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Development and cross-validation of sequencing-based assays for genotyping common polymorphisms of the *CXCL5* gene

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Abstract

Background: Epithelial neutrophil activating peptide (ENA-78) is encoded by the polymorphic *CXCL5* gene and is a recruiter and activator of neutrophils. Furthermore, ENA-78 may be involved in pathological inflammatory processes and variable drug responses.
Methods: To facilitate future disease–gene and pharmacogenetic investigation of ENA-78, we developed and cross-validated medium- to high-throughput genotyping assays for 2 commonly occurring *CXCL5* polymorphisms (rs352046 and rs425535). Furthermore, we compared allele and genotype frequencies in a U.S. population with those of a previously studied European population.
Results: There was 100% genotype concordance between the 2 methods used (Pyrosequencing® and TaqMan®). Variant allele frequencies for rs352046 were consistent between the U.S. (16%) and European (16%) populations, while the rs425535 variant allele was more than twice as high in the European cohort (38% vs. 16%). There was complete linkage of genotypes at both loci in our population.
Conclusions: The distribution of variant alleles for the 2 polymorphisms studied should be further evaluated in other populations. In addition, our data highlight the importance of assay validation using multiple platforms.

Keywords: ENA-78; CXCL5; Polymorphism; Genotype; Pyrosequencing; TaqMan

1. Introduction

Neutrophil activation is often a component of detrimental inflammatory processes underlying many diseases. Therefore, genotype determination for polymorphisms in genes related to neutrophil activation represents a potentially important tool in molecular diagnostics and pharmacogenetics. Epithelial neutrophil activating peptide (ENA-78) has been shown to be a recruiter of neutrophils and involved in their activation [1]. This C–X–C chemokine has been implicated in pulmonary disease, lung cancer, arthritis, and other pathological states [2–6]. Furthermore, we have recently showed ENA-78 production from endothelial cells to be reduced upon treatment with

Table 1
Genotype distributions and comparisons with previous reports

Genotype[a]	Pyrosequencing®/TaqMan®	Previously reported[b]
−156G→C	*N*=60	*N*=161
G/G	42 (70%)	113 (70%)
G/C	17 (28%)	43 (27%)
C/C	1 (2%)	5 (3%)
Allele		
G	84%	84%
C	16%	16%
398G→A	*N*=60	*N*=63
G/G	42 (70%)	20 (32%)
G/A	17 (28%)	38 (60%)
A/A	1 (2%)	5 (8%)
Allele		
G	84%	62%
A	16%	38%

[a] Genotypes were 100% concordant between methods.
[b] From [8].

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atorvastatin, suggesting a possible role for ENA-78 in cardiovascular drug responses [7].

ENA-78 is encoded by the *CXCL5* gene which has recently been shown to contain 2 single nucleotide polymorphisms (SNPs) with relatively high variant allele frequencies (q=0.14–0.38 in European populations) [8]. The first polymorphism is described as a G-to-C change at position −156 of the *CXCL5* promoter (rs352046), and the second polymorphism is a synonymous G-to-A SNP at nucleotide 398 of exon 2 (rs425535).

While the functional relevance of these SNPs is unknown, their high minor allele frequencies as well as their gene locations make them candidate SNPs for disease–gene association studies [9]. Furthermore, to push application of genotype-guided diagnostics and therapy forward, medium- to high-throughput assays must be available to serve as a rapid aid to research and practice. As such, we sought to develop and cross-validate assays using 2 sequencing-based methods for genotype determination of the described *CXCL5* −156G→C and 398G→A SNPs. Specifically, assays were developed for the non-gel, luciferase-based Pyrosequencing™ platform (Biotage, Uppsala, Sweden) and compared with commercially available assays for the fluorescence-based TaqMan® platform (Applied Biosystems, Foster City, USA). Furthermore, because of the paucity of data regarding population distribution of *CXCL5* variant alleles, we also sought to compare allele and genotype frequencies in a U.S. population with those of a European population.

