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研究成果の刊行物・別冊

The Cleft Palate-Craniofacial Journal 49(6) pp. 714–717 November 2012

ORIGINAL ARTICLE

No Evidence of Association Between 8q24 and Susceptibility to Nonsyndromic Cleft Lip With or Without Palate in Japanese Population

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Objective: **Recent genome-wide association studies identified susceptibility loci for nonsyndromic cleft lip with or without cleft palate (NSCL±P) on 8q24.21, 10q25.3, 13q31.1, 15q13.3, 17q22, and 18q22 in populations of European origin. The purpose of this study was to determine, using DNA samples, whether 8q24.21 was a susceptibility locus for the development of NSCL±P in Japanese patients.**

Methods: **We used DNA from 167 Japanese NSCL±P patients (45 cleft lip without cleft palate and 122 cleft lip with cleft palate patients) and 190 Japanese unaffected control individuals. We performed an association study using 13 single nucleotide polymorphisms (SNPs) selected on the 8q24.21 locus. Genotyping of each SNP was carried out by direct sequencing of genomic DNA. Additionally, a haplotype block was constructed using the selected SNPs.**

Results: **The 13 selected SNPs were successfully genotyped in 357 individuals. The *p* values obtained were not low enough to indicate a significant association between the haplotypes and the development of NSCL±P in this population.**

Conclusions: **Our results suggest that the 8q24.21 locus is not associated with susceptibility to NSCL±P in Japanese patients and provide further evidence that ethnicity is a strong factor in determining susceptibility loci, albeit using a limited number of samples. Further studies are needed to identify regions involved in the development of NSCL±P in the Japanese population.**

KEY WORDS: *association study, cleft lip with or without cleft palate, 8q24, susceptibility*

Cleft lip with or without cleft palate (CL±P) is one of the most common birth defects worldwide. Of variable phenotype, the incidence of this disease varies markedly depending on ethnicity, and the frequency of orofacial clefts in Japan is higher than that in other countries (Wyszynski et al., 1996; Murthy and Bhaskar, 2009).

CL±P comprises a heterogeneous group of congenital malformations and is typically classified into two categories: syndromic and nonsyndromic clefting. Genetic and environmental factors are involved in the pathogenesis of nonsyndromic cleft lip with or without cleft palate (NSCL±P). Much research into inherited factors for NSCL±P has been done in both animals and humans. Although many molecular genetic studies have identified genes or loci associated with CL±P, the underlying mechanism of this disease remains to be clarified (Murray and Schutte, 2004; Carinci et al., 2007; Jugessur et al., 2009; Marazita et al., 2009; Murthy and Bhaskar, 2009). Genome-wide association studies (GWAS) aim at identifying unknown disease factors not found by linkage analysis with existing loci and thus determining phenotypical correlations with various genotypes (Murthy and Bhaskar, 2009).

Recent GWAS have identified susceptibility loci for NSCL±P on 8q24.21, 10q25.3, 13q31.1, 15q13.3, and

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TABLE 1 Association Between Japanese NSCL±P* and Single Nucleotide Polymorphisms (SNPs) at 8q24.21

SNP	*Position (bp)*	*Alleles*	*Genotypes, Cases*	*MAF, Cases*	*Genotypes, Controls*	*MAF, Controls*	*P Value*	*OR (95% CI)*
rs11994831	129957663	T/C	7, 51, 109	0.195	7, 54, 129	0.179	.5919	1.109 (0.76–1.616)
rs7845615	129957976	C/T	16, 68, 83	0.299	16, 70, 104	0.268	.3595	1.165 (0.841–1.614)
rs17241253	129959370	C/T	0, 0, 167	0	0, 0, 574†	0	0	
rs1155582	129964203	G/C	3, 30, 134	0.108	4, 32, 154	0.105	.9133	1.027 (0.638–1.653)
rs1519851	129965001	T/C	0, 33, 134	0.099	4, 31, 155	0.103	.8655	0.9586 (0.588–1.563)
rs10956449	129965527	C/T	27, 73, 67	0.38	22, 86, 82	0.342	.2898	1.18 (0.869–1.602)
rs17819888	129970861	C/T	5, 49, 113	0.177	8, 36, 146	0.137	.1433	1.353 (0.902–2.031)
rs1157136	129971092	T/C	8, 58, 101	0.222	8, 55, 127	0.187	.2503	1.239 (0.86–1.784)
rs10956450	129971577	T/C	28, 74, 65	0.389	24, 84, 82	0.347	.2473	1.197 (0.883–1.624)
rs6470670	129982630	C/T	0, 30, 137	0.0898	5, 31, 154	0.108	.421	0.8159 (0.497–1.339)
rs1530300	129988640	T/C	1, 6, 160	0.024	0, 7, 183	0.0184	.6074	1.308 (0.469–3.645)
rs987525	130015336	C/A	1, 21, 145	0.0689	0, 20, 170	0.0526	.3634	1.331 (0.717–2.47)
rs12548036	130017064	G/T	2, 25, 140	0.0868	0, 26, 164	0.0684	.3577	1.295 (0.746–2.246)

* NSCL±P = nonsyndromic cleft lip with or without palate; MAF = minor allele frequency; OR = odds ratio; CI = confidence interval.
† 574 samples were analyzed for estimation of allele frequency.

