A

T C C A A C T A C G T A A A A T A T
205　　　211　　　217

Leukemic
cells

c.1141T>C p.C381R
Homozygous

T C C A A C T A T G T A A A A T A T
206　　　211　　　216　　　22:

Leukocytes
in remission

wild type

B

C T T A C A T C C C G G C A G G T A C
286　　291　　296　　301

JMML
cells

c.1222T>C p.W408R
Homozygous

C T T A C A T C C G G C A G G T A C
283　　289　　295

Buccal
cells

T/C

c.1222T>C p.W408R
Heterozygous

Fig. 1. *CBL* mutations identified in patients. (A) Sequencing charts of leukemic cells and peripheral blood at complete remission from Patient PL1. (B) Sequencing charts of JMML cells and buccal mucosa from Patient PL52.

CBL mutations are associated with an 11q-acquired uniparental disomy (aUPD) involving the *CBL* locus, which converts these mutations into a homozygous state [3]. An SNP array analyzed the difference in DNA between PB samples at disease onset (leukemia cells) and leukocytes in remission. The analysis revealed a UPD 11q13.1qter that contained the *CBL* locus only in the T-ALL sample (Table 1). In addition, homozygous deletions of 4q25, which encodes the *LEF1* gene, and of 9p21.3, including the region that encodes *CDKN2A*, were detected. A UPD at 9pter-p13.3 was also observed. The deletion of 14q11.2, which encodes *TCRA*, and the one-copy deletion in 7q34 (including *TCRB*) that were observed in the DNA from the T-ALL cells may be due to a TCR rearrangement. The effects of the gain of the immunoglobulin light chain at 2p11.2 and the gain at 17q12, which contains *CCL3L3*, *CCL4L2* and *TBC1D3*, are unknown.

3.4. ELK and c-Jun transactivation in cells expressing mutant CBL proteins

The *CBL* p.C381R mutation that was identified in one T-ALL patient has also been identified in a JMML patient and a single patient with MDS [5,23]. However, a functional analysis of p.C381R has not been performed.

A WT allele and the two *CBL* mutants, p.C381R and p.R420Q, were introduced in NIH 3T3 cells, and ELK transactivation was examined to elucidate the activation of the ERK pathway. The allele p.R420Q was used as a positive control because this mutant activates ERK [12]. ELK is a transcription factor that is phosphorylated by activated ERK and that binds the serum response element in the promoters of the immediate early genes, including c-FOS [24]. ELK transactivation was remarkably enhanced in cells expressing

Table 1
Genetic abnormalities of T-ALL at diagnosis.

Chromosomal sites	Copy number state (leukemia)	Copy number state (germline)	Loss/gain	Size (kb)	[a]Start_Linear_Position	[a]End_Linear_Position	Genes included in the region
11q13.1qter		2	UPD	69575.00	64877380	134452384	*CBL* and others
14q11.2	1	2	Loss	369.94	21660717	22030660	*TCRA, TCRD, TCR*
17q12	3	2	Gain	192.98	31460821	31653797	*CCL3L3, CCL4L2, TBC1D3*
2p11.2	4	3	Gain	460.63	88914227	89374858	*IGK@*
2p11.2	3	2	Gain	109.16	89753412	89862571	*IGK@*
4q25	0	2	Loss	104.82	109199454	109304271	*LEF1*
7q34	0	2	Loss	491.97	141711730	142203700	*TCRB*
9p11.2	3	2	Gain	127.88	44667843	44795721	
9p21.3	0	2	Loss	117.67	21864256	21981923	*CDKN2A*
9pterp13.3		2	UPD	33701.54	1	33701540	*CDKN2A* and others

Abbreviations: CBL, Cas-Br-M (murine) ecotropic retroviral transforming sequence; TCRA, T cell receptor alpha; TCRD, T cell receptor delta; CCL3L3, chemokine ligand 3-like 3; CCL4L2, chemokine ligand 4-like 2; TBC1D3, TBC1 domain family, member 3; IGK@, immunoglobulin kappa locus; LEF1, lymphoid enhancer binding factor 1; TCRB, T cell receptor beta; CDKN2A, cyclin-dependent kinase inhibitor 2A.

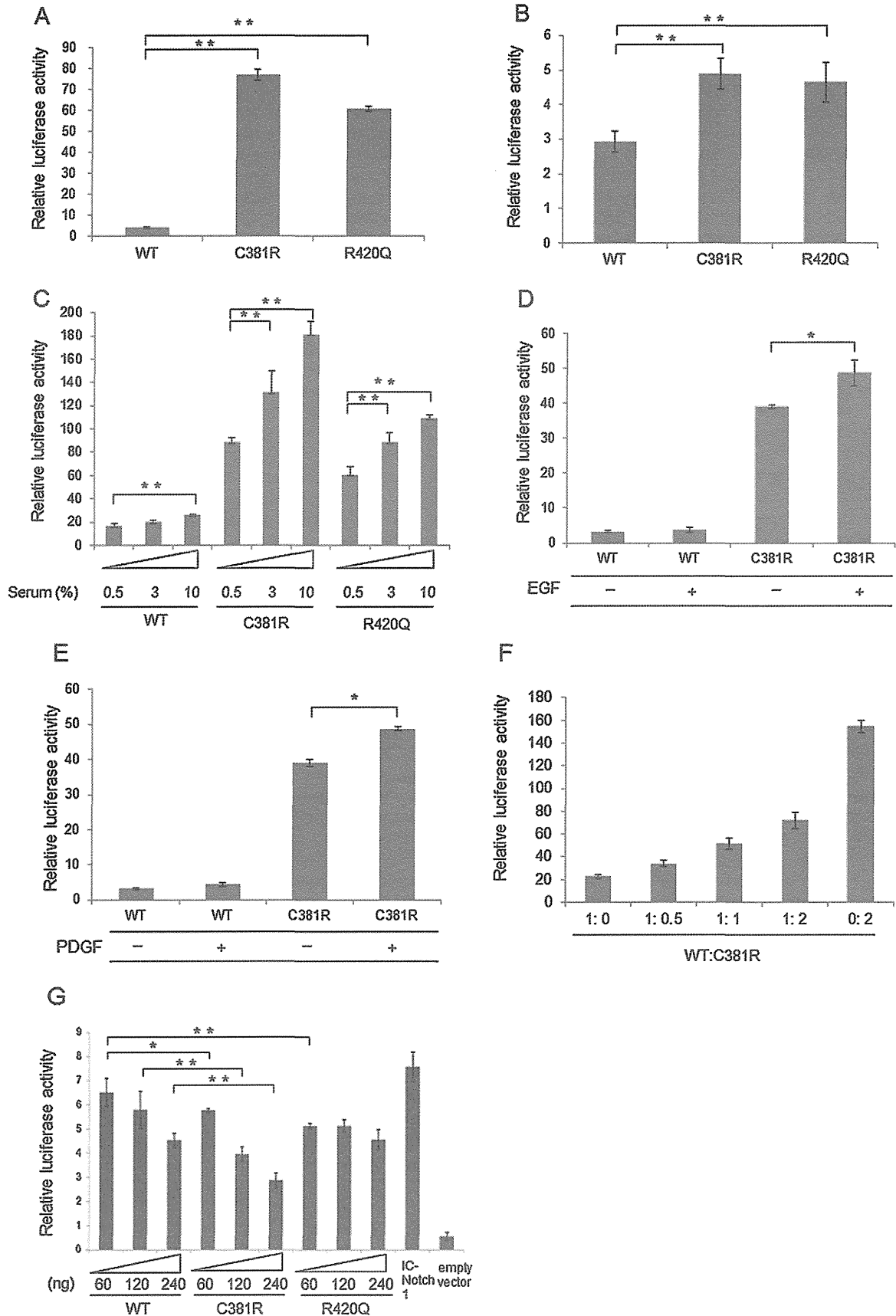[a] Denoted by NCBI 36 reference human genome (hg18).

