Supplemental Methods

Genotyping

MBL2

The target SNPs were amplified in multiplexed PCR reactions using a HotStar Taq Polymerase Kit (Qiagen) with the following conditions: 2.5mM $MgCl_2$, 0.25mM dNTPs, 10nmole of each primer and 0.25U of the polymerase and 50ng of genomic DNA. For some multiplexed PCR reactions 1X Q solution was added to increase the specificity of reaction (Table 2). The MJ Research PTC 225 Thermocycler conditions were as follows: initial activation at 95^0C for 15 min, followed by 32 cycles of 94^0C for 30 sec, 59^0C for 90 sec, 72^0C for 1 min. The amplicons were treated with mixture of Shrimp Alkaline Phosphatase and Exonuclease (1U/20μl reaction each) for 1hour at 37^0C and 95^0C for 5 min. The Allele Specific Primer Extension (ASPE) reactions were performed using Platinum *Tsp* Polymerase (Invitrogen) with 1.5 mM $MgCl_2$, 0.25 mM dNTPs (except biotin-dCTP at 0.1mM) and mixed ASPE primers (0.1pmole each, Table 2). The cycler conditions were as follows: initial denaturation at 95^0C for 2 min, followed by 16 cycles of 94^0C for 10 sec, annealing at 62^0C, decreasing 0.5^0C with each cycle for 1 min, extension at 72^0C for 30 sec, followed by 16 cycles of 94^0C for 10 sec, annealing at 55^0C for 1 min, extension at 72^0C for 30 sec. The ASPE reactions were hybridized with Luminex FlexMap100 beads (1250 of each bead/well) in 50μl 1X Wash Buffer (10X contains 2M NaCl, 1M Tris, 0.8% Triton X-100). Hybridization was performed 92^0C for 2 min followed by 37^0C for 30-60 min. The hybridized beads were filtered through a Millipore MultiScreen filter plate on a vacuum station and were washed once with 150μl of 1X Wash Buffer and developed with Streptavidin-Phycoerythrin (0.15μl /reaction) in 150μl of 1X Wash Buffer with shaking for 10 min. The reactions were analyzed on the Luminex LiquiChip Flowcytometry Reader. The raw

data were exported to the internal database where alleles were annotated using a proprietary algorithm.

The genotyping data generated by the method described above was verified by sequencing.

Measuring of MBL2 levels in plasma

The MBL2 LiquiChip assay was developed in house based on the 131-01 monoclonal antibodies (AntibodyShop). Since active MBL2 is only capable of binding Mannose in multimeric form and all missense mutations in the *MBL2* gene abolish the multimerization of the mutant MBL2 protein, we developed a bead based assay that utilizes the same antibody for binding (131-01) and detection (131-01B, biotin modified). To couple the LiquiChip Activated Beads (Qiagen, Cat. No: 922543) to the antibodies the bead stock was vortexed for 30 sec at full speed and another 30 min in the dark to completely resuspend the beads. 100 µl of LiquiChip Activated Beads suspension was pipetted into a 1.5 ml siliconized polypropylene copolymer reaction microtubes (Fisher Scientific, cat. no. 3544350). The antibody was diluted in coupling buffer (50 mM MES, pH 6.5) to a concentration of 0.2 mg/ml in a volume of 50 µl (10 µg protein) and was added to the beads. The beads were incubated for 2hr in the dark at room temperature with shaking. The bead suspension was centrifuged for 3 min at 10,000 x *g* and the supernatant was removed stepwise in small aliquots to minimize bead loss. Beads were washed by resuspension in 500 µl of PBS and after centrifugation for 3 min at 10,000 x *g* the supernatant was discarded. The beads were stored in 100 µl PBS/1% BSA at a concentration 1.25 x10^5 beads/ml at 4°C in the dark. Plasma samples were cleared by centrifugation for 5 min at full speed, the supernatant was additionally filtered through a Millipore MultiScreen filter plate by centrifugation at 3500rpm for 5 min. The samples were diluted at least 1:2, e.g. 50µl plasma plus 100µl of