17q22 in European (Birnbaum et al., 2009; Beaty et al., 2010; Mangold et al., 2010) and Mesoamerican populations (Rojas-Martinez et al., 2010). Another GWAS from Philadelphia also identified loci 8q24 and 18q22 for NSCL±P in individuals of European descent (Grant et al., 2009). Although few other studies have reported 8q24 as a locus in NSCL±P, this region has been well reported in epilepsy and tumor. The MYC enhancer region downstream of 8q24 was reported to be closely involved in the development of epilepsy (Zeidler et al., 1994; Morita et al., 1998; Wasserman et al., 2010). The aim of the present study was to determine whether 8q24, which showed a significant *p* value in two independent GWAS, was associated with susceptibility to NSCL±P in a Japanese population.

Materials and Methods

We used DNA collected from 167 Japanese NSCL±P patients (45 cleft lip without cleft palate patients and 122 cleft lip with cleft palate patients) at Nagasaki University Hospital and Tokyo Dental College Hospital. All diagnoses were based on examinations by well-trained plastic or oral surgeons. We also examined 190 Japanese unaffected adult control individuals at Nagasaki University Hospital. The study protocol was approved by the Committee for the Nagasaki University Ethical Committee on Human Genome and Gene Analysis.

Genotyping

We selected 13 single nucleotide polymorphisms (SNPs) (Table 1) located on 8q24.21 that were identified in a GWAS of Central European NSCL±P (Birnbaum et al., 2009). Among these, three SNPs (rs17241253, rs1530300, and rs987525) were reported to be the most significant (Birnbaum et al., 2009). The other 10 SNPs (rs11994831, rs7845615, rs1155582, rs1519851, rs10956449, rs17819888, rs1157136, rs10956450, rs6470670, and rs12548036) were chosen because their minor allele frequencies (MAF) were relatively high in the Japanese population. A search of the HapMap 3 database for the 13 SNPs targeted in this study in the Japanese yielded the following values: rs1530300, 0.012; rs987525, 0.042; and rs17241253, 0. Of the remaining SNPs, four were ~0.1 to ~0.3, while no data were available on the others. Genotyping of each SNP was carried out by direct sequencing of genomic DNA extracted from peripheral blood lymphocytes using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and primers designed from data at the University of California, Santa Cruz, Genome Browser Home (http://genome.ucsc.edu/). Polymerase chain reaction (PCR) was performed in 10 μL of reaction mixture with the DNA Thermal Cycler Model 9700 (Applied Biosystems, Applied Biosystems, Carlsbad, California, U.S.) under the appropriate conditions. The PCR products were subjected to Exonuclease I (Epicentre, Madison, WI) and shrimp alkaline phosphatase digestion (GE Healthcare UK Ltd., GE Healthcare UK Ltd., Little Chalfont, England) prior to sequencing. Direct sequencing was carried out using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems). Samples were purified with Sephadex G-50 (GE Healthcare UK Ltd., Little Chalfont, England) and electrophoresed on the Autosequencer Model 3130xl (Applied Biosystems, Carlsbad, California, U.S.). The sequences were aligned with ATGC software (Genetyx Corp., Tokyo, Japan), and the genotypes of the SNPs were determined by visual inspection.

Statistical Analysis

All statistical tests for association were carried out using PLINK (Purcell et al., 2007) and Haploview software (Barrett et al., 2005). We tested for deviation from the Hardy-Weinberg equilibrium and for association with the Cochran-Armitage trend test. The odds ratio (OR) and corresponding 95% confidence interval for each SNP were also calculated.

Results and Discussion

The 13 selected SNPs were successfully genotyped in 357 individuals. No significant departures from the Hardy-Weinberg

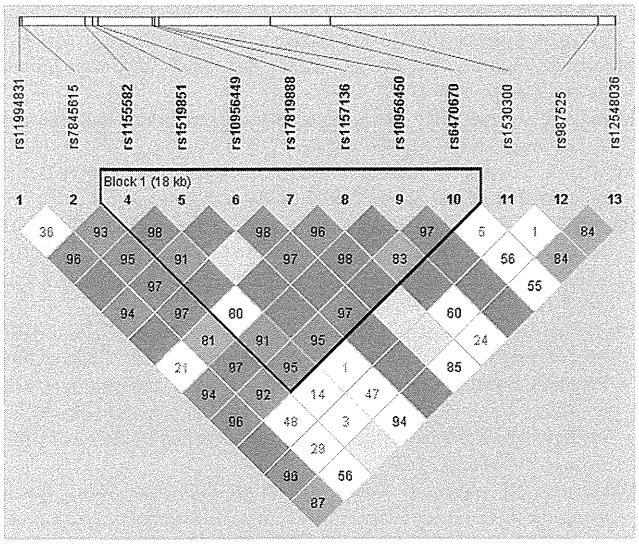

FIGURE 1 The linkage disequilibrium display was analyzed using Haploview software. Blocks show haplotypes constructed from the results of our analysis.

