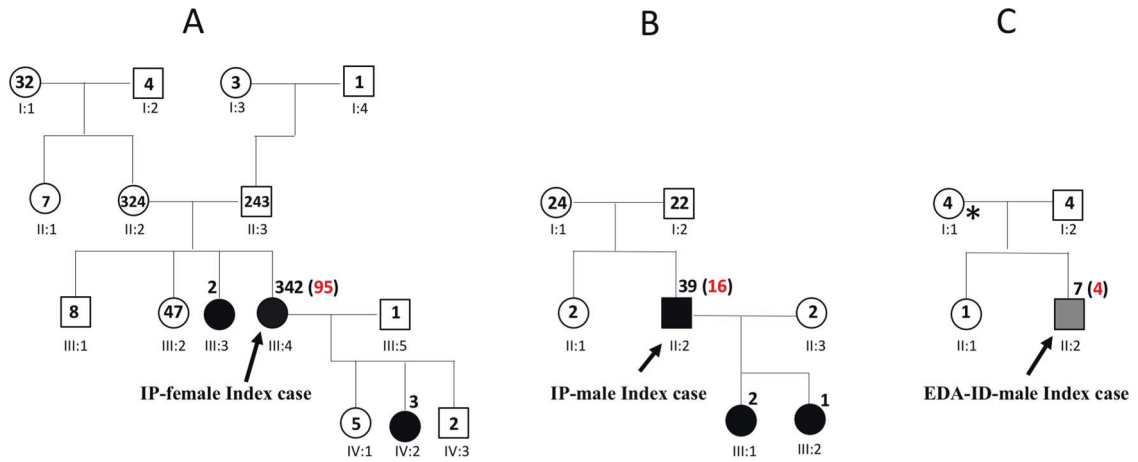

Fig. 4 Collection of IP (**a**), IP-male (**b**), and EDA-ID (**c**) index cases with relatives. In the figure the distribution of donors is shown in relation to the Index cases. The number of cases is indicated inside or near the pedigree symbol where possible. The number of repeated withdrawals is shown in red. All index cases (females and males, IP or EDA-ID) have deposited the DNA sample and a completed IP clinical questionnaire. For all relatives, we collected the DNA sample and only when they were affected we collected the clinical questionnaire. The arrows indicated index case. Asterisk: The mothers of EDA-ID are three healthy and one IP affected

Table 2 Spectrum of the disease groups

Disease groups	Case collection	IPHC collection[a]	IPGB pending	IPGB collection
IP-female index cases	342	233	63	46
IP-male index cases	39	33	0	6
EDA-ID male index cases	7	5	0	2
Total	388	271	63	54

[a]To be required for the permission for depositing in IPGB

Table 3 Spectrum of the genealogical data of each index case

Disease group	Case collection	DUOS (mother and index case)	TRIOS (parents and index case)	SINGLETON (only index case)
IP-female index cases	342	81	243	18
IP-male index cases	39	2	22	15
EDA-ID male index cases	**7**	0	4	3
Total	388	83(61)[a]	269(178)[a]	36

[a]Number of the index cases in simplex versus multiplex families

Italian population, we calculated the birth prevalence of IP female affected subjects from 2002 to 2017 (Table S6) and we observed that the mean birth prevalence is 1.6×10^{-5}, higher than the value of 1.2×10^{-5} reported in [2] as the estimated incidence of European cases. We noticed that the percentage of sporadic cases is 70%.

Discussion

In this paper, we have described the largest IP cohort in the world in terms of data and biological samples collection, the IPGB. To the best of our knowledge the IPGB is one of the largest rare-disease-oriented collections. All information collected in the IPGB is precious for the following reasons:

1. The IPGB collects trios (in some cases multiple generations) in order to preserve the genetic and phenotypic association between biological samples;
2. The IPGB produces and collects IP molecular test results, thus meaning that the samples and data of IP index cases can be harmonized for IP locus alterations;
3. The IPGB collects clinical data, updated over time, enabling genotype–phenotype studies;
4. The IPGB collects socio-demographic data, facilitating epidemiological study.

