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SPECIAL ARTICLE

A Quality Assessment Survey of SNP Genotyping Laboratories

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To survey the quality of SNP genotyping, a joint Nordic quality assessment (QA) round was organized between 11 laboratories in the Nordic and Baltic countries. The QA round involved blinded genotyping of 47 DNA samples for 18 or six randomly selected SNPs. The methods used by the participating laboratories included all major platforms for small- to medium-size SNP genotyping. The laboratories used their standard procedures for SNP assay design, genotyping, and quality control. Based on the joint results from all laboratories, a consensus genotype for each DNA sample and SNP was determined by the coordinator of the survey, and the results from each laboratory were compared to this genotype. The overall genotyping accuracy achieved in the survey was excellent. Six laboratories delivered genotype data that were in full agreement with the consensus genotype. The average accuracy per SNP varied from 99.1 to 100% between the laboratories, and it was frequently 100% for the majority of the assays for which SNP genotypes were reported. Lessons from the survey are that special attention should be given to the quality of the DNA samples prior to genotyping, and that a conservative approach for calling the genotypes should be used to achieve a high accuracy. Hum Mutat 27(7), 711–714, 2006.

KEY WORDS: SNP; genotyping; quality assessment

INTRODUCTION

SNP genotyping has gained widespread use in genetic association studies. Because findings from such studies are often difficult to replicate, concerns about the quality of the SNP genotyping results have been raised. These concerns are motivated because genotyping errors can have a deleterious effect on the statistical analyses in association studies of common, complex disorders [reviewed in Hattersley and McCarthy, 2005]. To address the concerns of the quality of SNP genotyping results, a joint Nordic quality assessment (QA) round was performed by SNP genotyping laboratories in the Nordic and Baltic countries. The round was initiated by the Finnish Genome Center and the SNP Technology Platform in Uppsala, and was coordinated by Labquality (www.labquality.fi), a Finnish nonprofit organization specialized in external QA schemes for clinical laboratories. The main purpose of the QA round was to survey the quality of the SNP

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genotyping work in the participating laboratories to promote good laboratory practices. The participating laboratories are research core facilities and have a strong track record in genotyping technology but are not regularly involved in similar proficiency testing/external quality assessment programs as clinical and forensic laboratories. Six Swedish, three Finnish, one Norwegian, and one Estonian laboratory participated in the project (Table 1).

MATERIALS AND METHODS

The QA round had a blinded design, according to which 47 DNA samples of unknown genotype from anonymous Finns were sent out to the participating laboratories by Labquality, together with a list of SNPs to be genotyped. The participating laboratories were encouraged to use their standard methods and procedures for SNP assay design and genotyping without additional attempts to verify their genotyping results. Either 18 or six SNPs were genotyped, depending on each participant's preference. One negative control sample (water) and one sample of poor DNA quality, according to its low ultraviolet (UV) absorbance ratio ($A_{260nm}/A_{280nm} = 1.4$) were included in the panel of samples. An aliquot of 700 ng of DNA extracted using the Gentra LS automated DNA extraction platform (Gentra Systems, Plymouth MN) and dried in microtiter plate wells was sent to each laboratory, to be used at a concentration appropriate for the genotyping method. To reflect an authentic genotyping project in a research core facility, the 18 SNPs had been randomly selected from dbSNP (www.ncbi.nlm.nih.gov/SNP) by a database expert who is not familiar with the details of the genotyping methods. The criteria applied for SNP selection were that the minor SNP allele frequency was between 0.3 and 0.5, and that the SNPs were located on different chromosomes and not in a repetitive region of the genome (Table 2). As can bee seen from Table 1, the methods used by the participating labs included all major platforms for small- to medium-size SNP genotyping. Four of the methods are based on hybridization with allele-specific oligonucleotide probes and seven methods use DNA polymerase-assisted primer extension. Details of the genotyping procedures in each laboratory are provided in Supplementary Appendix S1 (available online at http://www.interscience.wiley.com/jpages/1059-7794/suppmat).

The laboratories were encouraged to perform exactly those QA steps that are routinely used in their SNP genotyping procedure. In all laboratories, the QA procedures included control of the DNA samples using gel electrophoresis or by measuring the DNA concentration prior to genotyping, as well as a test of the performance of each SNP assay using control samples prior to genotyping the test samples, and inclusion of positive and negative control samples in the genotyping procedure. Most laboratories performed a check of Hardy-Weinberg equilibrium of the genotypes. Several of the laboratories operate with a genotype database and utilize a database or in-house developed software to assess the quality of the genotypes either visually or numerically. The genotypes assigned to the DNA samples at 18 SNP positions or at a subset of six SNP positions were submitted by the laboratories independently of each other to Labquality, by whom the data was analyzed without knowledge of the genotyping methods used in each laboratory. A consensus genotype that was considered to be the correct one for each DNA sample and SNP was determined by Labquality, and the results from each individual laboratory were compared to this consensus genotype. Labquality reported back only the genotyping results produced by a particular laboratory, together with the consensus genotype. The participating laboratories made their genotyping results available for publication after completion of the QA round.