161

Fig. 2. ELK, c-Jun, and HES1 transactivation in cells expressing mutant CBL proteins. The results are expressed as the mean and standard deviation of mean values from triplicate samples. **P < 0.01 and *P < 0.05 determined with Student's t-test. (A) ELK transactivation in cells with WT CBL and mutant CBL. (B) c-Jun transactivation in cells with WT CBL and mutant CBL. (C) ELK transactivation in NIH 3T3 cells transiently expressing WT CBL and C381R CBL in DMEM that contained the indicated concentrations of newborn calf serum (NCS). (D) For EGF stimulation, the ELK transactivation level in cells expressing p.C381R stimulated with EGF was significantly enhanced compared

p.C381R and p.R420Q in DMEM containing 10% NCS compared with WT *CBL*-transfected cells (Fig. 2A). The transactivation of the transcription factor c-Jun was examined in NIH 3T3 cells. Studies have shown that c-Jun activity is upregulated by the phosphorylation of c-Jun NH2-terminal kinases (JNK) [25]. In this case, c-Jun transactivation was significantly enhanced in cells expressing p.C381R and p.R420Q in DMEM containing 10% NCS (Fig. 2B). These results demonstrate that *CBL* mutants activate the ERK and JNK pathways, possibly via the upstream activation of RAS in the presence of serum.

ELK transactivation was examined in different NCS concentrations to evaluate the effect of serum concentration. ELK transactivation in cells expressing p.C381R and p.R420Q was enhanced in an NCS concentration-dependent manner (Fig. 2C). Significant ELK activation was observed in cells expressing p.C381R and p.R420Q in DMEM with 0.5% NCS. The effects of EGF and PDGF on ELK transactivation were examined in cells expressing WT *CBL* or p.C381R *CBL*. The ELK transactivation levels in cells expressing p.C381R that were stimulated with 100 ng/ml EGF (Fig. 2D) or 100 ng/ml PDGF (Fig. 2E) were significantly enhanced compared with those of unstimulated cells. However, EGF and PDGF stimulation did not significantly alter the ELK transactivation levels in cells expressing WT *CBL*. These results suggest that the p.C381R mutation constitutively activates the RAS pathway.

CBL mutations affect endogenous WT CBL in a dominant-negative manner [7]. NIH 3T3 cells were co-transfected with WT CBL and C381R to evaluate the effect of p.C381R on WT CBL. The hypertransactivation response that was induced by the CBL mutant was abolished by the co-transfection of WT CBL (Fig. 2F), suggesting the pathogenic importance of the WT *CBL* allele loss.

3.5. HES transactivation in cells expressing mutant CBL

HES1 is a target gene for NOTCH1. WT or mutant CBL constructs were transiently transfected in NIH 3T3 cells with the HES-Luc reporter and a constitutively active intracellular domain of NOTCH1 (ICN1) construct. ICN1 expression significantly increased the transactivation of HES (Fig. 2G, IC-NOTCH1 lane). The introduction of CBL WT or mutants significantly reduced the HES transactivation levels compared with cells expressing ICN1 (Fig. 2G). The HES1 transactivation levels in cells expressing p.C381R were significantly decreased compared with CBL WT-expressing cells.

4. Discussion

In this study, a homozygous p.C381R mutation and a UPD of the region that included *CBL* were identified in T-ALL cells, and a heterozygous germline p.W408R mutation was identified in one patient with JMML. An additional mutation analysis identified two *NOTCH1* mutations and homozygous deletions of *LEF1* and *CDKN2A* in T-ALL cells. A functional analysis revealed that cells expressing the p.C381R mutant constitutively transactivated ELK and c-Jun. Co-transfection of WT and the p.C381R mutation in NIH 3T3 cells revealed that WT inhibited the ELK-activating effects of p.C381R. The HES1 transactivation levels in cells expressing p.C381R were significantly decreased compared with CBL WT-expressing cells, suggesting that this *CBL* mutation plays a role in NOTCH signaling pathway.

CBL mutations are rare in ALL patients. Recently, mutations in *CBL* have been identified in 2 infant ALL patients with *MLL* gene

rearrangements [26]. Nicholson et al. analyzed the linker-RING domains of CBL in a cohort of 180 diagnostic and 46 relapsed ALL patients and identified deletions/insertions of *CBL*, including the splicing acceptor or donor site of exon 8 in three ALL samples [27]. *CBL* mutations in ALL may promote the proliferation of leukemia cells by activating the RAS pathway ([27] and our study). Alternatively, our HES-reporter assay in cells that expressed the *NOTCH1* constitutive active mutant showed that *CBL* p.C381R downregulated the NOTCH1 signaling pathway, suggesting that the *CBL* p.C381R mutation may contribute to leukemogenesis through interaction with NOTCH1. The relationship between CBL and NOTCH1 has not been elucidated, but one report has demonstrated that CBL promotes the ubiquitin-dependent lysosomal degradation of membrane-associated NOTCH1 [28]. In the case of NOTCH3, its interactions with pre-TCR lead to the recruitment and persistence of the CBL to the lipid rafts in thymocytes from mice expressing the constitutively active intracellular domain of NOTCH3, which suggests that CBL may regulate the NOTCH3 and pre-TCR relationship during T-cell leukemogenesis [29]. Further analysis will elucidate the role of the *CBL* mutation in T-ALL leukemogenesis.

Somatic and germline *CBL* mutations have been clustered in either the linker domain or the RING finger domain (Fig. 3). The loss of the ubiquitination of activated receptor tyrosine kinases is thought to contribute to the transforming potential of leukemia-associated mutant CBL proteins. The distributions of somatic and germline mutations were almost similar. However, Y371, which is a hot spot for *CBL* mutations in JMML, is rarely mutated in other myeloid malignancies [5]. The germline p.W408R mutation has been identified in a patient with JMML [5]. Individuals with germline *CBL* mutations display a variable combination of dysmorphic features, including mild hypertelorism, a short upturned nose, a deeply grooved philtrums and thick lips, which are reminiscent of the facial gestalt of NS [10]. Patient PL52, who had a germline p.W408R mutation, had normal development and no dysmorphic features at 15 months of age. However, her young age may have precluded any firm conclusions. Long-term follow-up examinations and an analysis of wider cohorts is necessary to further characterize the phenotypic spectrum that is associated with germline mutations in *CBL*.

The effect of mutant CBLs on ERK activation depends on the level of endogenous WT CBL [7,30]. Therefore, we examined ELK transactivation in NIH 3T3 cells, which have low endogenous CBL protein expression [31]. Our study demonstrated that ELK transactivation in cells expressing p.C381R decreased with increasing WT CBL expression. These results suggest that the p.C381R mutation functions in a dominant-negative manner or as a gain-of-function mutation.

In this study, SNP array analyses of samples from leukemia cells and leukocytes obtained from patients in remission revealed a copy number imbalance that was specific for leukemia cells. The homozygous deletion of the entire *LEF1* gene was identified in the T-ALL sample with the *CBL* mutation. LEF1 is a member of the lymphoid enhancer factor/T-cell factor family of DNA-binding transcription factors that interact with nuclear β-catenin in the WNT signaling pathway [32]. Monoallelic or biallelic *LEF1* microdeletions have been identified in 11% (5 of 47) of primary samples from the diagnostic specimens of 47 children with T-ALL, using high-resolution array comparative genomic hybridization [33]. The homozygous deletion of *CDKN2A* and