As an example, we observed that the median age of appearance of the first IP dermatological sign is at day 29.88 from birth that represents an important finding in terms of the clinical diagnosis of IP. Such analysis

Table 4 Distribution of phenotype of relatives

	Index cases	Affected relatives	Healthy relatives	Total
IP-female index cases	342			342
Mother		88	236	324
Father		0	243	243
Sister		2	47	49
Brother		0	8	8
Child of IP-index case		3	7	10
Maternal grandmother		3	29	32
Maternal grandfather		0	4	4
*Paternal*grandmother		0	3	3
Paternal grandfather		0	1	1
Aunts		0	7	7
IP-male index cases	39			39
Child of IP male-index case		3	0	3
Mother		0	24	24
Father		0	22	22
Sister		0	2	2
IP phenotype total	381	99	633	1113
IP cases husband and wife		0	3	3
EDA-ID male index cases	7			**7**
Mother		1	3	4
Father		0	4	4
Sister		0	1	1
EDA-ID phenotype total	7	1	8	16

extended to the other relevant signs of IP could be to set up a clinical follow-up protocol for IP patients to avoid psychological stress in the management of the disease. To realize this study of natural history of IP disease is essential the involvement of patients' organizations because they raise awareness, interest, and trust in biobanks. This has been instrumental in gaining the critical mass of samples essential for research into this rare disease. The IPGB is allied with three patients' associations, two of which are directly involved in the enrolment of participants and in raising funds for the biobank project (IPASSI and IPIF), with the third serving as an advisor and collaborator, fostering the dissemination of information about the IPGB and helping to fund the collateral research activity of IPGB staff members.

The role of the IPGB is not only to collect samples and data from patients with IP but also to use them for research purposes. The IPGB enables scientists in different countries to propose interdisciplinary collaborations on common research projects based on the complementary strengths and sharing of skills, with a clear benefit for patients. The IPGB aims to reduce the time period required for the recruitment of appropriate samples and data, a very complex phase especially in the case of rare diseases. The standardized collection of samples and information of the IPGB can be used in transnational research proposals to accelerate diagnosis or in omic or multiomic integrated approaches for the discovery of genetic and/or phenotypic biomarkers to predict the onset of severe forms of IP and for the development of personalized therapy. Access to the IPGB can be requested by submitting a research application to the IPGB Scientific Board, which consists of experts in the fields of IP, in epidemiology, human genetics, basic research, ethics, and health care law. The use and access regulations and policies comprise (1) general rules for cooperative studies, (2) forms and templates to submit the research proposals, (3) a well-defined process of decision, and (4) publication guidelines. The sample donors have transferred the right to use their biomaterials for scientific purposes to the IPGB, which reserves the right to use such samples until the time the sample donors should withdraw their consent.

In conclusion, a fundamental prerequisite for the construction of a valuable collection of samples was the agreement with patients' associations, which illustrated the operating rules to the patients and their families giving them the opportunity to express opinions, needs, and concerns. In addition, the IP patients' organizations have kept the associated families and referring clinicians informed of the IPGB's activities and policies, promoted the recruitment of patients and relatives, and raised funds to support the IPGB in order to improve research on rare disease.

Access to the IPGB

A research application to the IPGB Scientific Board, which includes a representative of the IP patients' organizations, has to be submitted. The final decision is made by the IPGB Steering Committee and by the representatives of the institution where the samples have been collected. Decision criteria are the project's feasibility, the clear indication of well-defined objectives and benefit for patients, the inclusion of the applicant within the IP research community, and a stringent scheduling. Appropriate expertise in the field of interest is therefore a prerequisite. Upon approval, collaborating research groups and institutions must agree to make their data available to research community for future studies upon completion and publication of their study.

Academic and noncommercial researchers are eligible to apply and to use samples and/or anonymized data.

Application and review procedures are in place. Researchers should present a sound scientific rationale for the proposed study, have a good research track record, and be supported by their institution. The following types of studies will be prioritized: studies testing or generating new hypotheses on the pathophysiology of IP/EDA-ID; studies improving diagnosis and phenotyping; and basic science studies, e.g., pharmacological in vitro studies, potentially leading to clinical trials of therapeutic approaches.

The release of data and/or samples will be enabled upon the signing of a materials transfer agreement and/or data transfer agreement, and any such researchers must not attempt to identify any individuals.

The IPGB has been planned to operate on a cost recovery basis to replace any released samples, thereby helping to ensure the long-term sustainability of the resource.

Additional information and application forms can be found on the Biobank website: http://ipgb.cnr.it.

Sustainability

Since January 2015 the IPGB was supported by the IPASSI and IPIF.

Bioresource location

The project is led and managed by the IP team, located within the IGB-ABT CNR. The participants' data are securely stored at the IPGB database in locked files and in a bespoke database. Deidentified biological samples are stored at the IPGB Biobank.

Bioresource contact: incontinentia.pigmenti@igb.cnr.it

Bioresource URL http://www.igb.cnr.it/ipgb

The IPGB Biobank is an IP project and, therefore, the bioresource's URL is linked to the IP group webpage. Researchers are encouraged to seek information through this site and/or contact the IP team.

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Author contributions Conceived and designed the research study: FF, MBL, and MVU. Conducted the collection: VV and DF. Analyzed the data: FF and MVU. Wrote the manuscript: FF, AP, and MVU.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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