RESULTS AND DISCUSSION

The overall result from the QA survey was excellent (Tables 2 and 3). Four out of eight laboratories submitted genotypes for all 18 SNPs genotyped. Two laboratories delivered results for 17 SNPs, one for 16 SNPs, and one for 15 SNPs. Three laboratories genotyped the smaller subset of six SNPs, and one laboratory analyzed four out of the six SNPs in this subset. The reason for the failed SNP assays included assay design failure due to a complex flanking sequence, unspecified reasons for poor quality of the genotyping signals, and in one case the reason for failure was an administrative misunderstanding. Most laboratories did not attempt to redesign failed SNP assays, although in a real situation this would be done for SNPs of key importance. No laboratory delivered any genotypes for the negative control sample. One of the

TABLE 1. Overview of Participating Laboratories and SNP Genotyping Procedures

	Laboratory or organization	Genotyping method	Amount of DNA used per reaction/SNP (ng)[a]	Number of SNPs attempted	Reference
A	Umeå CGR	Real-time PCR by the 5′ nuclease TaqMan assay	10	18	Livak [1999]
B	CGB, KI, Stockholm	Dynamic Allele-Specific Hybridization (DASH)	5	18	Jobs et al. [2003]
C	Folkhälsan, Helsinki	Real-time PCR by the 5′ nuclease TaqMan assay	10	6	Livak [1999]
D	SNP Platform, Uppsala[b]	Minisequenicng using GenomeLab SNPstream	4/0.4–4.0	18	Bell et al. [2002]
E	SNP Platform, Uppsala[b]	Minisequencing with fluorescence polarization detection (FP-TDI)	4	6	Chen et al. [1999]
F	CRC, KI Huddinge	MassARRAY MALDI-TOF-MS	2.5	18	Jurinke et al. [2002]
G	KTH, Stockholmb	Pyrosequencing	5	18	Ronaghi et al. [1998]
H	Clinical Chemistry, Malmö	Mass ARRAY MALDI-TOF-MS	2.5	6	Jurinke et al. [2002]
I	Asper Biotech, Tartu	Arrayed primer extension (APEX)	15	18	Kurg et al. [2000]
J	KTL, Helsinki	Allele-specific primer extension on microarrays	10/0.8–2.5	18	Pastinen et al. [2000]
K	Finnish Genome Center	Mass ARRAY MALDI-TOF-MS	6.6	18	Jurinke et al. [2002]
L	Radiumhospitalet, Oslo	NanoChip Electronic Microarray	3	6	Edman et al. [1997]

[a]The amount corresponds to the total amount of DNA and amount DNA per SNP in case multiplexed assays were used.
[b]Operated under a quality system at the time of the QA survey. The laboratories have thereafter received accreditation according to the standard ISO/IEC 17025 by the Swedish accreditation body SWEDAC.
CGR, Center for Genome Research; CGB, Center for Genomics and Bioinformatics; CRC, Clinical Research Centre; KI, Karolinska Institute; KTH, Royal Institute of Technology; KTL, National Public Health Institute.

TABLE 2a. SNPs Genotyped in the Nordic SNP Quality Assessment Project and the Success and Accuracy Values for Each Individual SNP in Each Individual Laboratory that Analyzed 18 SNPs