with unstimulated cells. (E) For PDGF stimulation, the ELK transactivation level in cells expressing p.C381R stimulated with 100 ng/ml PDGF was enhanced compared with unstimulated cells. (F) Co-transfection of WT CBL and C381R CBL. The hypertransactivation response induced by CBL p.C381R was abolished by the co-transfection of WT CBL. (G) Mutant CBL constructs in pCMV6 were transiently transfected in NIH 3T3 cells with the HES-Luc reporter and the intracellular NOTCH1 (ICN1) construct where appropriate.
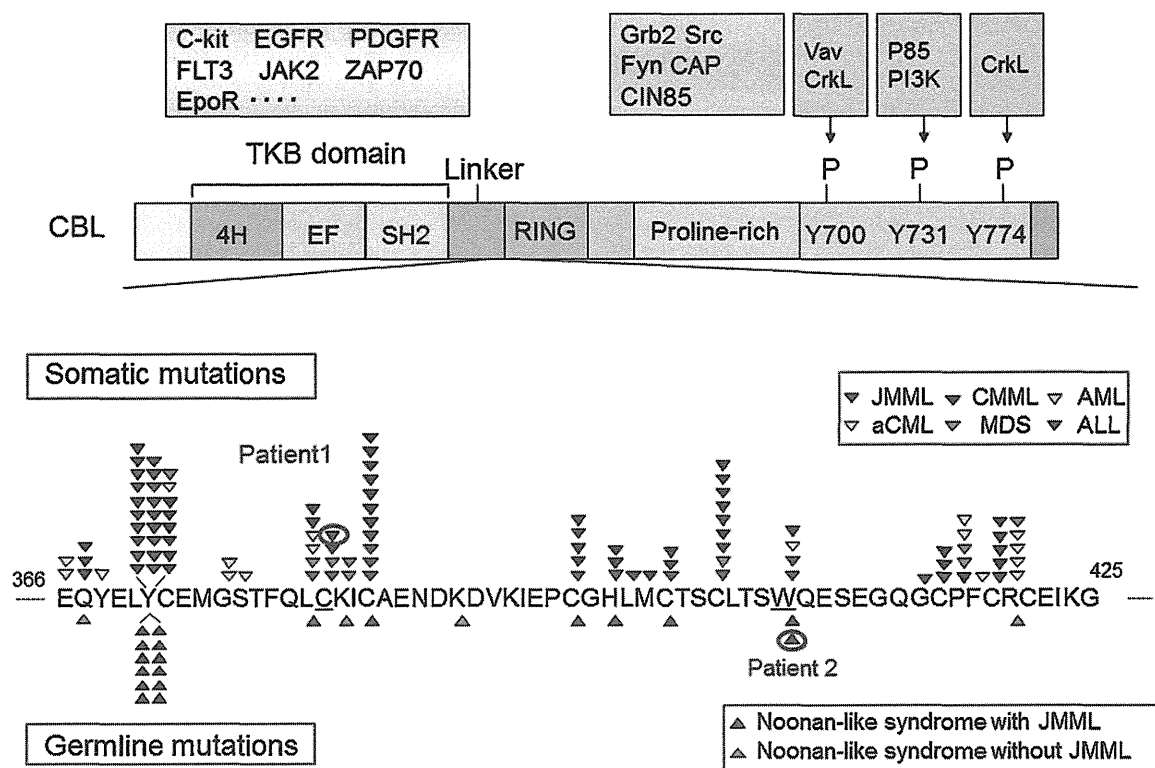
Fig. 3. The CBL structure and mutation spectrum. CBL comprises an N-terminal tyrosine kinase binding domain (TKB) connected by a linker to the RING-finger domain implicated in E2 enzyme binding. These domains are followed by a proline-rich region and a C-terminal portion containing tyrosine phosphorylation sites. The molecular interaction of CBL with cytokine receptors and other signaling molecules are also shown on top. The CBL mutations identified in hematologic malignancies partially overlap with those identified in the germline.

CDKN2B, which are frequently inactivated in various hematological malignancies [34], was also identified in the T-ALL sample. A comparison of the copy numbers of DNA samples from leukemia cells and germline DNA will help to highlight the abnormalities in leukemia.

In conclusion, we identified a CBL p.C381R mutation in leukemia cells from one patient with T-ALL. A functional analysis demonstrated that the mutation constitutively activated the RAS-MAPK pathway and inhibited the constitutive activation of the NOTCH signaling pathway. Further studies will be needed to determine the relationship between CBL and leukemogenesis.

Conflict of interest statement

All authors declare no competing financial interests.

Contributions. YS, YA and SK designed the research study; YS, HM, HM and TN performed the research; MI, TR, YS, ST and SK provided patients samples; YS, HM, HM and JPM analyzed the data; YS, YA and YM wrote the paper.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.leukres.2012.04.018.

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A Case of Almost Unilateral Focal Dermal Hypoplasia Resulting From a Novel Mutation in the *PORCN* Gene

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Focal dermal hypoplasia (FDH), also known as Goltz syndrome, is an X-linked dominant disorder of ecto-mesenchymal development. In general, FDH presents with characteristic linear streaks of hypoplastic dermis and various abnormalities in some organs (1). Although this disorder is normally lethal in male patients, approximately 10% of cases of FDH are male, and most represent post-zygotic mosaicism (2–4).

The molecular basis of FDH involves mutations in the *PORCN* (human porcupine locus MG61/PORC) gene (5). *PORCN* is a member of the porcupine (porc) gene family and is located on chromosome Xp11. 23 (1, 6). The gene is thought to encode an O-acyltransferase that catalyses cysteine N-palmitoylation and serine O-acylation in the endoplasmic reticulum that allows membrane targeting and secretion of several Wnt proteins that have key roles in embryonic tissue development, notably for fibroblast proliferation and osteogenesis (1, 7).

We describe here a case of FDH with a novel deletion mutation at exon 14 (c.1179_1193 del) detected by PCR-sequencing analysis of the entire coding sequences of the *PORCN* gene. Interestingly, in this case, the characteristic symptom was prominent in the patient's left hemibody. To the best of our knowledge, this is the second case report that reveals a mutation of the PORCN gene in a patient with almost unilateral FDH.

CASE REPORT

A 2-year-old Japanese girl visited our outpatient clinic for hypopigmented and atrophic linear skin lesions on the trunk and extremities. She had been surgically treated one year before for syndactyly of the left middle and ring finger. On her first visit to our hospital, physical examination disclosed hypo-pigmented patches of dermal hypoplasia distributed along Blaschko's lines on the arms, legs and trunk (Fig. 1). Interestingly, these symptoms in lesions were prominent on her left hemibody. Her hair was sparse and brittle and she had non-scarring alopecia. In addition, extensive dental caries was noted. From the above information, we diagnosed this patient as having almost unilateral FDH.

To confirm the diagnosis, we examined the *PORCN* gene for mutations. After receiving informed consent, DNA was extracted from the peripheral blood sample taken from affected individual using standard methods. Primers were designed to amplify individual exons and the flanking intron of the *PORCN* gene, as described previously (1). PCR-sequencing analysis of the entire coding sequences of *PORCN* revealed c.1179_1193 del mutation at exon 14 (Fig. 2). This variant was predicted to result in altered residues 394–412 and to produce a truncated protein (p.G394 fs X20). The sequence chromatograms showed low signal intensities at the site of mutation, indicating post-zygotic mutation. To verify the mosaic state, the PCR products were cloned into the pCR4 TOPO TA Cloning Vector (Invitrogen, Karlsruhe, Germany), transfected. *E. coli* clones were chosen and subjected to colony PCR, and PCR products from individual clones were sequenced. By DNA sequence analysis of selected clones we could assign the wild-type and the mutant sequence to either of the 2 banding patterns and identified a ratio of 5/26 (~19%; mutant vs. wild-type sequence).

DISCUSSION

FDH is characterized by linear and whorled lesions of hypoplastic dermis and variable abnormalities of organs including the bone, nails, hair, limbs and teeth (1–3). Cutaneous features that predominate at birth are red atrophic, linear lesions following Blaschko's lines

Fig. 1. Skin manifestation in the patient. (A) Hair loss on the left temporal scalp. (B, C) Small whitish depigmented spots, slightly depressed from the skin surface, distributed linearly on the left side of the armpit (B) buttocks and lower limbs (C).
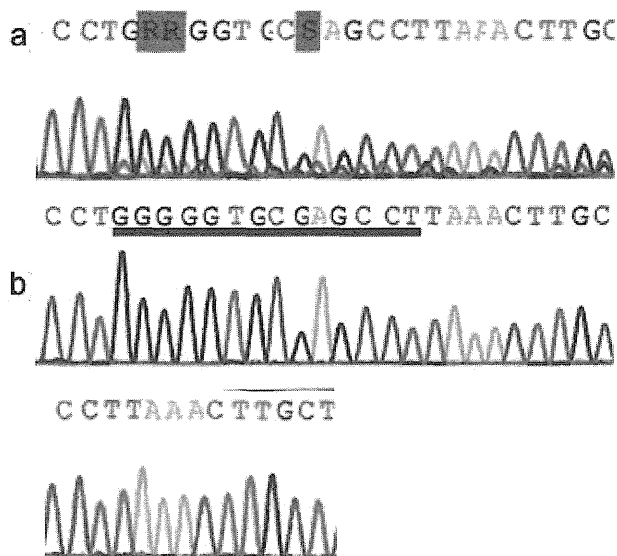
Fig. 2. Molecular basis of focal dermal hypoplasia (FDH). (a) Sequencing of exon 14 of the *PORCN* gene in the patient's DNA reveals c.1179_1193 del mutation. (b) Sequencing of control DNA shows the wild-type and mutant sequences.

and telangiectasias. It is presumed to be lethal in men who are fully hemizygous for a mutation in *PORCN* (4). FDH generally involves both sides of the body. Only 6 cases of unilateral FDH have been published (8–10). Recently, Maalouf et al. (8) reviewed all cases of unilateral FDH and found no side predeliction. One case was male (17%) and the other 5 were females (83%). Moreover, they described that gene sequence analysis on the *PORCN* gene detected a novel heterogeneous mutation in exon 10, c.854-855insACCTGAC [p.T285fsX316]. In addition, a substantial majority of cases of FDH have previously been reported to result from post-zygotic mutations (11). In the present case, sequencing analysis of the entire coding sequences of *PORCN* gene revealed a novel deletion mutation at exon 14 (c.1179_1193 del). As a ratio of 5/26 mutant vs. wild-type alleles was found, a post-zygotic mutation is the likely cause of the syndrome in our case. A germline mutation would have resulted in a 1:1 distribution of both alleles in functional X-chromosome-mosaicism as described previously by Bornholdt et al. (11). Although

we did not perform the X-chromosome inactivation analysis using DNA extracted from the affected and non-affected skin, the present case suggests that the deletion mutation at exon 14 might be connected with the unilateral involvement of FDH. To confirm our hypothesis, further case reports and molecular studies of patients with unilateral FDH are necessary.