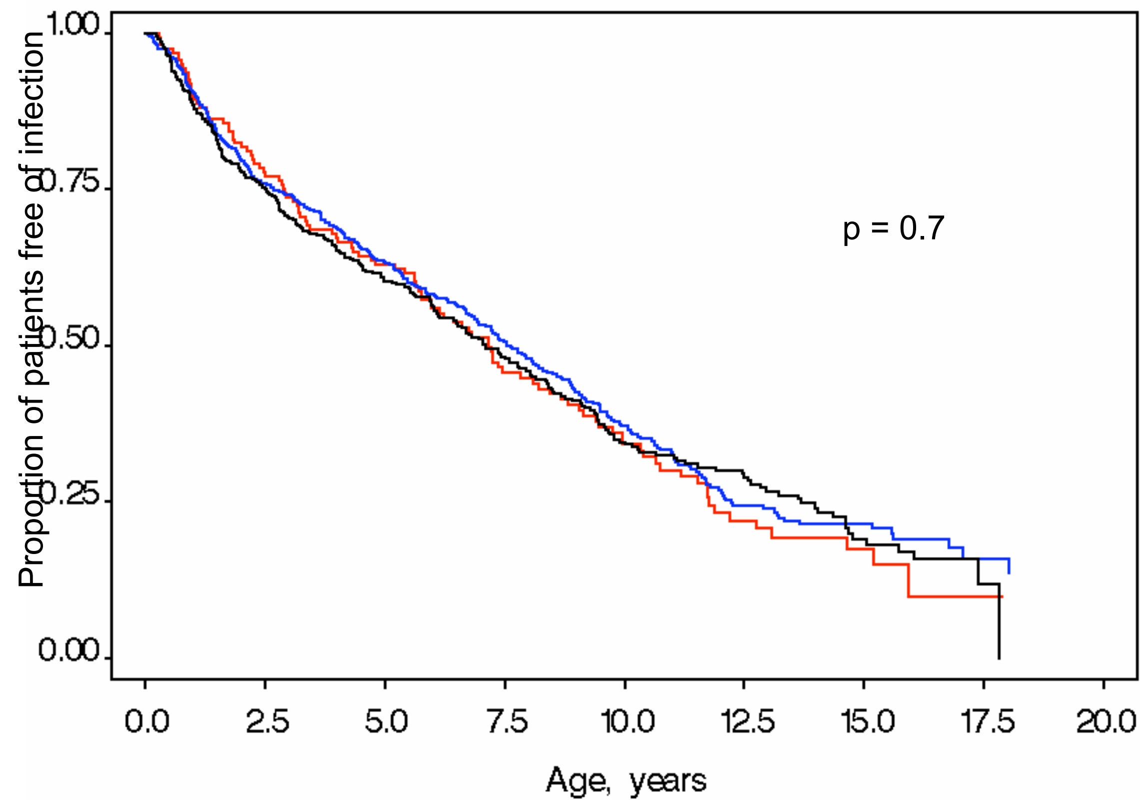
LiquiChip Human Serum Dilution Buffer (Qiagen, Cat No: 922300) and loaded on the Millipore MultiScreen filter plates. Next 20 µl beads mixture (diluted 1/20 in PBS/BSA, equivalent to 1250 beads per well) was added per well, mixed for 10 sec on a microplate shaker at 850rpm and incubated for 2 hours in the dark at room temperature while shaking. The beads were washed twice - drained on vacuum, resuspended in 150µl PBS-TW, vortexed and vacuumed again. The biotinylated secondary antibodies were diluted in PBS-TW (1:5000 for 131-01B) and 100µl of diluted antibodies was added to each well and incubated for 1.5 hours in the dark at room temperature on a microplate shaker at 850 rpm. The reactions were developed with addition of 100 ng Streptavidin–R-PE in a volume of 10 µl, incubated for 30 min in the dark. The reactions were analyzed on Luminex^{100} LiquiChip Reader, according to manufacturer instructions. Minimum of 100 beads were counted for each analyte and Mean Fluorescence Index (MFI) values were calculated by the software.

Supplemental References

Madsen HO, Garred P, Thiel S, Kurtzhals JA, Lamm LU, Ryder LP, Svejgaard A. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol. 1995 Sep 15;155(6):3013-20.

Taylor JD, Briley D, Nguyen Q, Long K, Iannone MA, Li MS, Ye F, Afshari A, Lai E, Wagner M, Chen J, Weiner MP. Flow cytometric platform for high-throughput single nucleotide polymorphism analysis. Biotechniques. 2001 Mar;30(3):661-6, 668-9.

Ye F, Li MS, Taylor JD, Nguyen Q, Colton HM, Casey WM, Wagner M, Weiner MP, Chen J. Fluorescent microsphere-based readout technology for multiplexed human single nucleotide polymorphism analysis and bacterial identification. Hum Mutat. 2001 Apr;17(4):305-16.