			Lab A		Lab B		Lab D		Lab F		Lab G		Lab I		Lab J		Lab K	
SNP#	rs#	Genomic position	Success (%)	Accuracy (%)	Success (%)	Accuracy (%)	Success (%)	Accuracy (%)	Success (%)	Accuracy (%)	Success (%)	Accuracy (%)	Success (%)	Accuracy (%)	Success (%)	Accuracy (%)	Success (%)	Accuracy (%)
1	rs746320	NT_004511.17:g.15286895C>G	95.7	100	100	100	0.0	–	97.9	100	100	89.4	97.9	100	95.7	100	95.7	100
2	rs2364731	NT_005403.15:g.28357621G>A	0.0	–	97.9	97.8	97.9	100	91.5	100	100	100	95.7	100	100	100	93.6	100
3	rs777886	NT_005612.14:g.10929882T>A	no design	–	95.7	100	95.7	100	100	100	100	100	97.9	100	95.7	100	91.5	100
4	rs4306954	NT_016354.17:g.29798285T>C	97.9	100	100	100	97.9	100	97.9	100	100	100	97.9	100	97.9	100	95.7	100
5	rs2219584	NT_010966.13:g.10560889G>T	95.7	100	100	100	100	100	97.9	100	100	100	97.9	100	97.9	100	95.7	100
6	rs2056218	NT_019546.15:g.7014731C>A	95.7	100	97.9	100	97.9	100	93.6	100	100	100	91.5	97.9	89.4	100	95.7	100
7	rs1403294	NT_008046.15:g.17563951A>G	95.7	100	97.9	100	97.9	100	95.7	100	100	100	97.9	97.9	95.7	97.9	95.7	100
8	rs3781058	NT_035040.4:g.1608795C>T	97.9	100	100	100	93.6	100	93.6	100	100	100	97.9	100	0.0	–	95.7	100
9	rs1395504	NT_033899.7:g.36291583G>T	95.7	100	97.9	100	97.9	100	93.6	100	100	100	87.2	91.5	97.9	100	93.6	100
10	rs617335	NT_033927.7:g.13580307C>G	95.7	100	100	97.9	97.9	100	97.9	100	100	100	97.9	100	91.5	100	95.7	100
11	rs7994365	NT_024524.13:g.23847954G>A	97.9	100	97.9	100	95.7	100	93.6	100	100	100	97.9	100	74.5	100	95.7	100
12	rs3742786	NT_026437.11:g.56372764G>A	0.0	–	0.0	–	100	100	97.9	100	100	100	97.9	100	0.0	–	95.7	100
13	rs1465471	NT_010498.15:g.22010576C>T	95.7	100	100	100	100	100	100	100	100	100	97.9	100	97.9	100	95.7	100
14	rs8110400	NT_011109.15:g.30106116C>A	95.7	100	100	97.9	97.9	100	93.6	100	100	100	97.9	100	97.9	100	95.7	100
15	rs1981431	NT_011362.9:g.9028366C>A	97.9	100	100	100	100	100	95.7	100	100	100	97.9	100	97.9	100	95.7	100
16	rs744533	NT_011362.9:g.26055927T>C	95.7	100	100	100	97.8	100	93.5	100	100	100	97.8	100	95.7	100	97.8	100
17	rs1500320	NT_008413.16:g.8355047G>A	93.6	100	100	100	97.9	100	87.2	100	100	100	97.9	100	95.7	100	95.7	100
18	rs139842	NT_011520.10:g.17663444T>C	93.6	100	100	100	97.9	100	100	97.9	100	100	97.9	97.9	97.9	100	95.7	100
Average			84.7	100	93.6	99.6	92.4	100	95.6	99.9	100	99.4	96.8	99.1	84.4	99.9	95.4	100

47 DNA samples included in the survey was intentionally of poor quality. Three laboratories (Labs I, K, and L) chose not to deliver any results for this sample, and two laboratories (Labs I and K) reported the poor quality of the sample. Another DNA sample appeared to be unintentionally contaminated, as noted by two of the laboratories (Labs B and K). Both laboratories alerted the organizers about this potential problem, and one of them (Lab K) chose not to deliver any results for the contaminated sample, whereas the other group (Lab B) flagged their results as unreliable for SNPs ♯10 and ♯14. Several other labs noticed the problem indirectly, and delivered fewer genotypes for the samples of poor quality. Apparently, the method used for calling the genotypes indicated a problem, and led to failing the results from these particular samples. This is seen as lower success rates in Tables 2 and 3. However, discrepant genotyping results for nine out of 18 SNPs were also delivered for these two poor samples by several laboratories (Table 3). The accuracy values given in Table 3 were calculated for each SNP, both including and omitting the two poor samples.

The overall genotyping accuracy achieved in the survey was excellent. Six out of the 11 laboratories delivered genotype data that were in full agreement with the consensus genotype. In three out of the five laboratories who reported genotypes that differed from the consensus genotype, the discrepant results were caused by a single SNP in each laboratory, and in one laboratory by single discrepancies in two SNPs. In general, these SNPs differed between laboratories, although in two cases they coincided. Three SNPs (♯1, ♯9, and ♯12) yielded discrepant results for more than a single sample. Tables 2 and 3 provides the success and accuracy values for each individual SNP in each individual lab. After reporting the genotype data to Labquality, the reason for the discrepant result for SNP ♯12 in Lab B turned out to be an additional SNP (rs 3742785) in the binding site for one of the PCR primers and this SNP was withdrawn from the summary results in Table 3. The reasons for the other discrepant results remain unknown, but in these cases no known SNPs (according to dbSNP) were located in the primer-binding sites. The average accuracy per SNP varied from 99.1 to 100% between the laboratories, and it was frequently 100% for the majority of the assays for which SNP genotypes were reported.