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Human Variome Project Country Nodes: Documenting Genetic Information within a Country[†]

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ABSTRACT: The Human Variome Project (http://www.humanvariomeproject.org) is an international effort aiming to systematically collect and share information on all human genetic variation. The two main pillars of this effort are gene/disease-specific databases and a network of Human Variome Project Country Nodes. The latter are nationwide efforts to document the genomic variation

reported within a specific population. The development and successful operation of the Human Variome Project Country Nodes are of utmost importance to the success of Human Variome Project's aims and goals because they not only allow the genetic burden of disease to be quantified in different countries, but also provide diagnosticians and researchers access to an up-to-date resource that will assist them in their daily clinical practice and biomedical research, respectively. Here, we report the discussions and recommendations that resulted from the inaugural meeting of the International Confederation of Countries Advisory Council, held on 12th December 2011, during the 2011 Human Variome Project Beijing Meeting. We discuss the steps necessary to maximize the impact of the Country Node effort for developing regional and country-specific clinical genetics resources and summarize a few well-coordinated genetic data collection initiatives that would serve as paradigms for similar projects.

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KEY WORDS: human variome project; country nodes; national/ethnic mutation databases; populations; genomic variation; genomics

Introduction

The Human Variome Project (HVP; http://www.humanvariomeproject.org) is an international initiative to systematically identify and document pathogenic and benign genomic variations worldwide. The project aims to extract, organize, and curate genome variation data from clinical, medical, and research laboratories. The ultimate goal of this project is to improve translational research strategies and clinical decision-making processes. The HVP is a collaborative consortium of internationally renowned scientists and healthcare professionals working on genomics that are organized into working groups around specific topics to produce standards, specify systems requirements, and address related issues [Cotton et al., 2007; Kaput et al., 2009]. The two main avenues of genome variation data collection are (1) gene/disease-specific collection, and (2) country-specific collection.

The HVP Country Nodes (Table 1) are key to the success of the HVP as they would allow: (1) data sharing among diagnostic laboratories and clinics in each country to support nationwide genetic testing services; (2) data archiving in National/Ethnic mutation databases [NEMDBs; Cotton et al., 2009; Patrinos, 2006] to estimate the genetic burden in each country, hence contributing to better targeting of healthcare planning and policy development; and (3) data sharing between NEMDBs and locus-specific databases (LSDBs) or central data repositories (e.g., NCBI and EBI), in a country-specific ethically compliant manner. Building capacity at the national level also provides a necessary platform for engagement across borders. However, there are many issues involved in establishing and, most importantly, running such a Node.

Here, we report the discussions and recommendations that resulted from the inaugural meeting of the International Confederation of Countries Advisory Council (ICCAC), held on 12th December 2011, during the 2011 HVP Beijing Meeting, in which all existing HVP Country Nodes were represented in addition to several human genetics societies (Box 1) to better pursue the HVP aims and goals through the HVP Country Nodes.

Human Variome Project Country Nodes in Practice

At present, 12 HVP Country Nodes are represented in the International Confederation of Countries Council of HVP (Table 1), which have been incorporated in HVP from 2010 and are particularly variable in their stage of development. The outline of establishing an HVP Country Node and the recommendations to collect country-specific genetic data are described elsewhere [Al Aama et al., 2011; Patrinos et al., 2011] and as such will not be discussed here.

The *Australian HVP Node* automatically collects the results of genetic tests performed by Australian Diagnostic laboratories. This genetic data set is stored within a secure repository, which can only be accessed by diagnostics laboratories and medical clinics to assist the diagnosis of patients [Al Aama et al., 2011]. Using this dataset, diagnostics laboratory staff is able to access the cumulative knowledge of every diagnostic laboratory in Australia. The molecular dataset is linked to clinical data housed at hospitals and clinics throughout Australia via the BioGrid service (http://www.biogrid.org.au) and can be accessed by researchers who have obtained approval from a Human Research Ethics Committee.

The *Austrian HVP Node* is at the moment part of the Austrian Human Genetic Society (Österreichische Gesellschaft für Humangenetik, ÖGH). In Austria, there is neither central registration of patient data nor centralized collection of genetic data. As such, the aim of the Austrian HVP Node will be to create a centralized national platform for sharing genetic knowledge and data and providing an easy way to submit variation data directly to international databases, potentially also of interim storage of such data.

The *Belgian HVP Node* is represented by the Belgium Society of Human Genetics, which has Board members from all eight centers offering genetic testing services in the country. The Belgian Plan for Rare Diseases was formulated under the chairmanship of emeritus Professor Jean-Jacques Cassiman. This foresees a National registry, which will be coordinated by the Ministry of Social Affairs and public Health. Presently, there are local disease-specific registries, and some laboratories are entering information into LSDBs. Centers of excellence in the field of cystic fibrosis, neuromuscular disorders, and metabolic diseases do exist and more are foreseen.

Table 1. Existing HVP Country Nodes (as of End of December 2011)

	Node	Node representative	URL	NEMDB[a]
1	Australian	Timothy Smith	http://www.hvpaustralia.org.au	Yes
2	Austrian	Martina Witsch-Baumgartner	http://www.oegh.at	N/A
3	Belgian	Thomy de Ravel	http://www.beshg.be	N/A
4	Chinese	Ming Qi	http://www.genomed.org/lovd	Yes
5	Cypriot	Andreas Hadjisavvas	N/A	N/A
6	Egyptian	Sherifa Ahmed Hamed	http://www.goldenhelix.org	Yes
7	Hellenic	George P. Patrinos	http://www.goldenhelix.org	Yes
8	Kuwaiti	Fahd Al Mulla	http://www.al-mulla.org	Yes
9	Malaysian	Zilafalil bin Alwi	http://1mhgvc.kk.usm.my	N/A
10	Nepalese	Tilak Shreshta	N/A	N/A
11	Spanish	María-Jesús Sobrido	N/A	N/A
12	Vietnamese	Chí Dũng Vũ	N/A	N/A

[a]Existence of a centralized HVP Country Node-specific NEMDB.
N/A, not yet available.

The *Chinese HVP Node* has a central role in the entire project. The Node members have already built several databases of genetic diseases, initially focusing on databases of ion-channel cardiac arrhythmias (LQTS1-12, including KCNQ1, KCNH2, SCN5A, KCNE1, KNCE2, KCNJ2, etc.), *BRCA1* and *BRCA2*, mismatch repair genes (*MMR*), and *APC* genes for breast cancer, Lynch syndrome, and familial adenomatous polyposis (FAP), respectively, in the Chinese population using the Leiden Open Variation Database (LOVD) format [Pan et al., 2011; Zhang et al., 2010; http://www.genomed.org/LOVD]. Data mining was performed by a group of students formed for this purpose using PubMed and some Chinese search engines to collect all the variants in these genes in the Chinese population.

The *Cypriot HVP Node* is in its early phase of development and is under the auspices of the Cyprus Society of Human Genetics. At present, only one institution is performing genetic testing at the diagnostic level, whereas many research laboratories are working on the genetics of rare and common diseases. The Cypriot HVP Node is also in the process of establishing a National Genetic database. Presently, the existence of an ETHNOS-based National Genetic database [Kleanthous et al., 2006; http://www.goldenhelix.org/server/cypriot], which is also expanded along the lines of the MED-GENET European Commission project, is expected to facilitate the establishment of the Cypriot HVP Node National Genetic database.