Supplemental Figure 1. The age at first infection with *P. aeruginosa* in the pediatric CF cohort (n=1003; <18.5 years) stratified by the *TGFB1* genotype of codon10, showed no effect of polymorphism in the *TGFB1* gene on the onset of infection (p = 0.7).

Supplemental Table 1. Primer sequences for the PCR amplification of DNA fragments containing four *MBL2* gene variants and for ASPE primers.

PCR primers	
MBL_ex1F	CCTGTAGCTCTCCAGGCATC
MBL_ex1R	CAGGCAGTTTCCTCTGGAAG
MBL_XY promoterF	CACCTGGGTTTCCACTCATT
MBL_XY promoterR	CCTTGTGACACTGCGTGACT
rs1800469/TGFb1(-509)F	GTTGAGTGACAGGAGGCTGCTT
rs1800469/TGFb1(-509)R	AGGCTGGGAAACAAGGTAGGAG
rs2241715/TGFb1(intron)F	CAATCCTCTTCTCCCCAACA
rs2241715/TGFb1(intron)R	TACTCAGCAAACCCCAAAGG
ASPE primers	
rs1800469/TGFb1(-509)T:12*	TACACTTTCTTTCTTTCTTTCTTTGCCTCCTGACCCTTCCATCCT
rs1800469/TGFb1(-509)C:94*	CTTTCTATCTTTCTACTCAATAATGCCTCCTGACCCTTCCATCCC
rs2241715/TGFb1(intron)C:6*	CTTTTACAATACTTCAATACAATCAGACAGACCTCCCGCCCTGGGAGAG
rs2241715/TGFb1(intron)G:20*	CTTTTACAATACTTCAATACAATCAGACAGACCTCCCGCCCTGGGAGAG
rs1800451/MBL57G:59*	TCATCAATCAATCTTTTTCACTTTACCTGGTTCCCCCTTTTCTC
rs1800451/MBL57A:30*	TTACCTTTATACCTTTCTTTTTACACCTGGTTCCCCCTTTTCTT
rs5030737/MBL52C:28*	CTACAAACAAACAAACATTATCAACTTCCCAGGCAAAGATGGGC
rs5030737/MBL52T:46*	TACATCAACAATTCATTCAATACACTTCCCAGGCAAAGATGGGT
rs1800450/MBL54C:12*	TACACTTTCTTTCTTTCTTTCTTTTTCCCCCTTTTCTYCCTTGGTGC
rs1800450/MBL54T:37*	CTTTTCATCTTTTCATCTTTCAATTTCCCCCTTTTCTYCCTTGGTGT
rs7096206/MBL_XY_C:30*	TTACCTTTATACCTTTCTTTTTACCCATTTCTTCTCACTGCCACC
rs7096206/MBL_XY_G:59*	TCATCAATCAATCTTTTTCACTTTCCATTTCTTCTCACTGCCACG

* Please note: 1. First 21 bp correspond to an anti-Zip sequence of code indicated by the last two digits in the end of ASPE primer name. 2. For tagging the ASPE primers TagIT™ program (TmBioscience) was used: https://tagit.luminexcorp.com/tagit/welcome.jsp. 3. All ASPE reactions were done in separate multiplexed to avoid primer-dimer formation, therefore the same codes for Zip tags were used.

Supplemental Table 2. Allelic, genotypic and diplotypic frequencies in the CF patient and parent cohorts