It can be concluded from the results of this survey that all participating laboratories meet the high standards expected from experienced scientific laboratories, core facilities, or genotyping services, and that their genotype data can be utilized with high confidence. This was the case, despite the fact that the number of samples and SNPs included in our survey was suboptimal in relation to the optimal throughput or level of automation of the routine workflow in the participating laboratories. No significant differences in accuracy or success rates were detected between the genotyping methods deployed, although it would be plausible that the methods would have different susceptibilities to sequence-dependent problems. Genotyping errors due to unknown SNPs or SNPs not allowed for in a primer- or probe-binding site is a potential pitfall, irrespective of the genotyping method used. Due to the low number of samples in our survey, systematic genotyping errors due to additional SNPs in primer- or probe-binding sites would have remained unnoticed based on departure from Hardy-Weinberg equilibrium. Although the quality control steps differed between the laboratories, as can be expected due to the different needs for different laboratory setups and methodologies, no single step in the quality control procedures of the individual laboratories explains the sporadic cases in which problems occurred. One of the results of the study is that special attention should be given to the quality of the DNA samples prior to genotyping. The results also show that a conservative approach for

TABLE 2b. SNPs Genotyped in the Nordic SNP Quality Assessment Project and the Success and Accuracy Values for Each Individual SNP in Each Individual Laboratory that Analyzed Six SNPs

			Lab C		Lab E		Lab H		Lab L	
SNP#	rs#	Genomic position	Success (%)	Accuracy (%)	Success (%)	Accuracy (%)	Success (%)	Accuracy (%)	Success (%)	Accuracy (%)
1	rs746320	NT.004511.17:g.15286895C>G	100	100	100	100	100	100	97.9	100
2	rs2364731	NT.005403.15:g.28357621G>A	100	100	93.6	100	100	100	97.9	100
3	rs777886	NT.005612.14:g.10929882T>A	100	100	87.2	100	100	100	89.4	97.9
4	rs4306954	NT.016354.17:g.29798285T>C	100	100	100	100	100	100	97.9	100
5	rs2219584	NT.010966.13:g.10560889G>T	97.9	100	97.9	100			97.9	97.9
6	rs2056218	NT.019546.15:g.7014731C>A	100	100	97.9	100			89.4	97.9
Average			99.6	100	96.1	100	100	100	95.0	98.9

TABLE 3. Overview of SNP Genotyping Success Rate and Accuracy

Laboratory	Success rate[a]	Number of SNPs with delivered genotypes	SNPs with failed genotypes[b]	SNPs with discrepancies compared to consensus[b]	Mean accuracy per SNP (%), including poor samples[c]	Mean accuracy per SNP (%), omitting poor samples[c]
A	84.7	15	#2, #3, #12		100	100
B	93.6	17	#12	#2, (#10[d], #14[d])	99.6	99.9
C	99.6	6			100	100
D	92.4	17	#1		100	100
E	96.1	6			100	100
F	95.6	18		(#18)	99.9	100
G	100	18		#1	99.4	99.4
H	100	4			100	100
I	96.8	18		#9, (#6, #7, #18)	99.1	99.4
J	84.4	16	#8, #12	#7	99.9	99.9
K	95.4	18			100	100
L	95.0	6		#5, #6, (#3)	98.9	99.3

[a]Percentage of delivered genotypes out of the maximal number of SNPs attempted in 47 samples.
[b]SNP designations as in Table 1. SNPs with discrepancies only in the samples of poor quality are given in parentheses.
[c]Accuracy in relation to the consensus genotype. Poor samples correspond to one intentionally poor sample and one unintentionally contaminated sample.
[d]Genotypes called but flagged as unreliable.

calling the genotypes should be used to achieve a high accuracy at the cost of a lower genotyping success rate.

All participating laboratories have expressed their interest in continuing comparative analysis between SNP methodologies and quality assessment procedures to learn from possible pitfalls to maintain good quality of their SNP genotyping. Such procedures are customary, and even mandatory, in clinical and forensic laboratories, but have not to our knowledge been available for research-based genotyping laboratories. Thus, new regular QA rounds for laboratories involved in large, costly genetic epidemiological studies are welcomed in the future.

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