The *Egyptian HVP Node* is currently run by the Egyptian Neurogenetic Disorders Consortium in Upper Egypt. Egypt has the largest population in the Arab world (about 85 million), with a high percentage (up to 40%) of consanguineous marriages. The Egyptian NEMDB is already available, using the ETHNOS software (http://www.goldenhelix.org/server/egyptian), along the lines of the MED-GENET European Commission project, and can be further expanded to form the basis of the Egyptian HVP Node. Also, the Egyptian National Registry project has been initiated in 2007 for the Genetic Disorders in the National Research Centre, while in the same year the DNA biobank for rare genetic diseases and for subsequent whole genome analysis studies has been established.

The *Hellenic HVP Node* was formally incorporated in the Human Variome Project in 2010. At present, the Hellenic HVP Node is supervised by a board of 11 geneticists from various Greek academic institutions, most of them sitting at the Board of the Hellenic Society of Human Genetics. The Hellenic HVP Node is endorsed by the Hellenic Bioscientists Association (PEV; http://www.pev.gr; content in Greek) and by the Hellenic Society of Human Genetics (http://www.sige.gr; content in Greek) and has been built around the Hellenic National Mutation frequency database, one of

the very first NEMDBs developed in 2005 [Patrinos et al., 2005]. The Hellenic NEMDB and the entire Hellenic HVP Node structure are hosted as a contribution to the HVP within the Golden Helix Server of the Golden Helix Institute of Biomedical Research (http://www.goldenhelix.org). The Hellenic NEMDB, such as the Cypriot and the Egyptian NEMDBs, is based on the ETHNOS software [van Baal et al., 2010]. The existence of the Hellenic NEMDB and the subsequent creation of the Hellenic HVP Node has not only promoted the collection of genomic variation in the Hellenic population but has also encouraged new studies to document the genetic heterogeneity of the most common genetic disorders in various parts of the country [Papachatzopoulou et al., 2010; Samara et al., 2007]. Also, board members of the Hellenic HVP Node participate in a nationwide study that have been recently initiated to critically ascertain the general public's awareness and healthcare professionals' opinion on genetics and genetic testing services in Greece [Mai et al., 2011; Pavlidis et al., 2012; Sagia et al., 2011].

The *Kuwaiti HVP Node* was incorporated into the Human Variome Project in 2010 in an attempt to propel local scientists and clinicians into the forefront of genetic research [Ozcelik et al., 2010; Tadmouri et al., 2009]. Currently, the Kuwaiti HVP Node is headed by two members from Kuwait University and the Kuwait Medical Genetic Center (KMGC), who aim at three major objectives: (1) Publicize the importance of HVP and the Kuwaiti HVP Node to the health of the Kuwaiti population and its impact on theranostics. To this end, the Kuwait Node members have recruited collaborators from KMGC Clinical geneticists and senior clinical scientists at the KMGC are now involved in the education of physicians, and the public through seminars and lectures directed toward the importance of the HVP effort. (2) Attract private and public funds for research. The Kuwaiti HVP Node members have initiated an important collaboration with Weill-Cornell Qatar to sequence 360 Arabs from all Gulf States and Lebanon using next generation sequencing technology. Also, the Kuwaiti HVP Node has initiated an ambitious collaboration with the Kuwait Medical Students Association entitled "Adopt a gene project" that encourages groups of medical students to attract private funding from local businesses and sequence a single gene per group and submit the data to the appropriate HVP databases. (3) Submit available data to international and already established databases [Tadmouri et al., 2006]. The National registry contains more than 37,000 files for families in Kuwait with diverse and rare genetic disorders. Transferring this information to international databases and the HVP in an ethically appropriate manner has been initiated by depositing the mutations found in gastrointestinal hereditary tumor syndromes to the INSIGHT database (http://www.insight-group.org).

The *Malaysian HVP Node* is coordinated by the 1Malaysian Human Genome Variation Consortium (1mhgvc), which consists of 60 researchers from 12 Malaysian universities and academic institutions. The research consortium was formed in mid 2010 and received support from the Genetics Society of Malaysia, the Malaysian Society of Human Genetics, the Medical Genetics Society of Malaysia, and the Malaysian Society of Bioinformatics and Computational Biology. Among the objectives of the research consortium is to create the human genome variation map of the major ethnic groups in Malaysia and to study its implications on ethical, legal, and social issues (ELSI); archeogenomics; forensics; and disease genome-wide association studies (GWAS). The Malaysian HVP Node was launched on 9 October, 2010 and during the event, a Malay whole-genome SNP database was launched and has grown in size since then. Currently, a total of 291,718 SNPs from 103 individuals representing the Kelantan, Champa, Banjar, Bugis, Kedah, and Jawa Malay subgroups has been deposited in the database. A mutation database has recently been added to the 1mhgvc databases. Currently, the database contains mutations of the *RB1* and the *MSX1* genes of the Malay patients and these are regularly updated with new mutations and new genes.

The *Spanish HVP Node* started in 2010, with the representative office in the Galician Foundation for Genomic Medicine (Santiago de Compostela) and under the auspices of the Board of the Spanish Society of Human Genetics. The first steps undertaken were directed to awareness raising and gathering of support among the genetics community and other relevant biomedical societies in Spain, mostly in meetings of the Spanish Societies of Neurology, Genetics, and Neuroscience, as well as talks to patient support groups. Also, in the fall of 2010 a document on the HVP was produced and presented to several Health and Science administrations, in which benefits, challenges, and solutions regarding collection of genetic variants by the HVP were described. The document contained also a Node development plan with steps and resources needed, as well as considerations on some legal and ethical aspects, with reference to the HVP recommendations [Povey et al., 2010]. The Regional Government of Galicia approved a two-year grant (2009–2010) to support the collection of genetic variation in neurological and psychiatric disorders in Galicia and, specifically, to contribute to the building of LSDBs and to the HVP. Among the next most important aims of the Spanish HVP Node are to join forces with the ongoing effort of the Spanish Registry for Rare Diseases and to contribute to catalyze the Latin American HVP Node.

The *Vietnamese HVP Node* currently includes only one hospital that has clinical genetics capacity. Also, registries for rare diseases exist in the country that need to be interconnected with the Vietnamese HVP Node. Molecular testing for some monogenic disorders and cancers are available in some centers in the two biggest cities of the country, namely, Hanoi and Ho Chi Minh City.

Finally, the *Nepalese HVP Node* is represented by an eight-member consortium that includes government representatives. There is active interaction with the Chinese HVP Node. To date, there is no established NEMDB in Nepal, although future plans include the development of such database in collaboration with other HVP Nodes that will also include research partnership, technical, and advanced academic training. To this end, there are some initial discussions with the Hellenic HVP Node to provide expertise to these disciplines, as a result of the Beijing International Confederation of Countries Advisory Council meeting. Also, initial funding from HVP coordinating office and the University Grant Commission in Nepal will greatly facilitate these efforts.

Other countries represented in the meeting were South Africa, the United Arab Emirates, Japan, Philippines, the United Kingdom, the United States, Czech Republic, and Finland, while the Latin America region was also represented. Some of these countries have relevant activities that will form the basis of the HVP Nodes. The above illustrates that although the existing HVP Country Nodes are, at present, at various stage of development, they have the potential to make significant contributions to the benefit of local societies, for example, by stratifying molecular diagnostics services, particularly in large heterogeneous populations or by increasing genetics awareness of various stakeholders. Also, the existence of these HVP Country Nodes can motivate the development of other HVP Country Nodes for countries interested to join this international initiative.

Discussion

Over the last decade, various initiatives have been established to capture and archive the genetic heterogeneity in various populations and ethnic groups worldwide (e.g., International HapMap project, 1000 Genomes Project, and Pan-Asian personal genomics initiative). NEMDBs, a large number of which reside in developing countries, aim to extend the effort and fulfil the need to create more comprehensive databases of genetic variants [Patrinos, 2006] by ensuring a thorough documentation of common and rare genetic diseases in each population. For developing countries, a set of recommendations have been recently proposed [Patrinos et al., 2011], that would enable developing countries to better orchestrate the process of capturing genetic variation data linked to pathologies.

One of the stated goals of HVP, as outlined during the inaugural [Melbourne, Australia; Cotton et al., 2007], planning [Costa-Brava, Spain; Kaput et al., 2009], and implementation meetings [Paris, France; Kohonen-Corish et al., 2010] is the realization of the HVP Country Nodes, possibly acting as the locomotive of the entire initiative. However, there are several issues that should be taken into consideration not only while establishing and managing an HVP Country Node but also while coordinating the entire effort. These issues, which have been discussed during the inaugural meeting of the International Confederation of Countries Advisory Council, are outlined below.