<table>
<tr><td></td><td colspan="6"></td><td colspan="6"></td></tr>
<tr><td colspan="13">MBL2 promoter variant X and Y</td></tr>
<tr><td></td><td colspan="6">Patients</td><td colspan="6">Parents</td></tr>
<tr><td>Allele</td><td colspan="3">X</td><td colspan="3">Y</td><td colspan="3">X</td><td colspan="3">Y</td></tr>
<tr><td>Frequency</td><td colspan="3">21.50</td><td colspan="3">78.50</td><td colspan="3">22.17)</td><td colspan="3">77.83</td></tr>
<tr><td colspan="13">MBL2 variants A, B,C and D</td></tr>
<tr><td></td><td colspan="6">Patients</td><td colspan="6">Parents</td></tr>
<tr><td>Allele</td><td colspan="3">0</td><td colspan="3">A</td><td colspan="3">0</td><td colspan="3">A</td></tr>
<tr><td>Frequency</td><td colspan="3">21.07</td><td colspan="3">78.93</td><td colspan="3">21.58</td><td colspan="3">78.42</td></tr>
<tr><td colspan="13">MBL2 XY genotypes</td></tr>
<tr><td></td><td colspan="6">Patients</td><td colspan="6">Parents</td></tr>
<tr><td>Genotype</td><td colspan="2">XX</td><td colspan="2">XY</td><td colspan="2">YY</td><td colspan="2">XX</td><td colspan="2">XY</td><td colspan="2">YY</td></tr>
<tr><td>Frequency
N (%)</td><td colspan="2">63
(4.52)</td><td colspan="2">473
(33.96)</td><td colspan="2">857
(61.52)</td><td colspan="2">27
(4.50)</td><td colspan="2">212
(35.33)</td><td colspan="2">361
(60.17)</td></tr>
<tr><td colspan="13">MBL2 A0 genotypes</td></tr>
<tr><td></td><td colspan="6">Patients</td><td colspan="6">Parents</td></tr>
<tr><td>Genotype</td><td colspan="2">00</td><td colspan="2">A0</td><td colspan="2">AA</td><td colspan="2">00</td><td colspan="2">A0</td><td colspan="2">AA</td></tr>
<tr><td>Frequency
N (%)</td><td colspan="2">52
(3.73)</td><td colspan="2">483
(34.67)</td><td colspan="2">858
(61.60)</td><td colspan="2">20
(3.33)</td><td colspan="2">219
(36.50)</td><td colspan="2">361
(60.17)</td></tr>
<tr><td colspan="13">MBL2 diplotypes</td></tr>
<tr><td></td><td colspan="6">Patients</td><td colspan="6">Parents</td></tr>
<tr><td>Diplotype</td><td>00_**</td><td>A0_XY</td><td>A0_YY</td><td>AA_XX</td><td>AA_XY</td><td>AA_YY</td><td>00_**</td><td>A0_XY</td><td>A0_YY</td><td>AA_XX</td><td>AA_XY</td><td>AA_YY</td></tr>
<tr><td>Frequency
N (%)</td><td>52
(3.73)</td><td>123
(8.83)</td><td>360
(25.84)</td><td>63
(4.52)</td><td>350
(25.13)</td><td>445
(31.95)</td><td>20
(3.33)</td><td>62
(10.33)</td><td>157
(26.17)</td><td>27
(4.50)</td><td>150
(25.00)</td><td>184
(30.67)</td></tr>
</table>

Supplemental Table 3. Pair-wise comparison of normalized MBL2 levels between CF patient and parents by the diplotype group.

Diplotype	Median and SD in parents	Median and SD in CF patients	Delta _median	p-value
00_YY	-1.14 ± 0.32	-0.98 ± 0.36	0.16	0.20
A0_XY	-1.13 ± 0.41	-0.86 ±0.70	0.27	<0.0001
A0_YY	-0.33 ± 0.59	0.03 ±0.70	0.36	<0.0001
AA_XX	0.21 ± 0.77	0.97 ± 0.70	0.76	0.0007
AA_XY	1.02 ± 0.57	1.46 ± 0.69	0.44	<0.0001
AA_YY	1.38 ± 0.58	1.68 ± 0.65	0.30	<0.0001

Supplemental Table 4. CFTR alleles in 1019 PI patients analyzed in the study.

Mutation	Number of chromosomes	Proportion (%)
ΔF508	1546	75.86
621+1G>T	57	2.80
G542X	42	2.06
N1303K	30	1.47
G551D	29	1.42
M1101K	24	1.18
ΔI507	20	0.98
711+1G>T	17	0.83
S489X	15	0.74
G85E	12	0.59
W1282X	11	0.54
R553X	10	0.49
Y1092X	9	0.44
1154insTC	7	0.34
2183AA>G	7	0.34
R1162X	7	0.34
3659delC	6	0.29
Q1313X	6	0.29
Q493X	6	0.29
4016insT	5	0.25
1717-1G>A	4	0.20
2184insA	4	0.20
3120+1G>A	4	0.20
3905insT	4	0.20
S4X	4	0.20
3876delA	3	0.15
P574H	3	0.15
R560T	3	0.15
S549N	3	0.15
1138insG	2	0.10
2184delA	2	0.10
3007delG	2	0.10
394delTT	2	0.10
406-1G>A	2	0.10
A559T	2	0.10
CFTRdele2-4	2	0.10
E585X	2	0.10
E60X	2	0.10
F508C	2	0.10
G480S	2	0.10
L1254X	2	0.10
Q220X	2	0.10
Q814X	2	0.10
R1066C	2	0.10
R1158X	2	0.10
R709X	2	0.10