2. Materials and methods

Allele frequencies were quantified for 60 consecutively enrolled healthy individuals, and concordance of genotypes was

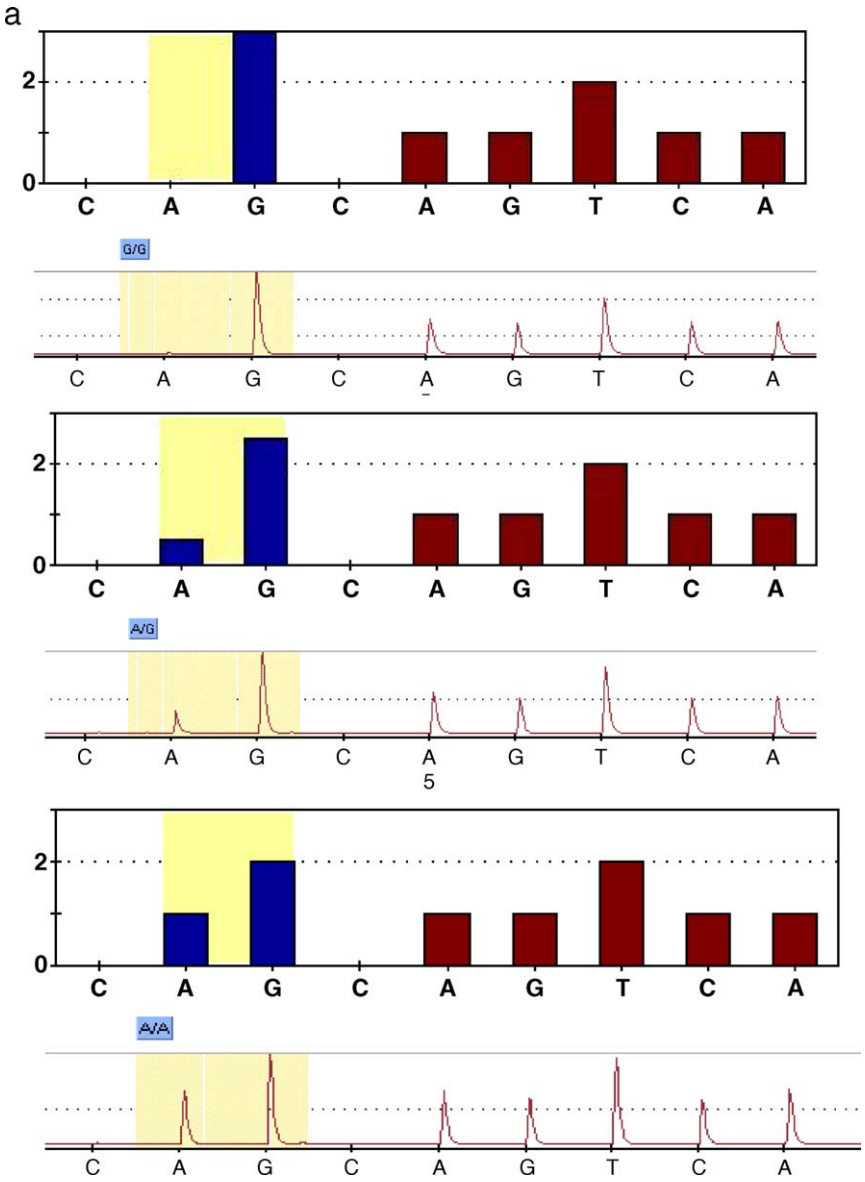

Fig. 1. Visual genotype outputs for *CXCL5* 398G→A polymorphism by 2 methods. a) Theoretical histogram and actual pyrograms for *CXCL5* 398G→A genotypes using Pyrosequencing®. b) Clustering of genotypes for *CXCL5* 398G→A polymorphism using TaqMan®.