Model HVP Country Nodes

There are substantial variations regarding the degree that existing HVP Country Nodes are developed and their activities deployed in the corresponding countries. Despite the fact that most of the HVP Country Nodes have only recently been officially admitted to HVP, some of them, such as the Australian, Chinese, Hellenic, and others have been built around existing structures, such as a NEMDB. However, it has been agreed that at the moment there is no model HVP Country Node because level of development of the Node, genetic, and cultural composition of each country is unique. Also, the International Confederation of Countries Advisory Council noted that the level of development of an HVP Country Node should not be the sole criterion to consider a Country Node as a model because the genetic and cultural composition of each country is unique. An important parameter for assessment of country efforts is whether the HVP Country Node has deployed its activities in concordance with the HVP guidelines and recommendations, as previously described [Al Aama et al., 2011; Patrinos et al., 2011]. Considering the situation in developed countries, such as the United Kingdom, where the genetic services operate in a very organized manner, each HVP Country Node could operate by establishing HVP country-specific councils in various places/cities rather than a Country Node as a whole. This model is consistent with the model adopted by the

European Pharmacogenomics for Every Nation Initiative (PGENI; http://www.pgeni.org) Regional Center with the adoption of PGENI Country Councils, consisting of scientists from the entire country and the number of Council members is largely dependent upon the population size [Mitropoulos et al., 2011]. In any case, defining the "model HVP Country Node" is a challenging goal and this issue can be, at least initially, resolved by establishing a consensus on minimum data content and system requirements, data sharing, and database model standards.

Areas Requiring Standards and Guidelines for Countries

The different areas requiring standards and guidelines for countries were also among the issues discussed during the meeting. The development and management of an HVP Country Node requires the adoption of certain standards, guidelines, and recommendations to assure reliability and validity of the data. An initial set of recommendations have been previously issued, particularly for developing countries [Patrinos et al., 2011], whereas some standards and recommendations already exist for other types of databases (http://www.gen2phen.org), relating to genotype and phenotype object models, mutation database models, LSDB-in-a-box platforms, a minimal content list, a data exchange format, a variation ontology, stable reference sequences for genome regions, and digital identifiers for databases, bioresources, and researchers [Celli et al., 2012; Vihinen et al., 2012; Webb et al., 2011]. There is a need to agree on standards, contents and data models for NEMDBs. This will allow data exchange and integration with other NEMDBs and databases, such as LSDBs, as well as development of software tools. An initial set of recommendations for the ideal content of an NEMDB has been previously suggested [Patrinos, 2006], based on which the *ETHNOS* NEMDB software was developed [van Baal et al., 2010], which is currently being upgraded [Tzimas et al., 2012]. Development of new software in every country would be unnecessary waste of resources and easily lead to large number of isolated databases, which cannot be connected to other resources. ETHNOS could be developed to become such software for NEMDBs. Selecting the latter option is preferred by the attendees of the HVP Council and by some HVP Country Nodes that already use the ETHNOS software for their NEMDBs. The meeting attendees also expressed preference to employ this software for the establishment of NEMDBs in other HVP Country Nodes. The adoption and upgrade of existing software will not only expedite the establishment of new HVP Country Nodes, but will also facilitate the development of existing NEMDBs, by their faster migration to the upgraded software version. We hope a standard will be agreed upon in the near future. Equally important is the assembly and curation of population-specific data collection to ensure that absolute frequencies of disease causing and clinically relevant genome variation are obtained for these populations, particularly those with a high consanguinity rate [Gialluisi et al., 2012].

Software and Systems Between NEMDBs and LSDBs and Strategies for Data Sharing

One of the most critical parameters for the operation of the HVP Country Nodes is the software and systems requirements to accommodate data deposition in each Node and, most importantly, the ability to share and exchange data among different HVP Country Nodes, LSDBs and central repositories. To this end, two main options exist: (1) A gigantic central NEMDB that will be developed for the needs of the HVP that would accommodate all popula-

tion/ethnic specific datasets, and (2) individual NEMDBs in each HVP Country Node that would be preferably based on the same interoperable Web-enabled platform. The FINDbase database, which was developed for clinically relevant genome variation allele frequencies [http://www.findbase.org; van Baal et al., 2007] may be used as a central unified system [Georgitsi et al., 2011a,b], since the software documents causative mutation and pharmacogenomic marker allele frequencies at a summary level. The interoperable NEMDB approach for data gathering and sharing, which is based on data warehousing principles, has several advantages: (1) databases can be managed and curated from each HVP Country Node representatives, (2) data homogeneity can be ensured since individual NEMDBs will function under the same software, (3) individual population differences, such as social, religious, ethnic differences, can be addressed by minimal software customization without affecting the key functionalities of the main system. Such an approach has already been successfully implemented in the Israeli NEMDB [Zlotogora et al., 2007, 2009], and (4) data gathering can be expedited by assuming a local rather than a central coordinating role. The latter approach has been previously suggested [Patrinos, 2006] and implemented in existing NEMDBs [Georgitsi et al., 2011b; van Baal et al., 2007]. NEMDB database interoperability is also possible not only among NEMDBs that are based on different platforms but also among NEMDBs and other types of databases, for example, LSDBs or central databases. To this end, Café Variome (http://www.cafevariome.org) has been designed to serve as an exchange portal for gene variant (mutation) data. This portal offers users a forum to announce, discover and acquire a comprehensive listing of observed neutral and disease-causing gene variants in patients, unaffected individuals and even populations and, as such, it enables holistic searching across various databases.

In addition to these suggestions and advances, the success of data gathering can be boosted with incentives for data submitters such as microattribution [Giardine et al., 2011] or by encouraging submission of population specific datasets to specialized database journals [Patrinos and Petricoin, 2009] or regulatory incentives [Cotton et al., 2009].

Patient Registries and Other Possible Synergies

Patient registries or clinical genetic databases are equally important aspects of the HVP Country Nodes as summary-level NEMDBs. Patient registries usually contain individual-level data including important genotype–phenotype information that are either maintained locally in hospitals and clinical centers or are big national and some time multicenter supranational initiatives, such as the CFTR2 project (http://www.cftr2.org). However, the establishment and maintenance of patient registries as individual-level clinical genetic databases is a far more demanding task than the development of summary-level NEMDBs. One of the most important challenges is the vital need to ensure patients' anonymity, which dictates removing or safeguarding (e.g., behind firewalls or by encoding) patients' personal information so that they cannot be linked back to their own detailed genotype and phenotype/clinical information. This is particularly important for rare disorders, where one's phenotypic information can reveal his/her identity. This parameter also touches upon the development of databases for clinical trials, which derives from the explosion of individualizing therapeutic interventions. Interconnecting patient registries with mutation databases has been recently discussed [Auerbach et al 2011; Ayme et al 2011], and this combination would be of utmost importance not only for common but also for rare diseases. In actual fact, few HVP Country Nodes, such as the Spanish, the Hellenic, and the Vietnamese HVP

Nodes plan or have already joined forces with the corresponding rare disorders registries.

Listing of Needs

To successfully maintain the existing HVP Country Nodes, to encourage the establishment of new Nodes, and to expand HVP and thereby deliver improved healthcare, additional steps need to be undertaken (summarized in Box 2)

First, a need exists to strengthen the role of the International Confederation of Countries Advisory Council since the Country Nodes will play a vital role in the efforts of the HVP. At the same time, each HVP Country Node and accompanying NEMDB, should bear an electronic seal of HVP as a quality feature, indicating that all necessary recommendations, standards, and guidelines are followed. A dedicated Committee or Working group from the International Confederation of Countries Advisory Council will be responsible to assign this quality feature and monitor the Country Node to ensure it continues to conform to the standards agreed. Reciprocally, the corresponding Human Genetics Societies should formally endorse their Country Node to be admitted to the International Confederation of Countries.

Also, careful consideration and planning of the long-term financial stability and sustainability of each HVP Country Node is an absolute requirement. Viable financial planning by the local HVP Country Node must accompany the country's application and possibly a detailed Strength/Weakness/Opportunities/Threat (SWOT) analysis to allow the HVP Country Node Council to evaluate the feasibility of the proposed approach and the ability of the applicants to successfully establish and manage the Node. Fund raising should be sought locally, such as from governments, national grants, charities, patients' organizations, or others. Similarly, the HVP could also facilitate fund raising efforts by providing grants for the start-up and the initial operation of the Node. Besides grants for start-up of Nodes, funding could also be provided centrally by HVP, for example, to upgrade the NEMDB software, to organize educational and/or outreaching activities, and so on. Funding assistance is particularly important for developing countries.