R75X	2	0.10
R785X	2	0.10
V520F	2	0.10
1078delT	1	0.05
1161delC	1	0.05
1249-27delTA	1	0.05
1288insTA	1	0.05
1525-1G>A	1	0.05
1717-1G>T	1	0.05
2622+1G>A	1	0.05
2789+5G>A	1	0.05
2951insA	1	0.05
3199del6	1	0.05
3447delG	1	0.05
3600G>A	1	0.05
3617delGA	1	0.05
3662delA	1	0.05
3850-3T>G	1	0.05
4022insT	1	0.05
4089delA	1	0.05
557delT	1	0.05
C276X	1	0.05
CFTRdele10	1	0.05
CFTRdele17a-18	1	0.05
CFTRdele2	1	0.05
G458V	1	0.05
H199R	1	0.05
I148T/3199del6	1	0.05
L1077P	1	0.05
L206W	1	0.05
L218X	1	0.05
L468P	1	0.05
Q483X	1	0.05
Q890X	1	0.05
R1070Q	1	0.05
S1118C	1	0.05
S466X	1	0.05
S549R	1	0.05
V1240G/R75Q	1	0.05
W1274X	1	0.05
Negative	63	3.09
TOTAL	2038	100.00

Overall distribution of 1019 CFTR genotypes:
ΔF508/ΔF508 = 611
ΔF508/Other = 324
Other/Other = 84
where "Other" is a mutation listed in the table or "negative".

Supplemental Table 5. Member institutions of the Canadian Consortium for CF Genetic Studies

Institution	Location
Alberta Children's Hospital	Calgary, Alberta, Canada
BC Children's Hospital	Vancouver, British Columbia, Canada
Centre Hospitalier de Gatineau.	Gatineau, Quebec, Canada
Centre Hospitalier de L'Universite	Laval, Quebec, Canada
Centre Hospitalier Regional de L'Outaouais	Hull, Quebec, Canada
Centre Hospitalier Regional de Rimouski	Rimouski, Quebec, Canada
Centre Universitaire de Sante de L'Estrie	Fleurimont, Quebec, Canada
Children's Hospital of Eastern Ontario	Ottawa, Ontario, Canada
Children's Hospital of Western Ontario	London, Ontario, Canada
Children's Hospital of Winnipeg - Pediatric CF Clinic	Winnipeg, Manitoba, Canada
Foothills Medical Centre - Adult CF Clinic	Calgary, Alberta, Canada
Grand River Hospital	Kitchener, Ontario, Canada
Hamilton Health Science Centre - Pediatric CF Clinic	Hamilton, Ontario, Canada
Hamilton Health Science Centre - Adult CF Clinic	Hamilton, Ontario, Canada
Health Sciences Centre-Adult CF Clinic	Winnipeg, Manitoba, Canada
Hopital de Chicoutimi	Chicoutimi, Quebec, Canada
Hopital Sainte-Justine	Montreal, Quebec, Canada
Hospital for Sick Children	Toronto, Ontario, Canada
Hospital Pediatric Clinic	Saskatoon, Saskatchewan, Canada
Hospitalier Rouyn-Noranda	Rouyn-Noranda, Quebec, Canada
Hotel Dieu de Montreal	Montreal, Quebec, Canada
Hotel Dieu Hospital	Kingston, Ontario, Canada
IWK Health Centre– Pediatric Clinic	Halifax, Nova Scotia, Canada
Janeway Children's Health Centre	St. John's, Newfoundland, Canada
Montreal Chest Institute	Montreal, Quebec, Canada
Montreal Children's Hospital	Montreal, Quebec, Canada
Ottawa Genera Hospital	Ottawa, Ontario, Canada
Queen Elisabeth Health Sciences Centre –Adult Clinic	Halifax, Nova Scotia, Canada
Regina General Hospital	Regina, Saskatchewan, Canada
Royal Jubilee Hospital	Victoria, British Columbia, Canada
Royal University Hospital - Adult Clinic	Saskatoon, Saskatchewan, Canada
Royal University Hospital - Pediatric Clinic	Saskatoon, Saskatchewan, Canada
St. John Regional Hospital.	St. John, NB, Canada
St. Michael's Hospital	Toronto, Ontario, Canada
St. Paul's Adult CF Clinic	Vancouver, British Columbia, Canada
Sudbury Regional Hospital	Sudbury, Ontario, Canada
University of Alberta Adult Clinic	Edmonton, Alberta, Canada
University of Alberta Pediatric Clinic	Edmonton, Alberta, Canada
Victoria General Hospital	Victoria, British Columbia, Canada