equilibrium were observed (all Hardy-Weinberg equilibria, $p > 0.1$). The results of the association analysis are shown in Table 1. For all 13 SNPs, the p values were above .1. The MAF of SNP rs17241253 was 0 in our analysis; therefore, neither a p value nor an OR could be calculated. Association mapping was performed, the linkage disequilibrium (LD) structure determined, and p values of haplotypes calculated with Haploview (Fig. 1, Table 2). SNP rs17241253 showed the highest OR in an earlier study by Birnbaum et al. (2009). However, a search of the HapMap database revealed that the MAF for rs17241253 was very low in the Japanese population. Although there is a strong correlation between the development of CL±P and a lower MAF value for rs17241253 in Europeans, only the TT homozygous genotype was found here, making this SNP not appropriate for CLP studies in the Japanese population. Therefore, we selected SNPs near rs17241253 for our haplotype analysis. Haplotype was defined as r-square > 0.8, and an association test was then performed by Haploview. No haplotype block including rs17241253 showed a statistically significant p value in our single SNP or haplotype association analysis. Moreover, rs11994831, rs7845615, rs1530300, rs987525, and rs12548036 were not included in this haplotype block.

We clearly verified that the LD block including rs17241253 was not associated with NSCL±P. The associated region reported by Birnbaum et al. (2009) was greater than 185 kb. This LD block including the associated region was divided into two definitive blocks in the European population. In contrast, very weak LD blocks were observed in Japanese and were not definitive when analyzed using the HapMap database (data not shown). The results of this study suggest that LD blocks including rs17241253 indicate no association with NSCL±P in the Japanese population. Our results suggest that rs1530300 and rs987525 are also not associated with NSCL±P in the Japanese population. This is similar to the results of Beaty et al. in other Asian populations (2010). These results indicate that an SNP may have a low association in Asians, even if it has a high association in Europeans.

TABLE 2 Haplotypes and the Result of Their Association Study*

Block	*Haplotype*	*Frequency*	*Case, Control Frequencies*	*P Value*
	GTCCTTC	0.626	0.602, 0.647	.2091
	GTTTCCC	0.151	0.170, 0.134	.1759
	CCTCTCT	0.094	0.087, 0.100	.5472
	GTTCTCC	0.056	0.060, 0.053	.6774
	GTTCCCC	0.046	0.045, 0.047	.8811

* LD Plot in Fig. 1 shows establishment of the combination between each haplotype.

The pathogenesis of NSCL±P is very complex, and both genetic and environmental factors are involved (Mossey et al., 2009). It is thought that many genes and proteins are involved in the pathogenesis of NSCL±P, and many genes and loci associated with NSCL±P have been reported (Beaty et al., 2010). While the results of this study provide no conclusive evidence of an association between specific loci and the development of NSCL±P in the Japanese population, the incidence of NSCL±P has been shown to vary depending on ethnicity: It is high in Asia and Latin America and low in Israel, South Africa, and Southern Europe (Mossey et al., 2009), for example. These differences may result from genetic background, and the Japanese population may have its own susceptibility gene or locus, such as MAFB on 20q12, IRF6 on 1q32.2, or ABCA4 on 1p22.1 (Beaty et al., 2010). These results suggest that it is difficult to apply findings from European populations to Asian populations. Further study is needed to identify regions involved in the development of NSCL±P in the Japanese population. The incidence of this disease is higher in Asian populations, making the identification of loci involved in its development all the more important.

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SPECIAL ARTICLE

Human Mutation

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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

†This article is dedicated to the memory of Professor David Rimoin, the first Chair of the Human Variome Project International Scientific Advisory Committee, whose support and overall contribution for the Human Variome Project was tireless and instrumental for its success.

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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.

Box 1. The Various National Genetics Societies and Regional Human Genetics Networks That Were Represented in the Inaugural Meeting of the ICCAC in Beijing (in Alphabetical Order)

(a) National Genetics Societies
- American Society of Human Genetics
- American College of Medical Genetics
- Austrian Society of Human Genetics
- Belgian Society of Human Genetics
- Hellenic Association of Medical Geneticists
- Spanish Society of Human Genetics

(b) Regional Genetics Networks and Societies
- African Society of Human Genetics
- Centre for Arab Genomic Studies
- European Society of Human Genetics
- Human Genetics Society of Australasia
- International Federation of Human Genetics Societies
- Latin American Network of Human Genetics Societies

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 population/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