Consortium members, particularly those from countries having close ties with international organizations like UNESCO and WHO, indicated the importance of these organizations, particularly when speaking to their own governments through Ministries of Health, and Ministries with mandates for science, research, and education. HVP has the status of an international nongovernmental organization in operational relations with UNESCO, which facilitates cooperation with the United Nations. UNESCO has some 56 field offices throughout the world with headquarters in Paris. In addition, UNESCO has a global network of national cooperating bodies known as National Commissions for UNESCO, set up by their respective governments. Presently, there are 197 National Commissions for UNESCO across the world, which operate for the purpose of associating their governmental and nongovernmental bodies in education, sciences, culture, and communication with the work of the Organization. HVP Country Nodes are encouraged to build relationships with their respective UNESCO National Commissions and field offices in areas of UNESCO's mandate. With regard to WHO, as it has a complex structure of approximately 150 country offices, 6 regional offices, and a headquarter in Geneva, building relationships with it are complex. With WHO having a core mandate focusing on public health and its governance being largely in the hands of Ministers

Box 2. Recommendations for Development of HVP Country Nodes

(a) Political measures
- Empowerment of the HVP International Confederation of Countries Advisory Council.
- Develop quality standards and follow their implementation. HVP Country Nodes would have a permission to show HVP seal as a quality feature, to indicate that all necessary recommendations, standards, and guidelines are followed.
- Formal endorsement of each country's application to become an HVP Country Node by the respective national Human Genetics and Genomics Society.
- Agreement on distribution of anonymized information.
- Advocacy with governments: (1) in particular Ministries of Health for awareness of and support for genetic- and genomic-related health in their national health plans; and (2) through Ministries responsible for education, science, and research and through the National Commissions for UNESCO in each country for awareness and support for capacity building in genomics and bioinformatics research and education.

(b) Financial measures
- Careful consideration and planning of a long-term sustainability upon application to become an HVP Country Node.
- Fund raising from local sources, such as national grants, philanthropic organizations, and patients' organizations.
- Financial support by the HVP International Coordinating Office for the start-up and the initial operation of the Country Node, particularly for developing countries.
- Financial support by the HVP Central office for software upgrade and maintenance, for organizing educational and outreaching activities.

(c) Other measures
- Tackling ethical, legal, and societal issues, particularly where patient registries are involved.
- Adopt a country-specific data gathering and sharing approach.
- Provision of education for the general public and healthcare professionals on issues relating to genetics and genomics.
- Development of standards, recommendations, and data models for NEMDBs and encouragement of their implementation to software products.

of Health, it is important for HVP Country Nodes to develop good relationships with their national Ministries of Health based on how Country Nodes can contribute to the health and well being of the populations they serve.

Media coverage in the national and international arena can add to highlighting the importance of the local HVP effort and improving public knowledge of genetics, genomics, and health. Careful consideration needs to be given to how Country Nodes can best be supported in managing positive relationships with various types of local media. A major aim for the next 12 months is to begin work toward globally agreed standards for databases as well as invite more countries to establish Nodes.

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Conclusions

The success of existing HVP Country Nodes and progress on multiple other planned national platforms underscores the potential value of the HVP Country Node concept. HVP Country Nodes would best be served by coordinating their efforts promoted by the International Confederation of Countries Advisory Council meeting, as described above. A crucial parameter that would guarantee the successful outcome of these efforts is collaborative work of partners from all over the world, which will save time and effort, improve knowledge generation for all, and will result in better local and global solutions for genomic medicine.

Overall, HVP Country Node operational guidelines will help expand the establishment of centers focusing on genome medicine, which will in turn facilitate the fulfillment of the HVP goals toward a comprehensive worldwide genome variation data collection.

Acknowledgments

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Clinical Reasoning: A young man with progressive subcortical lesions and optic nerve atrophy

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Clinical Reasoning: A young man with progressive subcortical lesions and optic nerve atrophy

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SECTION 1

The male patient is the third child of unrelated Japanese parents. His older sister had tachypnea and feeding difficulties, and died at 5 days of age. The patient was delivered at term (birthweight, 3.8 kg), following an unremarkable pregnancy. He presented with tachypnea, metabolic acidosis, and hyperammonemia (944 μmol $\cdot$ L^{-1}) at 6 days.

Questions for consideration:

1. What is the differential for infantile presentation of hyperammonemia in the neonatal period?
2. What laboratory tests would you pursue?

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SECTION 2

Hyperammonemia occurs in urea cycle disorders (e.g., ornithine transcarbamylase deficiency) and organic acidurias (e.g., methylmalonic aciduria, propionic aciduria, and isovaleric aciduria) and fatty acid oxidation defects (e.g., multiple acyl-CoA dehydrogenase deficiency). The existence of acidosis with ketosis indicates organic aciduria, whereas respiratory alkalosis is observed in urea cycle defects. Diagnosis is based on quantitative assay of amino acids and acylcarnitines from dried blood and organic acids in urine samples.

Case: part 2. Elevated levels of 2-methylcitric acid and 3-hydroxypropionic acid were found in the urine. The plasma propionic acid concentration was increased (4.5 $mg \cdot dL^{-1}$), and propionyl-CoA carboxylase (PCC) activity in fibroblasts was decreased (6.3 $pmol \cdot min^{-1} \cdot mg^{-1}$ protein, normal value: 292 [n = 4]). The patient was treated with exchange transfusion, peritoneal dialysis, high-calorie infusions, and a low-protein diet.

Questions for consideration:

1. What is the diagnosis?
2. How should these infants be treated in the acute period?
3. What treatment should be given long term?

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SECTION 3

Propionic aciduria is an autosomal recessive disease caused by a deficiency of PCC. PCC is a biotin-dependent enzyme that catalyzes the branched chain amino acids valine and isoleucine but not leucine; it also catalyzes methionine, threonine, and odd-chain fatty acids in the mitochondrial matrix. PCC is composed of α and β subunits, which are encoded by nuclear genes *PCCA* and *PCCB*, respectively. PCC deficiency causes accumulation of propionic acid, 3-hydroxypropionic acid, 2-methylcitric acid, and propionylglycine in blood, urine, and CSF.[1]

Clinical forms of propionic aciduria are described on the basis of the age at onset: neonatal and late onset. The neonatal-onset form is characterized by poor sucking, vomiting, failure to thrive, and progressive encephalopathy. Routine laboratory findings are metabolic acidosis, ketosis, lactic acidosis, hyperammonemia, leukocytopenia, thrombocytopenia, and anemia. The late-onset form is characterized by periodic vomiting to life-threatening hyperammonemia, psychomotor retardation, and other chronic symptoms.[1] Propionic aciduria is characterized by increased excretion of propionic acid, 3-hydroxy propionic acid, and 2-methylcitric acid in urine as well as elevated concentrations of propionyl-carnitine in blood serum or plasma. It is initially diagnosed based on enzymatic analysis of propionyl-CoA carboxylase activity in fibroblasts or leukocytes. Identification of the specific mutations in *PCCA* or *PCCB* is required to confirm the diagnosis.[1]

The increased 2-methylcitric acid and 3-hydroxypropionic acid levels and decreased propionyl-CoA carboxylase activity in this case indicated propionic aciduria. Mutation analysis revealed homozygosity for p.Thr428Ile in the *PCCB* gene, confirming propionic aciduria.

Emergency treatment for propionic aciduria involves low-protein, high-energy nutrition and rehydration. Almost all propionic aciduria patients show hyperammonemia, which results from inhibition of urea cycle enzymes by accumulated acyl-CoA esters. Some patients, especially those severely affected, require hemodialysis/hemofiltration. Sodium benzoate and carbamyl glutamate are used to treat secondary hyperammonemia.[2] Long-term management comprises low-protein diet and carnitine supplementation. Arginine and carnitine are administered for detoxification of toxic metabolites. Metronidazole is often administered to reduce production of propionic acid by gut bacteria.[1]

Case: part 3. After emergency intervention, the patient was treated with a low-protein diet and carnitine supplementation. During the first 5 years of life, he had several episodes of metabolic acidosis requiring hospitalization; however, he never showed metabolic decompensation thereafter. Despite nearly normal development at 4 years, he thereafter developed intellectual deficits that gradually deteriorated with age: his developmental quotient was 88 at the age of 4 and 74 at 6, while his IQ was 73 at the age of 7 and 54 at 15.

At 22 years, metronidazole administration was initiated to reduce propionic acid production by gut bacteria. Bilateral vision impairment was also detected during a regular health check-up. Ophthalmologic examination showed temporal pallor of the right eye and left optic nerve atrophy (figure 1). The patient's visual acuity was 20/200 in the right eye and 20/300 in the left eye. In both eyes, his visual fields showed central scotoma, and his visual evoked potential (VEP) displayed decreased amplitude. An electroretinogram showed normal findings, while optical coherence tomography revealed no retinal structure abnormalities. Within a year, his visual acuity decreased from 20/200 to hand motion in the right eye and from 20/300 to counting fingers in the left. He also had intention tremor, mild hyperammonemia, and elevated lactic acid levels, but no metabolic acidosis. Ophthalmologic examination results at 11 years were normal.

Brain MRI revealed symmetric lesions of the basal ganglia, including the caudate nucleus, putamen, and globus pallidus (figure 2A). At 23 years, no symptoms were present, but diffusion-weighted MRI of the brain showed subcortical lesions (figure 2, B–D). At 24 years, he showed acute reversible muscle weakness and dysarthria, comparable to pseudobulbar paralysis. Neurologic evaluation showed increased deep tendon reflexes on the left side of the body, while subsequent MRI of the brain revealed progression of the subcortical lesions (figure 2, E–G), without evidence of metabolic decompensation.

Figure 1   Fundal images
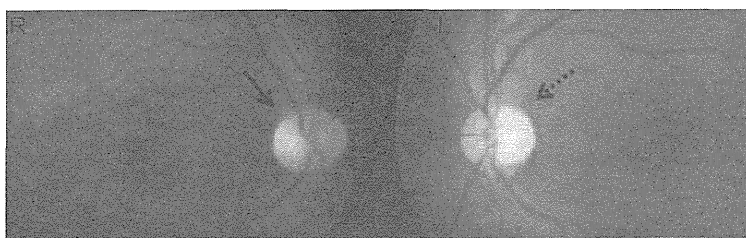
Temporal pallor in the right eye and optic nerve atrophy in the left.

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Figure 2   MRI
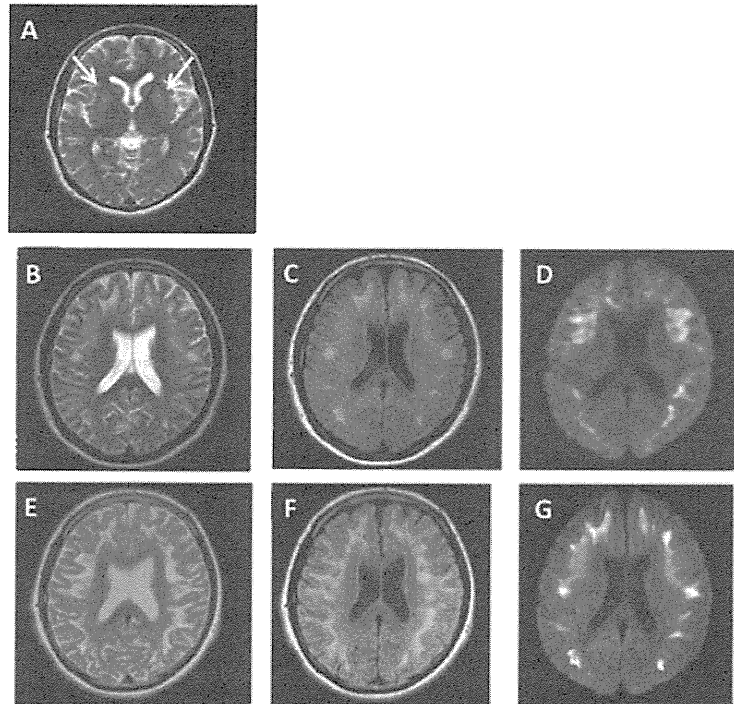
(A, B, E) T2-weighted images; the arrows in A indicate the high intensity of basal ganglia areas. (C, F) Fluid-attenuated inversion recovery. (D, G) Diffusion-weighted imaging, showing abnormal signals in subcortical lesions.

At 24 years, chest radiography showed an increased cardiothoracic ratio (65%) and pulmonary edema. Echocardiogram showed dilated hypokinetic left ventricle and a decrease in ejection fraction (48%), resembling dilated cardiomyopathy. The patient was administered furosemide, spironolactone, and carvedilol.

Questions for consideration:

1. Why did this patient's condition deteriorate even without metabolic acidosis crises?
2. What is the range of prognosis for neonatal-onset form propionic aciduria?
3. What type of monitoring will he need?

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SECTION 4

The accumulation of toxic organic acids causes cerebral stroke that cannot be accounted for by hypoxemia or vascular insufficiency: this neurologic event is termed metabolic stroke.[3] Toxic metabolites cause secondary mitochondrial dysfunction, which leads to metabolic stroke.[4] According to the recent "trapping hypothesis," the limited transport of toxic metabolites from the brain to the blood compartment leads to accumulation of toxic dicarboxylic acids in glutaric aciduria type I and methylmalonic aciduria.[5] Like propionic aciduria, methylmalonic aciduria is also a branched-chain amino acid disorder. For propionic aciduria patients, accumulation of the dicarboxylic acid 2-methylcitric acid seems likely; however, it has not yet been sufficiently documented.

Patients with propionic aciduria and methylmalonic aciduria often present with mental retardation, epilepsy, and extrapyramidal symptoms. Sixty percent of patients with propionic aciduria have an IQ lower than 75.[2] Symmetric lesions of the basal ganglia are the most frequently reported MRI changes in propionic aciduria and methylmalonic aciduria.[2] Subcortical white matter abnormality was additionally reported in 11.5% of patients with methylmalonic aciduria.[6] However, these findings have not been confirmed in propionic aciduria, probably because the number of patients is relatively small.

Compared to previously reported late-onset optic nerve atrophy in patients with methylmalonic aciduria and propionic aciduria,[7] our patient is the oldest. The previous report suggested that optic nerve atrophy observed in propionic aciduria and methylmalonic aciduria resembled Leber hereditary optic neuropathy (LHON),[7] as both showed optic nerve atrophy and normal retina. LHON is caused by 1 of 3 pathogenic mtDNA mutations at the nucleotide positions 11,778, 3,460, and 14,484, located in genes encoding the mitochondrial complex I subunits. Our patient carried none of these mutations. The common findings between optic nerve atrophy in propionic aciduria/methylmalonic aciduria and LHON suggest that secondary mitochondrial dysfunction leads to optic nerve atrophy in patients with propionic aciduria and methylmalonic aciduria. Optic nerve atrophy is age-dependent, but independent of metabolic control, other neurologic complications, and overall health status.[7] Therefore, we recommend regular ophthalmologic examination of patients with propionica aciduria and methylmalonic aciduria.

In many countries, propionic aciduria is targeted in newborn screening programs. About 60% of patients diagnosed through newborn screening were already symptomatic and less than 10% remained asymptomatic.[8] Even though newborn screening diagnosis does not positively correlate with a milder clinical course or better neurologic outcome,[8] it is important from the viewpoint of earlier diagnosis and decreased early mortality.

According to genotype and phenotype correlation analysis, certain null mutations are related to neonatal onset, while certain missense mutations are related to the late-onset form.[9] Although late-onset patients have higher survival rates compared to neonatal-onset patients,[9] both face the risk of relapses of life-threatening episodes of metabolic decompensation and risk of death or further neurologic damage.

PCC plays a role mainly in the liver; therefore, liver transplantation has been considered an alternative therapy.[2] Liver transplantation minimizes further metabolic acidosis and improves the quality of life. However, various complications, including basal ganglia lesions, cardiomyopathy, and optic nerve atrophy, were reported in patients without metabolic decompensation.[2,7] Even after liver transplantation, stroke-like episodes or cardiomyopathy was reported.[10]

Thus, conventional management is insufficient to improve the long-term prognosis for propionic aciduria patients, indicating the need for novel therapeutic approaches based on a better understanding of the pathophysiology.

AUTHOR CONTRIBUTIONS

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DISCLOSURE

The authors report no disclosures relevant to the manuscript. **Go to Neurology.org for full disclosures.**

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